ISOPYRAZAM (249)

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EXPLANATION

Isopyrazam is a broad-spectrum foliar fungicide belonging to the chemical class of ortho-substituted phenyl amides. It controls a wide range of fungal pathogens including *Septoria tritici*, *Puccinia recondita* and *Puccinia striformis* on wheat, *Pyrenophora teres*, *Rhynchosporium secalis* and *Ramularia collocygni* on barley, *Puccinia recondita* on rye and triticale and *Pyrenophora avenae* on oats. It also controls *Mycospaerella fijiensis* on banana.

Isopyrazam contains two diastereoisomers designated *syn*- and *anti*-isomers. Both of these isomers are biologically active and the specification for technical isopyrazam covers the range of *syn*:*anti* isomer ratios from 70:30 to 100:0.

Isopyrazam was first registered in 2010 and, since then, it has been registered in eight countries by the time of the current Meeting.

It was identified as a priority new compound at the Forty-second Session of the CCPR in 2010 (ALINORM 10/33/24, para. 167) for evaluation by the 2011 JMPR. The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, processing and farm animal feeding.

The specification of isopyrazam has not been developed by the Joint FAO/WHO Meeting on Pesticide Specification yet.

IDENTITY

ISO common name: Isopyrazam

Isopyrazam consists of the *syn*-isomer and the *anti*-isomer in a range of 70:30 to 100:0 ratios. Each of these isomers is a racemate of two enantiomers.

Chemical name

IUPAC: *syn*-isomer: 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide
anti-isomer: 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide

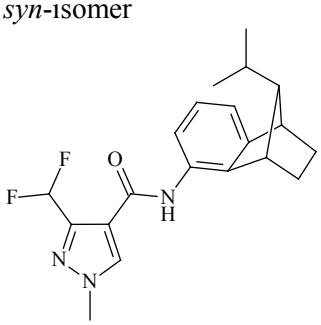
CAS: *syn*-isomer: 1H-pyrazole-4-carboxamide,3-(difluoromethyl)-1-methyl-N-[(1R,4S,9R)-1,2,3,4-tetrahydro-9-(1-methylethyl)-1,4-methanonaphthalen-5-yl]-,rel-
anti-isomer: 1H-pyrazole-4-carboxamide, 3-(difluoromethyl)-1-methyl-N-[(1R,4S,9S)-1,2,3,4-tetrahydro-9-(1-methylethyl)-1,4-methanonaphthalen-5-yl]-, rel-

CAS Registry No.: 881685-58-1
syn-isomer: 683777-13-1
anti-isomer: 683777-14-2

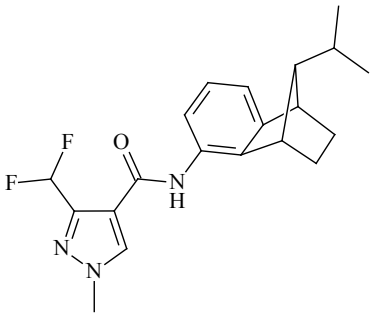
CIPAC No.: Not applicable

Synonyms for active substance: Isopyrazam
Consists of two isomers with the following codes:
syn-isomer: SYN534969
anti-isomer: SYN534968

Structural formula: *syn*-isomer



anti-isomer



Molecular formula: $C_{20}H_{23}F_2N_3O$

Molecular weight: 359.4

Physical and chemical properties

Pure active ingredient

Property	Test material	Results	Reference
Appearance:	*syn*-isomer:	White, crystalline powder	Das R., 2007a
	anti-isomer:	White, crystalline powder	Das R., 2007b
Odour:	*syn*-isomer:	Odourless	Das R., 2007a
	anti-isomer:	Odourless	Das R., 2007b
Melting point:	*syn*-isomer:	130.2°C	Geoffroy A., 2007a
	anti-isomer:	144.5°C	Geoffroy A., 2007b
Temperature of decomposition:	Isopyrazam:	Stable at room temperature, see boiling point (above) for temperature of decomposition.	Williams C., 2008
Relative density:	Isopyrazam:	1.332 g/cm^3 at 20°C	Weissenfeld M., 2008a
Vapour pressure:	*syn*-isomer:	$2.4 \cdot 10^{-7}$ Pa at 20°C and $5.6 \cdot 10^{-7}$ Pa at 25°C	Geoffroy A., 2007c
	anti-isomer:	$2.2 \cdot 10^{-8}$ Pa at 20°C and $5.7 \cdot 10^{-8}$ Pa at 25°C	Geoffroy A., 2007d
Henry's Law constant at 25°C calculation):	*syn*-isomer:	$1.9 \cdot 10^{-4}$ Pa · m^3/mol	Stulz J., 2008
	anti-isomer:	$3.7 \cdot 10^{-5}$ Pa · m^3/mol	

Property	Test material	Results		Reference
Solubility in water at 25 °C (unbuffered)	*syn*-isomer:	1.05 mg/L		Weissenfeld M., 2008b
	anti-isomer:	0.55 mg/L		Weissenfeld M., 2008c
	The active substance is a neutral molecule without dissociation and therefore shows no pH dependency of the water solubility.			
n-Octanol/ water partition coefficient (log P_{ow})	*syn*-isomer:	4.1		Weissenfeld M., 2008g
	anti-isomer:	4.4		Weissenfeld M., 2008h
Hydrolysis:		25 °C	50 °C	
pH 4: pH 5 pH 7: pH 9:	Isopyrazam:	- Stable for 30 days Stable for 30 days Stable for 30 days	Stable for 5 days Stable for 5 days Stable for 5 days Stable for 5 days	Berdat T., 2006
Direct photo-transformation of purified active substance in water (pH 7, 25°C)	Isopyrazam:	In natural water: First order kinetics DT_{50} 4.5 days Corresponding to: DT_{50} 16.3 days Based on spring sunlight in Tokyo In pH7 buffer: First order kinetics DT_{50} 54.3 days Corresponding to: DT_{50} 176 days Based on spring sunlight in Tokyo Two major discrete degradates were formed, namely CSAA798670 and CSCC210616.		Kuet S., 2008
Quantum yield of direct photo-transformation:	Isopyrazam:	1.01×10^{-5} For geographical latitudes in summer sunlight: 82 days at 30°N and 40°N 84 days at 50°N.		Wardrope L., 2008
Dissociation in water:	*syn*-isomer:	No pK_a was found in the range of 1.0 to 12.0.		Martin N., 2007a
	anti-isomer:	No pK_a was found in the range of 1.0 to 12.0.		Martin N., 2007b
Estimated photochemical oxidative degradation:	No test material used	The atmospheric oxidation of isopyrazam by hydroxyl radicals was estimated by calculation according to Atkinson. The estimated half-life is 2.29 hours.		Hayes S., 2007
UV absorption in methanol:	*syn*-isomer:	Solution / wavelength [nm] / Molar extinction coefficient [L/mol × cm] Neutral 225 17576 250 9895 295 1341 Acidic 225 17439 250 9789 295 1332 Basic 225 17328 250 9948 295 1471 No absorption maximum between 340 nm and 750 nm was observed.		Oggenfuss P., 2008

Property	Test material	Results	Reference
	anti-isomer (99.6%):	Solution wavelength [nm] Molar extinction coefficient [L/mol × cm] Neutral 225 16378 250 10480 295 1306 Acidic 225 16088 250 10222 295 1230 Basic 225 16905 250 11934 295 2265 No absorption maximum between 340 nm and 750 nm was observed.	

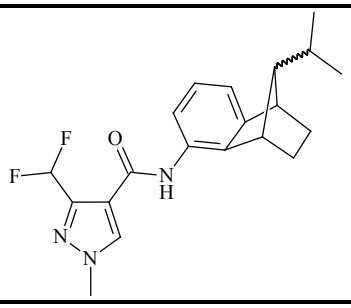
Technical material

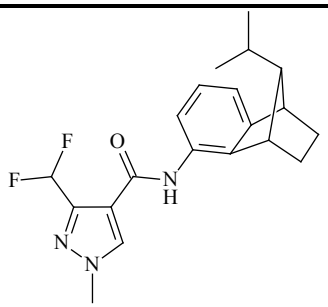
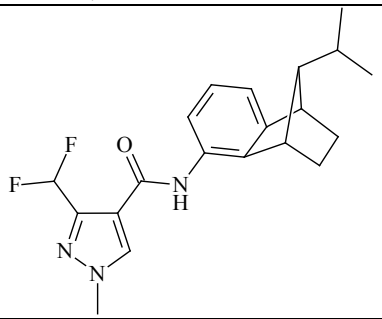
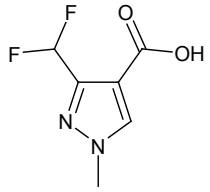
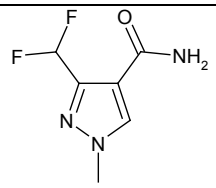
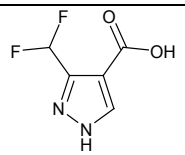
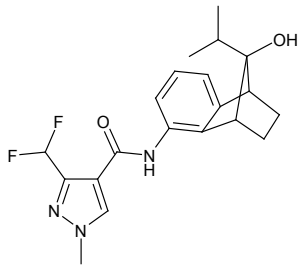
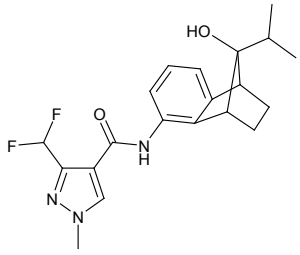
Property	Results		Reference
Appearance:	Isopyrazam:	Off-white powder	Das R., 2008
Odour:	Isopyrazam:	Odourless	Das R., 2008
Flammability:	Isopyrazam:	Not flammable	Jackson W.A., 2008a
Explosive properties:	Isopyrazam:	Not explosive under effect of heat, mechanical shock or friction	Jackson W.A., 2008c
Surface tension:	Isopyrazam:	63.1 mN/m at 19.8 °C	Weissenfeld M., 2008k
Solubility in organic solvents at 25 °C			
	Isopyrazam:	Hexane 1.17 g/L Acetone 314 g/L Methanol 119 g/L Ethyl acetate 179 g/L Octanol 44.1 g/L Toluene 77.1 g/L Dichloromethane 330 g/L	Weissenfeld M., 2008f
Oxidizing properties:	Isopyrazam:	Not an oxidising substance	Jackson W.A., 2008d
Formulations:	Emulsifiable concentrate (EC) formulation containing 125 g ai/L alone Emulsifiable concentrate (EC) formulation containing 62.5 g ai/L isopyrazam and 187.5 g ai/L cyprodinil Soluble concentrate (SC) formulation containing 125 g ai/L isopyrazam and 90 g ai/L epoxiconazole		

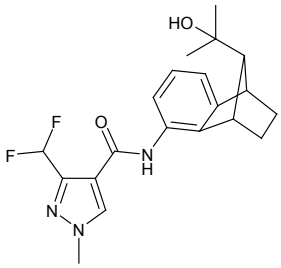
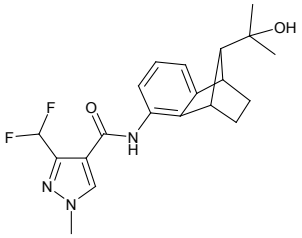
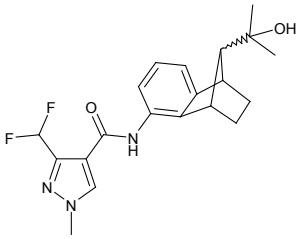
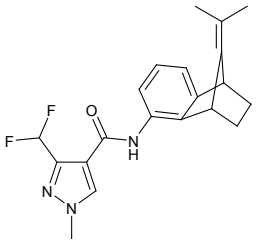
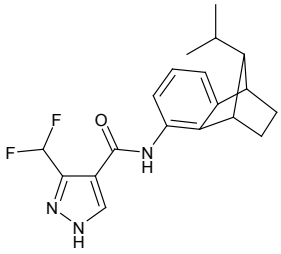
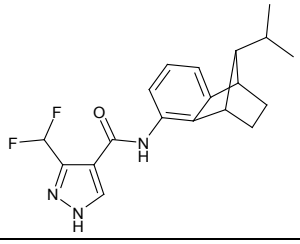
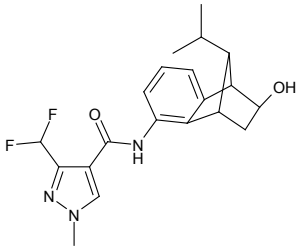
METABOLISM AND ENVIRONMENTAL FATE

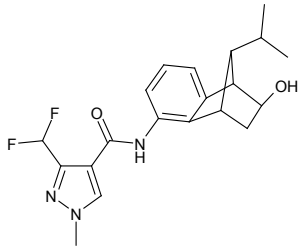
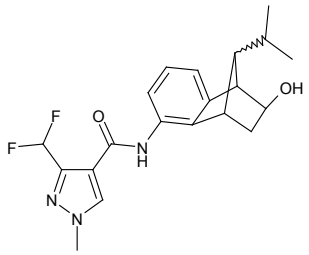
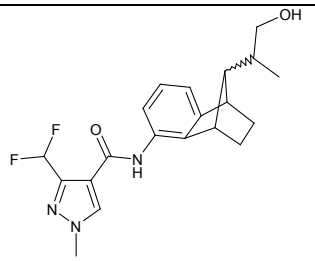
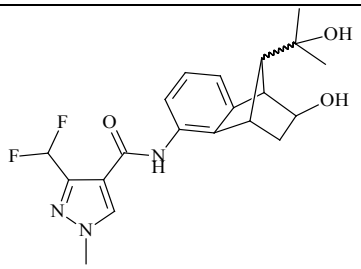
The following links manufacturer code name and structure or description of the compounds appearing in the various metabolism and environmental fate studies.

Structure of compounds appearing in metabolism and environmental fate studies

Common name or Code Number	Description/ Denomination (IUPAC name)	Metabolite found in	Structure
Isopyrazam	A mixture of 3-(difluoromethyl)-1-methyl-N-[(1RS,4SR,9RS)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-N-[(1RS,4SR,9SR)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide	Parent, isopyrazam	

Common name or Code Number	Description/ Denomination (IUPAC name)	Metabolite found in	Structure
Syn-isomer	(3-(difluoromethyl)-1-methyl-N-[(1RS,4SR,9RS)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methano-naphthalen-5-yl]pyrazole-4-carboxamide) (*syn*-diastereomers)	Parent, *syn*-isomer	
Anti-isomer	(3-(difluoromethyl)-1-methyl-N-[(1RS,4SR,9SR)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methano-naphthalen-5-yl]pyrazole-4-carboxamide) (*anti*-diastereomers)	Parent, *anti*-isomer	
CSAA798670	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid	Plants (wheat, grape, lettuce, rotational crops) Soil (via photolysis) Water (via photolysis) Outdoor aquatic system	
CSCC210616	3-difluoromethyl-1-methyl-1H-pyrazole-4-amide	Plants (rotational crops) Soil (via photolysis) Water (via photolysis) Outdoor aquatic system	
CSCD465008	3-difluoromethyl-1H-pyrazole-4-carboxyic acid	Plants (grape, lettuce, rotational crops) Soil	
CSCD120604	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid ((S)-9-hydroxy-9-isopropyl-(1RS,4SR,9RS)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide (*syn*-diastereoisomers)	Plants (wheat, lettuce, rotational crops)	
CSCD120605	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid ((R)-9-hydroxy-9-isopropyl-(1RS,4SR,9SR)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide (*anti*-diastereoisomers)	Plants (wheat, rotational crops)	

Common name or Code Number	Description/ Denomination (IUPAC name)	Metabolite found in	Structure
CSCD459488	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxyic acid [9-(1-hydroxyl-1-methyl-ethyl)-(1RS,4SR,9RS)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl]amide (*syn*-diastereoisomer)	Plants (wheat, grape, lettuce, rotational crops) Soil Water-sediment Outdoor aquatic system	
CSCD459489	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxyic acid [9-(1-hydroxyl-1-methyl-ethyl)-(1RS,4SR,9SR)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl]amide (*anti*-diastereoisomer)	Plants (grape, rotational crops) Soil	
CSCD460260	A mixture of 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid [9-(1-hydroxy-1-methyl-ethyl)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl]-amide (*syn*- and *anti* -diastereoisomers)	Soil	
CSCC230729	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (9-isopropylidene-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide	Plants (wheat, lettuce rotational crops)	
CSCD539372	3-difluoromethyl-1H-pyrazole-4-carboxylic acid (9-isopropyl-(1RS,4SR,9RS)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide (*syn*-diastereoisomers)	Rat Plants (wheat, grape, lettuce, rotational crops) Soil	
CSCD539391	3-difluoromethyl-1H-pyrazole-4-carboxylic acid (9-isopropyl-(1RS,4SR,9SR)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide (*anti*-diastereoisomers)	Rat Plants (wheat, grape, rotational crops) Soil	
CSCD658108	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxyic acid (2-hydroxy-9-isopropyl-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)- amide (*syn*-diastereoisomers)	Livestock (goat) Outdoor aquatic system	

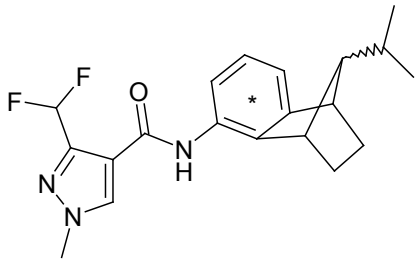
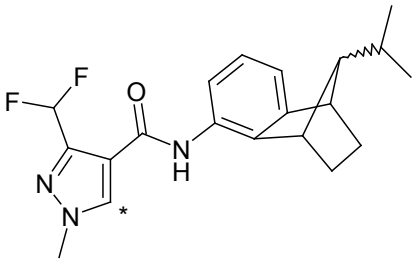
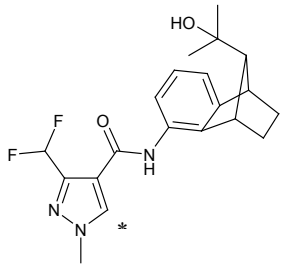
Common name or Code Number	Description/ Denomination (IUPAC name)	Metabolite found in	Structure
CSCD658109	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxyic acid (2-hydroxy-9-isopropyl-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)- amide (*anti*-diastereoisomers)	Livestock (goat) Outdoor aquatic system	
CSCD563692	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxyic acid (2-hydroxy-9-isopropyl-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)- amide (*syn*- and *anti* -diastereomers)	Rat Livestock (goat) Plants (wheat, grape, lettuce, rotational crops) Outdoor aquatic system	
CSCD563691	Hydroxy isopyrazam–Intermediate in formation of carboxylic acid	Rat Plants (wheat)	
CSCD656800	A mixture of 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid [2-hydroxy-9-(1-hydroxy-1-methyl-ethyl)-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl]-amide (*syn*- and *anti* -diastereoisomers)	Livestock (goat, hen, rat) Plants (grape, wheat, lettuce)	

Note: Those metabolites with substitution at undefined position are not included in the above list.

Radio-labelled Isopyrazam Used in Metabolism Studies

Both *syn*- and *anti*-isomers are biologically active and the specification for technical isopyrazam covers the range of *syn*:*anti* isomer ratios from 70:30 to 100:0. In keeping with this specification, livestock metabolism, crop metabolism studies have been conducted to cover the range of isomer ratios from 70:30 to 95:5.

The hen and goat metabolism studies on parent isopyrazam employed three different batches of radio-chemical. Both phenyl- and pyrazole-labelled isopyrazam were used at a *syn/anti* ratio of 95:5. Additionally, [phenyl-(U)-^{14}C] isopyrazam was used at a *syn/anti* ratio of 70:30 for direct comparison.

[phenyl-(U)-^{14}C] isopyrazam	[pyrazole-5-^{14}C] isopyrazam
	
[pyrazole-5-^{14}C] CSCD459488	
	

(*) ^{14}C position

In the goat metabolism study with CSCD459488, only a single test material, [pyrazole-5-^{14}C] CSCD459488, was used. The compound is, by definition, the *syn* diastereomer.

The wheat metabolism study employed three different batches of radio-chemical. Both phenyl- and pyrazole-labelled isopyrazam were used at a *syn/anti* ratio of 95:5. Additionally, [phenyl-(U)-^{14}C] isopyrazam was used at a *syn*/*anti* ratio of 70:30 for direct comparison.

In the grape and lettuce metabolism studies, the two test materials, [phenyl-(U)-^{14}C] isopyrazam and [pyrazole-5-^{14}C] isopyrazam, were used at a single *syn*/*anti* ratio of 70:30. The wheat study had shown no significant differences between the metabolism at a *syn/anti* ratio of 95:5 and at a *syn*/*anti* ratio of 70:30. Therefore, to maximise the possibility of finding radio-labelled components derived from *anti*-isopyrazam, the two subsequent metabolism studies were conducted using isopyrazam at a *syn*/*anti* ratio of 70:30 (i.e., at the highest proposed concentration of the *anti*-isomer).

Where residue levels in mg/kg are presented in the following study summaries, these refer to parent isopyrazam equivalents.

The radiolabelled test materials used in the crop metabolism studies are described in Table 1.

Table 1 Label position and *syn*:*anti* ratio of radio-labelled compounds used in the metabolism studies

Study	Radio-labelled compound	*Syn*:*anti* ratio	
		Phenyl-labelled	Pyrazole-labelled
Lactating Goat Study 1	Isopyrazam	95.1 : 4.9 69 : 31	95.7 : 4.3
Lactating Goat Study 2	CSCD459488	-	100 : 0
Laying Hen	Isopyrazam	95.1 : 4.9 69 : 31	95.7 : 4.3
Wheat	Isopyrazam	96.4 : 3.6 70.4 : 29.6	95.4 : 4.6
Grapes	Isopyrazam	69.5 : 30.5	69.1 : 30.9
Lettuce	Isopyrazam	69.7 : 30.3	69.3 : 30.7
Confined Rotational Crops	Isopyrazam	96.4 : 3.6	95.4 : 4.6

Animal metabolism

The Meeting received information on the results of studies on lactating goats and laying hens.

Metabolism studies on laboratory animals including rats were reviewed in the framework of toxicological evaluation by the current JMPR. When radio-labelled isopyrazam was administered once at 1 or 75 mg/kg bw to rats, approximately 70% of the dose was absorbed. Most of the absorbed dose was excreted within 24 h after administration, 65–90% via bile and the rest via urine. Highest residues were identified in the liver, kidney, thyroid and adrenals. After repeated dosing, no accumulation of radioactivity was observed in rats. There were no significant differences between the toxicokinetic parameters of *syn*- and *anti*-isomers. The predominant metabolic pathway for isopyrazam or its N-demethylated metabolite is hydroxylation in the bicyclic-isopropyl moiety, followed by further oxidation to form carboxylic acid and/or to give rise to multiple hydroxylated metabolites with subsequent formation of glucuronic acid or sulphate conjugates.

Lactating goats

Three lactating goats were used for Study 1 and each goat was orally given a capsule with a separate radiolabelled form of isopyrazam (Lowrie C., Mackinnon I. 2008). The radioactive compounds used were [pyrazole-5-^{14}C] isopyrazam and [phenyl-U-^{14}C]isopyrazam with *syn/anti* ratios approximately 95:5 and [phenyl-U-^{14}C] isopyrazam with a *syn/anti* ratio approximately 69:31. The nominal dose rate was 30 ppm in dietary dry matter and dosing was carried out for 7 consecutive days. Actual dose rates based on dry matter consumed were 45, 31 and 29 mg/kg for pyrazole 96:4, phenyl 95:5 and phenyl 69:31, respectively. Milk, urine and faeces were collected daily. The goats were sacrificed approximately 16 hours after the final dose and tissues taken for quantification and analysis.

Total radioactive residue (TRR) in liquid samples were determined directly by liquid scintillation counter (LSC) with automatic quench correction by external standard-channels ratio. TRR in solid samples was determined also by LSC after combustion using a sample oxidizer.

The recovered radioactivity for all three goats was greater than 79.5% of the administered dose. The majority of the radioactivity was excreted in urine and faeces (Table 2).

Table 2 Radioactive Residues in Excreta and Tissues of Lactating Goats Treated with ^{14}C-isopyrazam at 30 ppm for 7 days

Matrix	% TAR [a]		
	Pyrazole 96:4	Phenyl 95:5	Phenyl 69:31
Faeces	62.0	62.5	60.1
Urine	12.3	12.9	11.9
Tissues and milk	0.56	0.68	0.57
Blood	0.07	0.06	0.04
Bile	0.05	0.38	0.05
GI Tract	7.6	7.3	6.7
Cage wash	0.52	0.31	0.17
Total	83.1	84.1	79.5

[a] Percent of total administered dose

The highest residues were found in the liver and the lowest in the fat, but residues represented low quantities in all commodities (Table 3). Quantification of total daily radioactivity for milk indicated that a plateau was reached within 2-3 days. Plateau levels were 0.065, 0.055 and 0.052 mg/kg for pyrazole 95:5, phenyl 95:5 and phenyl 69:31 respectively.

Table 3 Radioactive Residues in Milk and Tissues of Lactating Goats Treated with ^{14}C-isopyrazam at 30 ppm for 7 Days

Matrix	Radioactive Residue, mg/kg		
	Pyrazole 96:4	Phenyl 95:5	Phenyl 69:31
Milk [a]	0.076	0.059	0.055
Liver	0.612	0.604	0.331
Kidney	0.189	0.143	0.174
Muscle	0.032	0.022	0.023
Fat	0.012	0.016	0.020

[a] TRR in Day 5 a.m. milk sample

Tissue and milk samples were extracted with solvents for analysis. In all samples more than about 75% of the extracts were recovered. Unextractable residues in tissues, other than kidney, and milk were ≤ 0.021 mg/kg while in kidney the highest unextractable residue was 0.089 mg/kg.

Comparison of chromatographic profiles from the pyrazole 95:5 and phenyl 95:5 labelled experiments indicated that there was no cleavage between the rings. Extracts from the phenyl 69:31 experiment showed some extra degree of complexity relative to the 95:5 treatments. Full details of metabolites identified in milk and tissues are given in Table 4.

Table 4 Characterisation and Identification of Radioactive Residues in Milk and Tissues of Lactating Goats Treated with ^{14}C-isopyrazam at 30 ppm in dietary dry matter for 7 days

[Pyrazole-5-^{14}C] isopyrazam (96:4 *syn*:*anti* Ratio)										
Components	Milk [a]		Liver		Kidney		Muscle		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Extracted:										
Isopyrazam	0.4	0.0003	0.7	0.0043	0.1	0.0002	2.2	0.0007	39.6	0.0047
CSCD656800	25.1	0.0192	17.0	0.1041	20.0	0.0379	40.5	0.0130	nd	nd
(as conjugates)			(14.5)	(0.0888)	(6.6)	(0.0125)				
CSCD658108	nd	nd	11.3	0.0692	nd	nd	nd	nd	nd	nd
(as conjugates)			(11.3)	(0.0692)						
CSCD658109	nd	nd	0.8	0.0049	nd	nd	nd	nd	nd	nd
(as conjugates)			(0.8)	(0.0049)						
CSCD626401	nd	nd	2.8	0.0172	nd	nd	nd	nd	nd	nd
(as conjugates)			(2.2)	(0.0135)						
CSCD591489	nd	nd	4.0	0.0245	nd	nd	nd	nd	nd	nd
CSCD610195*	nd	nd	trace	trace	nd	nd	nd	nd	nd	nd
Aqueous Unknowns	26.1	0.0187	17.0	0.1026	37.5	0.0711	31.4	0.0103	39.3	0.0045
Organo soluble Unknowns	20.1	0.0150	29.1	0.1479	18.5	0.0353				
Others	10.0	0.0091	13.3	0.0832	16.0	0.0299	18.3	0.0097	19.3	0.0023
Subtotal	81.7	0.0623	96.0	0.5579	92.1	0.1744	92.4	0.0337	98.2	0.0115
Unextracted	1.8	0.0014	2.0	0.0126	4.7	0.089	6.8	0.0022	nd	nd
[Phenyl-U-^{14}C] isopyrazam (95:5 *syn*:*anti* Ratio)										
TRR Extracted:										
Isopyrazam	0.1	0.0001	< 0.1	< 0.0001	0.2	0.0003	4.0	0.0009	51.0	0.0082
CSCD656800	31.7	0.0187	13.9	0.0839	24.7	0.0354	43.8	0.0097	nd	Nd
(as conjugates)			(13.6)	(0.0821)	(8.1)	(0.0116)				
CSCD658108	nd	nd	20.0	0.1207	nd	nd	nd	nd	nd	Nd
(as conjugates)			(19.9)	(0.1201)						
CSCD658109	nd	nd	1.0	0.0060	nd	nd	nd	nd	nd	Nd
(as conjugates)			(1.0)	(0.0060)						
CSCD626401	nd	nd	0.2	0.0012	nd	nd	nd	nd	nd	Nd
(as conjugates)			(< 0.1)	(< 0.0001)						
CSCD591489	nd	nd	2.3	0.0139	nd	nd	nd	nd	nd	Nd
(as conjugates)			(1.7)	(0.0103)						
Aqueous Unknowns	19.5	0.0121	17.0	0.1026	22.8	0.0328	26.7	0.0060	23.3	0.0036
Organo soluble Unknowns	30.0	0.0179	28.7	0.1731	20.9	0.0293				
Others	16.8	0.0090	10.9	0.0660	7.6	0.0110	13.5	0.0015	13.1	0.0022
Subtotal	98.1	0.0578	94.0	0.5674	76.2	0.1088	88.0	0.0181	87.4	0.0140
Unextracted	2.7	0.0016	3.0	0.0183	10.6	0.0153	11.9	0.0026	nd	Nd
[Phenyl-U-^{14}C] isopyrazam (69:31 *syn*:*anti* Ratio)										
Isopyrazam	1.4	0.0008	1.9	0.0063	0.6	0.0010	8.6	0.0019	50.4	0.0101
CSCD656800	14.7	0.0081	6.2	0.0205	13.2	0.0230	29.2	0.0066	nd	Nd
(as conjugates)			(4.6)	(0.0152)	(4.1)	(0.0071)				
CSCD658108	nd	nd	4.5	0.0149	nd	nd	nd	nd	nd	Nd
(as conjugates)			(4.3)	(0.0142)						
CSCD658109	nd	nd	3.3	0.0109	nd	nd	nd	nd	nd	Nd
(as conjugates)			(3.0)	(0.0099)						
CSCD626401	nd	nd	2.0	0.0060	nd	nd	nd	nd	nd	Nd
(as conjugates)			(0.4)	(0.0013)						
CSCD591489	nd	nd	6.1	0.0202	nd	nd	nd	nd	nd	Nd
(as conjugates)			(1.0)	(0.0033)						
CSCD610195[b]	nd	nd	trace	trace	nd	nd	nd	nd	nd	Nd

[Pyrazole-5-^{14}C] isopyrazam (96:4 *syn:anti* Ratio)										
Components	Milk [a]		Liver		Kidney		Muscle		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Extracted:										
CSCD610196[b]	nd	nd	trace	trace	nd	nd	nd	nd	nd	Nd
Aqueous Unknowns	10.3	0.0061	18.6	0.0614	14.5	0.0252	45.6	0.0103	35.5	0.0071
Organo soluble Unknowns	39.1	0.0214	28.3	0.0938	28.3	0.0489				
Others	9.4	0.0049	14.7	0.0486	19.8	0.0349	12.3	0.0028	-1.0	-0.0003
Subtotal	74.9	0.0413	85.6	0.2826	76.4	0.1330	95.7	0.0216	84.9	0.0169
Unextracted	2.4	0.0013	6.3	0.0210	5.9	0.0103	4.6	0.0010	nd	Nd

[a] milk sample in the morning of day 5

[b]–detected by MS only.

nd–Not detected

Metabolites identified in liver and kidney were present entirely or partially in a conjugated form.

Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

In milk, isopyrazam was identified as a minor component (maximum 1.4% TRR, 0.0008 mg/kg). The major component was identified by co-chromatography and mass spectrometry as the dihydroxylated metabolite CSCD656800. It accounted for 14.7–31.7% TRR (0.0081–0.0192 mg/kg). The remaining radioactive residue in all samples comprised multiple minor components, none of which exceeded 0.01 mg/kg.

In liver, unchanged isopyrazam was detected only at extremely low levels and accounted for a maximum of 1.9% TRR and 0.0063 mg/kg. The dihydroxylated metabolite CSCD656800 was a significant component in liver and accounted for 6.2–17.0% TRR (0.0205–0.1041 mg/kg), dependent upon label type. The majority of this metabolite existed as a glucuronide or sulphate conjugate. Monohydroxylated metabolites were identified as CSCD658108 and CSCD658109, the *syn* and *anti* forms of a bicyclic ring secondary alcohol. As expected, the *syn* form, CSCD658108, predominated and accounted for up to 20.0% TRR (0.1207 mg/kg). The corresponding *anti* diastereoisomer, which represented a maximum of 3.3% TRR (0.0109 mg/kg), occurred at highest levels in liver from the phenyl 69:31 experiment. Other monohydroxylated metabolites, CSCD610195 and CSCD610196, which are *syn* and *anti* diastereoisomers of primary alcohols arising from hydroxylation of the isopropyl group, were detected at trace levels by mass spectrometry but could not be quantified by this method. Further oxidation at the same site led to carboxylic acids CSCD626401 and CSCD591489 (both *syn* stereochemistry). The former accounted for up to 2.8% TRR and the latter up to 6.1% TRR. Both metabolites existed predominantly as conjugates. Chromatographic profiles of liver were highly complex and large numbers of minor metabolites were found in all samples. No individual component exceeded either 10% TRR or 0.05 mg/kg.

Unextracted material remaining in the liver extraction debris was investigated further. Significant proportions of the residual radioactivity were released following treatment with protease enzymes and radioactivity remaining in the debris represented < 10% TRR and < 0.05 mg/kg in all samples. The solubilised components were profiled by HPLC and found to be composed of multiple minor metabolites.

Analysis of kidney samples detected very low levels of unchanged isopyrazam (maximum 0.6% TRR, 0.0010 mg/kg). The principal metabolite was CSCD656800 which accounted for 13.2–24.7% TRR, (0.0230–0.0379 mg/kg). In all samples, approximately one third of the total was present in a conjugated form. The remainder of the residue comprised a large number of minor metabolites, the most significant of which accounted for 8.9% TRR, but this was equivalent to only 0.0169 mg/kg.

In muscle, isopyrazam accounted for up to 8.6% TRR and 0.0019 mg/kg. The dihydroxylated metabolite CSCD656800 predominated, representing 29.2–43.8% TRR (0.0066–0.0130 mg/kg). Also in muscle extract, there were many minor components, all of which were significantly less than 0.01 mg/kg.

In fat, unchanged isopyrazam represented a higher proportion of the residue than in other tissues (39.6–51.0% TRR). However, as the overall accumulation of radioactivity in fat was very low, the highest concentration of parent compound was only 0.0101 mg/kg. Many other components were detected at significantly lower concentrations. The highest percentage contribution of an individual component was 12.1% TRR but no unknown metabolite exceeded 0.0022 mg/kg.

In order to further understand the potential metabolic pathway, a representative urine sample (day 7) from each goat was analysed by LC-MS with concurrent radiodetection. Dihydroxylation and trihydroxylation of parent were clearly evident. Many other metabolites contained a carboxylic acid which arose from oxidation of a methyl group on the isopropyl substituent. Carboxylic acids of generic structure CSCD662024, and hydroxy carboxylic acids were characterised. In addition, there was evidence for N-demethylation of the pyrazole ring to form N-demethylated carboxylic acids (generic structure CSCD676318) and N-demethylated hydroxy carboxylic acids.

A goat metabolism study 2 was conducted following oral treatment of lactating goats via gelatin capsules with CSCD459488 which is the main metabolite in wheat, grapes and lettuce (Strathdee A., Vance C. 2008). One lactating goat was dosed with [pyrazole-5-^{14}C] CSCD459488 for 7 days at a nominal rate of 12 ppm in dietary dry matter intake. The actual dose rate achieved was approximately 19 mg/kg bw/day. Milk, urine and faeces were collected daily. The goat was sacrificed approximately 12 hours after the administration of the final dose and tissues taken *post mortem* for quantification and analysis.

Total radioactive residue (TRR) in liquid samples were determined directly by liquid scintillation counter (LSC) with automatic quench correction by external standard-channels ratio. TRR in faeces and tissue samples was determined also by LSC after combustion using a sample oxidizer.

Table 5 shows radioactive residues in excreta and tissues. The majority of the administered radioactivity was excreted in the faeces (56.6%) and urine (30.3%). The total radioactive recovery was 97.3%. The radioactive residues determined in the milk and tissues are summarized in Table 5.

Table 5 Radioactive Residues in excreta and tissues of Lactating Goats Treated with ^{14}C-CDCD459488 at 12 ppm in dietary dry matter for 7 days

Matrix	% of Dosed Radioactivity Recovered from Goat
Faeces	56.6
Urine	30.3
Milk	0.48
Liver	0.26
Kidney	0.02
Muscle	0.16
Fat	0.04
Gastrointestinal Tract and Contents	8.68
Cage wash	0.77
Total	97.3

Table 6 Total Radioactive Residues (TRR) in Milk and Tissue Samples from a Lactating Goat Treated with CDCD459488 at 12 ppm in dietary dry matter for 7 days

Matrix	Radioactive Residue (mg/kg equiv. CSCD459488)	
	By direct quantification	By summation of extracts and debris radioactivity
Milk (128 h)[a]	0.123	0.116
Milk plateau concentration [b]	0.08	Not analysed
Liver	0.457	0.440
Kidney	0.246	0.245
Muscle	0.038	0.036
Fat	0.007	0.007

[a] 128 h milk (day 6 pm) sample was extracted and profiled by 2D-TLC and HPLC-MS.

[b] Milk reached a plateau at Day 2 with a mean concentration of 0.08 mg/kg achieved on the plateau level.

With the exception of milk, which was analysed directly, figures are derived from quantification of solvent extracts and residual debris.

The highest residue of 0.440 mg/kg was found in the liver but accounted for only 0.26% of the administered dose. Residue levels in other commodities such as kidney, muscle and fat were low and in total accounted for < 0.20% of the administered dose. Radioactive residues in milk (TRRs per 24 hour period) plateaued at a level of 0.08 mg/kg after approximately 2 days of dosing.

All tissue samples and a representative milk sample were extracted with solvents and analysed chromatographically. In excess of 85% of the radioactivity in milk, muscle and fat was extractable with solvents. Extractability of kidney and liver was slightly lower (89.25% TRR and 83.57% TRR respectively) and some further work was conducted on the debris samples. Summaries of the metabolites identified in milk and tissues are presented in Table 7.

Table 7 Characterisation and Identification of Radioactive Residues in Milk and Tissues in a Goat Treated with CDCD459488 at 12 ppm in dry matter for 7 days

Components	Milk[a]		Liver		Kidney		Muscle		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Extracted:										
CSCD459488 (as conjugated)	0.16	< 0.001	1.47 (1.08)	0.007 (0.005)	0.11 (0.11)	< 0.001 (< 0.001)	0.26	< 0.001	6.23	< 0.001
CSCD656800 (as conjugated)	33.32	0.039	35.97 (26.65)	0.159 (0.118)	36.70 (0.00)	0.090 (< 0.001)	55.97	0.021	35.63	0.002
Dihydroxylated metabolite (as conjugated)	2.86	0.003	5.21 (3.35)	0.023 (0.015)	10.97 (3.73)	0.027 (0.009)	nd	nd	nd	Nd
Trihydroxylated metabolite (as conjugated)	20.63	0.024	nd	nd	nd	nd	nd	nd	nd	Nd
Aqueous Unknowns	7.40	< 0.006	13.90	0.059	6.55	0.0015	24.94	0.008	42.29	0.002
Organo soluble Unknowns	14.74	0.017	10.71	0.042	12.07	0.025				
Others	6.61	0.009	13.03	0.063	17.75	0.047	9.25	0.002	9.74	< 0.001
Subtotal	85.72	0.092	80.29	0.353	84.15	0.1905	90.42	0.031	93.89	0.004
Unextracted	4.09	0.005	3.28	0.015	6.35	0.015	3.34	0.001	3.42	< 0.001

nd- Not detected.

Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

CSCD459488 was detected in all commodities but only at very low levels (maximum 1.47% TRR, 0.007 mg/kg). In liver and kidney a high proportion of this residue was in a conjugated form. The principal metabolite was CSCD656800, which accounted for > 33%TRR in all samples and > 0.1 mg/kg in both liver and kidney. In liver a large proportion existed as a glucuronide or sulphate conjugate. This metabolite arises from hydroxylation of CSCD459488 on a methylene group of the bicyclic ring system. Since CSCD459488 has *syn* stereochemistry, it is likely that CSCD656800 also exists in the *syn* form, although this was not confirmed.

Analysis of milk by mass spectrometry established the presence of two further components which were characterised as a dihydroxylated and a trihydroxylated metabolite. The trihydroxylated compound accounted for 20.63% TRR, 0.024 mg/kg and the dihydroxylated for 2.86% TRR, 0.003 mg/kg. Co-chromatography confirmed the presence of the dihydroxylated compound in liver and kidney, where a significant proportion was present as a conjugate. It is reasonable to suppose that both the dihydroxylated and trihydroxylated compounds retain the hydroxyl group present in CSCD459488 and then undergo further hydroxylation at one or two other sites.

Chromatographic analysis of extracts of all commodities demonstrated the presence of multiple minor components additional to those identified. In liver and kidney the largest of these components, which were more polar than CSCD459488, represented 3.43% TRR (0.015 mg/kg) and 2.09% TRR (0.005 mg/kg) respectively. In other commodities all minor components accounted individually for < 0.01 mg/kg.

The liver and kidney debris, which contained residues accounting for 16.42% TRR (0.072 mg/kg) and 10.75% TRR (0.026 mg/kg) respectively, were treated with protease enzymes.

This resulted in solubilisation of approximately half of the liver debris leaving 7.96% TRR (0.035 mg/kg) in the solid. Similar results were obtained for kidney, where 6.35% TRR (0.015 mg/kg) remained unextracted. The solubilised material from both samples was chromatographed but remained at the origin of the TLC.

An overall metabolic pathway for isopyrazam in the lactating goat shows no major differences between the metabolic profiles of the three radiolabelled experiments. Comparison of the pyrazole 95:5 and phenyl 95:5 labelled experiments indicated that there was no cleavage of the amide. The phenyl 70:30 experiment showed some extra degree of complexity relative to the 95:5 treatments, but there was reasonable evidence to suggest that the additional components, most of which were relatively minor, were due simply to higher contributions of *anti* diastereoisomers, which were chromatographically resolved from their corresponding *syn* forms.

The primary mechanisms for the proposed biotransformation pathway of isopyrazam in the lactating goat are hydroxylation of the bicyclic ring and isopropyl group, with further oxidation of primary alcohols to carboxylic acids. *N*-demethylation of the pyrazole ring was evident from analysis of urine but metabolites of this type were not identified in tissues or milk. The principal metabolite CSCD656800, which was identified as a significant proportion of the residue in milk and all tissues except fat, resulted from dihydroxylation of parent. Monohydroxylated metabolites and carboxylic acids were also identified in liver.

Proposed metabolic pathway of CSCD656800

A proposed metabolic pathway for CSCD459488 in the lactating goat is presented in Figure 1**.** The primary mechanism is hydroxylation of CSCD459488 on a methylene group of the bicyclic ring system in order to generate CSCD656800, a dihydroxylated metabolite of isopyrazam (i.e., monohydroxylated CSCD459488). It is reasonable to suppose that both the dihydroxylated and trihydroxylated isopyrazam compounds retain the hydroxyl group present in CSCD459488 and then undergo further hydroxylation at one or two other sites.

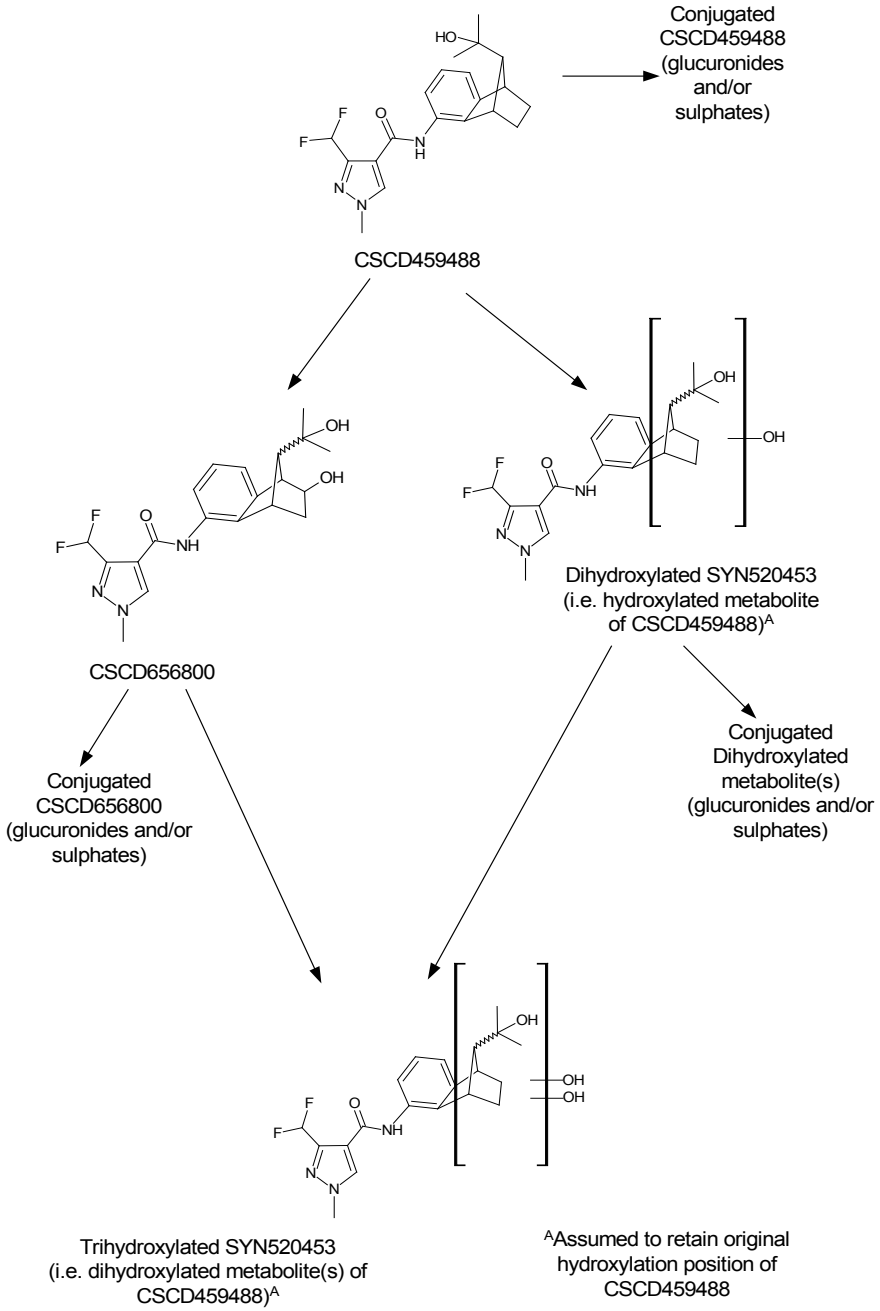


Figure 1 Proposed Metabolic Pathway for CSCD459488 in Lactating Goats

Laying Hens

Two groups, each consisting of five laying hens, were orally dosed separately via capsules with either [pyrazole-5-^{14}C] isopyrazam or [phenyl-U-^{14}C] isopyrazam with a *syn:anti* ratio of approximately 95:5 at a nominal rate of 11 ppm in dietary dry matter for 14 consecutive days. A third group of laying hens was dosed with 69:31 *syn:anti* ^{14}C-isopyrazam radiolabelled in the phenyl ring, also at a nominal rate of 11 mg/kg dietary dry matter for 14 consecutive days. An actual dose rate of 11ppm in dietary dry matter was achieved in all cases. Eggs and excreta were collected daily. The hens were sacrificed approximately 16 hours after the final dose and tissues taken *post mortem.*

Total radioactive residue (TRR) in liquid samples were determined directly by liquid scintillation counter (LSC) with automatic quench correction by external standard-channels ratio. TRR in solid samples was determined also by LSC after combustion using a sample oxidizer.

The average radioactive balance for all three dose groups was > 90.8 % of the administered dose (Table 8).

Table 8 Radioactive Residues in excreta and tissues of Laying Hens Treated with ^{14}C-isopyrazam at 11 ppm in dry matter for 14 days

Matrix	Radioactive residue (% of TAR)		
	Pyrazole 96:4	Phenyl 95:5	Phenyl 69:31
Excreta	90.0	88.3	93.3
Egg Whites, Egg Yolks, Liver, Muscle, Peritoneal Fat, Skin and Attached Fat	0.13	0.20	0.13
Blood	0.01	0.01	0.01
GI Tract	0.73	0.92	0.84
Cage wash	0.67	1.21	0.74
Total	91.6	90.8	95.0

Composite egg samples, yolk and white, were prepared in all three experiments by combination of samples collected on days 7 to 14 since the plateau was reached after 7 days. Composite liver, skin and attached fat and peritoneal fat samples were prepared in all three experiments. The composite egg whites and yolks were extracted with combinations of acetonitrile/water and methanol/water. Combined extracts were profiled by HPLC and/or 2D-TLC with some extracts requiring clean up by elution through a C18 SPE column prior to analysis.

The composite liver samples were extracted using combinations of acetonitrile/water and acetone. Extracts were eluted through a C18 SPE column using varying proportions of methanol and water and then analysed by HPLC. Significant radioactivity remained unextracted in the liver debris after the initial solvent extraction and therefore the post extraction solids (PES) were subjected to enzyme hydrolysis employing protease (*Streptomyces griseus*, type XIV bacterial). The resulting hydrolysates were loaded on to various SPE columns and eluted with water/methanol prior to HPLC analysis. The PES remaining after enzyme hydrolysis of the 95:5 treatments were hydrolysed using 0.1N HCl. The resulting hydrolysates were also subjected to C18 SPE clean up as appropriate prior to HPLC analysis.

The composite skin and attached fat and peritoneal fat samples were extracted with dichloromethane and hexane. The extracts were subject to either a hexane and acetonitrile/water partition or a SPE procedure eluting with either acidified or basified MTBE prior to HPLC analysis.

The day 13 excreta from the hens treated with phenyl 95:5 isopyrazam was subjected to enzyme hydrolysis employing protease (*Streptomyces griseus*, type XIV bacterial) and the resulting hydrolysate subjected to a polymer SPE procedure. The remaining solid was extracted with acetonitrile/water and methanol and combined with the SPE eluate and analysed by mass spectrometry.

The highest tissue residues were found in the liver and the lowest in muscle, but residues represented low mg/kg quantities in all commodities (Table 9). Radioactive residues in eggs (TRRs per 24 hour period) reached plateau after approximately seven days of dosing. In yolks, radioactive residues reached plateau after approximately seven days (0.039–0.080 mg/kg) and plateau was achieved after approximately 2–3 days in egg whites (0.017–0.024 mg/kg).

Table 9 Radioactive Residues in Composite Tissue and Egg Samples from Laying Hens Treated with ^{14}C-isopyrazam at 11 ppm in dietary dry matter for 14 days

Radiolabel Position and Isomer Ratio	Matrix	TRR (mg/kg)	
		By Direct Quantification	By Summation of Extractable and Non-Extractable Radioactivity
[Pyrazole-5-^{14}C] isopyrazam (96:4 *syn:anti* Ratio).	Egg White [a]	0.017	0.016
	Egg Yolk [a]	0.039	0.043
	Liver	0.119	0.164
	Muscle	0.004	na
	Skin and Attached Fat	0.008	0.010

Radiolabel Position and Isomer Ratio	Matrix	TRR (mg/kg)	
		By Direct Quantification	By Summation of Extractable and Non-Extractable Radioactivity
	Peritoneal Fat	0.011	0.009
[Phenyl-U-^{14}C] isopyrazam (95:5 *syn:anti* Ratio).	Egg White [a]	0.017	0.017
	Egg Yolk [a]	0.042	0.050
	Liver	0.119	0.119
	Muscle	0.005	na
	Skin and Attached Fat	0.011	0.015
	Peritoneal Fat	0.019	0.021
[Phenyl-U-^{14}C] isopyrazam (69:31 *syn:anti* Ratio).	Egg White [a]	0.024	0.024
	Egg Yolk [a]	0.080	0.085
	Liver	0.143	0.149
	Muscle	0.006	na
	Skin and Attached Fat	0.020	0.013
	Peritoneal Fat	0.020	0.019

na = Not analysed

[a] = Composite egg samples, Days 7-14, were prepared after plateau was achieved in whole eggs.

All composite tissue and egg samples with TRRs ≥ 0.01 mg/kg were extracted with solvents and analysed. The extractable radioactivity of the composite egg whites was ≥ 89.6% TRR; in the egg yolks ≥ 86.3% TRR; in the liver ≥ 85.6% TRR; in the skin and attached fat ≥ 55.6% TRR; and in the peritoneal fat ≥ 70.1% TRR. The majority of the radioactivity from the liver post extraction solid (PES) was released following treatment with protease enzymes (51.0–65.2 %TRR). Further release with 0.1M HCl resulted in a maximum of 12.4% TRR, 0.018 mg/kg remaining in the PES. Chromatographic profiling of the extracts indicated that they were composed of multiple minor metabolites.

Parent isopyrazam was detected at low levels in all fat samples and also in egg yolks (pyrazole 96:4 and phenyl 69:31) and liver (pyrazole 96:4). In all cases it accounted for < 0.01 mg/kg but represented the highest percentage of the TRR in peritoneal fat (21.4 %TRR).

There were multiple metabolites in each of the eggs and tissues samples with no single metabolite greater than 0.012 mg/kg. Chromatograms were highly complex and indicated that the metabolites were more polar than the parent.

Further isolation and analysis procedures resulted in the identification of three metabolites, which were considered to represent the most significant components in one or more tissues. Two of these metabolites were dihydroxylated and designated as CSCD656800 and hydroxy CSCD459489. The third metabolite was an unsaturated carboxylic acid. CSCD656800 was identified in both the phenyl 95:5 and pyrazole 96:4 egg whites and yolks. It was characterized as being present in the phenyl 69:31 egg whites and yolks as well as in liver extracts from all three experiments. Hydroxy CSCD459489 was identified in phenyl 69:31 egg whites and yolks and characterized as being present at low levels in liver extracts from all three experiments. This compound was shown to have *anti* stereochemistry hence its identification in the 69:31 samples was consistent with the increased levels of *anti* isopyrazam dosed in this experiment. The unsaturated acid metabolite was identified in phenyl 69:31 egg yolks but accounted for 9.2 %TRR and 0.008 mg/kg. It was characterized as being present in the phenyl 95:5 and phenyl 69:31 liver extracts as well as phenyl 69:31 egg yolks. A detailed summary of the metabolites identified and their respective levels are presented in Table 10 below.

Table 10 Characterisation and Identification of Radioactive Residues in Tissues and Egg Samples from Laying Hens Treated with ^{14}C-isopyrazam at 11 mg/kg for 14 days

[Pyrazole-5-^{14}C] Isopyrazam (96:4 *syn:anti* Ratio)										
Components	Egg White		Egg Yolk		Liver		Skin and Attached Fat		Peritoneal Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Extracted:										

[Pyrazole-5-^{14}C] Isopyrazam (96:4 *syn*:*anti* Ratio)										
Components	Egg White		Egg Yolk		Liver		Skin and Attached Fat		Peritoneal Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Isopyrazam	nd	nd	3.4	0.001	0.2	< 0.001	9.2	0.001	2.5	< 0.001
CSCD656800	6.8	0.001	11.5	0.005	1.5	0.002	nd	nd	nd	nd
Hydroxy CSCD459489	nd	nd	nd	nd	1.5	0.002	nd	nd	nd	nd
Unsaturated Acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Others	82.8	0.014	72.9	0.031	96.7	0.159	67.2	0.07	67.6	0.006
Subtotal	89.6	0.015	87.8	0.037	99.9a	0.163a	76.4	0.071	70.1	0.006
Unextracted	1.0	< 0.001	18.0	0.008	8.9	0.015	3.7	< 0.001	26.6	0.002
[Phenyl-U-^{14}C] isopyrazam (95:5 *syn*:*anti* Ratio)										
Components	Egg White		Egg Yolk		Liver		Skin and Attached Fat		Peritoneal Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Extracted:										
Isopyrazam	nd	nd	nd	nd	nd	nd	13.9	0.002	21.4	0.004
CSCD656800	29.0	0.005	10.8	0.005	1.6	0.002	nd	nd	nd	nd
Hydroxy CSCD459489	nd	nd	nd	nd	1.1	0.001	nd	nd	nd	nd
Unsaturated Acid	nd	nd	nd	nd	3.3	0.004	nd	nd	nd	nd
Others	68.2	0.011	83.5	0.042	88.5	0.105	59.7	0.009	65.5	0.014
Subtotal	97.2	0.016	94.3	0.047	94.5a	0.112a	73.6	0.011	86.9	0.018
Unextracted	2.8	0.001	11.9	0.006	nd	nd	26.3	0.004	4.5	0.001
[Phenyl-U-^{14}C] isopyrazam (69:31 *syn*:*anti* Ratio)										
Components	Egg White		Egg Yolk		Liver		Skin and Attached Fat		Peritoneal Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Extracted										
Isopyrazam	nd	nd	4.9	0.004	nd	nd	7.6	0.001	9.3	0.002
CSCD656800	15.9	0.004	6.6	0.006	1.0	0.001	nd	nd	nd	nd
Hydroxy CSCD459489	17.1	0.004	14.0	0.012	1.6	0.002	nd	nd	nd	nd
Unsaturated Acid	5.7	0.001	9.2	0.008	6.1	0.009	nd	nd	nd	nd
Others	64.8	0.016	51.6	0.044	76.9	0.115	48.0	0.006	83.0	0.016
Subtotal	103.5	0.025	86.3	0.074	85.6a	0.127a	55.6	0.007	92.3	0.018
Unextracted	3.1	0.001	11.6	0.010	12.4	0.018	19.2	0.002	4.7	0.001

[a] Summation of TRR extracted, protease hydrolysate and HCl hydrolysate

nd–Not detected

The metabolism of isopyrazam in the laying hen was shown to be complex but with no major differences between the metabolic profiles of the three radiolabelled experiments. However, the samples from the phenyl 69:31 experiment showed some extra degree of complexity relative to the 95:5 treatments. It is proposed that the additional components were due to the higher contributions of the *anti* diastereoisomers in the 69:31 treatment. All metabolites identified in the study contained both the pyrazole and phenyl moieties, thus showing no indication of amide cleavage.

The primary mechanisms for the proposed biotransformation pathway of isopyrazam in the laying hen are hydroxylation of the isopropyl group and bicyclic portion of the molecule. Further oxidation to carboxylic acids (methyl of isopropyl group) and dehydration were observed in eggs and tissues. Conjugates of dihydroxylated metabolites (glucuronides and sulphates), a trihydroxy metabolite and an N-demethylated dihydroxy metabolite were observed in the excreta.

The metabolic pathway in laying hens and lactating goats was similar to the one in the rat. The overall metabolism of isopyrazam in animals proceeded by the following processes:

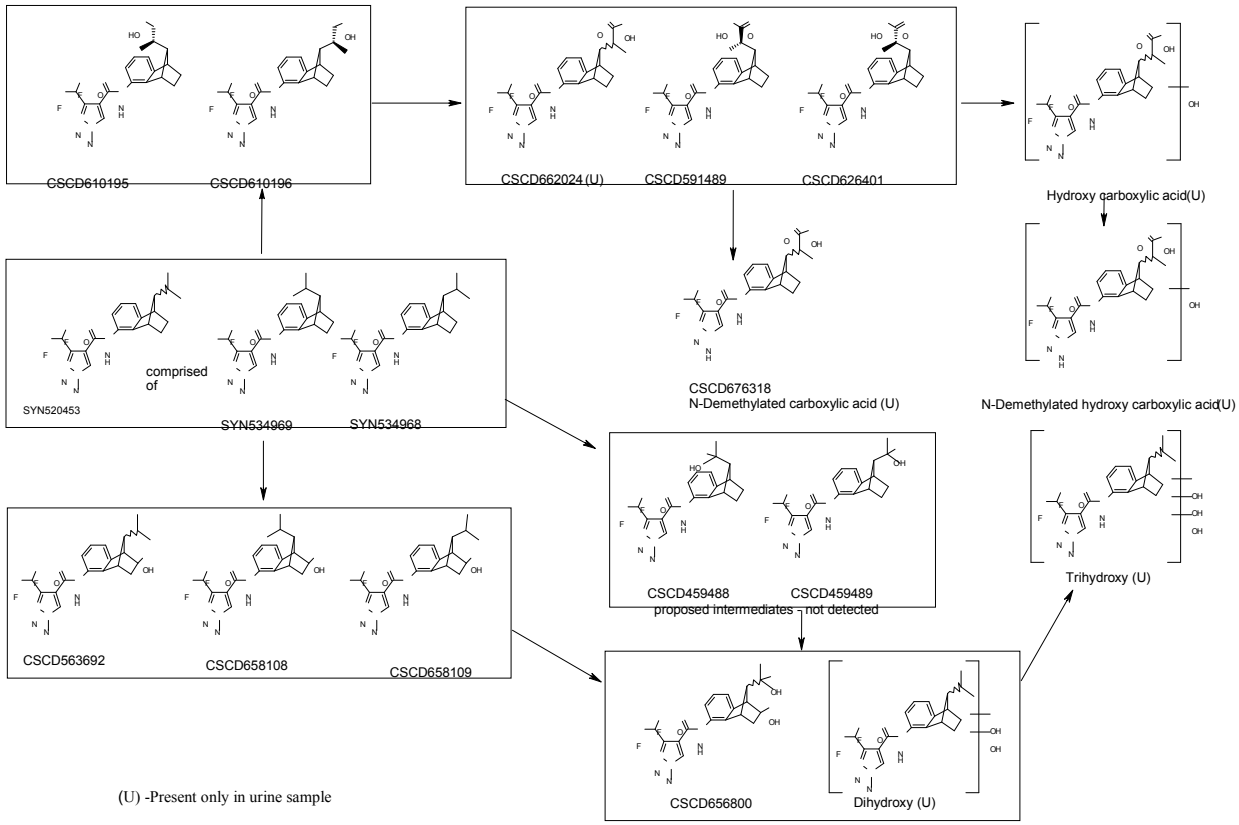
Hydroxylation of the bicyclic ring and isopropyl group

N-demethylation of the pyrazole ring

Oxidation of primary alcohols to form carboxylic acids and/or to give rise to multiple hydroxyl moieties

Formation of glucuronic acid or sulphate conjugates

The proposed metabolic pathway for isopyrazam in lactating goats and laying hens is shown in Figure 2.



Note:- Unspecified hydroxylation positions, as indicated by brackets, are restricted to the isopropyl and bicyclo ring moieties

Figure 2 Proposed Metabolic Pathways for isopyrazam in Lactating Goat and Laying Hens

Plant Metabolism

The Meeting received information on the fate of isopyrazam after foliar application on three different crops (wheat, grapes and lettuce) representative of the cereal, fruit and leafy crop group.

Wheat

A wheat metabolism study was conducted following three foliar spray applications of SYN530453, under glasshouse conditions, at Bracknell, Berkshire in the UK (Booth J., Goodwin S. 2007). The study was designed to quantify the total radioactive residue levels in appropriate crop parts (i.e., forage, straw (including husks) and grain) and to determine the extractability and nature of the residues.

Wheat (variety Tybalt) was treated three times with [phenyl-U-^{14}C] or [pyrazole-5-^{14}C] isopyrazam (*syn/anti* ratios approximating to 96:4) or [phenyl-U-^{14}C] isopyrazam (*syn/anti* ratio approximating to 70:30), at a nominal recommended application rate of 125 g ai/ha (equivalent to a seasonal rate of 375 g ai/ha). The radiochemicals were formulated as an emulsifiable concentrate

containing 100 g/L isopyrazam (EC 100, A14421C) and were applied as three foliar spray applications at growth time-points approximating to BBCH 31, BBCH 39 and BBCH 69. Plants were grown and treated in pots of soil under glasshouse conditions. For each radiolabel experiment, samples of forage were taken 13 days after the second application (BBCH growth stage 55–59). Straw and grain were harvested at maturity 46–48 days after the final application.

Forage samples were homogenised in the presence of liquid nitrogen. The husks were separated from the grain and combined with the mature straw before homogenization in the presence of liquid nitrogen. Grain was homogenized separately.

Aliquots of the homogenised wheat commodities (i.e., forage, straw (including husks) and grain) were initially analysed by direct combustion/LSC to determine the total radioactive residue in each commodity. Sub-samples of the homogenised wheat commodities (i.e., forage, straw (including husks) and grain) were then homogenised in the presence of solvents (e.g., acetonitrile, acetonitrile/water (80:20 v/v), acetonitrile/water (30:70 v/v), water and acetone). The solid and liquid phases were separated by centrifugation and aspiration allowing radio-assay of the solid and liquid phases to be carried out by combustion and LSC respectively. The total radioactive residue was derived from the summation of the radioactivity present in the extracts and debris.

Characterisation of the radioactive components in sample extracts was derived from their chemical and physical properties based primarily on partitioning and chromatographic behaviour. Identification of the radioactive components was carried out by TLC-co-chromatography with reference standards in both normal and reverse phase systems where possible. LC-MS/MS was used where appropriate to confirm the identification of metabolites present.

Total radioactive residues (TRRs) in wheat commodities were at a maximum level of 6.56 mg/kg in forage samples rising to a maximum level of 22.49 mg/kg in straw. TRRs in grain were much lower at a maximum residue level of 0.057 mg/kg. The TRR values for all three wheat commodities, determined by summing the radioactivity present in the extracts and the debris after initial extraction, were in good agreement with those derived by direct quantification. TRR values determined by summation of the radioactivity present in the extracts and the debris after initial extraction are used to express residue levels from this point forwards.

The total radioactive residues (TRRs) found in the wheat commodities are summarised in Table 11.

Table 11 Total Radioactive Residues in Wheat Samples after Treatment with Radiolabelled isopyrazam

Radiolabel Position & Isomer Ratio	Plant Part	Total Radioactive Residue (mg/kg) [a]	
		By Direct Combustion and LSC Quantfication[1]	By Summation of Extracted and Unextracted Radioactivity
Phenyl-U-^{14}C *syn/anti* 96:4	Forage	7.088	6.525
	Straw	20.844	22.491
	Grain	0.058	0.056
Pyrazole-5-^{14}C *sn/anti* 96:4	Forage	6.175	6.253
	Straw	20.189	20.011
	Grain	0.059	0.057
Phenyl-U-^{14}C *syn/anti* 70:30	Forage	4.749	4.933
	Straw	14.083	13.370
	Grain	0.031	0.033

[a] mg of isopyrazam equivalents per kg of plant sample

Very high solvent extractability (> 90 % TRR) was achieved for forage and straw samples for each radiolabelled experiment. Slightly lower solvent extractability (> 76% TRR) was achieved for grain samples for each radiolabelled experiment.

No further work was performed on the debris from any of the wheat commodities following solvent extraction.

Tables 12 to Table 14 summarise the identification and characterization of the residues in wheat extracts. The profile of radioactive components present in forage, straw (including husks) and grain were very similar.

Parent compound, isopyrazam, was the major residue detected in all three commodities which amounted to a maximum level of 89.0% TRR (5.807 mg/kg) in forage and a maximum level of 68.7% TRR (15.451 mg/kg) in straw. isopyrazam residues in grain were significantly lower, at a maximum level of 65.6% TRR (0.0365 mg/kg). Analysis of the combined extracts from phenyl (*syn/anti* ratio 70:30) labelled forage and straw indicated no significant change in the *syn/anti* ratio of isopyrazam relative to the original applied material.

No metabolites in grain exceeded 5.6% TRR (0.0032 mg/kg). The most significant metabolite was identified as the tertiary alcohol CSCD459488, at a maximum residue level in straw corresponding to 1.941 mg/kg (9.7% TRR). CSCD459488 was also observed at lower levels in forage (maximum of 0.150 mg/kg, 2.4% TRR) and grain (maximum of 0.0008 mg/kg, 1.4% TRR). Also identified were a secondary alcohol CSCD563692 and a primary alcohol CSCD563691, which were at their maximum residue levels in straw, corresponding to 0.760 mg/kg (3.8% TRR) and 0.540 mg/kg (2.7% TRR). A further metabolite was identified by mass spectrometry as a dihydroxylated metabolite of isopyrazam at a maximum residue level in straw corresponding to 0.495 mg/kg (2.2% TRR). Major proportions of these four metabolites were released after enzyme hydrolysis indicating the presence of these compounds as carbohydrate conjugates.

A number of additional components were identified, at trace levels, as the N-demethylated parent compounds (CSCD539372 and CSCD539391) and the unsaturated product CSCC230729 which was assumed to arise via dehydration of an alcohol. The half-molecule pyrazole acid CSAA798670 was also observed, indicating some very limited cleavage of the amide bond.

These results demonstrate that unchanged parent isopyrazam comprises the majority of the residue in wheat. The principal metabolism steps are hydroxylation of the isopropyl group and hydroxylation of a methylene group of the bicyclic ring. The compounds containing the alcohol functionality showed a potential to form sugar conjugates. Limited cleavage of the amide bond between the two aromatic rings resulted in the formation of one metabolite exclusive to the pyrazole label. No phenyl specific metabolites were detected.

Table 12 Summary of Identification and Characterisation of Residues in Isopyrazam-Treated Wheat Forage Samples

Components	Phenyl-U-^{14}C (*syn/anti* 96:4)		Pyrazole-5-^{14}C (*syn/anti* 96:4)		Phenyl-U-^{14}C (*syn/anti* 70:30)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam	89.0	5.807	78.8	4.927	91.4[a]	4.504[a]
CSCD459488	0.9 (0.1)	0.059	2.4 (0.7)	0.150	0.4	0.020
CSCD563692	0.6 (0.6)	0.039	1.6 (1.4)	0.100	0.2 (0.2)	0.010
CSCD563691	0.1 (0.1)	0.007	0.2 (0.1)	0.013	< 0.1	< 0.001
CSCD539372[b]	< 0.1	< 0.007	nd	nd	< 0.2	< 0.010
CSCD539391[b]	nd	nd	nd	nd	< 0.1	< 0.005
CSCC230729[b]	nd	nd	< 0.1	< 0.006	nd	nd
CSAA798670	nd	nd	0.7	0.044	nd	nd
Dihydroxy (Diol)	0.4 (0.3)	0.026	0.7 (0.4)	0.044	< 0.1	< 0.001
Organosoluble Unknowns	4.3[c]	0.281[c]	6.5[d]	0.406[d]	3.2[e]	0.158[e]
Aqueous soluble unknowns	5.0[f]	0.326[f]	8.9[f]	0.557[g]	3.4[h]	0.168[h]
Baseline	0.7	0.046	1.5	0.094	0.9	0.044
Unassigned	nd	nd	0.3	0.019	0.3	0.015
Organosoluble Fraction (not	0.1	0.007	0.1	0.006	0.2	0.010

Components	Phenyl-U-^{14}C (*syn/anti* 96:4)		Pyrazole-5-^{14}C (*syn/anti* 96:4)		Phenyl-U-^{14}C (*syn/anti* 70:30)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
analysed)						
Unextracted	1.0	0.065	1.3	0.081	0.5	0.025
Total	102.2	6.670	103.1	6.447	100.7	4.968

[a]–2D-TLC chromatography shows the presence of both *syn*-isomer and *anti*-isomer) at 64.1% TRR; 3.162 mg/kg and 27.2% TRR; 1.342 mg/kg respectively.

[b]–These compounds were present at only trace levels. Accurate quantification by TLC was difficult to achieve and the figures quoted are almost certainly an exaggeration of the true residue levels–as indicated by the 'less than' (<) qualifiers. The compounds have, nevertheless, been included in the identification since they were detected by LC-MS/MS.

[c]–Comprised of at least 28 components none greater than 1.3% TRR; 0.085 mg/kg

[d]–Comprised of at least 20 components none greater than 1.9% TRR; 0.119 mg/kg

[e]–Comprised of at least 18 components none greater than 1.0% TRR; 0.049 mg/kg

[f]–Comprised of at least 8 components none greater than 3.1% TRR; 0.202 mg/kg

[g]–Comprised of at least 7 components none greater than 5.9% TRR; 0.369 mg/kg

[h]–Comprised of at least 8 components none greater than 2.0% TRR; 0.099 mg/kg

nd–Not detected

Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

Table 13 Summary of Identification and Characterisation of Residues in Isopyrazam-Treated Wheat Straw (including Husks) Samples

Components	Phenyl-U-^{14}C (*syn/anti* 96:4)		Pyrazole-5-^{14}C (*syn/anti* 96:4)		Phenyl-U-^{14}C (*syn/anti* 70:30)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam	68.7	15.451	60.7	12.147	64.0[a]	8.557[a]
CSCD459488	7.3 (5.2)	1.642	9.7 (7.0)	1.941	7.6 (6.5)	1.016
CSCD563692	2.9 (2.3)	0.652	3.8 (3.4)	0.760	2.8 (2.2)	0.374
CSCD563691	2.1 (1.6)	0.472	2.7 (2.4)	0.540	1.8 (1.6)	0.241
CSCD539372[b]	< 0.2	0.045	nd	nd	< 0.3	< 0.04
CSCD539391[b]	< 0.1	0.022	nd	nd	nd	nd
CSCC230729[b]	nd	nd	< 0.3	< 0.060	< 0.3	< 0.04
CSAA798670	nd	nd	0.1	0.020	nd	nd
Dihydroxy (Diol)	2.2 (1.8)	0.495	1.6 (1.4)	0.320	1.5 (1.3)	0.201
Organosoluble Unknowns	5.0[c]	1.125[c]	4.4[d]	0.880[d]	7.5[e]	1.003[e]
Aqueous soluble unknowns	5.9[f]	1.327[f]	6.3[g]	1.261[d]	5.6[h]	0.749[h]
Baseline	0.8	0.180	1.0	0.200	1.2	0.160
Unassigned	0.2	0.045	0.3	0.060	0.3	0.040
Unextracted	3.8	0.855	4.6	0.921	3.1	0.414
Total	98.9	22.311	95.2	19.110	95.4	12.835

[a] 2D-TLC chromatography shows the presence of both *syn*-isomer and *anti*-isomer; however the resolution achieved does not allow for accurate quantification of the two individual isomers. LC-MS/MS was used to confirm the *syn/anti* ratio of Isopyrazam.

[b] These compounds were present at only trace levels. Accurate quantification by TLC was difficult to achieve and the figures quoted are almost certainly an exaggeration of the true residue levels–as indicated by the 'less than' (<) qualifiers. The compounds have, nevertheless, been included in the identification since they were detected by LC-MS/MS.

[c] Comprised of at least 16 components none greater than 1.1% TRR; 0.247 mg/kg

[d] Comprised of at least 17 components none greater than 0.5% TRR; 0.100 mg/kg

[e] Comprised of at least 24 components none greater than 3.8% TRR; 0.508 mg/kg

[f] Comprised of at least 24 components none greater than 0.9% TRR; 0.202 mg/kg

[g] Comprised of at least 14 components none greater than 1.1% TRR; 0.220 mg/kg

[h] Comprised of at least 15 components none greater than 0.9% TRR; 0.120 mg/kg

nd–Not detected

Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

Table 14 Summary of Identification and Characterisation of Residues in Isopyrazam-Treated Wheat Grain Samples

Components	Phenyl-U-^{14}C (*syn/anti* 96:4)		Pyrazole-5-^{14}C (*syn/anti* 96:4)		Phenyl-U-^{14}C (*syn/anti* 70:30)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam	65.6	0.0365	53.3	0.0303	63.2[a]	0.0206[a]
CSCD459488	1.2	0.0007	1.4	0.0008	1.3	0.0004
CSCD563692	nd	nd	0.5	0.0003	0.5	0.0002
CSCD563691	nd	nd	nd	nd	nd	nd
CSCD539372	nd	nd	nd	nd	nd	nd
CSCC230729	nd	nd	nd	nd	nd	nd
Dihydroxy (Diol)	nd	nd	2.4	0.0013	nd	nd
Unknowns	nd	nd	5.6[b]	0.0032[b]	nd	nd
Baseline	12.0	0.0067	11.9	0.0068	7.9	0.0026
Unassigned	nd	nd	0.2	0.0001	0.5	0.0001
Organosoluble fraction (not analysed)	0.3	0.0002	0.2	0.0001	0.5	0.0002
Aqueous soluble fraction (not analysed)	0.8	0.0004	0.3	0.0002	0.7	0.0002
Unextracted	10.5	0.0058	14.4	0.0079	21.4	0.0070
Total	90.4	0.0503	90.2	0.0510	96.0	0.0313

[a] 2D-TLC chromatography shows the presence of both *syn*-isomer and *anti*-isomer; however the resolution achieved does not allow for accurate quantification of the two individual isomers.

[b] Comprised of at least 1 component

nd–Not detected

Grapes

A grape metabolism study was conducted after a single foliar spray application of isopyrazam in the field in the UK (Chapleo S. 2008). [Phenyl-U-^{14}C] and [pyrazole-5-^{14}C] isopyrazam (*syn/anti* ratio approximately 70:30) were formulated as suspension concentrates (formulation code A15309E containing 250 g/L isopyrazam) and applied separately as a foliar spray to grape vines (variety Syrah). A single application was made, at a nominal application rate of 400 g ai/ha. Samples of mature grapes and vine leaves were harvested 21 days after application. Stalks were removed from grapes and discarded and the fresh weight of each sample taken. Approximately 30% of the bulk grape sample produced from each radiolabelled experiment was washed with solvent to provide samples enriched with surface residue radioactivity. These solvent washes were produced to aid characterisation / identification of residues, should they be required during subsequent analysis of unwashed grape and leaf samples (the two test commodities) in the analytical phase of the study.

Grapes and leaves were homogenized in the presence of solid carbon dioxide pellets. Following sublimation of the carbon dioxide, the total radioactive residues (TRRs) were determined by direct combustion/LSC of the homogenized samples. Grapes were extracted into acetonitrile:water (60:40 v/v) using homogenization. Vine leaves were extracted into acetonitrile, acetonitrile/water (80:20, v/v), acetonitrile/water (30:70, v/v) and water in succession in the same manner. Extracts were analysed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Following extraction of homogenised samples, the total radioactive residues (TRRs) in grapes and leaves were determined by the summation of the radioactivity present in the extracts and the remaining debris. The leaf aqueous fractions were incubated with pectinase (30 °C, 18 h) partitioned with diethyl ether and analysed by TLC.

Identification of the radioactive components in sample extracts was by co-chromatography with reference standards.

The TRRs in unwashed grapes were 0.128 and 0.147 mg/kg for the [phenyl-(U)-^{14}C] and [pyrazole-5-^{14}C] isopyrazam labelled experiments respectively. The TRR in the vine leaves were 10.98 and 3.76 mg/kg for the [phenyl-U-^{14}C] and [pyrazole-5-^{14}C] isopyrazam labelled experiments respectively. TRRs found in the grapes and leaves are summarized in Table 15.

Table 15 Total Radioactive Residues in Grapes Berry and Leaf Samples after Treatment with Radiolabelled isopyrazam

Radiolabel Position	Plant Part	TRR (mg/kg)	
		By Direct Combustion and LSC Quantification	By Summation of Extracted and Unextracted Radioactivity
Phenyl-U-^{14}C	Berries	0.156	0.128
	Leaves	10.973	10.979
Pyrazole-5-^{14}C	Berries	0.147	0.147
	Leaves	3.768	3.759

The extractability of radioactive residues from grapes using acetonitrile:water (60:40 v/v) and from vine leaves using acetonitrile, acetonitrile/water (80:20, v/v), acetonitrile/water (30:70, v/v) and water was examined. The majority of the radioactivity in the grapes was extractable using acetonitrile:water (60:40 v/v) accounting for 98.0–98.4% TRR leaving 1.4–1.8% TRR unextracted. Most of the radioactivity (about 100% TRR) in the leaves was also extractable using acetonitrile/water mixtures leaving 1.7–1.8% TRR unextracted.

Table 16 summarises the identification and characterization of the residues in the extracts of grape samples. Table 17 summarises the identification and characterization of the residues in leaf samples. The main figures are the sum of both the free and conjugated forms. The figures in parentheses indicate the percentage of the TRR that is present as the conjugated forms.

Parent isopyrazam was identified as the major component of the residue from both labels ranging 89.4–90.3% TRR (maximum 0.131 mg/kg) in grapes and 86.4–91.2% TRR (maximum 10.013 mg/kg) in leaves. The *syn/anti* ratio of isopyrazam in grapes and leaf fractions was confirmed by HPLC as approximating 70:30 indicating no significant change in ratio compared to that of the applied radiochemical.

The most significant metabolites detected were the hydroxylated metabolites CSCD563692 (secondary alcohol) and CSCD610195 (primary alcohol), individually present at maximum residue levels of 1.7% TRR (0.002 mg/kg) in grapes and 4.2% TRR (0.158 mg/kg) in leaves. A tertiary alcohol CSCD459488 with *syn* stereochemistry was detected at maximum residue levels of 1.4% TRR (0.002 mg/kg) in grapes and 2.4% TRR (0.110 mg/kg) in leaves. The equivalent *anti* form of the tertiary alcohol (CSCD459489) was detected at trace levels in leaves. Additional metabolites identified at low levels were the dihydroxylated metabolite CSCD656800 (leaves) and N-demethylated parent (CSCD539372 and/or CSCD539391–leaves).

There were no significant differences between the metabolic profiles of the two radiolabelled experiments although trace levels of the half-molecule pyrazole acid metabolites CSCD465008 (grapes and leaves) and CSAA798670 (grapes) were detected in the pyrazole-labelled experiment. No phenyl-specific metabolites were observed. Metabolites containing either an alcohol or carboxylic acid functionality were found in both free and conjugated forms within the leaves.

The principal metabolism steps of isopyrazam involve hydroxylation of the isopropyl group and/or the bicyclic ring. A minor metabolic transformation involved N-demethylation of the pyrazole ring. Only very minor levels of the half molecule pyrazole acids, CSAA798670 and CSCD465008, were observed indicating that cleavage of the amide bond between the two aromatic rings was not a significant metabolic transformation. Some of the metabolites, which contained either an alcohol or carboxylic acid functionality, also existed as conjugates.

Table 16 Summary of Identification and Characterisation of Residues in isopyrazam-treated Grape Berry Samples

Component	Phenyl-U-^{14}C		Pyrazole-5-^{14}C	
	% TRR	Residue (mg/kg)	% TRR	Residue (mg/kg)
Isopyrazam	90.3	0.116	89.4	0.131
CSCD459488	0.8	0.001	1.4	0.002
CSCD563692 [a]	0.7	0.001	1.7	0.002
CSCD610195 [a]	0.4	< 0.001		
CSCD465008	nd	nd	0.2	< 0.001
CSAA798670	nd	nd	0.5	0.001
Unassigned [b]	0.3	< 0.001	1.1	0.002
Baseline [c]	0.1	< 0.001	0.5	0.001
Remainder [d]	3.2	0.004	0.2	< 0.001
Aqueous Fraction [e]	2.2	0.003	3.4	0.005
Unextracted	1.8	0.002	1.4	0.002
Total	99.8	0.127	99.8	0.146

[a] Additional 2D-TLC was undertaken to resolve these metabolites to allow quantification (phenyl label only), values for the pyrazole label represent the sum of both components

[b] In each case, one discrete component was detected

[c] Baseline material on TLC plate

[d] Areas of the chromatogram which could not be assigned to discrete radioactive components

[e] Aqueous fraction remaining after diethyl ether partitioning

nd–not detected

Table 17 Summary of Identification and Characterisation of Residues in Isopyrazam-treated Grape Leaf Samples

Component	Phenyl-U-^{14}C		Pyrazole-5-^{14}C	
	% TRR	Residue (mg/kg)	% TRR	Residue (mg/kg)
Isopyrazam	91.2	10.013	86.4	3.248
CSCD459488	1.0	0.110	2.4	0.090
	(0.8)	(0.088)	(1.9)	(0.071)
CSCD459489	0.2	0.022	0.5	0.019
	(0.1)	(0.011)	(0.3)	(0.011)
CSCD563692 [a]	1.0	0.110	4.2	0.158
	(0.8)	(0.088)	(3.6)	(0.135)
CSCD610195 [a]	0.4	0.048		
	(0.4)	(0.044)		
CSCD539391/ CSCD539372[e]	0.1	0.011	0.1	0.004
CSCD656800	0.3	0.033	0.7	0.026
	(0.2)	(0.022)	(0.4)	(0.015)
CSCD465008[f]	nd	nd	1.4	0.053
			(1.0)	(0.038)
Unassigned [b]	0.9	0.099	1.7	0.064
Baseline [c]	0.4	0.044	0.7	0.027
Remainder [d]	2.5	0.274	1.9	0.071
Organosoluble Extract [e]	0.7	0.077	0.5	0.019
Aqueous Extract [f]	0.1	0.011	0.1	0.004
Aqueous Hydrolysate [f]	0.1	0.011	0.2	0.008
Unextracted	1.7	0.187	1.8	0.068
Total	100.6	11.050	102.6	3.859

[a]–Additional 2D-TLC was undertaken to resolve these metabolites to allow quantification (phenyl label only), values for the pyrazole label represent the sum of both components

[b]–Phenyl label–at least 8 discrete components, none greater than 0.3% TRR (0.033 mg/kg). Pyrazole label–at least 4 discrete components, none greater than 0.6% TRR (0.023 mg/kg).

[c]–Baseline material on TLC plate

[d]–Areas of the chromatogram which could not be assigned to discrete radioactive components

[e]–Aqueous acetonitrile extract

[f]–Aqueous fraction remaining after diethyl ether partitioning of post-pectinase hydrolysates
Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

Lettuce (grown outdoor)

Butterhead lettuce (variety Mona) was treated three times separately with [phenyl-U-^{14}C] and [pyrazole-5-^{14}C] isopyrazam, at a nominal recommended application rate of 125 g ai/ha (corresponding to a seasonal rate of 375 g ai/ha) (Rosenwald J. 2008). The radiochemicals were formulated as EC 100 (A14421D) and applied as three foliar spray applications at growth time-points approximating BBCH < 40, BBCH 42 and BBCH 46. Plants were grown and treated in plots under outdoor conditions. For each radiolabel experiment, samples of lettuce foliage were taken three days after the last application. Samples of lettuce foliage were also taken from both radiolabelled experiments at maturity, 14 days after the final application.

The total radioactive residues in lettuce foliage were initially determined by direct combustion of homogenized samples. Sub-samples of homogenised lettuce were extracted in the presence of solvents (e.g., acetonitrile and acetonitrile/water (80:20 v/v). The total radioactive residue was derived from the summation of the radioactivity present in the extracts and debris.

Liquid-liquid partitions were carried out between two immiscible solvent phases (diethyl ether and aqueous fractions) in order to separate unconjugated metabolites (diethyl ether phase) from predominantly conjugated forms of the metabolites (aqueous phase). The phases were then separated and the partition / separation procedure repeated twice. The organic phases were combined prior to analyses. Aqueous fractions were submitted to enzymatic and acidic hydrolysis to release primary metabolites from their corresponding conjugates. Resulting extracts were analysed either by TLC and/or HPLC using reference standards for co-chromatography.

The total radioactive residues (TRRs) reached a maximum of 1.555 and 1.538 mg/kg in lettuce harvested 3 days after the last application for phenyl and pyrazole radiolabelled experiments, respectively. The TRRs in lettuce harvested at maturity, 14 days after the final application, were significantly lower and were 0.311 mg/kg and 0.221 mg/kg for phenyl and pyrazole radiolabelled experiments, respectively.

The total radioactive residues found in lettuce foliage are summarised in Table 18.

Table 18 Total Radioactive Residues in Lettuce Foliage Samples after Treatment with Radiolabelled isopyrazam.

Radiolabel Position	Harvest date after the last application	Total radioactive residue (mg/kg)	
		By summation of extract and debris	By direct combustion and LSC
Phenyl-U-^{14}C	3 days	1.555	1.613
	14days	0.311	0.316
Pyrazole-5-^{14}C	3 days	1.538	1.471
	14 days	0.221	0.217

A total of 82.5% (phenyl label) and 80.1% (pyrazole label) of the total radioactive residue (TRR) were extracted from lettuce harvested 3 days after the final application. In mature lettuce, the extractable residue was 96.1% (phenyl label) and 114.8% (pyrazole label) of the TRR.

Tables 19 and 20 Table summarise the identification and characterization of the residues in lettuce extracts derived at 3 days after the last application .

Chromatographic analysis of the extracted radioactivity in each case showed two major radiocomponents to predominate. These were characterised as the parent compound (isopyrazam) and polar conjugates of its metabolites.

Following chromatographic analysis of pre-and post-hydrolysis fractions, unchanged isopyrazam was the major radiocomponent (1st harvest: phenyl label–66.2% TRR, 1.029 mg/kg; pyrazole label–71.1% TRR, 1.093 mg/kg and 2nd harvest: phenyl label 34.8% TRR; 0.108 mg/kg,

pyrazole-5-^{14}C; 45.3% TRR; 0.100 mg/kg). The tertiary alcohol CSCD459488 arising from hydroxylation of the isopropyl group was a significant metabolite which occurred almost entirely in a conjugated form (1st harvest: phenyl label–1.4% TRR, 0.022 mg/kg; pyrazole label–0.6% TRR, 0.009 mg/kg. 2nd harvest: phenyl label–17.1% TRR, 0.053 mg/kg; pyrazole label–14.1% TRR, 0.031 mg/kg).

Other minor metabolites characterised in lettuce from both radiolabelled experiments at the first harvest were CSCD539372, CSCD610195 and/or CSCD563692 and a component characterised to be a dihydroxylated metabolite of isopyrazam by its similar Rf in two dimensional TLC to that observed in the isopyrazam wheat metabolism study. Collectively, these accounted for ≤ 4.6% TRR (≤ 0.072 mg/kg). Individual amounts of these metabolites did not exceed 3.9% TRR (0.019 mg/kg). An additional minor metabolite, CSCD465008 (0.7% TRR; 0.011 mg/kg) derived only from lettuce of the pyrazole labelled experiment was also identified. No phenyl-specific metabolites were observed.

Minor metabolites characterised to be present in lettuce of both radiolabelled experiments of the 2nd harvest time point and collectively accounting for ≤ 14.7% TRR (≤ 0.045 mg/kg) were CSCD573363, CSCD120604, CSCD539372, CSCD610195 and/or CSCD563692 and the dihydroxylated metabolite also observed in the 1st harvest time point lettuce. Individual amounts of these metabolites did not exceed 5.8% TRR (0.018 mg/kg). Two additional minor metabolites, CSAA798670 (3.7% TRR; 0.008 mg/kg) and CSCD465008 (1.0% TRR; 0.002 mg/kg), derived only from lettuce of the pyrazole labelled experiment, were also identified. No phenyl-specific metabolites were observed.

The results indicated that parent compound is the dominant residue in lettuce and that there were no significant differences between the metabolic profiles of the two radiolabelled experiments.

The figures in the table are the total radioactive residue for each metabolite reported, expressed as mg/kg isopyrazam equivalents per kg lettuce forage. The main figures are the sum of both the free and conjugated forms. The figures in parentheses indicate the percentage of the TRR that is present as the conjugated forms.

Table 19 Summary of Identification and Characterisation of Residues in Lettuce Foliage Harvested 3 Days after the Last Application

Components	Phenyl-U-^{14}C		Pyrazole-5-^{14}C	
	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam	66.2	1.029	71.1	1.093
CSCD459488	1.4	0.022	0.6	0.009
	(1.4)	(0.022)	(0.6)	(0.009)
CSCD610195 /CSCD563692[a]	0.7	0.011	0.2	0.003
	(0.7)	(0.011)	(0.2)	(0.003)
CSCD539372	< 0.1	< 0.001	0.1	0.002
	(< 0.1)	(< 0.001)	(0.1)	(0.002)
CSCD465008[b]	na	na	0.7	0.011
	(na)	(na)	(0.7)	(0.011)
Dihydroxylated metabolite [c]	3.9	0.061	2.3	0.036
	(1.2)	(0.019)	(0.5)	(0.008)
Conjugates [d]	8.9	0.138	4.9	0.075
Others (unassigned)[e]	1.4	0.022	0.2	0.003
Unextracted [f]	3.1	0.048	3.5	0.054
Total	85.6	1.331	83.6	1.286

[a] A radiolabelled reference standard of CSCD563692 was used.

[b] A metabolite structure retaining only the pyrazole ring of isopyrazam.

[c] A radiocomponent, characterised by its position on 2D-TLC relative to that of a wheat metabolite to be a dihydroxylated metabolite of isopyrazam (positions of hydroxylation unknown).

[d] Characterised as polar sugar conjugates remaining unhydrolysed in the analysis.

[e] Other unassigned residues, comprising at least two discrete radiocomponents, no single one of which was greater than 2.7% TRR (0.042 mg/kg) in the [phenyl-U-^{14}C] radiolabelled experiment or greater than 1.8% TRR (0.028 mg/kg) in the [pyrazole-5-^{14}C] radiolabelled experiment.

[f] Radioactivity remaining unextracted from the lettuce debris following solvent extraction.

na–Not applicable

Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

Table 20 Summary of Identification and Characterisation of Residues in Lettuce Foliage Harvested 14 Days after the Last Application

Components	Phenyl-U-^{14}C		Pyrazole-5-^{14}C	
	% TRR	mg/kg	% TRR	mg/kg
isopyrazam	34.8	0.108	45.3	0.100
CSCD459488	17.1	0.053	14.1	0.031
	(16.2)	(0.050)	(14.1)	(0.031)
CSCD610195 /CSCD563692[a]	5.8	0.018	4.7	0.011
	(5.8)	(0.018)	(4.7)	(0.011)
CSCD573363	1.6	0.005	0.8	0.002
	(1.6)	(0.005)	(0.8)	(0.002)
CSCD539372	0.7	0.002	0.8	0.002
	(0.7)	(0.002)	(0.8)	(0.002)
CSCD120604	2.6	0.008	1.9	0.004
	(2.6)	(0.008)	(1.9)	(0.004)
CSCD465008[b]	na	na	1.0	0.002
	(na)	(na)	(1.0)	(0.002)
CSAA798670[b]	na	na	3.7	0.008
	(na)	(na)	(3.7)	(0.008)
Dihydroxylated metabolite [c]	4.0	0.012	3.7	0.008
	(4.0)	(0.012)	(3.7)	(0.008)
CSCD230729[d]	2.8	0.009	2.8	0.006
	(2.8)	(0.009)	(2.8)	(0.006)
Conjugates [e]	16.0	0.049	19.4	0.042
Others (unassigned)[f]	0.5	0.002	1.8	0.004
Unextracted [g]	10.2	0.032	14.8	0.033
Total	96.1	0.298	114.8	0.253

[a] A radiolabelled reference standard of CSCD563692 was used.

[b] A metabolite structure retaining only the pyrazole ring of isopyrazam.

[c] A radiocomponent, characterised by its position on 2D-TLC relative to that of a wheat metabolite to be a dihydroxylated metabolite of isopyrazam (positions of hydroxylation unknown).

[d] Deemed not to be a true metabolite but an artefact produced during a strong acid hydrolysis procedure.

[e] Characterised as polar sugar conjugates remaining unhydrolysed in the analysis.

[f] Other unassigned residues, comprising at least one discrete radiocomponent 0.5% TRR (0.002 mg/kg) in the [phenyl-U-^{14}C] radiolabelled experiment and at least two discrete radiocomponents in the [pyrazole-5-^{14}C]radiolabelled experiment, no single one of which was greater than 1.2% TRR (0.003 mg/kg).

[g] Radioactivity remaining unextracted from the lettuce debris following solvent extraction.

na–Not applicable

Figures in parentheses indicate the percentage of the TRR that is present as conjugated forms

The principal metabolism of isopyrazam in lettuce involves hydroxylation of the isopropyl group or hydroxylation of the bicyclic ring. A minor metabolic transformation involved N-demethylation of the pyrazole ring.

Only minor levels of the half molecule pyrazole acids, CSAA798670 and CSCD465008, were observed in this study indicating that cleavage of the amide bond between the two aromatic rings is not a significant metabolic transformation of isopyrazam in lettuce.

Metabolic pathway of isopyrazam in plants

Metabolism studies of isopyrazam in three different crop groups (cereal–wheat, leafy crop–lettuce and fruit–grape) have provided an understanding of the biotransformation of this compound in food and feed items. The metabolic routes determined in the three crops were predominantly the same and consequently the metabolism of isopyrazam in all three crops is considered comparable. The available

plant metabolism studies are therefore deemed sufficient to support the use of isopyrazam on all crops.

The extent of metabolism of isopyrazam was related to the time interval between application and harvest and also to the growth stage of the crop at the time of application but in all cases unchanged parent accounted for a very significant proportion of the residue, ranging from a minimum of 34.8% TRR in lettuce to approximately 90% TRR in grapes and vine leaves. Measurement of the *syn/anti* ratio of isopyrazam in crop commodities indicated that there was no significant change in ratio from that of the original test material.

The predominant metabolic reaction in all three crops was hydroxylation of the saturated hydrocarbon moiety to produce alcohols. The principal site of reaction was on the isopropyl side chain and resulted in the *syn* tertiary alcohol CSCD459488. Very minor amounts of the corresponding *anti* tertiary alcohol, CSCD459489, were detected, but only in vine leaves. These compounds underwent further reaction with naturally occurring carbohydrate moieties in the crops, hence were present primarily as polar conjugates. The exocons were released via hydrolysis under enzymic or acidic conditions. CSCD459488 accounted for up to 17.1% TRR, 0.053 mg/kg in lettuce, 9.7% TRR, 1.941 mg/kg in wheat straw and 2.4% TRR, 0.090 mg/kg in vine leaves. It was also present at detectable levels in all other commodities (grapes, wheat forage and grain). CSCD459488 was the only primary crop metabolite which exceeded 10% of the TRR. There was evidence for very low levels of metabolite CSCC230729, which is a dehydration product of CSCD459488 and CSCD459489, in wheat and lettuce. Trace amounts (< 0.3% TRR) of CSCC230729 appeared to be produced in wheat but in lettuce, where it accounted for up to 2.8% TRR in post-hydrolysis fractions, it was believed to be an artefact created by dehydration under acidic conditions.

Other alcohol metabolites were also identified in all three crop studies but generally at much lower levels than CSCD459488. Oxidation of a terminal methyl of the isopropyl substituent, leading to primary alcohol metabolites, was evident. Substitution at this position introduced a further chiral centre into the molecule hence there were four possible diastereoisomers; two *syn* and two *anti*. In the wheat metabolism study a primary alcohol, CSCD563691, representing a maximum of 2.7% TRR in straw after hydrolysis of conjugates, was identified but without any definition of stereochemistry. Subsequent provision of all four primary alcohol diastereoisomers as synthetic reference standards demonstrated that the wheat primary alcohol corresponded to CSCD610195, one of the pair of *syn* diastereoisomers. CSCD610195 was also detected at low levels in both the grape and lettuce studies following hydrolysis of conjugates. Accurate quantification was difficult in view of overlap with another unrelated alcohol (CSCD563692) in most available chromatographic systems. In the lettuce study, hydrolysis of a sample from the second harvest released 5.8% TRR, 0.018 mg/kg of a mixture of CSCD610195 and the unrelated alcohol. Chromatographic analysis of the same lettuce sample suggested the presence of trace levels of CSCD573363 (1.6% TRR, 0.005 mg/kg), one of the *anti* diastereoisomers of the primary alcohol.

A further site of oxidation observed in all three crop studies was one of the methylene carbons of the bicyclic ring system. The resulting secondary alcohol metabolite, which was also present primarily as a conjugate, was identified as CSCD563692. Although the stereochemistry could be defined at the site of hydroxylation, it was not possible to establish whether the isopropyl substituent was in the *syn* and/or *anti* position. CSCD563692 accounted for a maximum of 3.8% TRR, 0.76 mg/kg in wheat straw (highest mg/kg value), 5.8% TRR, 0.018 mg in lettuce (highest % TRR value but unresolved from CSCD610195) and was also present in vine leaves and grapes.

A more polar metabolite, also observed at low levels in all three studies, was established to be a dihydroxylated product. In the grape study (vine leaves), the component of interest, which represented a maximum of only 0.7% TRR, was shown to co-chromatograph with a standard of CSCD656800. The positions of hydroxylation correlate with the tertiary alcohol CSCD459488 and secondary alcohol CSCD563692 discussed previously. Although samples from the wheat and lettuce studies were not co-chromatographed with the standard, the available data strongly suggest that the same dihydroxylated metabolite was present in all three studies. This metabolite was observed at a

maximum of 4% TRR, 0.012 mg/kg in lettuce and 2.2% TRR, 0.495 mg/kg in wheat straw following hydrolysis of polar conjugates.

There may be potential for hydroxylation of other sites on the hydrocarbon moiety of isopyrazam based upon the co-chromatography of the *syn* tertiary alcohol CSCD120604 with a minor component in the lettuce sample from the second harvest, however this was present at levels well below the normal identification triggers (max 2.6% TRR, 0.008 mg/kg). The presence of CSCD120604 and its *anti* diastereoisomer CSCD120605 were also proposed from MS analysis of wheat straw but levels were too low for detection by TLC.

There was some evidence for other biotransformation pathways in crops, but the resulting metabolites were present as extremely minor components and were identified as a consequence of reference standard availability, rather than to meet guideline requirements. N-Demethylation of the pyrazole ring resulted in trace levels of CSCD539372 and/or SYN539391 in all three studies. Cleavage of the amide functionality between the two aromatic rings resulted in trace levels of the pyrazole acid CSAA798670 in all crops and the equivalent N-demethylated acid (CSCD465008) in the grape and lettuce studies. There was no evidence for metabolites containing exclusively the phenyl ring.

In summary, the wheat, lettuce and grape metabolism studies all exhibit the same biotransformation pathways for isopyrazam:

- Hydroxylation of the saturated hydrocarbon moiety to produce a range of alcohol products, with the tertiary alcohol CSCD459488 as the principal metabolite
- Subsequent conjugation to naturally occurring carbohydrates
- N-demethylation of the pyrazole ring (minor pathways)
- The proposed metabolism of isopyrazam in plants is summarised in Figure 3.

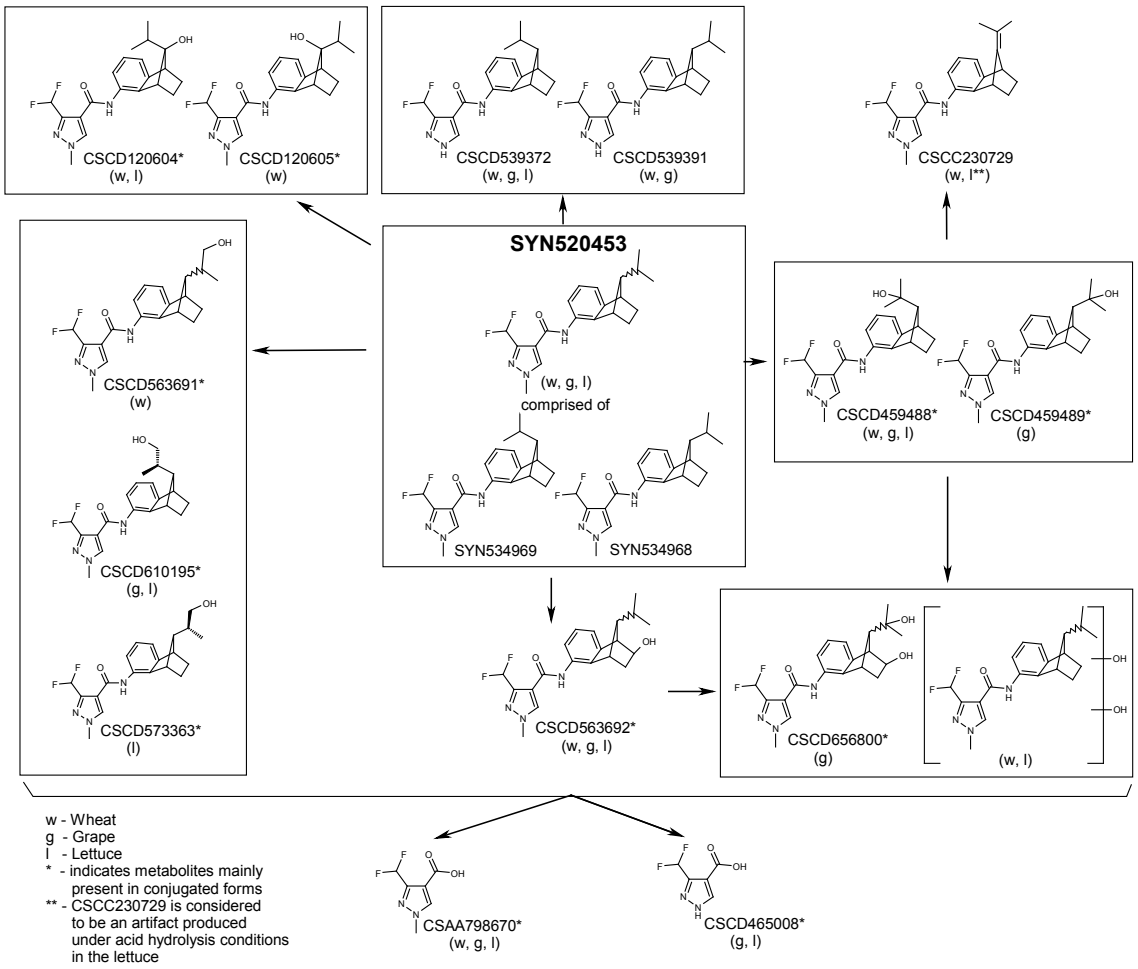


Figure 3 Proposed Metabolic Pathway for isopyrazam in Crops

Environmental fate in soil

The Meeting received information on hydrolysis and residues in succeeding crops as well as, aerobic soil metabolism, photodegradation in soil, soil dissipation, absorption and desorption and ready biodegradability, some of which are summarised in the Physical and Chemical Properties Section. Since isopyrazam is a fungicide with foliar application, studies on hydrolysis and residues in succeeding crops are relevant for the current review.

Metabolism and distribution in succeeding crops

A confined study has been conducted to examine the metabolism and distribution of isopyrazam in succeeding crops (Evans J. 2008). [Pyrazole-5-^{14}C] isopyrazam and [Phenyl-U-^{14}C] isopyrazam (*syn/anti* ratio of both radiochemical approximately 96/4) were formulated to a specification approximating EC 100 (A14421C). A single application of the formulated radiochemicals was made in separate experiments to containers of sandy loam soil at a nominal rate of 375 g ai/ha, i.e., higher than the proposed maximum annual application rate of 250 g ai/ha.

The commodities derived from the harvested crops collected from each of the experiments are summarised in Table 24. At each rotational interval of 30, 90 and 300 days after application (DAA), representative cereal (spring wheat: variety "Tybalt"), leafy (lettuce: variety "Voyager") and root (turnip: variety "Tokyo cross") crops were sown into the treated soil. All crops were grown under glasshouse conditions and harvested at maturity (turnip: 40–43 days after sowing (DAS), lettuce: 47–65 DAS, wheat: 88–98 DAS). Harvested crops were separated into commodities representative of food and feed (wheat: straw and grain; lettuce: foliage only; turnip: foliage and root as separate

commodities). Intact aerial samples of immature wheat were also harvested at two early time points simulating forage (BBCH 30–31; 33 DAS; derived from all rotational intervals), and hay (BBCH 77–83; 65–71 DAS; derived from 90 and 300 day plant back intervals only). Homogenised samples of all commodities were prepared for analysis. Soil cores (to a depth of 20cm) were also taken at the time of harvesting mature wheat from the 30-day plant-back interval (118 DAA).

Table 21 Commodities taken as test samples

Crop	Growth stage at harvest	Sample taken	Rotational sowing interval (DAA)	Harvest timepoint (DAA)	Harvest timepoint (DAS)
Wheat	BBCH 30-31	Forage	30	63	33
			90	123	33
			300	333	33
	BBCH 83	Hay	90	161	71
	BBCH 77–83		300	365	65
	Maturity	Straw Soil	30	118	88
			90	188	98
			300	398	98
		Grain	30	118	88
			90	188	98
			300	398	98
Turnip	Maturity	Foliage	30	70	40
			90	133	43
			300	342	42
		Root	30	70	40
			90	133	43
			300	342	42
Lettuce	Maturity	Foliage	30	77	47
			90	147	57
			300	365	65

The total radioactive residues present in crop commodities from all plant back intervals of both radiolabelled experiments are given in Table 22. TRR values were obtained from both direct analysis (i.e., combustion analysis/LSC) and from the summation of the radioactivity present in individual extracts plus that remaining in plant debris following extraction, and these are quoted separately in these tables. Both sets of data obtained from the two methods were in good agreement indicating that there were no losses of volatile radioactive components during extraction. The latter values are considered the more reliable because of the larger sample sizes taken for analysis and were therefore used as the definitive TRR for the purposes of calculating residue values (mg/kg) and percentages of the total radioactive residue values (% TRR) associated with analytical fractions.

Total radioactive residues (TRR, expressed as mg of isopyrazam equivalents per kg of commodity) were highest in the foliage-derived commodities from wheat; forage (≤ 0.154 mg/kg), hay (≤ 0.398 mg/kg) and straw (≤ 1.02 mg/kg), residues increasing with increased maturity of the commodity. The initial high residues observed in these 30-day plant-back commodities were shown to decrease gradually in the later 90 and 300-day plant-back commodities (wheat straw–pyrazole: 1.02 mg/kg decreasing to 0.728 mg/kg; phenyl: 0.801 mg/kg decreasing to 0.409 mg/kg, wheat hay–pyrazole: 0.398 mg/kg decreasing to 0.234 mg/kg; phenyl: 0.354 mg/kg decreasing to 0.261 mg/kg, wheat forage–pyrazole: 0.154 mg/kg decreasing to 0.120 mg/kg; phenyl: 0.073 mg/kg decreasing to 0.062 mg/kg).

Much lower TRRs were observed in wheat grain (≤ 0.0226 mg/kg) and commodities from other crops: turnip root (≤ 0.0183 mg/kg), turnip foliage (≤ 0.0532 mg/kg) and lettuce (≤ 0.023 mg/kg).

All commodities with TRRs > 0.01 mg/kg were extracted with solvents and analysed. Very high solvent extractability (88.0% TRR to 97.7% TRR) was achieved for all analysed commodities except for wheat grain where slightly lower residue extractability was observed (57.8% TRR to 83.0% TRR). Extracted residues were, as appropriate, further fractionated and characterised by aqueous/organic partition, pectinase enzyme hydrolysis and mineral acid hydrolysis. Residues present

in the principal fractions were subjected to thin layer chromatography/bioimage analysis for quantification and identification / characterisation by comparison with authentic reference standards of isopyrazam and its metabolites.

Table 22 Total Radioactive Residues from Rotational Crop Samples Grown in Soil Treated with Pyrazole-5-^{14}C Labelled Isopyrazam and Phenyl-U-^{14}C Labelled Isopyrazam

Commodity	Plant Back Interval (Days)	TRR (mg/kg)	
		Pyrazole-5-^{14}C	Phenyl-U-^{14}C
Wheat forage	30	0.1535	0.0730
	90	0.1507	0.0565
	300	0.1203	0.0622
Wheat hay	30	nh	nh
	90	0.3975	0.3539
	300	0.2341	0.2613
Wheat straw	30	1.0209	0.8005
	90	0.9477	0.7056
	300	0.7278	0.4094
Wheat grain	30	0.0226	0.0124
	90	0.0222	na
	300	0.0178	na
Turnip root	30	0.0183	0.0159
	90	na	na
	300	na	na
Turnip foliage	30	0.0508	0.0268
	90	0.0532	na
	300	0.0421	0.0107
Lettuce	30	0.0230	0.0096
	90	0.0315	na
	300	0.0181	na

na–Not applicable
nh–Not harvested

Table 23 to Table 25 summarise the identification and characterization of residues in the wheat straw, wheat forage and wheat hay. The residue profiles of wheat straw, hay and forage were all very similar. Parent isopyrazam was present at only minor levels, attaining maximum levels in straw of ≤ 0.0316 mg/kg and maximum percentage TRR in forage (≤ 9.28% TRR). Quantification of the *syn/anti* ratio in isopyrazam residues of wheat straw (93/7) and forage (95/5) revealed little or no change in the isomer ratio relative to that of the applied radiochemical (96/4).

The tertiary alcohol metabolite, CSCD459488, was the major identified residue in all three commodities, attaining maximum residue levels in wheat straw (≤ 0.165 mg/kg; ≤ 22.2% TRR) and the largest proportion of the total radioactive residue in wheat hay (≤ 0.090 mg/kg; ≤ 25.3%). Other significant metabolites in straw were the secondary alcohol, CSCD563692, the primary alcohol, CSCD610195 (both present at ≤ 0.057 mg/kg; ≤ 6.62% TRR) and, in the pyrazole labelled samples, the pyrazole acids, CSCD465008 (≤ 0.038 mg/kg; ≤ 3.90% TRR) and CSAA798670 (≤ 0.039 mg/kg; ≤ 3.85% TRR). A further radiocomponent was characterised to be one or more dihydroxylated metabolites of isopyrazam (≤ 0.042 mg/kg; ≤ 5.69% TRR). Trace levels of the N-demethylated parent CSCD539372/CSCD539391 and tertiary alcohol isomer pairs CSCD120604/CSCD120605 were detected. A pyrazole-specific metabolite, the amide CSCC210616 was also detected. High proportions of the alcohols and acids occurred as carbohydrate conjugates and were released after pectinase enzyme and/or mineral acid hydrolysis.

Isopyrazam and all of the metabolites identified in the wheat foliage were also shown to be present in wheat grain, turnip root, turnip foliage, and lettuce but at much lower levels.

Residues of isopyrazam (≤ 0.0001 mg/kg; ≤ 0.36% TRR) and its principal metabolites in wheat grain (CSAA798670, CSCD645008 and CSCD459488) were all present at very low or trace levels (individually ≤ 0.0018 mg/kg; ≤ 6.96% TRR).

Residues of isopyrazam present in turnip foliage, turnip root and lettuce never exceeded 0.0055 mg/kg. The principal metabolites of isopyrazam in these commodities were the N-desmethyl pyrazole acid, CSCD465008, in turnip foliage (≤ 0.0254 mg/kg; ≤ 47.67% TRR) and lettuce (≤ 0.0111 mg/kg; ≤ 35.25% TRR) and the pyrazole acid metabolite, CSAA798670, in turnip root (13.53% TRR; 0.0025 mg/kg). Levels of the tertiary alcohol metabolite, CSCD459488, in these commodities never exceeded 0.0012 mg/kg or 5.25% TRR.

In conclusion, the study showed that following application of isopyrazam to soil and subsequent aging of the treated soil for periods of up to 300 days, uptake of residues into rotational crops was most significant in wheat straw, wheat hay and wheat forage. Much lower uptake of residues was observed in wheat grain, turnip root, turnip foliage and lettuce.

The proposed biotransformation pathway for isopyrazam in rotational crops involved hydroxylation of the isopropyl group, hydroxylation of the bicyclic ring and N-demethylation of the pyrazole ring. The presence of the pyrazole half-molecule acids CSCD465008 and CSAA798670 in crop commodities indicates that cleavage of the amide linkage of isopyrazam is a significant process in succeeding crops. These metabolites were only observed at trace or negligible levels in the foliar applied isopyrazam primary crop metabolism studies, indicating that their greater prominence in rotational crops is most probably the result of initial cleavage of the amide bond and demethylation in the soil followed by uptake of the corresponding pyrazole half-molecule acids into crops.

Table 23 Summary of Identification and Characterisation of Residues in Wheat Straw Samples Grown in Soil Treated with ^{14}C-isopyrazam

Commodity	Wheat straw											
Plant back interval (days)	30				90				300			
Radiolabelled experiment	Pyrazole-5-^{14}C		Phenyl-U-^{14}C		Pyrazole-5-^{14}C		Phenyl-U-^{14}C		Pyrazole-5-^{14}C		Phenyl-U-^{14}C	
Component	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam [a]	3.10	0.0316	3.77	0.0301	3.27	0.0310	3.50	0.0247	2.25	0.0164	1.26	0.0051
CSCD459488	15.84	0.1617	17.20	0.1377	17.42	0.1650	22.15	0.1563	8.31	0.0605	12.02	0.0492
CSCD459489 [b]	0.13	0.0013							0.21	0.0015		
CSCD610195 [b]	3.20	0.0327	5.97	0.0478	5.97	0.0566	6.62	0.0467	1.24	0.0090	1.16	0.0048
CSCD563692 [b]	3.01	0.0308							0.86	0.0063		
CSCD539372 + CSCD539391 [c]	0.38	0.0039	0.56	0.0045	0.25	0.0024	0.32	0.0023	0.19	0.0014	0.13	0.0005
CSCD120604 + CSCD120605 [c]	0.21	0.0021	0.32	0.0026	0.37	0.0035	0.61	0.0042	0.15	0.0011	0.29	0.0011
Dihydroxy metabolite(s) [d]	3.49	0.0356	4.21	0.0337	4.39	0.0415	5.69	0.0402	1.33	0.0097	2.15	0.0089
CSCC230729 [e]	0.28	0.0029	0.78	0.0062	0.02	0.0002	0.01	0.0001	0.19	0.0014	0.51	0.0021
CSCC210616 [f]	0.82	0.0084	na	na	0.47	0.0045	na	na	0.35	0.0025	na	na
CSAA798670 [f]	3.85	0.0393	na	na	3.27	0.0310	na	na	2.85	0.0207	na	na
CSCD465008 [f]	3.72	0.0380	na	na	3.90	0.0369	na	na	3.58	0.0262	na	na
Organosoluble unknowns	13.22	0.1343	12.21	0.0977	13.09	0.1244	15.48	0.1090	11.54	0.0839	7.93	0.0322
Water soluble unknowns	15.83	0.1617	18.01	0.1442	17.30	0.1638	20.05	0.1415	35.63	0.2591	42.73	0.1749
Baseline	8.49	0.0867	14.27	0.1143	8.54	0.0810	10.68	0.0753	2.97	0.0216	15.82	0.0649
Remainder	7.04	0.0719	2.72	0.0218	2.10	0.0199	2.30	0.0163	6.79	0.0495	1.55	0.0064
Unextracted	10.46	0.1068	8.35	0.0668	10.80	0.1024	7.60	0.0536	12.00	0.0873	8.61	0.0352
Total	93.07	0.9497	88.37	0.7074	91.16	0.8641	95.01	0.6702	90.44	0.6581	94.16	0.3853

na: Not applicable to this radiolabelled experiment (see superscript 6).

[a] *Syn*/*anti* isomer ratio determined in the 30 day plant back [pyrazole-5-^{14}C] labelled analysis was found to be 93/7.

[b] Quantification of the individual levels of these metabolites undertaken in [pyrazole-5-^{14}C] labelled 30 day and 300 day plant back interval straw residue analyses only.

[c] Unable to fully resolve these metabolites in all fractions analysed therefore the value quoted is for the sum of the two metabolites.

[d] Characterised to be one or more "dihydroxy" metabolites based on similar Rf in all 2D-TLC solvent systems to that of a

dihydroxy metabolite of isopyrazam isolated from a *C. Elegans* fungal culture.

[e] Deemed not a true metabolite of isopyrazam but an artefact formed by the dehydration of appropriately substituted monohydroxylated metabolites (e.g., CSCD459488) under the mineral acid conditions of hydrolysis employed to release these metabolites from their conjugates.

[f] Metabolites structures retaining only the pyrazole ring of the parent molecule. Residues values correspond only to [pyrazoly-5-^{14}C] labelled experiment.

Table 24 Summary of Identification and Characterisation of Residues in Wheat Forage Samples Grown in Soil Treated with ^{14}C-isopyrazam

Commodity	Wheat forage											
Plant back interval (days)	30				90				300			
Radiolabelled experiment	Pyrazole-5-^{14}C		Phenyl-U-^{14}C		Pyrazole-5-^{14}C		Phenyl-U-^{14}C		Pyrazole-5-^{14}C		Phenyl-U-^{14}C	
Component	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam [a]	3.96	0.0060	9.28	0.0067	2.34	0.0035	6.81	0.0039	3.09	0.0037	3.90	0.0024
CSCD459488	12.83	0.0197	17.68	0.0129	12.28	0.0186	22.40	0.0127	12.75	0.0153	24.29	0.0151
CSCD610195 + CSCD563692 + CSCD459489 [b]	0.56	0.0009	0.98	0.0007	2.45	0.0037	5.92	0.0033	0.41	0.0005	0.78	0.0005
CSCD539372 + CSCD539391 [c]	0.14	0.0002	0.74	0.0006	0.26	0.0004	0.40	0.0002	0.32	0.0003	0.27	0.0001
CSCD120604 + CSCD120605 [c]	0.33	0.0005	0.60	0.0004	0.31	0.0004	0.58	0.0003	0.40	0.0005	0.53	0.0003
Dihydroxy metabolite(s) [d]	0.38	0.0006	1.72	0.0012	2.07	0.0031	5.27	0.0030	0.92	0.0011	1.32	0.0007
CSCC230729 [e]	0.51	0.0008	0.26	0.0002	N/D	N/D	0.05	<0.0001	1.01	0.0012	2.07	0.0013
CSCC210616 [f]	2.18	0.0033	na	na	1.40	0.0021	na	na	1.00	0.0012	na	na
CSAA798670 [f]	8.01	0.0123	na	na	12.25	0.0185	na	na	5.44	0.0066	na	na
CSCD465008 [f]	7.51	0.0114	na	na	21.59	0.0325	na	na	11.88	0.0143	na	na
Organosoluble unknowns	6.12	0.0075	13.17	0.0095	8.18	0.0127	17.63	0.0100	5.11	0.0060	8.84	0.0051
Water soluble unknowns	30.89	0.0474	25.11	0.0183	10.22	0.0154	17.98	0.0101	32.05	0.0385	40.69	0.0253
Baseline	10.12	0.0156	22.02	0.0160	10.08	0.0152	18.70	0.0104	9.26	0.0110	9.41	0.0060
Remainder	7.33	0.0112	3.79	0.0027	3.25	0.0048	4.90	0.0028	3.43	0.0041	1.44	0.0009
Unanalysed extracts	0.51	0.0008	1.15	0.0008	3.07	0.0046	1.26	0.0007	2.98	0.0036	1.90	0.0012
Unextracted	2.62	0.0040	3.19	0.0023	4.89	0.0074	3.85	0.0022	2.94	0.0035	2.32	0.0014
Total	94.00	0.1422	99.69	0.0723	94.64	0.1429	105.75	0.0596	92.99	0.1114	97.76	0.0603

na: Not applicable to this radiolabelled experiment (see note [f]).

[a] *Syn*/*anti* isomer ratio determined in the 30 day plant back [pyrazole-5-^{14}C] labelled analysis was found to be 95/5.

[b] Unable to fully resolve these metabolite isomers in all fractions therefore the value quoted is for the sum of the three isomers.

[c] Unable to fully resolve these metabolite isomers in all fractions therefore the value quoted is for the sum of the two isomers.

[d] Characterised to be one or more "dihydroxy" metabolites based on similar Rf in all 2D-TLC solvent systems to that of a dihydroxy metabolite of isopyrazam isolated from a *C. Elegans* fungal culture.

[e] Deemed not a true metabolite of isopyrazam but an artefact formed by the dehydration of appropriately substituted monohydroxylated metabolites (e.g. CSCD459488) under the mineral acid conditions of hydrolysis employed to release these metabolites from their conjugates.

[f] Metabolites structures retaining only the pyrazole ring of the parent molecule. Residues values correspond only to [pyrazoly-5-^{14}C] labelled experiment.

Table 25 Summary of Identification and Characterisation of Residues in Wheat Hay Samples Grown in Soil Treated with

Commodity	Wheat hay							
Plant back interval (days)	90				300			
Radiolabelled experiment	Pyrazole-5-^{14}C		Phenyl-U-^{14}C		Pyrazole-5-^{14}C		Phenyl-U-^{14}C	
Component	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Isopyrazam [a]	2.83	0.0113	2.65	0.0093	1.11	0.0026	1.90	0.0050
CSCD459488	13.81	0.0549	25.32	0.0896	4.69	0.0110	10.52	0.0274
CSCD610195 + CSCD563692 + CSCD459489 [b]	4.49	0.0178	7.96	0.0282	0.38	0.0009	0.68	0.0018
CSCD539372 + CSCD539391 [c]	0.36	0.0014	0.27	0.0009	0.18	0.0004	0.28	0.0008
CSCD120604 + CSCD120605 [c]	0.43	0.0017	0.49	0.0018	0.14	0.0003	0.25	0.0006
Dihydroxy metabolite(s) [d]	3.84	0.0153	8.41	0.0298	0.67	0.0016	1.29	0.0033
CSCC230729 [e]	0.02	0.0001	0.04	0.0001	0.26	0.0006	0.74	0.0019
CSCC210616 [f]	0.50	0.0020	na	na	0.38	0.0008	na	na
CSAA798670 [f]	4.97	0.0198	na	na	1.18	0.0028	na	na
CSCD465008 [f]	8.16	0.0324	na	na	2.37	0.0057	na	na
Organosoluble unknowns	9.94	0.0398	18.11	0.0640	3.29	0.0073	6.23	0.0165
Water soluble unknowns	16.89	0.0674	12.58	0.0445	46.61	0.1091	44.60	0.1165
Baseline	11.55	0.0458	18.57	0.0657	10.26	0.0240	8.80	0.0229
Remainder	2.09	0.0082	2.88	0.0101	2.22	0.0052	1.61	0.0043
Unanalysed extracts	1.68	0.0067	2.86	0.0101	1.73	0.0041	1.62	0.0042
Unextracted	8.13	0.0323	5.12	0.0181	10.95	0.0256	7.75	0.0203
Total	89.69	0.3569	105.26	0.3722	86.42	0.202	86.27	0.2255

na: Not applicable to this radiolabelled experiment (see superscript 6).

[a] *Syn/anti* isomer ratio measurement not undertaken in hay analyses.

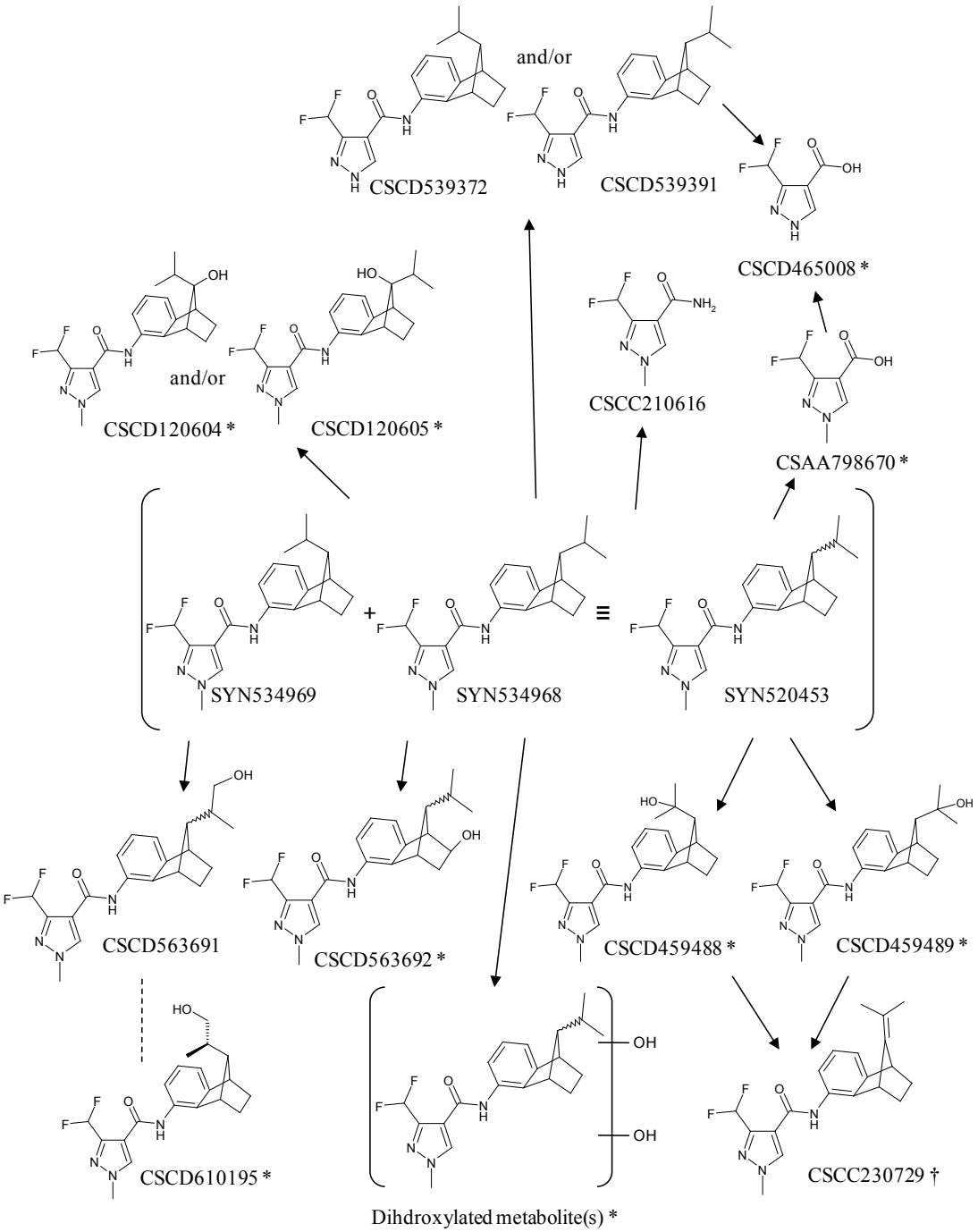
[b] Unable to fully resolve these metabolites in all fractions analysed therefore the value quoted is for the sum of all three metabolites.

[c] Unable to fully resolve these metabolites in all fractions analysed therefore the value quoted is for the sum of all two metabolites.

[d] Characterised to be one or more "dihydroxy" metabolites based on similar Rf in all 2D-TLC solvent systems to that of a dihydroxy metabolite of isopyrazam isolated from a *C. Elegans* fungal culture.

[e] Deemed not a true metabolite of isopyrazam but an artefact formed by the dehydration of appropriately substituted monohydroxylated metabolites (e.g. CSCD459488) under the mineral acid conditions of hydrolysis employed to release these metabolites from their conjugates.

[f] Metabolites structures retaining only the pyrazole ring of the parent molecule. Residues values correspond only to [pyrazolyl-5-^{14}C] labelled experiment.



* Significant amounts in carbohydrate conjugated form.
† Minor component, present predominantly as an artefact (dehydration of appropriately substituted hydroxy metabolites e.g. CSCD459488, under strong mineral acid hydrolysis conditions).

Figure 4 Proposed Metabolic Pathway of Isopyrazam in Rotational Crops

Residues in Succeeding Crops

Four field trials were conducted in Europe to investigate the magnitude of residues of isopyrazam and its metabolites in succeeding or rotational crops.

The specification for technical isopyrazam covers the range of *syn*:*anti* isomer ratios from 70:30 to 100:0. However, the radio-labelled rotational crop study had shown only very low residues of the *anti*-isomers of parent isopyrazam and the hydroxy metabolite CSCD459489. Therefore, to maximise the possibility of detecting these compounds in the field rotational crop studies, all four trials were conducted using the formulation having a *syn/anti* ratio of (approximately) 70:30 (i.e., at the highest proposed concentration of the *anti*-isomer).

Isopyrazam was applied three times to primary crops of wheat at a nominal rate of 125 g ai/ha, giving a total application rate of 375 g ai/ha. This is significantly higher than the proposed use pattern of two applications at 125 g ai/ha, a seasonal rate of 250 g ai/ha.

In the field succeeding crop studies, analysis was performed for those compounds identified as potentially significant on the basis of the radio-labelled succeeding crop study: isopyrazam (as separate *anti*-isomer and *syn*-isomer), CSCD459488, CSCD459489, CSCD465008 and CSAA798670.

Two field crop rotation trials were carried out in France, Southern Europe, during 2007 and 2008 to determine any residues of isopyrazam and its major metabolites in crops grown in an area previously treated with isopyrazam (Bell A. 2010a). Three applications of isopyrazam as a 125 g/L EC formulation were made to a primary crop of winter wheat at a rate of 125 g ai/ha with a spray interval of 10 days (last application at BBCH 31 or 32). Rotational crops of a small grain, a root crop and a leafy vegetable were planted at 30 or 32, 61 or 63, and 372 or 382 days after application and sampled at growth stages applicable to immature and mature commodities. Representative crops used were barley, carrot and spinach. The rotational crops were maintained according to normal agricultural practices.

Crops sampled at immature stages were barley forage at BBCH 32 or 33, barley whole plant at BBCH 59 or 71 and immature spinach at BBCH 43. All three crops, barley, carrot and spinach, were sampled at maturity Residues of *anti*-isomer, *syn*-isomer, CSCD459489 and CSAA798670 were all less than the limit of quantification of the method (0.005 mg/kg for *anti*-isomer, *syn*-isomer, CSCD459489 and 0.01 mg/kg for CSAA798670), in all the following crops and at both plant-back intervals.

In crops from the first plant-back interval (30 or 32 days), residues of CSCD459488 between < 0.005 and 0.010 mg/kg were found in immature spinach. The residues in mature spinach leaves were < 0.005 to 0.006 mg/kg. There were no detectable residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 32–33) residues were 0.011 and 0.015 mg/kg. In whole barley plants sampled at BBCH 59 and 71, residues of CSCD459488 were 0.018 and 0.010 mg/kg. Residues in barley grain at maturity were < 0.005 mg/kg and in straw, residues were 0.029 and 0.054 mg/kg. Residues of CSCD465008 were found at < 0.01 and 0.01 mg/kg in immature spinach (BBCH 43) and 0.01 and 0.02 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg. In carrot leaves the residues were 0.04 and 0.07mg/kg. In barley forage and whole plants, residues were < 0.01 mg/kg. In mature barley grain the residues of CSCD465008 were < 0.01 mg/kg and in barley straw, the residues were < 0.01 mg/kg.

In crops from the second plant-back interval (61 or 63 days), residues of CSCD459488 between 0.005 and 0.006 mg/kg were found in immature spinach. The residues in mature spinach leaves were < 0.005 and 0.008 mg/kg. There were no residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 32-33) residues were 0.009 and 0.020 mg/kg. In whole barley plants sampled at BBCH 59 and 71, residues of CSCD459488 were 0.034 and 0.007 mg/kg. Residues in mature barley grain were < 0.005 to 0.007 mg/kg and in straw, residues were found at 0.024 and 0.052 mg/kg. Residues of CSCD465008 were found at 0.02 mg/kg in immature spinach (BBCH 43) and 0.01 and 0.04 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 and 0.01 mg/kg. In carrot leaves the residues were 0.05 and 0.15 mg/kg. In barley forage and whole plants, residues were < 0.01 to 0.02 mg/kg. In mature barley grain the residues of CSCD465008 were < 0.01 mg/kg and in barley straw, the residues were < 0.01 and 0.04 mg/kg.

In crops from the third plant-back interval (372 or 382 days), residues of CSCD459488 were <.005 mg/kg in immature spinach. The residues in mature spinach leaves were < 0.005 and

0.006 mg/kg. There were no residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 32-33) residues were 0.006 and 0.009 mg/kg. In whole barley plants sampled at BBCH 59 and 71, residues of CSCD459488 were 0.011 and 0.007 mg/kg. Residues in mature barley grain were < 0.005 and 0.008 mg/kg and, in straw, residues were found at 0.034 and 0.049 mg/kg. Residues of CSCD465008 were found at < 0.01 and 0.02 mg/kg in immature spinach (BBCH 43) and < 0.01 and 0.02 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg. In carrot leaves the residues were 0.01 and 0.07 mg/kg. In barley forage and whole plants, residues were < 0.01 mg/kg. In mature barley grain the residues of CSCD465008 were < 0.01 mg/kg and, in barley straw, the residues were < 0.01 mg/kg.

Another field crop rotation trial was carried out in France, Northern Europe during 2007 and 2008 to determine any residues of isopyrazam and its major metabolites in crops grown in an area previously treated with isopyrazam (Bell A. 2010b). Three applications of isopyrazam as a 125 g/L EC formulation were made to a primary crop of winter wheat at a rate of 125 g ai/ha with a spray interval of 9 days (last application at BBCH 32). Rotational crops of small grain, root crop and leafy vegetables were planted at 28, 60 and 354 days after application and sampled at growth stages applicable to immature and mature commodities. Representative crops used were barley, carrot and spinach. The rotational crops were maintained according to normal agricultural practices.

The crops were sampled at immature stages (barley forage at BBCH 31, barley whole plant at BBCH 59 and immature spinach at BBCH 42–43). All three crops were sampled at maturity. The samples were analysed for isopyrazam (as separate isomers *anti*-isomer and *syn*-isomer) and its metabolites CSCD459488, CSCD459489, CSCD465008 and CSAA798670. Residues of *anti*-isomer, CSCD459489 and CSAA798670 were all less than the limit of quantification of the method (0.005 mg/kg for *anti*-isomer, *syn*-isomer, CSCD459489 and 0.01 mg/kg for CSAA798670), in all the following crops and at both plant-back intervals. Residues of *syn*-isomer were less than the limit of quantification of the method (0.005 mg/kg), except at the 28-day plant-back interval (PBI), where residues of 0.006 mg/kg were observed in the carrot root and barley forage samples and residues of 0.005 mg/kg occurred in the barley whole plants.

In crops planted 28 days after the final application, residues of CSCD459488 in immature spinach were < 0.005 mg/kg. The residues in mature spinach leaves were < 0.005 mg/kg. There were no detectable residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 30-33) the residue was 0.006 mg/kg. In whole barley plants sampled at BBCH 59, the residue of CSCD459488 was 0.007 mg/kg. The residue in barley grain was < 0.005 mg/kg, in straw, the residue was 0.017 mg/kg. The residues of CSCD465008 were < 0.01 in immature spinach (BBCH 41-43) and 0.02 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg. In carrot leaves the residue was 0.02 mg/kg. In barley forage and whole plant residues were < 0.01 mg/kg. In barley grain and straw the residues were < 0.01 mg/kg.

In crops planted 60 days after the final application, residues of CSCD459488 found in immature spinach were 0.009 mg/kg. The residues in mature spinach leaves were 0.008 mg/kg. There were no residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 30–33) the residue was 0.008 mg/kg. In barley whole plant sampled at BBCH 59, the residue of CSCD459488 was 0.008 mg/kg. The residue in barley grain was < 0.005 mg/kg, in straw, the residue was 0.018 mg/kg. The residues of CSCD465008 were found at 0.01 mg/kg in immature spinach (BBCH 41–43) and 0.02 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg. In carrot leaves the residue was 0.03 mg/kg. In barley forage, whole plants, grain and straw the residues were < 0.01 mg/kg.

In crops planted 354 days after the final application, residues of CSCD459488 found in immature spinach (BBCH 42) were < 0.005 mg/kg. There were no residues of CSCD459488 in carrot roots or leaves, or barley forage, whole plant or grain. In barley straw, the residue was 0.011 mg/kg. Residues of CSCD465008 were found at < 0.01 mg/kg in immature spinach. The residues in carrot roots and leaves were < 0.01 mg/kg. In barley forage, whole plants, grain and straw the residues were < 0.01 mg/kg. Mature spinach leaves were not sampled.

In addition, a field crop rotation trial was carried out in Germany in Northern Europe during 2007 and 2008 to determine any residues of isopyrazam and its major metabolites in crops grown in an area previously treated with isopyrazam (Simon P. 2009). Three applications of isopyrazam as a 125 g/L EC formulation were made to a primary crop of winter wheat at a rate of 125 g ai/ha with a spray interval of 8 and11 days (last application at BBCH 30-31). Rotational crops of small grain, root crop and leafy vegetables were planted 30, 59 and 373 (in the case of spinach, 408) days after application and sampled at growth stages applicable to immature and mature commodities. Representative crops used were barley, carrots and spinach. The rotational crops were maintained according to normal agricultural practices.

Crops sampled at immature stages were barley forage at BBCH 30-31 or 30-32, barley whole plant at BBCH 59 or 55-61 and immature spinach at BBCH 41-42 or 17/51. All three crops were sampled at maturity. Residues of *anti*-isomer, *syn*-isomer, CSCD459489 and CSAA798670 were all less than the limit of quantification of the method (0.005 mg/kg for *anti*-isomer, *syn*-isomer, CSCD459489 and 0.01 mg/kg for CSAA798670), in all the following crops and at both plant-back intervals.

In crops planted 30 days after the final application, residues of CSCD459488 in immature spinach were 0.006 mg/kg. The residues in mature spinach leaves were 0.005 mg/kg. There were no residues of CSCD459488 above 0.005 mg/kg in carrot roots, and in carrot leaves the residue was 0.005 mg/kg. In barley forage (BBCH 30–33) the residue was 0.022 mg/kg. In whole barley plants sampled at BBCH 59, the residue of CSCD459488 was 0.006 mg/kg. The residue in barley grain was < 0.005 mg/kg and in straw the residue was 0.036 mg/kg. The residues of CSCD465008 were < 0.01 mg/kg in immature spinach (BBCH 41-43) and 0.02 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg and in carrot leaves the residue was 0.03 mg/kg. In barley forage and whole plants, residues were 0.01 and < 0.01 mg/kg, respectively. In barley grain and straw the residues were < 0.01 mg/kg.

In crops planted 59 days after the final application, residues of CSCD459488 found in immature spinach were 0.011 mg/kg. The residues in mature spinach leaves were 0.015 mg/kg. There were no residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 30–33) the residue was 0.042 mg/kg. In whole barley plants sampled at BBCH 59, the residue of CSCD459488 was 0.034 mg/kg. The residue in barley straw was 0.031 mg/kg, the corresponding grain sample was not collected because the crop was insufficiently developed. CSCD465008 residues of 0.02 mg/kg were found in immature spinach (BBCH 41–43) and 0.06 mg/kg in spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg. In carrot leaves the residue was 0.06 mg/kg. In barley forage and whole plant, residues were 0.01 and < 0.01 mg/kg, respectively. In barley straw at normal commercial harvest, the CSCD465008 residue was < 0.01 mg/kg; the corresponding grain sample was not available because the crop was insufficiently developed.

In crops planted 373 (carrot and barley) or 408 (spinach) days after the final application, residues of CSCD459488 found in immature spinach were < 0.005 mg/kg. The residues in mature spinach leaves were 0.005 mg/kg. There were no residues of CSCD459488 in carrot roots or leaves. In barley forage (BBCH 30) the residue was 0.009 mg/kg. In whole barley plants sampled at BBCH 55–59, the residue of CSCD459488 was < 0.005 mg/kg. The residue in barley straw (BBCH 92) was 0.008 mg/kg, and, in the corresponding grain, was < 0.005 mg/kg. CSCD465008 residues were not found in immature spinach (BBCH 41–43) or spinach leaves sampled at normal harvest (BBCH 49). Residues in carrot roots were < 0.01 mg/kg but in carrot leaves the residue was 0.02 mg/kg. In barley forage and whole plant, residues were < 0.01 mg/kg. In barley grain and straw at normal commercial harvest, the CSCD465008 residue was < 0.01 mg/kg.

Residues of isopyrazam, CSCD459488 and CSCD465008 found in field succeeding crops are summarised in Table 26, as their median and highest values

Table 26 Summary of residues found in succeeding crops in four field studies

Crop/part	Plant back interval (days)	Residue (mg/kg)					
		Isopyrazam*		CSCD459488		CSCD465008	
		median	highest	median	highest	median	highest
Spinach							
Immature leaves	28, 30 or 32	< 0.01	< 0.01	0.006	0.010	< 0.01	0.01
	59, 60, 61 or 63	< 0.01	< 0.01	0.008	0.011	0.02	0.02
	354, 372, 382 or 408**	< 0.01	< 0.01	< 0.005	< 0.005	< 0.01	0.02
Leaves	28, 30 or 32	< 0.01	< 0.01	0.005	0.006	0.02	0.02
	59, 60 or 63	< 0.01	< 0.01	0.008	0.015	0.03	0.06
	372, 382 or 408**	< 0.01	< 0.01	0.005	0.006	< 0.01	0.02
Carrot							
Roots	28, 30 or 32	< 0.01	0.01	< 0.005	< 0.005	< 0.01	< 0.01
	59, 60 or 63	< 0.01	< 0.01	< 0.005	< 0.005	< 0.01	0.01
	354, 372, 373 and 382	< 0.01	< 0.01	< 0.005	< 0.005	< 0.01	< 0.01
Leaves	28, 30 or 32	< 0.01	< 0.01	< 0.005	0.005	0.04	0.07
	59, 60 or 63	< 0.01	< 0.01	< 0.005	< 0.005	0.06	0.15
	354, 372, 373 and 382	< 0.01	< 0.01	< 0.005	< 0.005	0.02	0.07
Barley							
Forage/Plant	28, 30 or 32	< 0.01	0.01	0.011	0.022	< 0.01	0.01
	59, 60 or 63	< 0.01	< 0.01	0.015	0.042	< 0.01	0.02
	354, 372, 373 and 382	< 0.01	< 0.01	0.007	0.011	< 0.01	< 0.01
Grain	28, 30 or 32	< 0.01	< 0.01	< 0.005	< 0.005	< 0.01	< 0.01
	60 or 63	< 0.01	< 0.01	< 0.005	0.007	< 0.01	< 0.01
	354, 372, 373 and 382	< 0.01	< 0.01	< 0.005	0.008	< 0.01	< 0.01
Straw	28, 30 or 32	< 0.01	< 0.01	0.033	0.054	< 0.01	< 0.01
	59, 60 or 63	< 0.01	< 0.01	0.028	0.052	< 0.01	0.04
	354, 372, 373 and 382	< 0.01	< 0.01	0.023	0.049	< 0.01	< 0.01

*–Sum of *anti*-isomer and *syn*-isomer

** Spinach sown 373 days after the last application did not emerge and therefore it was sown again 408 days after the last application.

METHODS OF RESIDUE ANALYSIS

Analytical Methods

Analytical methods have been developed and validated for the determination of the active substance isopyrazam in target crops and animal products (eggs, milk, muscle, liver, kidney and fat). An overview of analytical methods considered is presented in Table 27.

Table 27 Methods for the determination of residues of isopyrazam in commodities of plant and animal origin

Matrix tested	Analyte	Method	LOQ (mg/kg)	Method No. Reference
Residue trials				
Barley, Wheat	*anti*-isomer *syn*-isomer	GC-MS/MS	0.005 0.005	GRM006.01A (Draft) Clur P., Crook S.J., Elliott A. (2008a)
Barley, Ryegrass, Spinach, Tomato, Orange, Potato, Lentils, Sunflower seeds	*anti*-isomer *syn*-isomer	LC-MS/MS	0.005 0.005	GRM006.01A Clur P., Crook S.J., Elliott A. (2008b) *Validation*: Elliott A. (2008)

Matrix tested	Analyte	Method	LOQ (mg/kg)	Method No. Reference
Barley, Apple, Carrot, Spinach, Potato, Oil seed rape seed Lentil, Tomato, Bran, Bread, Beer	*anti*-isomer *syn*-isomer	LC-MS/MS	0.005 0.005	GRM006.01B Hargreaves S.L. (2008a) *Validation*: Eversfield S., Morriss A. (2008a)
Barley, Apple, Carrot, Spinach, Oil seed rape seed, Lentils, Bran, Bread, Beer	CSCD459488 CSCD459489	LC-MS/MS	0.005 0.005	GRM006.03A Crook S. (2008a) *Validation*: Morriss A. (2008a)
Barley, Carrot, Spinach	CSCD465008 CSAA798670	LC-MS/MS	0.01 0.01	GRM006.08A Hargreaves S.L. (2008b) *Validation*: Morriss A. (2008b)
Barley, Carrot, Spinach	CSCD465008 CSAA798670	LC-MS/MS	0.01 0.01	GRM006.08B Hargreaves S.L. (2009)
Animal products	*anti*-isomer *syn*-isomer	LC-MS/MS	0.005 0.005	GRM006.09A Crook S. (2008b) *Validation*: Ferguson L. (2008a)
Animal products	CSAA798670 (common moiety method: Isopyrazam + metabolites)	LC-MS/MS	0.005	GRM006.10A Crook S. (2008c) *Validation*: Ferguson L. (2008b) *ILV*: Lakaschus S. (2008)
Enforcement				
Wheat, Apple, Oilseed rape seed	*anti*-isomer *syn*-isomer	GC-MSD	0.005 0.005	DFG-S19 *Validation*: Lakaschus S., Gizler A.S. (2008a)
Wheat, Apple, Oilseed rape seed	*anti*-isomer *syn*-isomer	LC-MS/MS	0.005 0.005	DFG-S19 *Validation*: Lakaschus S., Gizler A.S. (2009a) *ILV*: Schulz H. (2008)
Apple, Wheat, Oilseed rape seed	*anti*-isomer *syn*-isomer	LC-MS/MS	0.005 0.005	DFG-S19 *Validation*: Bacher R. (2009)
Animal products	*anti*-isomer *syn*-isomer	LC-MS/MS	0.0025 0.0025	DFG-S19 *Validation*: Bacher R. (2008) *ILV*: Lakaschus S., Gizler A.S. (2008b)

Methods recommended for enforcement are DFG-S19 for crops and animal substracts. Since the methods include a derivation step for the analytical separation of the parent as the separate diastereomers by GC-MSD and LC-MS/MS, no multi-residue method is presented here.

Plant commodities

Analytical methods have been developed for the determination of residues of isopyrazam and relevant metabolites in crops. Methods for parent isopyrazam determine residues as the separate *anti*-isomer and *syn*-isomer, each with a Limit of Quantification (LOQ) of 0.005 mg/kg, to give an overall LOQ of 0.01 mg/kg for isopyrazam when the two isomers are summed up. Methods for metabolites have varying LOQs of either 0.005 or 0.01 mg/kg.

Residue analytical method GRM006.01A

The method GRM006.01A at its draft stage2 included extraction by homogenisation, clean-up by solid-phase extraction (SPE) and determination by GC-MS/MS. In order to address problems of the GC-MS/MS determination step, method was revised to use slightly different SPE clean-up conditions and determination by LC-MS/MS. The new LC-MS/MS procedure has been formally issued as method GRM006.01A(Clur P., Crook S.J., Elliott A., 2008a).

Isopyrazam is determined as separate *anti*-isomer and *syn*-isomer. Cereal samples are extracted by homogenisation with acetonitrile:water at different ratios. To extract cereal grain and whole plants, acetonitrile:water (80:20 v/v) is used whereas for cereal straw, acetonitrile:water (50:50 v/v) is used. Extracts are centrifuged and aliquots (equivalent to 0.1 g sample) are diluted with water. Clean-up is performed by solid-phase extraction (SPE) using Phenomenex Strata-X cartridges followed by Bond-Elut NH2 cartridges. Final determination is by LC-MS/MS with two MS/MS transitions (Crook S., 2008).

The limit of quantification for *anti*-isomer and *syn*-isomer residues in crop commodities using method GRM006.01A was established at 0.005 mg/kg for each isomer corresponding to 0.01 mg/kg for isopyrazam.

Analytical method GRM006.01A has been validated for the determination of residues of isopyrazam analysed as the isomers *anti*-isomer and *syn*-isomer in crops (Elliott A., 2008). Control samples were analysed in duplicate. Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) for each isomer and in quintuplet at a higher fortification level (barley grain 0.1 mg/kg, barley straw 2.5 mg/kg, ryegrass forage 2.5 mg/kg, spinach leaves 0.5 mg/kg, tomato fruit 0.5 mg/kg, orange fruit 0.5 mg/kg, potato tuber 0.05 mg/kg, lentils 0.1 mg/kg and sunflower seeds 0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions on all matrices tested. No significant matrix effects were observed and non-matrix standards were used for quantification. The recoveries obtained are detailed in Tables 28 to 31.

Table 28 Recovery results obtained during validation of Method GRM006.01A for *anti*-isomer in Crops. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	108, 95, 112, 91, 97	5	101	9	91-112
	0.1	96, 91, 96, 90, 105	5	95	6	90-105
	Overall		10	98	8	90-112
Barley straw	0.005*	93, 87, 99, 93, 99	5	94	5	87-99
	2.5	75, 74, 84, 76, 82	5	78	6	74-84
	Overall		10	86	11	74-99
Ryegrass forage	0.005*	98, 103, 111, 111, 93	5	103	8	93-111
	2.5	93, 105, 100, 101, 108	5	101	6	93-108
	Overall		10	102	7	93-111
Spinach Leaves	0.005*	84, 88, 75, 86, 96	5	86	9	75-96
	0.5	86, 95, 92, 91, 87	5	90	4	86-95
	Overall		10	88	7	75-96
Tomato fruit	0.005*	96, 94, 104, 102, 94	5	98	5	94-104
	0.5	93, 94, 97, 91, 95	5	94	2	91-97
	Overall		10	96	4	91-104
Orange fruit	0.005*	104, 97, 4, 82, 92	5	93	7	82-104
	0.5	93, 94, 89, 86, 96	5	92	4	86-96
	Overall		10	93	7	82-104
Potato tuber	0.005*	100, 106, 102, 100, 104	5	103	2	100-106
	0.05	92, 94, 101, 101, 100	5	97	4	92-101
	Overall		10	100	4	92-106
Lentils	0.005*	94, 89, 78, 83, 88	5	87	7	78-94
	0.1	70, 78, 68, 71, 88	5	75	11	68-88
	Overall		10	81	11	68-94
Sunflower seeds	0.005*	108, 107, 108, 94, 72	5	98	16	72-108
	0.1	104, 96, 103, 101, 98	5	100	3	96-104
	Overall		10	99	11	72-108

*–Limit of quantification, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 29 Recovery results obtained during validation of Method GRM006.01A for *anti*-isomer in Crops. Confirmatory transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	105, 89, 111, 89, 93	5	97	10	89-111
	0.1	94, 91, 96, 91, 103	5	95	6	91-103
	Overall		10	96	8	89-111
Barley straw	0.005*†	95, 90, 98, 95, 88	5	93	5	88-98
	2.5	75, 73, 84, 76, 82	5	78	6	73-84
	Overall		10	86	11	73-98
Ryegrass forage	0.005*	81, 100, 107, 100, 91	5	96	11	81-107
	2.5	93, 105, 98, 100, 104	5	100	5	93-105
	Overall		10	98	8	81-107
Spinach Leaves	0.005*	98, 93, 88, 82, 98	5	92	7	82-98
	0.5	88, 93, 88, 90, 89	5	90	2	88-93
	Overall		10	91	5	82-98
Tomato fruit	0.005*	100, 89, 102, 102, 100	5	99	5	89-102
	0.5	89, 95, 98, 93, 97	5	94	4	89-98
	Overall		10	97	5	89-102
Orange fruit	0.005*	103, 91, 102, 90, 88	5	95	7	88-103
	0.5	91, 92, 91, 86, 98	5	92	5	86-98
	Overall		10	93	6	86-103
Potato tuber	0.005*	109, 101, 102, 114, 112	5	108	5	101-114
	0.05	98, 100, 101, 102, 102	5	101	2	98-102
	Overall		10	104	5	98-114
Lentils	0.005*	82, 88, 78, 83, 100	5	86	10	78-100
	0.1	69, 80, 69, 73, 92	5	77	12	69-92
	Overall		10	82	12	69-100
Sunflower seeds	0.005*	104, 97, 96, 96, 70	5	92	14	70-104
	0.1	101, 93, 110, 98, 102	5	101	6	93-110
	Overall		10	97	11	70-110

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 30 Recovery results obtained during validation of Method GRM006.01A for *syn*-isomer in Crops. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	102, 94, 107, 84, 89	5	95	10	84-107
	0.1	92, 91, 98, 92, 101	5	95	5	91-101
	Overall		10	95	7	84-107
Barley straw	0.005*	90, 91, 93, 87, 83	5	89	4	83-93
	2.5	72, 74, 85, 74, 79	5	77	7	72-85
	Overall		10	83	9	72-93
Ryegrass forage	0.005*	88, 101, 107, 108, 107	5	102	8	88-108
	2.5	99, 102, 100, 100, 105	5	101	2	99-105
	Overall		10	102	6	88-108
Spinach Leaves	0.005*	83, 91, 87, 89, 90	5	88	4	83-91
	0.5	74, 82, 84, 83, 88	5	82	7	74-88
	Overall		10	85	6	74-91
Tomato fruit	0.005*	103, 101, 98, 91, 89	5	96	6	89-103
	0.5	92, 96, 95, 96, 94	5	95	2	92-96
	Overall		10	96	5	89-103
Orange fruit	0.005*	94, 91, 105, 94, 101	5	97	6	91-105
	0.5	96, 95, 96, 95, 97	5	96	1	95-97
	Overall		10	96	4	91-105
Potato tuber	0.005*	101, 100, 101, 100, 103	5	101	1	100-103
	0.05	92, 92, 90, 96, 90	5	92	3	90-96
	Overall		10	97	5	90-103
Lentils	0.005*	92, 94, 86, 86, 94	5	90	5	86-94

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	0.1	71, 78, 69, 69, 91	5	76	12	69-78
	Overall		10	83	12	69-94
Sunflower seeds	0.005*	92, 90, 97, 89, 66	5	87	14	66-97
	0.1	99, 96, 103, 100, 106	5	101	4	96-106
	Overall		10	94	12	66-106

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 31 Recovery results obtained during validation of Method GRM006.01A for *syn*-isomer in Crops. Primary transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	101, 106, 86, 104	5	100	8	86-106
	0.1	92, 89, 97, 93, 99	5	94	4	89-99
	Overall		10	97	7	86-106
Barley straw	0.005*	83, 86, 86, 90, 78	5	85	5	78-90
	2.5	73, 74, 85, 74, 79	5	77	7	73-85
	Overall		10	81	7	73-90
Ryegrass forage	0.005*	84, 99, 111, 105, 100	5	100	10	84-111
	2.5	100, 101, 99, 100, 105	5	101	2	99-105
	Overall		10	100	7	84-111
Spinach Leaves	0.005*	93, 88, 98, 97, 91	5	93	4	88-98
	0.5	84, 93, 90, 89, 91	5	89	4	84-93
	Overall		10	91	5	84-98
Tomato fruit	0.005*	94, 96, 97, 101, 90	5	96	4	94-101
	0.5	92, 97, 95, 95, 95	5	95	2	92-97
	Overall		10	95	3	92-101
Orange fruit	0.005*	102, 97, 109, 90, 96	5	99	7	90-109
	0.5	95, 96, 97, 94, 97	5	96	1	94-97
	Overall		10	97	5	90-109
Potato tuber	0.005*	104, 97, 95, 97, 102	5	99	4	95-104
	0.05	93, 88, 90, 96, 90	5	91	3	88-96
	Overall		10	95	5	88-104
Lentils	0.005*	90, 98, 93, 84, 86	5	90	6	84-98
	0.1	72, 78, 71, 71, 90	5	76	11	71-90
	Overall		10	83	12	71-90
Sunflower seeds	0.005*	92, 93, 98, 92, 64	5	88	15	64-98
	0.1	99, 96, 104, 101, 104	5	101	3	96-104
	Overall		10	94	12	64-104

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

The linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range from 2.5 pg to 2500 pg injected on column (equivalent to 0.00005 µg/mL to 0.05 µg/mL standards when using an 50 µL injection volume) and was found to be linear. The correlation coefficients (R^2) of the calibration curves were > 0.997.

The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each crop tested during method validation were < 20%.

Isopyrazam (*anti*-isomer and *syn*-isomer) has been shown to be efficiently extracted from cereal matrices under the conditions used in method GRM006.01A (*Booth J, Goodwin SL, 2007, T003918-05-REG*).

Method GRM006.01A has been demonstrated to be a reliable and accurate procedure for the determination of isopyrazam as *anti*-isomer and *syn*-isomer to a limit of quantification of 0.01 mg/kg in crop matrices, using commercially available laboratory equipment and reagents. Barley grain,

barley straw, ryegrass forage, spinach leaves, tomato fruit, orange fruit, potato tuber, lentils and sunflower seed have been used as example crops.

Residue analytical method GRM006.01B

Method GRM006.01B for the determination of isopyrazam in crops was significantly simpler than GRM006.01A. The clean-up steps using SPE were replaced by simple dilution. Isopyrazam is determined as separate *anti*-isomer and *syn*-isomer. Samples are extracted by homogenisation with acetonitrile:water (50:50 v/v for dry matrices such as straw, and 80:20 v/v for other crops). Extracts are centrifuged and aliquots (equivalent to 0.2 g sample) are diluted with methanol:water (50:50 v/v). Final determination is by high performance liquid chromatography with triple-quadrupole mass-spectrometric detection (LC-MS/MS) with two MS/MS transitions (Hargreaves S., 2008a).

The limit of quantification of the method is 0.005 mg/kg for *anti*-isomer and *syn*-isomer.

Analytical method GRM006.01B has been validated for the determination of residues of isopyrazam analysed as the isomers *anti*-isomer and *syn*-isomer in crops (Eversfield S., Morriss A., 2008a). Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) for each isomer and in quintuplet at ten times the LOQ (0.05 mg/kg). Higher fortification levels were validated in quintuplet for apples (2 mg/kg), lentils (0.1 mg/kg), barley forage (2.5 mg/kg), barley grain (0.3 mg/kg) and barley straw (10 mg/kg). Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions on all matrices tested. No significant matrix effects were observed and non-matrix standards were used for quantification. The recoveries obtained are detailed in Table 32 to Table 35.

Table 32 Recovery results obtained during validation of Method GRM006.01B for *anti*-isomer in Crops and Processed Commodities. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	109, 100, 103, 107, 98	5	103	4.5	98-109
	0.05	120, 94, 98, 110, 102	5	105	9.9	94-120
	0.3	99, 107, 101, 125, 99	5	106	10.4	99-125
	Overall		15	105	8.1	94-125
Barley forage	0.005*	89, 102, 99, 108, 93	5	98	7.6	89-108
	0.05	104, 92, 94, 94, 88	5	94	6.2	88-104
	2.5	95, 99, 98, 103, 104	5	100	3.7	95-104
	Overall		15	97	6.1	88-108
Barley straw	0.005*	74, 78, 68, 73, 74	5	73	4.9	68-78
	0.05	68, 71, 74, 70, 65	5	70	4.8	65-74
	10	89, 93, 97, 94, 102	5	95	5.1	89-102
	Overall		15	79	15.3	65-102
Apple	0.005*	110, 110, 110, 970[#], 110	4	110[#]	0.0[#]	110-110
	0.05	92, 111, 114, 106, 110	5	107	8.1	92-114
	2.0	97, 99, 104, 100, 93	5	99	4.1	93-104
	Overall		14	105[#]	6.9[#]	92-114
Carrot	0.005*	89, 94, 92, 98, 97	5	94	3.9	89-97
	0.05	107, 109, 109, 107, 108	5	108	0.9	107-109
	Overall		10	101	7.7	89-109
Spinach	0.005*	91, 90, 104, 107, 109	5	100	9.0	90-109
	0.05	103, 112, 107, 102, 112	5	107	4.4	102-112
	Overall		10	104	7.5	90-112
Potato	0.005*	93, 94, 100, 100, 99	5	97	3.5	93-100
	0.05	106, 107, 104, 102, 101	5	104	2.5	101-107
	Overall		10	101	4.5	93-107
Oil seed rape seed	0.005*	84, 84, 91, 93, 92	5	89	5.0	84-93
	0.05	91, 88, 79, 86, 84	5	86	5.3	84-91
	Overall		10	87	5.2	84-93
Lentil	0.005*	99, 100, 111, 111, 90	5	102	8.7	90-111
	0.05	102, 106, 96, 94, 98	5	99	4.9	94-106
	0.1	105, 106, 107, 103, 92	5	103	6.0	92-107
	Overall		15	101	6.4	90-111

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Tomato	0.005*	105, 103, 114, 110, 111	5	109	4.1	103-114
	0.05	109, 111, 112, 107, 110	5	110	1.8	107-114
	Overall		10	109	3.0	103-112
Bran	0.005*	98, 97, 114, 104, 100	5	103	6.7	97-114
	0.05	105, 115, 101, 109, 95	5	105	7.3	95-115
	Overall		10	104	6.7	95-115
Bread	0.005*	100, 105, 114, 112, 108	5	108	5.2	100-114
	0.05	111, 100, 104, 113, 104	5	106	5.1	100-113
	Overall		10	107	4.9	100-114
Beer	0.005*	104, 104, 102, 103, 101	5	103	1.3	101-104
	0.05	111, 109, 103, 107, 101	5	106	3.9	101-111
	Overall		10	105	3.3	101-111

*–Limit of quantification, defined by the lowest validated fortification level
#–Excluding one data point determined as an outlier using Grubbs' test
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 33 Recovery results obtained during validation of Method GRM006.01B for anti-isomer in Crops and Processed Commodities. Primary transition m/z 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	107, 107, 103, 100, 98	5	103	3.9	98-107
	0.05	116, 94, 102, 111, 106	5	106	8.0	94-116
	0.3	99, 103, 109, 103, 99	5	103	4.0	99-109
	Overall		15	104	5.5	94-116
Barley forage	0.005*	92, 97, 98, 101, 95	5	97	3.5	92-101
	0.05	106, 95, 95, 95, 94	5	97	5.2	94-106
	2.5	97, 109, 95, 96, 93	5	98	6.5	93-109
	Overall		15	97	4.9	92-109
Barley straw	0.005*	85, 74, 76, 74, 66	5	75	9.0	66-85
	0.05	70, 74, 78, 72, 69	5	73	4.9	69-78
	10	83, 87, 94, 95, 100	5	92	7.4	83-100
	Overall		15	80	13.0	66-100
Apple	0.005*	108, 109, 105, 960[#], 112	4	109[#]	2.7[#]	105-112
	0.05	88, 105, 111, 100, 110	5	103	9.1	88-111
	2.0	102, 106, 101, 103, 103	5	103	1.8	101-106
	Overall		14	105[#]	5.8[#]	88-112
Carrot	0.005*	79, 81, 76, 90, 79	5	81	6.6	76-90
	0.05	109, 112, 114, 110, 112	5	111	1.7	109-114
	Overall		10	96	17.1	76-114
Spinach	0.005*	91, 91, 108, 103, 107	5	100	8.4	91-108
	0.05	104, 112, 109, 106, 115	5	109	4.1	104-115
	Overall		10	105	7.6	91-115
Potato	0.005*	82, 88, 104, 90, 98	5	92	9.4	82-104
	0.05	104, 104, 103, 101, 102	5	103	1.3	101-104
	Overall		10	98	8.2	82-104
Oil seed rape seed	0.005*	90, 88, 92, 102, 94	5	93	5.8	88-102
	0.05	94, 91, 79, 87, 86	5	87	6.5	79-94
	Overall		10	90	6.7	79-102
Lentil	0.005*	108, 100, 112, 111, 89	5	104	9.2	89-112
	0.05	98, 104, 97, 96, 97	5	98	3.3	96-104
	0.1	114, 96, 112, 117, 93	5	103	10.4	93-117
	Overall		15	103	8.5	89-117
Tomato	0.005*	109, 102, 105, 112, 106	5	107	3.6	102-112
	0.05	110, 110, 112, 109, 110	5	110	1.0	109-112
	Overall		10	109	3.0	102-112
Bran	0.005*	95, 95, 108, 99, 95	5	98	5.7	95-108
	0.05	101, 114, 95, 104, 92	5	101	8.5	92-114
	Overall		10	100	7.0	92-114
Bread	0.005*	105, 104, 108, 103, 117	5	107	5.3	103-117

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	0.05	109, 100, 107, 111, 104	5	106	4.1	100-111
	Overall		10	107	4.5	100-117
Beer	0.005*	109, 101, 95, 100, 98	5	101	5.2	95-109
	0.05	113, 113, 104, 104, 104	5	108	4.6	104-113
	Overall		10	104	5.8	95-113

*–Limit of quantification, defined by the lowest validated fortification level
#–Excluding one data point determined as an outlier using Grubbs' test
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 34 Recovery results obtained during validation of Method GRM006.01B for *syn*-isomer in Crops and Processed Commodities. Primary transition *m/z* 360 → 320.

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	107, 99, 109, 104, 106	5	105	3.6	99-109
	0.05	118, 98, 101, 110, 103	5	106	7.6	98-118
	0.3	100, 100, 101, 98, 96	5	99	2.0	96-101
	Overall		15	103	5.6	96-118
Barley forage	0.005*	88, 97, 100, 103, 97	5	97	5.8	88-103
	0.05	102, 96, 97, 94, 93	5	96	3.6	93-102
	2.5	105, 95, 96, 101, 99	5	99	4.1	95-105
	Overall		15	98	4.4	88-105
Barley straw	0.005*	77, 74, 69, 76, 72	5	74	4.4	69-77
	0.05	67, 71, 75, 71, 70	5	71	4.0	67-75
	10	84, 83, 89, 93, 92	5	88	5.2	83-93
	Overall		15	78	11.1	67-93
Apple	0.005*	110, 106, 110, 970#, 114	4	110#	3.0#	106-114
	0.05	84, 111, 108, 95, 106	5	101	11.1	84-111
	2.0	106, 102, 106, 100, 101	5	103	2.7	100-106
	Overall		14	104#	7.3#	84-114
Carrot	0.005*	91, 91, 104, 100, 99	5	97	6.0	91-104
	0.05	106, 107, 109, 104, 103	5	106	2.3	103-109
	Overall		10	101	6.2	91-109
Spinach	0.005*	93, 93, 104, 109, 108	5	101	7.8	93-109
	0.05	101, 110, 104, 102, 114	5	106	5.3	102-114
	Overall		10	104	6.7	93-114
Potato	0.005*	89, 89, 100, 93, 96	5	93	5.1	89-100
	0.05	102, 107, 105, 101, 101	5	103	2.6	101-107
	Overall		10	98	6.4	89-107
Oil seed rape seed	0.005*	64, 70, 84, 78, 79	5	75	10.6	64-84
	0.05	79, 84, 72, 75, 76	5	77	5.9	72-79
	Overall		10	76	8.2	64-84
Lentil	0.005*	96, 98, 113, 110, 91	5	102	9.3	91-113
	0.05	98, 106, 98, 94, 95	5	98	4.8	94-106
	0.1	113, 114, 102, 111, 111	5	110	4.3	102-114
	Overall		15	103	7.8	91-114
Tomato	0.005*	103, 103, 116, 109, 110	5	108	5.0	103-116
	0.05	106, 109, 111, 106, 109	5	108	2.0	106-111
	Overall		10	108	3.6	103-116
Bran	0.005*	94, 96, 116, 108, 97	5	102	9.2	94-116
	0.05	102, 114, 103, 105, 97	5	104	6.0	97-114
	Overall		10	103	7.4	94-116
Bread	0.005*	93, 104, 118, 107, 108	5	106	8.5	93-118
	0.05	105, 98, 106, 116, 108	5	107	6.1	98-116
	Overall		10	106	6.9	93-118
Beer	0.005*	108, 104, 99, 103, 93	5	101	5.6	93-108
	0.05	106, 103, 94, 96, 91	5	98	6.4	91-106
	Overall		10	100	5.9	91-108

*–Limit of quantification, defined by the lowest validated fortification level
#–Excluding one data point determined as an outlier using Grubbs' test
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 35 Recovery results obtained during validation of Method GRM006.01B for *syn*-isomer in Crops and Processed Commodities. Primary transition m/z 360 → 244.

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	107, 97, 103, 100, 104	5	102	3.8	97-107
	0.05	114, 96, 100, 108, 103	5	104	6.7	96-114
	0.3	111, 104, 109, 104, 109	5	107	3.0	104-111
	Overall		15	105	4.9	96-114
Barley forage	0.005*	93, 98, 103, 104, 92	5	98	5.6	92-104
	0.05	104, 94, 94, 91, 92	5	95	5.5	91-104
	2.5	100, 94, 96, 101, 99	5	98	3.0	94-101
	Overall		15	97	4.7	91-104
Barley straw	0.005*	80, 78, 75, 82, 80	5	79	3.3	75-82
	0.05	66, 70, 76, 70, 69	5	70	5.2	66-76
	10	82, 83, 93, 94, 90	5	88	6.3	82-94
	Overall		15	79	10.9	66-94
Apple	0.005*	105, 110, 109, 919#, 110	4	109#	2.2#	105-110
	0.05	85, 105, 106, 95, 103	5	99	9.0	85-106
	2.0	104, 105, 109, 108, 103	5	106	2.4	103-109
	Overall		14	104#	6.5#	85-110
Carrot	0.005*	91, 87, 107, 104, 98	5	97	8.7	87-107
	0.05	107, 108, 106, 104, 102	5	105	2.3	102-108
	Overall		10	101	7.1	87-108
Spinach	0.005*	88, 91, 109, 109, 107	5	101	10.3	91-109
	0.05	103, 110, 105, 102, 114	5	107	4.7	102-114
	Overall		10	104	8.0	91-114
Potato	0.005*	91, 94, 102, 97, 104	5	98	5.5	91-104
	0.05	101, 108, 103, 101, 98	5	102	3.6	98-108
	Overall		10	100	5.0	91-108
Oil seed rape seed	0.005*	68, 70, 88, 78, 82	5	77	10.8	68-88
	0.05	77, 82, 71, 74, 77	5	76	5.4	71-82
	Overall		10	77	8.1	68-88
Lentil	0.005*	99, 98, 111, 110, 90	5	102	8.7	90-111
	0.05	96, 106, 97, 94, 92	5	97	5.6	92-106
	0.1	110, 114, 107, 107, 104	5	108	3.5	104-114
	Overall		15	102	7.5	90-114
Tomato	0.005*	101, 95, 113, 111, 105	5	105	7.0	95-113
	0.05	104, 111, 115, 107, 110	5	109	3.8	104-115
	Overall		10	107	5.7	95-115
Bran	0.005*	96, 96, 108, 100, 98	5	100	5.0	96-108
	0.05	102, 112, 102, 103, 95	5	103	5.9	95-112
	Overall		10	101	5.4	95-112
Bread	0.005*	95, 106, 116, 105, 101	5	105	7.4	95-116
	0.05	101, 97, 108, 116, 109	5	106	7.0	97-116
	Overall		10	105	6.8	95-116
Beer	0.005*	102, 105, 95, 97, 95	5	99	4.5	95-105
	0.05	104, 103, 93, 97, 93	5	98	5.4	93-104
	Overall		10	98	4.7	93-105

*–Limit of quantification, defined by the lowest validated fortification level
#–Excluding one data point determined as an outlier using Grubbs' test
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

The linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range from 0.004 ng to 16 ng injected on column (equivalent to 0.00005 μg/mL to 0.002 μg/mL standards when using an 80 μL injection volume) and was found to be linear. This is equivalent to a range of 50% LOQ to 20 LOQ. The correlation coefficients (R^2) of the calibration curves were > 0.999.

The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each crop tested during method validation were < 20%.

The extraction conditions were unchanged from method GRM006.01A and, therefore, isopyrazam (*anti*-isomer and *syn*-isomer) has been shown to be efficiently extracted from cereal matrices under the conditions used in method GRM006.01B (*Booth J, Goodwin SL, 2007, T003918-05-REG*) which is reported in the metabolism study in wheat.

Method GRM006.01B has been demonstrated to be a reliable and accurate procedure for the determination of isopyrazam as *anti*-isomer and *syn*-isomer in crop matrices to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents. Barley grain, barley forage, barley straw, apple, carrot, spinach, potato, oil seed rape (seed), lentil, tomato, bran, bread and beer have been used as example commodities.

Residue analytical method GRM006.03A

GRM006.03 is the method used for data-generation for CSCD459488 and CSCD459489 in crops (Crook S., 2008a).Samples are extracted by homogenisation with acetonitrile:water (80:20 v/v). Extracts are centrifuged and aliquots (equivalent to 0.09 g sample) are hydrolysed with 0.1 M HCl at 60°C for 3 hours. Aliquots are then diluted with acetonitrile and water. Final determination is by LC-MS/MS with two transitions.

The limit of quantification of the method is 0.005 mg/kg for CSCD459488 and CSCD459489.

Analytical method GRM006.03A has been validated for the determination of residues of CSCD459488 and CSCD459489 in crops (Morriss A., 2008a). Control samples were analysed in duplicate. Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) for each isomer and in quintuplet at ten times the LOQ (0.05 mg/kg). Higher fortification levels were validated in quintuplet for barley forage (1 mg/kg) and barley straw (1 mg/kg and 2 mg/kg). Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions on all matrices tested with a few exceptions where the mean recovery was slightly above 110%. No significant matrix effects were observed and non-matrix standards were used for quantification. The recoveries obtained are detailed in Table 36 to Table 39.

Table 36 Recovery results obtained during validation of Method GRM006.03A for CSCD459488 in Crops and Processed Commodities. Primary transition *m/z* 374 → 156

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	88, 102, 94, 97, 93	5	95	5.5	88–102
	0.05	93, 99, 99, 97, 97	5	97	2.5	93–99
	Overall		10	96	4.2	88–102
Barley Forage	0.005*	106, 106, 109, 110, 109	5	108	1.7	106–110
	0.05	108, 105, 107, 102, 105	5	105	2.2	102–108
	0.5	106, 110, 104, 103, 123	5	109	7.5	103–123
	1.0	95, 93, 101, 92, 107	5	98	6.5	92–107
	Overall		20	105	6.4	92–123
Barley straw	0.005*	111, 114, 106, 104, 114	5	110	4.2	104–114
	0.05	100, 98, 75, 109, 108	5	98	14.0	75–109
	1.0	89, 96, 86, 94, 106	5	94	8.2	86–106
	2.0	97, 101, 102, 104, 98	5	100	2.9	97–104
	Overall		20	101	9.6	75–114
Apple	0.005*	82, 93, 94, 97, 97	5	93	6.7	82–97
	0.05	99, 105, 35[#], 105, 95	4	101[#]	4.9[#]	95–105
	Overall		9	96[#]	7.2[#]	82–105
Carrot	0.005*	114, 109, 112, 107, 110	5	110	2.4	107–114
	0.05	116, 114, 113, 110, 107	5	112	3.2	107–116
	Overall		10	111	2.8	107–116
Spinach	0.005*	87, 104, 103, 92, 108	5	99	9.0	87–108
	0.05	98, 99, 100, 100, 95	5	98	2.1	95–100
	Overall		10	99	6.2	87–108
Oil Seed Rape Seed	0.005*	103, 77, 65, 93, 97	5	87	18.0	65–103
	0.05	105, 85, 72, 106, 83	5	90	16.4	72–106

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	Overall		10	89	16.3	65–106
Lentils	0.005*	73, 81, 81, 79, 80	5	79	4.2	73–81
	0.05	79, 82, 84, 89, 86	5	84	4.5	79–89
	Overall		10	81	5.3	73–89
Bran	0.005*	77, 79, 87, 102, 97	5	88	12.4	77–102
	0.05	101, 109, 100, 85, 95	5	98	9.0	85–109
	Overall		10	93	11.4	77–109
Bread	0.005*	108, 120, 105, 106, 115	5	111	5.8	105–120
	0.05	103, 108, 105, 100, 104	5	104	2.8	100–108
	Overall		10	107	5.5	100–120
Beer	0.005*	93, 98, 97, 98, 96	5	96	2.2	93–98
	0.05	102, 100, 103, 99, 101	5	101	1.6	99–103
	Overall		10	99	3.0	93–103

*–Limit of quantification, defined by the lowest validated fortification level
#–Outlier as determined by Dixon's test, excluded from calculations
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 37 Recovery results obtained during validation of Method GRM006.03A for CSCD459488 in Crops and Processed Commodities. Confirmatory transition *m/z* 374 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	96, 104, 92, 104, 82	5	96	9.6	82–104
	0.05	89, 96, 97, 94, 93	5	94	3.3	89–97
	Overall		10	96	4.2	82–104
Barley Forage	0.005*	107, 101, 108, 110, 105	5	106	3.2	101–110
	0.05	109, 103, 104, 105, 109	5	106	2.7	103–109
	0.5	107, 105, 98, 99, 121	5	106	8.7	98–121
	1.0	97, 89, 103, 89, 107	5	97	8.4	89–107
	Overall		20	104	7.0	89–121
Barley straw	0.005*	109, 119, 117, 117, 132	5	119	7.0	109–132
	0.05	101, 100, 73#, 108, 108	4	104#	4.2#	100–108
	1.0	91, 98, 91, 91, 105	5	95	6.6	91–105
	2.0	97, 102, 100, 104, 100	5	101	2.6	97–104
	Overall		19	105#	10.2#	91–132
Apple	0.005*	82, 93, 96, 97, 98	5	93	7.0	82–98
	0.05	100, 100, 26#,97, 97	4	99#	1.8#	97–100
	Overall		9	96#	5.8#	82–100
Carrot	0.005*	107, 111, 109, 109, 109	5	109	1.3	107–111
	0.05	116, 115, 115, 112, 111	5	114	1.9	111–116
	Overall		10	111	2.7	107–116
Spinach	0.005*	89, 109, 98, 94, 100	5	98	7.6	89–109
	0.05	90, 94, 92, 94, 93	5	93	1.8	90–94
	Overall		10	95	6.1	89–109
Oil Seed Rape Seed	0.005*	101, 76, 70, 97, 96	5	88	15.9	70–101
	0.05	107, 86, 73, 102, 83	5	90	15.5	73–107
	Overall		10	89	14.9	70–107
Lentils	0.005*	74, 78, 85, 87, 84	5	82	6.6	74–87
	0.05	84, 83, 93, 99, 92	5	90	7.4	83–99
	Overall		10	86	8.5	74–99
Bran	0.005*	80, 77, 86, 99, 98	5	88	11.5	77–99
	0.05	105, 106, 103, 87, 97	5	100	7.9	87–106
	Overall		10	94	11.2	77–106
Bread	0.005*	108, 110, 115, 111, 115	5	112	2.8	108–115
	0.05	111, 111, 106, 107, 105	5	108	2.6	105–111
	Overall		10	110	3.1	105–115
Beer	0.005*	99, 95, 98, 103, 99	5	99	2.9	95–103
	0.05	100, 103, 102, 102, 103	5	102	1.2	100–103
	Overall		10	100	2.7	95–103

*–Limit of quantification, defined by the lowest validated fortification level
#–Outlier as determined by Dixon's test, excluded from calculations
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 38 Recovery results obtained during validation of Method GRM006.03A for CSCD459489 in Crops and Processed Commodities. Primary transition *m/z* 374 → 156

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	101, 103, 97, 98, 94	5	99	3.6	94–103
	0.05	94, 100, 99, 97, 97	5	97	2.4	94–100
	Overall		10	98	2.9	94–103
Barley Forage	0.005*	109, 103, 108, 116, 110	5	109	4.3	103–116
	0.05	106, 103, 106, 100, 103	5	104	2.4	100–106
	0.5	108, 101, 98, 99, 122	5	106	9.4	98–122
	1.0	96, 90, 103, 90, 107	5	97	7.9	90–107
	Overall		20	104	7.4	90–122
Barley straw	0.005*	111, 118, 107, 115, 129	5	116	7.2	107–129
	0.05	99, 95, 79, 117, 106	5	99	14.2	79–117
	1.0	90, 98, 88, 91, 104	5	94	7.1	88–104
	2.0	96, 99, 104, 109, 101	5	102	4.9	96–109
	Overall		20	103	11.5	79–129
Apple	0.005*	80, 90, 90, 99, 99	5	92	8.6	80–99
	0.05	107, 107, 68, 107, 106	5	107	0.5	106–107
	Overall		9	98	9.9	80–107
Carrot	0.005*	105, 103, 104, 97, 105	5	103	3.3	97–105
	0.05	109, 106, 107, 106, 106	5	107	1.2	106–109
	Overall		10	105	3.0	97–109
Spinach	0.005*	87, 101, 96, 102, 98	5	97	6.2	87–102
	0.05	95, 98, 98, 105, 100	5	99	3.7	95–105
	Overall		10	98	5.0	87–105
Oil Seed Rape Seed	0.005*	89, 83, 75, 100, 102	5	90	12.7	75–102
	0.05	106, 87, 74, 102, 83	5	90	14.8	74–106
	Overall		10	90	13.0	74–106
Lentils	0.005*	94, 106, 97, 98, 98	5	99	4.5	94–106
	0.05	97, 95, 103, 97, 101	5	99	3.3	95–103
	Overall		10	99	3.7	94–106
Bran	0.005*	80, 75, 90, 95, 101	5	88	12.1	75–101
	0.05	101, 109, 103, 83, 92	5	98	10.4	83–109
	Overall		10	93	11.9	75–109
Bread	0.005*	113, 113, 107, 106, 114	5	111	3.4	107–114
	0.05	103, 110, 103, 104, 103	5	105	2.9	103–110
	Overall		10	108	4.2	103–114
Beer	0.005*	100, 100, 101, 101, 103	5	101	1.2	100–103
	0.05	102, 102, 101, 102, 104	5	102	1.1	101–104
	Overall		10	102	1.2	100–104

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 39 Recovery results obtained during validation of Method GRM006.03A for CSCD459489 in Crops and Processed Commodities. Confirmatory transition *m/z* 374 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.005*	88, 96, 90, 81, 82	5	87	7.0	81–96
	0.05	97, 99, 102, 97, 96	5	98	2.4	96–102
	Overall		10	93	7.8	81–102
Barley Forage	0.005*	103, 109, 109, 100, 102	5	105	4.0	100–109
	0.05	112, 110, 109, 106, 104	5	108	3.0	104–112
	0.5	102, 110, 110, 101, 122	5	109	7.7	101 -122
	1.0	92, 91, 102, 92, 105	5	96	6.8	91–105
	Overall		20	105	7.2	91–122

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley straw	0.01*	105, 103, 102, 98, 108	5	103	3.6	98–108
	0.05	99, 97, 76, 111, 104	5	97	13.5	76–111
	1.0	88, 98, 87, 90, 105	5	94	8.2	87–105
	2.0	102, 108, 100, 106, 98	5	103	4.0	98–108
	Overall		20	99	8.5	76–111
Apple	0.005*	79, 90, 92, 100, 98	5	92	9.0	79–100
	0.05	101, 98, 22#, 93, 91	4	96#	4.8#	91–101
	Overall		9	94#	7.3#	79–101
Carrot	0.005*	116, 98, 119, 109, 119	5	112	8.0	98–119
	0.05	108, 104, 107, 106, 103	5	106	2.0	103–108
	Overall		10	109	6.5	98–119
Spinach	0.005*	87, 105, 100, 95, 92	5	96	7.3	87–105
	0.05	95, 96, 96, 93, 95	5	95	1.3	93–96
	Overall		10	95	5.0	87–105
Oil Seed Rape Seed	0.005*	90, 74, 67, 101, 97	5	86	17.2	67–101
	0.05	108, 89, 72, 107, 83	5	92	17.0	72–108
	Overall		10	89	16.5	67–108
Lentils	0.005*	85, 98, 94, 96, 97	5	94	5.6	85–98
	0.05	97, 97, 104, 98, 100	5	99	3.0	97–104
	Overall		10	97	5.0	85–104
Bran	0.005*	80, 76, 89, 94, 94	5	87	9.5	76–94
	0.05	102, 109, 101, 85, 93	5	98	9.4	85–109
	Overall		10	92	11.0	76–109
Bread	0.005*	110, 113, 106, 106, 109	5	109	2.7	106–113
	0.05	109, 111, 108, 104, 103	5	107	3.2	103–111
	Overall		10	108	2.9	103–113
Beer	0.005*	101, 97, 100, 100, 100	5	100	1.5	97–101
	0.05	105, 103, 103, 101, 106	5	104	1.9	101–106
	Overall		10	102	2.6	97–106

*–Limit of quantification, defined by the lowest validated fortification level
#–Outlier as determined by Dixon's test, excluded from calculations
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

The linearity of the LC-MS/MS detector response for CSCD459488 and CSCD459489 was tested over the range from 0.15–30 pg injected on column (equivalent to 0.000005–0.001 μg/mL standards when using a 30 μL injection volume) and was found to be linear. The correlation coefficients (R^2) of the calibration curves were > 0.999.

The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each crop tested during method validation were < 20%.

CSCD459488 has been shown to be efficiently extracted from cereal matrices under the conditions used in method GRM006.03A (*Booth J, Goodwin SL, 2007, T003918-05-REG*) which is reported inthe metabolism study in wheat. Because of its low abundance in the radio-labelled samples, the extractability efficiency of CSCD459489 could not be directly assessed but may be assumed to be similar to that of CSCD459488.

Method GRM006.03A has been demonstrated to be a reliable and accurate procedure for the determination of CSCD459488 and CSCD459489 to a limit of quantification of 0.005 mg/kg for each analyte in crop matrices, using commercially available laboratory equipment and reagents. Barley grain, barley forage, barley straw, apple, carrot, spinach, potato, oil seed rape (seed), lentil, tomato, bran, bread and beer have been used as example commodities.

Residue analytical method GRM006.08A

GRM006.08 is the method used for data-generation for CSCD465008 and CSAA798670 in crops. Samples are extracted by homogenisation with acetonitrile:water (80:20 v/v). Extracts are centrifuged and aliquots (equivalent to 0.1 g sample) are evaporated then buffered at pH 5 and hydrolysed with pectinase at 37 °C for 16–20 hours. Samples are partitioned with hexane, then acidified and taken

through a solid phase extraction (SPE) clean-up procedure, using Waters Oasis HLB cartridges. The SPE cartridges are washed with water and CSCD465008 and CSAA798670 are eluted with acetonitrile:water (50:50 v/v). The column eluates are evaporated to remove the acetonitrile and then diluted with water. Final determination is by high-performance liquid chromatography using triple-quadrupole mass-spectrometric detection (LC-MS/MS) with two MS/MS transitions (Hargreaves S., 2008b).

The limit of quantification of the method is 0.01 mg/kg for both CSCD465008 and CSAA798670.

Analytical method GRM006.08A has been validated for the determination of residues of CSCD465008 and CSAA798670 in crops (Morriss A., 2008b). Control samples were analysed in duplicate. Fortified samples were analysed in quintuplet at the LOQ (0.01 mg/kg) and in quintuplet at ten times the LOQ (0.1 mg/kg). In general, acceptable overall mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions of both analytes on the matrices tested. Exceptions to this were recovery of CSCD465008 from barley forage fortified at 0.01 mg/kg (mean recovery 69%, %RSD 12.5%) and CSCD465008 from carrot leaf fortified at 0.01 mg/kg (mean recovery 67%, %RSD 11.4%). Although the mean percent recovery at the LOQ is outside the acceptable range, the overall mean recovery data for CSCD465008 from both barley forage and carrot leaf were within the acceptable range. The method is therefore considered to be valid and fit for purpose. Quantification was performed using non-matrix standards, except for straw and spinach matrices where matrix suppression >10% was observed for both analytes. Matrix-matched standards were used to compensate for these effects. The recoveries obtained are detailed in Table 40 to Table 43.

Table 40 CSCD465008 recovery data obtained during method validation. Primary transition *m/z* 161 → 141

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.01*	76, 67, 78, 76, 80	5	75	6.6	67-80
	0.1	80, 77, 81, 76, 78	5	78	2.6	76-81
	Overall		10	77	5.1	67-81
Barley Forage	0.01*	77, 62, 68, 59, 78	5	69	12.5	59-78
	0.1	85, 85, 83, 81, 81	5	83	2.4	81-85
	Overall		10	76	12.5	59-85
Barley straw†	0.01*	79, 73, 70, 74, 73	5	74	4.4	70-79
	0.1	79, 80, 88, 82, 78	5	81	4.9	78-88
	Overall		10	78	6.8	70-88
Carrot leaves	0.01*	74, 54, 70, 69, 66	5	67	11.4	54-74
	0.1	85, 84, 89, 81, 84	5	85	3.4	81-89
	Overall		10	76	14.5	54-89
Carrot root	0.01*	80, 71, 72, 68, 92	5	77	12.6	68-92
	0.1	73, 74, 91, 93, 80	5	82	11.4	73-93
	Overall		10	79	11.9	68-93
Spinach†	0.01*	75, 64, 79, 77, 83	5	76	9.4	64-79
	0.1	82, 80, 83, 87, 83	5	83	3.1	80-87
	Overall		10	79	8.0	64-87

*–Limit of quantification, defined by the lowest validated fortification level
†–Matrix matched standards were used for quantification of barley straw and spinach recoveries
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 41 CSCD465008 recovery data obtained during method validation. Confirmatory transition *m/z* 161 → 66

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.01*	75, 68, 80, 77, 79	5	76	6.3	68-79
	0.1	81, 78, 80, 75, 80	5	79	3.0	75-81
	Overall		10	77	5.0	68-81

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley Forage	0.01*	78, 60, 67, 63, 82	5	70	13.7	60-82
	0.1	80, 84, 82, 80, 80	5	81	2.2	80-84
	Overall		10	76	11.6	60-84
Barley straw†	0.01*	81, 72, 76, 71, 72	5	74	5.6	71-81
	0.1	78, 81, 88, 82, 78	5	81	5.0	78-88
	Overall		10	78	6.9	71-88
Carrot leaves	0.01*	72, 53#, 69, 70, 68	4	70#	2.4#	68-72
	0.1	77, 83, 89, 82, 85	5	83	5.3	77-89
	Overall		9	77#	10.1#	68-89
Carrot root	0.01*	76, 72, 76, 67, 89	5	76	10.7	67-89
	0.1	73, 75, 91, 94, 81	5	83	11.3	73-94
	Overall		10	79	11.4	67-94
Spinach†	0.01*	75, 67, 82, 73, 83	5	76	8.7	67-83
	0.1	83, 81, 85, 88, 84	5	84	3.1	81-88
	Overall		10	80	8.0	67-88

*–Limit of quantification, defined by the lowest validated fortification level
†–Matrix matched standards were used for quantification of barley straw and spinach recoveries
#–Rejected as an outlier by Dixon's test
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 42 CSAA798670 recovery data obtained during method validation. Primary transition *m/z* 175 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.01*	89, 84, 92, 83, 90	5	88	4.5	83-92
	0.1	91, 88, 98, 88, 92	5	91	4.5	88-98
	Overall		10	90	4.8	83-98
Barley Forage	0.01*	84, 83, 86, 79, 89	5	84	4.4	79-89
	0.1	90, 93, 89, 86, 88	5	89	2.9	86-93
	Overall		10	87	4.6	79-93
Barley straw [a]	0.01*	97, 114, 93, 98, 98	5	100	8.1	93-114
	0.1	91, 91, 98, 89, 88	5	91	4.3	88-98
	Overall		10	96	7.9	88-114
Carrot leaves [b]	0.01*	80, 76, 74, 47#, 70	4	75#	4.2#	70-80#
	0.1	90, 87, 93, 85, 89	5	89	3.4	85-93
	Overall		9	83#	9.6#	70-93
Carrot root	0.01*	88, 84, 78, 84, 86	5	84	4.5	78-88
	0.1	86, 101, 102, 99, 92	5	96	7.1	86-102
	Overall		10	90	9.1	78-102
Spinach [a,c]	0.01*	81, 79, 89, 83, 88	5	84	5.2	79-89
	0.1	81, 77, 85, 87, 84	5	83	4.7	77-87
	Overall		10	83	4.7	77-89

*–Limit of quantification, defined by the lowest validated fortification level.
#–Excluded as an outlier by Grubb's test.
[a] Matrix matched standards were used for quantification of barley straw and spinach recoveries.
[b] Mean residue in the control specimens of carrot leaves was >30% LOQ (0.0036 mg/kg). The reagent blank showed a residue of 0.0017 mg/kg. When the mean control residue was corrected for the reagent blank value, the mean control residue was < 30% LOQ and was accepted. Recoveries were corrected for the mean control residue of 0.0036 mg/kg as the worst case.
[c] One spinach control showed an anomalous residue value and was therefore treated as an outlier and discarded. The other spinach control sample residue was less than 30% LOQ.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table 43 CSAA798670 recovery data obtained during method validation. Confirmatory transition *m/z* 161 → 111

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Barley grain	0.01*	99, 85, 97, 83, 100	5	93	8.8	83-100
	0.1	96, 87, 99, 86, 93	5	92	6.1	86-99
	Overall		10	93	7.1	83-100
Barley Forage	0.01*	70, 88, 91, 86, 93	5	86	10.7	86-93
	0.1	91, 94, 88, 87, 87	5	89	3.4	87-94
	Overall		10	88	7.7	86-94
Barley straw [a]	0.01*	110, 112, 109, 107, 105	5	109	2.5	105-112
	0.1	87, 90, 97, 93, 86	5	91	5.0	86-97
	Overall		10	100	10.2	86-112
Carrot leaves	0.01*	75, 72, 74, 64, 79	5	73	7.6	64-79
	0.1	92, 86, 90, 86, 90	5	89	3.0	86-92
	Overall		10	81	11.6	64-92
Carrot root	0.01*	90, 75, 80, 76, 95	5	83	10.7	75-95
	0.1	83, 101, 100, 98, 92	5	95	7.9	83-101
	Overall		10	89	11.1	75-101
Spinach [a, b]	0.01*	73, 70, 92, 81, 82	5	80	10.8	70-92
	0.1	82, 77, 86, 86, 84	5	83	4.5	77-86
	Overall		10	81	8.0	70-92

*–Limit of quantification, defined by the lowest validated fortification level.

[a] Matrix matched standards were used for quantification of barley straw and spinach recoveries.

[b] One spinach control showed an anomalous residue value and was therefore treated as an outlier and discarded. The other spinach control sample residue was less than 30% LOQ.

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

The linearity of the LC-MS/MS detector response for CSCD465008 was tested in the range from 0.0075 ng to 0.75 ng injected on column (equivalent to 0.0005 µg/mL to 0.05 µg/mL standards when using a 15 µL injection volume) and was found to be linear. This is equivalent to a range of 50% LOQ–50 × LOQ.

The linearity of the LC-MS/MS detector response for CSCAA798670 was tested in the range from.0.005 ng to 0.5 ng injected on column (equivalent to 0.0005 µg/mL to 0.05 µg/mL standards when using a 10 µL injection volume) and was found to be linear. This is equivalent to a range of 50% LOQ–50 × LOQ.

The correlation coefficients (R^2) of the calibration curves were at least 0.999.

The relative standard deviations (RSDs) of recoveries of CSCD465008 and CSAA798670 at each fortification level and overall for each crop tested during method validation were < 20% for both transitions monitored.

CSCD465008 and CSAA798670 have been shown to be efficiently extracted from cereal matrices under the conditions used in method GRM006.08A (*Evans J, 2008, T003919-05-REG*) which is reported in the metabolism study in a succeeding crops.

Method GRM006.08A has been demonstrated to be a reliable and accurate procedure for the determination of CSCD465008 and CSAA798670 to a limit of quantification of 0.01 mg/kg each in crop matrices, using commercially available laboratory equipment and reagents. Barley grain, barley forage, barley straw, carrot leaf, carrot root and spinach have been used as example crops.

Residue analytical method GRM006.08B

Method GRM006.08B for the determination of CSCD465008 and CSAA798670 in crops is up-dated from GRM006.008A to include a viability check for the pectinase enzyme (Hargreaves S., 2009). The method is otherwise unchanged, so the analytical procedures are identical to those of GRM006.08A. Therefore, the validation data obtained for method GRM006.08A are equally applicable to GRM006.08B. No additional validation of the revised method was necessary.

Samples are extracted by homogenisation with acetonitrile:water (80:20 v/v). Extracts are centrifuged and aliquots (equivalent to 0.1 g sample) are evaporated then buffered at pH 5 and hydrolysed with pectinase at 37 °C for 16–20 hours. Samples are partitioned with hexane, then acidified and taken through a solid phase extraction (SPE) clean-up procedure, using Waters Oasis HLB cartridges. The SPE cartridges are washed with water and CSCD465008 and CSAA798670 are eluted with acetonitrile:water (50:50 v/v). The column eluates are evaporated to remove the acetonitrile and then diluted with water. Final determination is by high-performance liquid chromatography using triple-quadrupole mass-spectrometric detection (LC-MS/MS) with two MS/MS transitions.

The limit of quantification of the method is 0.01 mg/kg for both CSCD465008 and CSAA798670.

Method GRM006.08B has been demonstrated to be a reliable and accurate procedure for the determination of CSCD465008 and CSAA798670 to a limit of quantification of 0.01 mg/kg each in crop matrices, using commercially available laboratory equipment and reagents.

Multi-Residue analytical method DFG-S19

The DFG method S19 is a multi-residue method investigated for the monitoring of isopyrazam in crops. DFG method S19 is a multi-residue method suitable for the determination of residues of isopyrazam (analysed as the isomers *anti*-isomer and *syn*-isomer) in crops. The extraction of isopyrazam residues from wheat whole plant is performed according to extraction Module E1. The extraction of isopyrazam residues from wheat grain is performed according to Module E2. The extraction of isopyrazam residues from apples is performed according to extraction Module E3 and the extraction of isopyrazam residues from oil seed rape seed is performed using Module E7. Extracts are cleaned-up using gel permeation chromatography (GPC) followed by silica mini gel column chromatography (Module C1). Residues of isopyrazam are quantified using gas chromatography with mass selective detection (GC-MSD) in the selected ion monitoring mode (SIM)(Lakaschus S., Gizler A.S., 2008a). Alternatively, LC-MS/MS using multiple reaction monitoring (MRM) with either positive or negative ionisation may be used for final determination(Lakaschus S., Gizler A.S., 2009a).

The limit of quantification of the method is 0.005 mg/kg for *anti*-isomer and *syn*-isomer.

During validations of method S19, four crops were fortified with isopyrazam (as the isomers *anti*-isomer and *syn*-isomer). Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg for each isomer) and in quintuplet at a higher fortification level (0.3 mg/kg for wheat grain, 5.0 mg/kg for wheat whole plant, 1.0 mg/kg for apple and 0.1 mg/kg for oilseed rape seed). Control samples were analysed in duplicate. The crops are representative of the four crop types, i.e., dry crops (wheat grain), high water content crops (wheat whole plant), high fat content crops (oil seed rape seed) and high acid content crops (apples).

Lakaschus S. and Gizler A.S. (2008a) reported that using GC-MSD final determination, acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for all three ions monitored. No significant matrix effects were observed and non-matrix standards were used for quantification except for wheat grain and oil seed rape seed at the LOQ where matrix-matched calibration standards were used for quantification. The recoveries obtained are detailed in Table 44 to Table 49.

Lakaschus S.and Gizler A.S. (2009a) also reported that using LC-MS/MS final determination, acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for the two ion transitions monitored. No significant matrix effects were observed and non-matrix standards were used for quantification. The recoveries obtained are detailed in Table 50 to Table 53.

Table 44 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using GC-MSD final determination. Ion *m/z* 331

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	102, 98, 92, 102, 92	5	97	5.2	92–102
	0.3	90, 93, 93, 90, 100	5	93	4.4	90–100
	Overall		10	95	5.0	90–102
Wheat Whole Plant	0.005*	88, 84, 88, 108, 94	5	92	10	84–108
	5.0	89, 99, 109, 96, 96	5	98	7.4	89–109
	Overall		10	95	8.4	84–109
Apple	0.005*	110, 106, 82, 86, 90	5	95	13	82–110
	1.0	97, 94, 100, 108, 89	5	98	7.3	89–108
	Overall		10	96	10	82–110
Oilseed rape seed	0.005*	104, 106, 122, 106, 102	5	108	7.4	102–122
	0.1	90, 91, 99, 93, 93	5	93	3.7	90–99
	Overall		10	101	10	90–122

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 45 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using GC-MSD final determination. Ion *m/z* 344

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	86, 98, 92, 84, 96	5	91	6.7	84–98
	0.3	83, 83, 90, 93, 100	5	90	8.0	83–100
	Overall		10	91	7.0	83–100
Wheat Whole Plant	0.005*	92, 110, 98, 84, 114	5	100	12	84–114
	5.0	87, 88, 104, 94, 91	5	93	7.3	87–104
	Overall		10	96	10	84–114
Apple	0.005*	84, 98, 88, 90, 82	5	88	7.0	82–98
	1.0	113, 104, 102, 113, 89	5	104	9.5	89–113
	Overall		10	96	12	82–113
Oilseed rape seed	0.005*	96, 92, 100, 88, 86	5	94	4.8	86–100
	0.1	90, 82, 90, 87, 86	5	87	3.8	82–90
	Overall		10	91	5.8	82–100

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 46 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using GC-MSD final determination. Ion *m/z* 359

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	98, 106, 114, 98, 110	5	105	6.8	98–114
	0.3	83, 87, 107, 117, 123	5	103	17	83–123
	Overall		10	104	12	83–123
Wheat Whole Plant	0.005*	98, 98, 108, 104, 120	5	106	8.6	98–120
	5.0	88, 91, 106, 95, 95	5	95	7.2	88–106
	Overall		10	100	9.4	88–120
Apple	0.005*	108, 98, 98, 94, 102	5	100	5.3	94–108
	1.0	105, 98, 101, 112, 94	5	102	6.8	94 -112
	Overall		10	101	5.8	94–112
Oilseed rape seed	0.005*	78, 92, 94, 88, 76	5	86	9.5	76–94
	0.1	88, 98, 83, 77, 87	5	87	8.9	77–98
	Overall		10	86	8.7	76–98

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 47 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using GC-MSD final determination. Ion *m/z* 331

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	112, 98, 98, 94, 96	5	100	7.2	94–112
	0.3	97, 90, 103, 107, 103	5	100	6.6	90–107
	Overall		10	100	6.5	90–112
Wheat Whole Plant	0.005*	94, 90, 96, 84, 92	5	91	5.5	84–96
	5.0	88, 96, 105, 96, 94	5	96	6.4	88–105
	Overall		10	94	6.4	84–105
Apple	0.005*	108, 106, 100, 94, 94	5	100	6.5	94–108
	1.0	94, 90, 93, 102, 86	5	93	6.4	86–102
	Overall		10	97	7.3	86–108
Oilseed rape seed	0.005*	94, 102, 112, 104, 84	5	99	11	84–112
	0.1	80, 80, 83, 84, 82	5	82	2.2	80–84
	Overall		10	91	13	80–112

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 48 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using GC-MSD final determination. Ion *m/z* 344

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	110, 112, 114, 110, 102	5	110	4.2	102–114
	0.3	93, 100, 110, 90, 97	5	98	7.9	93–110
	Overall		10	104	8.2	93–114
Wheat Whole Plant	0.005*	106, 98, 106, 110, 98	5	104	4.8	98–110
	5.0	89, 95, 108, 98, 94	5	97	7.2	89–108
	Overall		10	100	7.0	89–110
Apple	0.005*	110, 110, 98, 80, 94	5	98	13	80–110
	1.0	101, 98, 94, 102, 92	5	97	4.5	92–102
	Overall		10	98	9.0	80–110
Oil seed rape seed	0.005*	104, 72, 92, 76, 108	5	90	18	72–108
	0.1	96, 94, 82, 81, 99	5	90	9.2	81–99
	Overall		10	90	13	72–108

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 49 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using GC-MSD final determination. Ion *m/z* 359

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	108, 114, 114, 114, 110	5	112	2.5	108–114
	0.3	93, 93, 103, 103, 103	5	99	5.5	93–103
	Overall		10	106	7.6	93–114
Wheat Whole Plant	0.005*	92, 80, 90, 72, 96	5	86	12	72–96
	5.0	87, 88, 103, 93, 90	5	92	7.1	87–103
	Overall		10	89	9.0	72–103
Apple	0.005*	106, 106, 98, 98, 100	5	102	4.0	98–106
	1.0	96, 93, 94, 101, 90	5	95	4.3	90–101
	Overall		10	98	5.4	90–106
Oilseed rape seed	0.005*	108, 98, 100, 100, 92	5	100	5.8	92–108
	0.1	86, 88, 84, 90, 87	5	87	2.6	84–90
	Overall		10	93	8.4	84–108

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 50 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination. Primary Transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	96, 106, 70, 100, 91	5	93	15	70–106
	0.3	70, 78, 81, 70, 84	5	77	8.3	70–84
	Overall		10	85	16	70–106
Wheat Whole Plant	0.005*	71, 72, 72, 72, 69	5	71	1.8	69–72
	5.0	93, 88, 80, 75, 78	5	83	9.0	75–93
	Overall		10	77	10	69–93
Apple	0.005*	90, 98, 98, 92, 85	5	93	5.9	85–98
	1.0	99, 79, 65, 67, 94	5	81	19	65–99
	Overall		10	87	15	65–99
Oilseed rape seed	0.005*	86, 71, 63, 74, 58	5	70	15	58–86
	0.1	75, 76, 74, 76, 91	5	78	9.1	74–91
	Overall		10	74	13	58–91

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 51 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination. Confirmatory Transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	102, 103, 66, 94, 101	5	93	17	66–103
	0.3	70, 83, 80, 70, 83	5	77	8.7	70–83
	Overall		10	85	17	66–103
Wheat Whole Plant	0.005*	69, 74, 69, 84, 72	5	74	8.4	69–84
	5.0	91, 89, 80, 74, 77	5	82	9.1	74–91
	Overall		10	78	10	69–91
Apple	0.005*	84, 89, 118, 96, 76	5	93	17	76–118
	1.0	94, 74, 61, 63, 92	5	77	20	61–94
	Overall		10	85	20	61–118
Oilseed rape seed	0.005*	95, 77, 63, 82, 67	5	77	17	63–95
	0.1	82, 81, 79, 81, 96	5	84	8.2	79–96
	Overall		10	80	13	63–96

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 52 Recovery results obtained during validation of Method S19 for syn-isomer in Crops using LC-MS/MS final determination. Primary Transition m/z 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	93, 90, 57, 94, 94	5	86	19	57–94
	0.3	69, 81, 88, 70, 85	5	79	11	69–88
	Overall		10	82	16	57–94
Wheat Whole Plant	0.005*	72, 75, 65, 77, 75	5	73	6.4	65–77
	5.0	90, 85, 80, 72, 76	5	81	8.8	72–90
	Overall		10	77	9.1	65–90
Apple	0.005*	89, 103, 110, 94, 83	5	96	11	83–110
	1.0	92, 79, 67, 66, 94	5	80	17	66–94
	Overall		10	88	16	66–110
Oilseed rape seed	0.005*	82, 72, 64, 82, 65	5	73	12	64–82
	0.1	82, 81, 76, 79, 92	5	82	7.3	76–92
	Overall		10	78	11	64–92

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 53 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination. Confirmatory Transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	84, 99, 56, 90, 89	5	84	19	56–99
	0.3	67, 80, 80, 69, 83	5	76	10	67–83
	Overall		10	80	16	56–99
Wheat Whole Plant	0.005*	67, 73, 68, 81, 73	5	72	7.6	67–81
	5.0	84, 81, 78, 73, 74	5	78	5.9	73–84
	Overall		10	75	7.6	67–84
Apple	0.005*	88, 104, 92, 104, 85	5	95	9.4	85–104
	1.0	97, 82, 65, 69, 97	5	82	18	65–97
	Overall		10	88	15	65–104
Oilseed rape seed	0.005*	86, 73, 58, 78, 60	5	71	17	58–86
	0.1	77, 79, 73, 74, 87	5	78	7.2	73–87
	Overall		10	75	13	58–87

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

During independent laboratory validation of DFG method S19 (Schultz H., 2008), wheat grain and oilseed rape seed matrices were fortified at the proposed limit of quantification (0.005 mg/kg for *anti*-isomer and *syn*-isomer) and at a higher level. The independent laboratory validation was performed using DFG method S19 with final determination using GC-MSD monitoring three ions (wheat grain only) and also using final determination by LC-MS/MS monitoring three transitions. The recoveries obtained from GC-MSD determination are detailed in Tables 54 to 59, and the recoveries obtained from LC-MS/MS determination are detailed in Tables 60 to 66.

Table 54 Recovery results obtained during independent validation of Method S19 for *anti*-isomer in Crops using GC-MSD final determination. Ion *m/z* 359

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	116, 87, 92, 130, 89	5	103	18.7	87–130
	0.05	90, 88, 86, 93, 83	5	88	4.3	83–93
	Overall		10	95	15.9	83–130

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 55 Recovery results obtained during independent validation of Method S19 for *anti*-isomer in Crops using GC-MSD final determination. Ion *m/z 3*16

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	35, 36, 19, 55, 72	5	43	47.1	19–72
	0.05	82, 84, 74, 76, 76	5	78	4.3	74–84
	Overall		10	61	23.1	19–84

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 56 Recovery results obtained during independent validation of Method S19 for *anti*-isomer in Crops using GC-MSD final determination. Ion *m/z 159*

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	145, 110, 102, 145, 72	5	115	27.0	72–145
	0.05	97, 93, 85, 91, 83	5	90	6.4	83–97
	Overall		10	102	24.3	72–145

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 57 Recovery results obtained during independent validation of Method S19 for *syn*-isomer in Crops using GC-MSD final determination. Ion *m/z* 359

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	127, 90, 92, 114, 107	5	106	15.5	90–127
	0.05	86, 88, 88, 92, 78	5	86	5.5	86–92
	Overall		10	96	15	86–127

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 58 Recovery results obtained during independent validation of Method S19 for *syn*-isomer in Crops using GC-MSD final determination. Ion *m/z 3*16

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	132, 112, 103, 111, 103	5	115	31	103–132
	0.05	87, 93, 87, 92, 81	5	90	5.8	81–93
	Overall		10	102	24.8	81–132

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 59 Recovery results obtained during independent validation of Method S19 for *syn*-isomer in Crops using GC-MSD final determination. Ion *m/z 159*

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	96, 106, 105, 105, 102	5	103	4.0	96–106
	0.05	96, 99, 97, 98, 77	5	93	10.0	77–99
	Overall		10	98	8.5	77–106

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 60 Recovery results obtained during independent validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination. Primary Transition *m/z* 358 → 131

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	84, 78, 84, 83, 80	5	82	3.2	78–84
	0.05	84, 85, 88, 82, 86	5	85	2.6	82–88
	Overall		10	83	3.5	78–88
Oilseed rape seed	0.005*	91, 94, 97, 97, 101	5	94	5.7	91–101
	0.05	99, 83, 97, 97, 94	5	94	6.8	83–99
	Overall		10	94	5.9	83–101

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 61 Recovery results obtained during independent validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination. Confirmatory Transition *m/z* 358 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	79, 80, 86, 79, 77	5	80	4.3	77–86
	0.05	86, 87, 91, 84, 84	5	86	3.3	84–91
	Overall		10	83	5.3	77–91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Oilseed rape seed	0.005*	96, 97, 98, 92, 108	5	98	6.0	92–108
	0.05	105, 92, 99, 101, 96	5	99	5.0	92–105
	Overall		10	98	5.2	92–108

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 62 Recovery results obtained during independent validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination. Confirmatory Transition *m/z* 358 → 111

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	86, 80, 88, 81, 81	5	83	4.3	80–88
	0.05	83, 86, 85, 84, 89	5	85	2.7	83–89
	Overall		10	84	3.6	80–89
Oilseed rape seed	0.005*	103, 110, 97, 100, 109	5	104	5.4	97–110
	0.05	101, 91, 98, 103, 97	5	98	4.7	91–103
	Overall		10	101	5.7	91–110

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 63 Recovery results obtained during independent validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination. Primary Transition *m/z* 358 → 131

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	83, 77, 86, 80 , 78	5	81	4.6	77–86
	0.05	86, 87, 87, 84, 85	5	86	1.5	84–87
	Overall		10	83	4.5	77–87
Oilseed rape seed	0.005*	91, 94, 97, 87, 101	5	94	5.7	87–101
	0.05	99, 83, 97, 97, 94	5	94	6.8	83–99
	Overall		10	94	5.9	83–101

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 64 Recovery results obtained during independent validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination. Confirmatory Transition *m/z* 358 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	83, 81, 83, 83, 80	5	82	1.7	80–83
	0.05	81, 84, 89, 87, 85	5	85	3.6	81–89
	Overall		10	84	3.4	80–89
Oilseed rape seed	0.005*	96, 97, 98, 92, 108	5	98	6.0	92–108
	0.05	105, 92, 99, 101, 96	5	99	5.0	92–105
	Overall		10	98	5.2	92–108

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 65 Recovery results obtained during independent validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination. Confirmatory Transition *m/z* 358 → 111

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Wheat grain	0.005*	79, 77, 83, 83, 75	5	79	4.5	77–83
	0.05	84, 87, 91, 83, 85	5	86	3.7	83–91
	Overall		10	83	5.7	77–91
Oilseed rape seed	0.005*	103, 110, 97, 100, 109	5	104	5.6	97–110

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	0.05	101, 91, 98, 103, 97	5	98	4.6	91–103
	Overall		10	101	5.7	91–110

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

GC-MSD detection in the SIM mode monitoring three ions is considered to be highly specific. The independent validation initially used GC-MSD in the SIM mode monitoring three ions but for some confirmatory ions the data at the LOQ were qualitative only. Subsequent analysis was, therefore, carried out by LC-MS/MS monitoring three transitions. LC-MS/MS detection using either positive or negative multiple reaction monitoring (MRM) is also considered to be highly specific.

The linearity of the GC-MSD detector response for *anti*-isomer and *syn*-isomer was tested over the range 0.0042 µg/mL to 0.5 µg/mL (*m/z* = 359), 0.0063 µg/mL to 0.5 µg/mL (*m/z* = 331 and 344) when using a 1 µL injection volume and was found to be linear (correlation coefficients, $R^2 > 0.9999$ in each case). This is equivalent to a range of 30% LOQ (*m/z* = 359) and 50% LOQ (*m/z* = 331 and 344) to 20% above the highest concentrations in the final extracts. The linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range from 0.5 to 75.5 ng/mL (equivalent to 0.0075 ng to 1.1325 ng when using a 15 µL injection volume) and was found to be linear ($R^2 > 0.999$). During independent validation the linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range from 0.075 to 20.18 ng/mL (equivalent to 0.0015 ng to 0.04 ng when using a 20 µL injection volume) and was found to be linear ($R^2 > 0.999$).

During independent validation using wheat grain matrix with final determination by GC-MSD, recovery of *syn*-isomer was within the acceptable range of between 70% and 110% with the exception of ion *m/z* 316 which was marginally above this level. Recovery of *anti*-isomer was within the acceptable range of between 70% and 110% with the exception of ion 159 which was marginally above this level and ion 316 which was qualitative only (mean recovery 43% at the lower fortification level). During independent validation using wheat grain and oilseed rape seed matrices fortified in quintuplet at the LOQ and in quintuplet at a higher fortification level with final determination by LC-MS/MS all *anti*-isomer and *syn*-isomer recovery was within the acceptable range of between 70% and 110%.

During independent validation using wheat grain matrix with final determination by GC-MSD relative standard deviations (RSDs) at the higher validation level were all < 20%, however, at the LOQ for all ions monitored for *anti*-isomer and for ion *m/z* 316 for *syn*-isomer relative standard deviations (RSDs) were > 20% (47% at the lower fortification level for *m/z* 316). The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each crop tested during method validation using both GC-MSD and LC-MS/MS detection and independent validation using LC-MS/MS final determination were < 20%. Thus, the method has satisfactory repeatability when LC-MS/MS determination is employed, but not with GC-MSD determination.

An independent laboratory validation was conducted and demonstrated acceptable reproducibility when LC-MS/MS determination is employed, but not with GC-MSD determination.

The Meeting received a further validation data of the method from the manufacturer (Bacher R., 2009). The validation of the method DFG S19 was performed as described in the guideline SANCO/825/00 rev.7 (17/03/04), for a series of recovery experiments by fortifying control (untreated) specimens of apple, wheat grain, whole plant of wheat, and oilseed rape seed. Residues of *anti*-isomer and *syn*-isomer were extracted using extraction module E1 (apple, whole wheat plants), E2 (wheat grain) or E7 (oilseed rape seed) of the DFG Method S 19 [1]. The extracts were further cleaned up by gel-permeation chromatography (GPC), followed by silica column fractionation (module C1). LC-MS/MS monitoring three mass transitions (in negative mode; m/z 358 → 131, m/z 358 → 111, and m/z 358 → 91) was used for determination and confirmation of both analytes.

The limit of quantification of the method is 0.005 mg/kg for *anti*-isomer and *syn*-isomer.

Samples of the four plant materials representative of the crop types were fortified (5 replicates per matrix and fortification level) with *anti*-isomer and *syn*-isomer at the LOQ (0.005 mg/kg per analyte) and at a higher fortification level indicative for expected residues for each commodity (for each analyte: 1.0 mg/kg for apple, 0.30 mg/kg for wheat grain, 5.0 mg/kg for whole plant of wheat, and 0.10 mg/kg for oilseed rape seed). An additional two replicate samples per matrix were kept untreated, serving as controls.

Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for all three transitions monitored. No significant matrix effects were observed and non-matrix standards were used for quantification except for wheat grain and oilseed rape seed at the LOQ where matrix-matched calibration standards were used for quantification. The results are summarised in Tables 66 to Table 71, below.

Table 66 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination: Transition *m/z* 358 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Apple	0.005*	88, 73, 77, 76, 73	5	77	8	73–88
	1.0	90, 92, 84, 80, 83	5	86	6	80–92
	Overall		10	81	9	73–92
Wheat grain	0.005*	86, 90, 106, 91, 115	5	98	13	86–115
	0.30	117, 107, 103, 100, 100	5	105	7	100–117
	Overall		10	101	10	86–117
Wheat whole plant	0.005*	100, 100, 104, 115, 106	5	105	6	100–115
	5.0	82, 87, 84, 82, 84	5	84	2	82–87
	Overall		10	94	13	82–115
Oilseed rape seed	0.005*	78, 73, 85, 77, 78	5	78	6	73–85
	0.1	97, 107, 102, 101, 115	5	104	7	97–115
	Overall			91	16	73–115

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 67 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination: Transition *m/z* 358 → 111

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Apple	0.005*	86, 72, 79, 77, 72	5	77	7	72–86
	1.0	90, 94, 85, 79, 84	5	86	7	79–94
	Overall		10	82	9	72–94
Wheat grain	0.005*	89, 92, 106, 93, 114	5	99	11	89–114
	0.30	117, 106, 96, 103, 100	5	104	8	96–117
	Overall		10	102	9	89–117
Wheat whole plant	0.005*	97, 97, 103, 111, 102	5	102	5	97–111
	5.0	83, 87, 86, 83, 84	5	84	2	83–87
	Overall		10	93	11	83–111
Oilseed rape seed	0.005*	81, 71, 79, 77, 79	5	77	5	71–81
	0.1	99, 109, 102, 103, 105	5	104	4	99–109
	Overall		10	90	16	71–109

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 68 Recovery results obtained during validation of Method S19 for *anti*-isomer in Crops using LC-MS/MS final determination: Transition *m/z* 358 → 131

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Apple	0.005*	82, 71, 78, 71, 71	5	75	7	71–82

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	1.0	91, 94, 84, 80, 84	5	87	7	80–94
	Overall		10	81	10	71–94
Wheat grain	0.005*	86, 90, 103, 92, 129	5	100	17	86–129
	0.30	116, 102, 108, 106, 100	5	106	6	102–116
	Overall		10	103	12	86–129
Wheat whole plant	0.005*	98, 101, 102, 106, 105	5	102	3	98–106
	5.0	82, 87, 85, 83, 84	5	84	2	82–87
	Overall		10	93	11	82–106
Oilseed rape seed	0.005*	78, 72, 77, 75, 79	5	76	3	72–79
	0.1	98, 105, 103, 109, 103	5	104	4	98–109
	Overall		10	90	16	72–109

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 69 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination: Transition *m/z* 358 → 91

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Apple	0.005*	85, 79, 81, 82, 79	5	81	3	79–85
	1.0	104, 101, 97, 95, 99	5	99	3	95–104
	Overall		10	90	11	79–104
Wheat grain	0.005*	91, 84, 92, 105, 104	5	95	9	84–105
	0.30	98, 94, 89, 85, 84	5	90	7	84–98
	Overall		10	93	8	84–105
Wheat whole plant	0.005*	89, 93, 96, 102, 89	5	94	6	89–102
	5.0	87, 92, 91, 88, 88	5	89	2	87–92
	Overall		10	92	5	87–102
Oilseed rape seed	0.005*	75, 65, 77, 73, 77	5	73	7	65–77
	0.1	105, 97, 103, 105, 102	5	102	3	97–105
	Overall		10	88	18	65–105

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 70 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination: Transition *m/z* 358 → 111

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Apple	0.005*	85, 78, 80, 77, 80	5	80	4	77–85
	1.0	104, 101, 97, 95, 99	5	99	4	95–104
	Overall		10	89	12	77–104
Wheat grain	0.005*	90, 83, 92, 104, 104	5	95	10	83–104
	0.30	97, 94, 88, 85, 84	5	90	6	84–97
	Overall		10	92	8	83–104
Wheat whole plant	0.005*	89, 93, 95, 100, 89	5	93	5	89–100
	5.0	87, 92, 92, 87, 89	5	89	3	87–92
	Overall		10	91	4	87–100
Oilseed rape seed	0.005*	74, 73, 84, 77, 83	5	78	6	73–84
	0.1	104, 102, 102, 103, 100	5	102	1	100–104
	Overall		10	90	15	73–104

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 71 Recovery results obtained during validation of Method S19 for *syn*-isomer in Crops using LC-MS/MS final determination: Transition *m/z* 358 → 131

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Apple	0.005*	85, 80, 81, 80, 79	5	81	3	79–85
	1.0	105, 101, 98, 96, 100	5	100	3	96–105
	Overall		10	90	11	79–105
Wheat grain	0.005*	90, 84, 92, 106, 106	5	96	11	84–106
	0.30	98, 94, 89, 86, 84	5	90	6	84–98
	Overall		10	93	9	84–106
Wheat whole plant	0.005*	90, 93, 96, 102, 90	5	94	5	90–102
	5.0	87, 93, 92, 88, 89	5	90	3	87–93
	Overall		10	92	5	87–102
Oilseed rape seed	0.005*	73, 68, 80, 75, 81	5	76	7	68–81
	0.1	103, 101, 102, 104, 101	5	102	1	101–104
	Overall		10	89	16	68–104

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Analysis by LC-MS/MS monitoring two (or, in this case, three) transitions is considered highly specific.

Linear calibration functions at five different concentrations ranging from 0.05 ng/mL or 0.10 ng/mL up to 10 ng/mL (apple, whole plant of wheat, wheat grain), or 20 ng/mL (oilseed rape seed) were used to evaluate the extracts. Correlation coefficients (r) were ≥ 0.99 for all plant materials and both analytes and the three MS/MS transitions monitored. The lower margin of the linearity test was equal to 20% of the LOQ (exception: oilseed rape seed: 30% of LOQ). The upper margin was higher than the concentrations in the final extracts by at least 20%.

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg for each isomer) and in quintuplet at higher fortification levels (0.3 mg/kg in wheat grain, 5.0 mg/kg in wheat plant, 1.0 mg/kg in apples, 0.1 mg/kg in oilseed rape seed). Acceptable mean recoveries of between 70% and 110% were found for *anti*-isomer and *syn*-isomer for the three transitions using LC-MS/MS detection on all matrices tested.

For all plant matrices investigated, for each fortification level and for all MS/MS transitions monitored, the average recoveries were in the range of 73% to 106%, and the relative standard deviations (RSD) were ≤ 17%.

This additional validation study demonstrated acceptable reproducibility when LC-MS/MS determination is employed.

DFG Method S19 was successfully validated and independently validated for the analysis of isopyrazam (analysed as the isomers *anti*-isomer and *syn*-isomer) residues in crops. The method used for both validation and independent validation was the same (the same modules of the S19 method and the same negative-ion LC-MS/MS detection system were employed, monitoring the same LC-MS/MS transitions). An LOQ of 0.005 mg/kg for each isomer was established, corresponding to 0.01 mg/kg for isopyrazam. Therefore, DFG method S19 may reliably be used for post-registration control and monitoring of isopyrazam residues in crop commodities. Either GC-MSD or LC-MS/MS may be used for final quantification of residues, but only the negative-ion LC-MS/MS procedure has received full validation and ILV.

Animal commodities

Residue analytical method GRM006.09A

GRM006.09 is a data-generation method for isopyrazam (as *anti*-isomer and *syn*-isomer) in commodities of animal origin.

Isopyrazam is determined as separate *anti*-isomer and *syn*-isomer. Samples are extracted by homogenisation with acetonitrile:water (80:20 v/v). Extracts are centrifuged and aliquots (equivalent to 0.02 g sample) are diluted with water. Final determination is by LC-MS/MS with two MS/MS transitions (Crook S., 2008b).

The limit of quantification of the method is 0.005 mg/kg for *anti*-isomer and *syn*-isomer in animal matrices.

Analytical method GRM006.09A has been validated for the determination of residues of isopyrazam analysed as the isomers *anti*-isomer and *syn*-isomer in animal matrices (Ferguson L., 2008a). Control samples were analysed in duplicate. Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) for each isomer and in quintuplet at ten times the LOQ (0.05 mg/kg). Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions on all matrices tested. No significant matrix effects were observed and non-matrix standards were used for quantification. The recoveries obtained are detailed in Tables 72 to Table 75.

Table 72 Recovery results obtained during validation of Method GRM006.09A for *anti*-isomer in Animal Matrices. Primary transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)*	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Eggs	0.005	85.4, 88.8, 89.9, 89.9, 93.7	5	90	3.3	85.4–93.7
	0.05	89.0, 84.2, 85.4, 85.6, 88.6	5	87	2.4	84.2–89.0
	Overall	-	10	88	3.3	84.2–93.7
Milk	0.005	89.4, 82.2, 91.7, 84.6, 90.2	5	88	4.6	82.2–91.7
	0.05	97.9, 89.6, 91.3, 91.0, 91.9	5	92	3.5	89.6–97.9
	Overall	-	10	90	4.7	82.2–97.9
Muscle	0.005	93.2, 107, 106, 92.1, 102	5	100	6.9	92.1–107
	0.05	103, 96.8, 92.8, 97.0, 93.1	5	97	4.3	92.8–103
	Overall	-	10	98	5.7	92.1–107
Liver	0.005	96.5, 103, 99.2, 92.6, 103	5	99	4.5	92.6–103
	0.05	89.5, 79.0, 82.4, 79.8, 84.5	5	83	5.1	79.0–89.5
	Overall	-	10	91	10.2	79.0–103
Kidney	0.005	92.5, 107, 100, 91.8, 95.9	5	98	6.5	91.8–107
	0.05	105, 85.8, 93.4, 94.0, 91.7	5	94	7.2	85.8–105
	Overall	-	10	96	6.7	85.8–107
Fat	0.005	91.2, 92.6, 97.8, 90.9, 95.7	5	94	3.2	90.9–97.8
	0.05	87.2, 92.7, 88.1, 87.2, 94.6	5	90	3.8	87.2–94.6
	Overall	-	10	92	3.9	87.2–97.8

*–Limit of quantification, defined by the lowest validated fortification level (0.005 mg/kg in all matrices).
Residues in control samples were less than 30% of the LOQ.

Table 73 Recovery results obtained during validation of Method GRM006.09A for *anti*-isomer in animal matrices. Confirmatory transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)*	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Eggs	0.005	81.2, 91.0, 93.7, 92.6, 93.6	5	90	5.8	81.2–93.7
	0.05	87.1, 84.3, 84.6, 83.8, 87.7	5	86	2.1	83.8–87.7
	Overall	-	10	88	5.1	81.2–93.7
Milk	0.005	90.7, 76.8, 83.7, 85.5, 83.9	5	84	5.9	76.8–90.7
	0.05	98.5, 89.9, 86.2, 89.5, 95.2	5	92	5.4	86.2–98.5
	Overall	-	10	88	7.0	76.8–98.5
Muscle	0.005	88.8, 101, 109, 92.1, 96.5	5	97	8.0	88.8–109
	0.05	103, 96.7, 94.2, 95.6, 95.5	5	97	3.8	94.2–103
	Overall	-	10	97	5.9	88.8–109
Liver	0.005	89.7, 108, 94.6, 86.5, 99.1	5	96	9.0	86.5–108
	0.05	89.3, 80.4, 82.2, 80.7, 87.2	5	84	4.8	80.4–89.3
	Overall	-	10	90	9.8	80.4–108

Matrix	Fortification Level (mg/kg)*	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Kidney	0.005	95.4, 108, 99.7, 103, 92.0	5	100	6.3	92.0–108
	0.05	102, 85.6, 91.6, 91.1, 92.5	5	93	6.5	85.6–102
	Overall	-	10	96	7.2	85.6–108
Fat	0.005	95.7, 99.5, 101, 91.4, 98.3	5	97	3.9	91.4–101
	0.05	88.1, 93.4, 87.9, 87.0, 92.7	5	90	3.3	87.0–93.4
	Overall	-	10	94	5.4	87.0–101

*–Limit of quantification, defined by the lowest validated fortification level (0.005 mg/kg in all matrices).
Residues in control samples were less than 30% of the LOQ.

Table 74 Recovery results obtained during validation of Method GRM006.09A for *syn*-isomer in Animal Matrices. Primary transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)*	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Eggs	0.005	93.9, 91.6, 87.0, 94.6, 85.7	5	91	4.4	85.7–94.6
	0.05	86.2, 83.7, 83.3, 89.4, 84.9	5	86	2.9	83.3–89.4
	Overall	-	10	88	4.7	83.3–94.6
Milk	0.005	76.3, 79.7, 73.1, 76.8, 72.5	5	76	3.9	72.5–79.7
	0.05	85.9, 90.4, 85.0, 87.6, 81.9	5	86	3.7	81.9–90.4
	Overall	-	10	81	7.7	72.5–90.4
Muscle	0.005	95.6, 101, 96.6, 89.2, 100	5	97	4.9	89.2–101
	0.05	97.3, 92.0, 89.4, 95.5, 91.5	5	93	3.4	89.4–97.3
	Overall	-	10	95	4.5	89.2–101
Liver	0.005	82.5, 97.6, 85.4, 75.2, 100	5	88	11.9	75.2–100
	0.05	76.0, 77.4, 76.9, 75.8, 76.3	5	77	0.9	75.8–77.4
	Overall	-	10	82.	11.3	75.2–100
Kidney	0.005	95.5, 108, 111, 100, 107	5	104	6.1	95.5–111Ŧ
	0.05	89.8, 88.0, 90.2, 84.7, 87.9	5	88	2.5	84.7–90.2
	Overall	-	10	96	10.0	84.7–111Ŧ
Fat	0.005	90.1, 94.4, 92.2, 86.2, 83.2	5	89	5.1	83.2–94.4
	0.05	83.4, 84.3, 82.5, 80.6, 84.5	5	83	1.9	80.6–84.5
	Overall	-	10	86	5.3	80.6–94.4

*–Limit of quantification, defined by the lowest validated fortification level (0.005 mg/kg in all matrices).
Residues in control samples were less than 30% of the LOQ.

Table 75 Recovery results obtained during validation of Method GRM006.09A for *syn*-isomer in Animal Matrices. Confirmatory transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)*	Recovery (%)	n	Mean (%)	CV (%)	Range (%)
Eggs	0.005	92.5, 88.1, 93.0, 94.9, 92.3	5	92	2.7	88.1–94.9
	0.05	85.1, 85.9, 79.9, 88.1, 85.1	5	85	3.6	79.9–88.1
	Overall	-	10	89	5.3	79.9–94.9
Milk	0.005	72.3, 62.4, 81.2, 77.2, 76.2	5	74	9.7	62.4–81.2Ŧ
	0.05	92.3, 83.4, 85.0, 86.7, 81.6	5	86	4.8	81.6–92.3
	Overall	-	10	80	10.5	62.4–92.3Ŧ
Muscle	0.005	93.4, 98.7, 90.3, 89.8, 94.8	5	93	3.9	89.8–98.7
	0.05	96.4, 92.4, 88.0, 96.3, 89.2	5	93	4.2	88.0–96.4
	Overall	-	10	93	3.9	88.0–98.7
Liver	0.005	90.7, 105, 80.4, 78.6, 98.8	5	91	12.7	78.6–105
	0.05	75.9, 79.4, 76.3, 76.2, 77.8	5	77	1.9	75.9–79.4
	Overall	-	10	84	12.6	75.9–105
Kidney	0.005	97.1, 110, 110, 101, 105	5	104	5.3	97.1–110
	0.05	90.0, 89.8, 89.5, 84.5, 86.4	5	88	2.8	84.5–90.0
	Overall	-	10	96	9.9	84.5–110
Fat	0.005	86.9, 84.3, 89.3, 82.5, 83.4	5	85	3.3	82.5–89.3
	0.05	83.7, 87.1, 85.9, 83.4, 84.9	5	85	1.8	83.4–87.1
	Overall	-	10	85	2.5	82.5–89.3

*–Limit of quantification, defined by the lowest validated fortification level (0.005 mg/kg in all matrices). Residues in control samples were less than 30% of the LOQ.

The linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range from 0.0025 ng to 0.1 ng injected on column (equivalent to 0.05 ng/mL to 2.0 ng/mL standards when using an 50 µL injection volume) and was found to be linear. This is equivalent to a range of 50% LOQ to 20 × LOQ. The correlation coefficients (R^2) of the calibration curves were > 0.999.

The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each crop tested during method validation were < 20%.

Isopyrazam was shown to be efficiently extracted from animal matrices under the conditions used in method GRM006.09A (*Melville S, 2008*).

Table 76 Comparison of Radioactive Residue Extractability using Residue Analytical Method and Exhaustive Extraction

Commodity	Study	Radioactive Residues Extracted	
		%TRR	Residue (mg/kg)
Liver	Metabolism [a]	88.5	0.542
	Residue [b]	78.1	0.479
Milk	Metabolism [a]	98.1	0.075
	Residue [b]	96.3	0.073

[a] Data generated from goat metabolism Study (Charles River Ref No. 212271)

[b] Mean recovery from two extractability experiments

Method GRM006.09A has been demonstrated to be a reliable and accurate procedure for the determination of isopyrazam as *anti*-isomer and *syn*-isomer to a limit of quantification of 0.005 mg/kg each in animal matrices, using commercially available laboratory equipment and reagents. Bovine milk, muscle, kidney, liver, fat and hen eggs have been used as example matrices.

Residue analytical method GRM006.10A

GRM006.10 is the method used for determination of isopyrazam residues in commodities of animal origin, as the common moiety CSAA798670. The method captures residues of parent isopyrazam and any metabolites hydrolysable to CSAA798670. To allow for the possible use of this method for residue monitoring (Crook S., 2008c), an independent laboratory validation study has been performed as well as the initial validation study.

Samples are extracted by homogenisation with acetonitrile:water (80:20 v/v). Extracts are centrifuged and aliquots (equivalent to 0.4 g sample) are evaporated to dryness. Potassium hydroxide solution (12M) is added and the samples heated (100 ºC, 3 hours) to hydrolyse isopyrazam and structurally related metabolites to CSAA798670. After hydrolysis, samples are cooled and diluted. Concentrated hydrochloric acid is then added to acidify the extracts. Samples are then taken through a solid phase extraction (SPE) clean-up procedure, using Waters Oasis HLB cartridges. The SPE cartridges are washed with water and CSAA798670 is eluted with 50:50 v/v acetonitrile:water. The column eluates are evaporated to remove the acetonitrile and then diluted with water. Final determination is by high performance liquid chromatography with triple-quadrupole mass-spectrometric detection (LC-MS/MS) with two MS/MS transitions.

The limit of quantification for CSAA798670 residues in animal matrices using method GRM006.10A was established at 0.01 mg/kg (expressed as isopyrazam equivalents) or 0.005 mg/kg CSAA798670 in both the validation and independent validation.

Analytical method GRM006.10A has been validated for the determination of residues of isopyrazam and structurally-related metabolites analysed as CSAA798670 in animal matrices (Freguson L., 2008b). Control samples were analysed in duplicate. Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) and in quintuplet at ten times the LOQ

(0.05 mg/kg). Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions on all matrices tested, except in milk, muscle liver and fat at the LOQ where the mean recovery was marginally higher than 110% and milk, liver and fat at the LOQ for the confirmatory transition where data was qualitative only. This was considered acceptable because the methodology is necessarily complex. Variable matrix effects were observed and matrix-matched standards were used for quantification in all cases. The recoveries obtained are detailed in Table s 77 and Table 78.

Table 77 Recovery Results Obtained During Validation of Method GRM006.10A for CSAA798670 in Animal Matrices. Primary transition *m/z* 175 → 91

Matrix	Fortification Level* (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Eggs	0.005**	92.7, 87.7, 70.3, 98.6, 74.5	5	85	14.2	70.3–98.6
	0.15	85.2, 40.8†, 71.8, 82.9, 84.1	4	81†	7.7†	71.8–85.2
	Overall	-	9	83†	11.5†	70.3–98.6
Milk	0.005**	117, 109, 109, 111, 124	5	114	5.8	109–124
	0.15	106, 109, 107, 105, 105	5	106	1.5	105–109
	Overall	-	10	110	5.4	105–124
Muscle Tissue	0.005**	115, 117, 119, 118, 116	5	117	1.5	115–119
	0.10	101, 102, 98.8, 104, 105	5	102	2.4	98.8–105
	Overall	-	10	110	7.3	98.8–119
Liver	0.005**	110, 108, 120, 121, 128	5	117	7.0	108–128
	1.25	97.2, 97.7, 101, 106, 113	5	103	6.6	97.2–113
	Overall	-	10	110	9.4	97.2–128
Kidney	0.005**	108, 107, 94.8, 97.8, 106	5	103	5.7	94.8–108
	0.50	66.0, 105, 102, 115, 108	5	99	19.2	66.0–115
	Overall	-	10	101	13.3	66.0–115
Fat	0.005**	120, 118, 119, 123, 122	5	120	1.9	118–123
	0.10	98.4, 95.5, 96.0, 97.7, 95.4	5	97	1.4	95.4–98.4
	Overall	-	10	108	11.6	95.4–123

*–Expressed as CSAA798670
**–Limit of quantification, defined by the lowest validated fortification level
†–Atypical value omitted from calculations.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 78 Recovery Results Obtained During Validation of Method GRM006.10A for CSAA798670 in Animal Matrices. Confirmatory transition *m/z* 175 → 111

Matrix	Fortification Level* (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Eggs	0.005**	101, 94.5, 94.8, 106, 86.6	5	97	7.7	86.6–101
	0.15	83.4, 40.7†, 72.6, 83.9, 82.8	4	81†	6.7†	72.6–83.9
	Overall	-	9	90†	11.7†	72.6–101
Milk	0.005**	LOQ not quantifiable for confirmatory transition				
	0.15	101, 101, 102, 103, 97.6	5	101	2.1	97.6–103
	Overall	-	5	101	2.1	97.6–103
Muscle Tissue	0.005**	91.0, 106, 95.0, 111, 98.3	5	100	8.1	91.0–111
	0.10	92.4, 93.7, 93.9, 99.1, 96.9	5	95	2.9	92.4–99.1
	Overall	-	10	98	6.4	91.0–111
Liver	0.005**	LOQ not quantifiable for confirmatory transition				
	1.25	100, 101, 105, 111, 118	5	107	7.0	100–118
	Overall	-	5	107	7.0	100–118
Kidney	0.005**	93.0, 87.6, 92.9, 83.4, 97.7	5	91	6.1	83.4–97.7
	0.50	64.7, 102, 99.6, 114, 106	5	97	19.5	64.7–114
	Overall	-	10	94	14.4	64.7–114
Fat	0.005**	LOQ not quantifiable for confirmatory transition				
	0.10	94.7, 93.0, 90.7, 97.4, 91.0	5	93	3.0	90.7–97.4
	Overall	-	5	93	3.0	90.7–97.4

*–Expressed as CSAA798670
**–Limit of quantification, defined by the lowest validated fortification level

†–Atypical value omitted from calculations
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

An independent laboratory validation of method GRM006.10A was performed, in which milk and liver matrices were fortified at the proposed limit of quantification (0.01 mg/kg expressed as isopyrazam equivalents or 0.005 mg/kg as CSAA798670) and at a higher level (Lakaschus S., 2008). The recoveries obtained are detailed in Tables 79 and 80.

Table 79 Recovery Results Obtained During Independent Validation of Method GRM006.10A for CSAA798670 in Animal Matrices. Primary transition *m/z* 175 → 91

Matrix	Fortification Level* (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.005**	102, 98, 91, 94, 98	5	97	4	91–102
	0.15	96, 96, 94, 100, 93	5	96	3	93–100
	Overall	-	10	96	3	91–102
Liver	0.005**	98, 100, 100, 98, 104	5	100	2	98–104
	1.2	88, 88, 89, 91, 99	5	91	5	88–99
	Overall	-	10	96	6	88–104

*–Expressed as CSAA798670
**–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 80 Recovery Results Obtained During Independent Validation of Method GRM006.10A for CSAA798670 in Animal Matrices. Confirmatory transition *m/z* 175 → 111

Matrix	Fortification Level* (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.005**	LOQ not quantifiable for confirmatory transition				
	0.15	76, 80, 79, 90, 78	5	81	7	76–90
	Overall	-	5	81	7	76–90
Liver	0.005**	102, 100, 96, 106, 128	5	106	12	96–128
	1.2	88, 87, 89, 90, 98	5	90	5	87–98
	Overall	-	10	98	12	87–128

*–Expressed as CSAA798670
**–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Some low level interference was observed in milk, liver and fat at the LOQ for the confirmatory transition where data was qualitative only. Liver was quantifiable at the LOQ in the ILV, however, low level interference was observed for milk.

The linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested in the range from 7.5 pg to 6375 pg injected on column (equivalent to 0.10 ng/mL to 85.0 ng/mL standards when using a 75 µL injection volume) and was found to be linear. The correlation coefficients (R^2) of the calibration curves were > 0.999. The detector response in the ILV was shown to be linear over the range from 8 pg to 1000 pg (equivalent to 0.4 ng/mL to 50 ng/mL) and from 20 pg to 1000 pg (equivalent to 1.0 ng/mL to 50 ng/mL) for diluted liver samples) when using a 20 µL injection volume). This was at least ± 20% of the concentration of the samples analysed. The correlation coefficients (R^2) of the calibration curves were > 0.999.

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) for each isomer and in quintuplet at ten times the LOQ (0.05 mg/kg). In the initial method validation, acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested, except in milk, muscle liver and fat at the LOQ for the primary transition where the mean recovery was marginally higher than 110% and milk, liver and fat at the LOQ for the confirmatory transition where data was qualitative only. During independent validation, all recovery data was within the acceptable range of 70% to 110%, except in milk at the LOQ for the confirmatory transition where data was qualitative only.

The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each crop tested during method validation and during independent validation were < 20%.

Isopyrazam and structurally related metabolites have been shown to be efficiently extracted from animal matrices under the conditions used in method Draft GRM006.10A (*Melville S, 2008, Report No. 29401*). For further details of the extraction of isopyrazam and structurally related metabolites from radio-labelled metabolism samples please refer to Section 2.1.3.

An independent laboratory validation of method GRM006.10A was conducted and demonstrated acceptable reproducibility as required in EU guidance (*SANC0 825/00 rev. 7*).

Method GRM006.10A has been demonstrated to be a reliable and accurate procedure for the determination of isopyrazam and structurally related metabolites as the moiety CSAA798670 in animal matrices, using commercially available laboratory equipment and reagents. The limit of quantification is 0.005 mg/kg CSAA798670, or 0.01 mg/kg isopyrazam equivalents. Bovine milk, muscle, kidney, liver, fat and hen eggs have been used as example matrices.

Multi-Residue analytical method DFG-S19

The DFG method S19 is a multi-residue method investigated for the monitoring of isopyrazam in animal commodities.

DFG method S19 is a multi-residue method suitable for the determination of residues of isopyrazam (analysed as the isomers *anti*-isomer and *syn*-isomer) in foodstuffs of animal origin. The extraction of isopyrazam residues from egg,bovine muscle, liver, and kidney is performed according to extraction Module E1. The extraction of isopyrazam residues from fat is performed according to Module E6 and the extraction of isopyrazam residues from milk is performed according to extraction Module E8. Extracts of all matrices except milk are cleaned-up using gel-permeation chromatography (GPC). Residues of isopyrazam are quantified using high performance liquid chromatography with triple-quadrupole mass-spectrometric detection (LC-MS/MS) monitoring two transitions.

The limit of quantification for *anti*-isomer and *syn*-isomer residues in animal commodities using method S19 was established at 0.0025 mg/kg for each isomer, corresponding to 0.005 mg/kg for isopyrazam.

During validation of method S19, six matrices were fortified with isopyrazam (as the isomers *anti*-isomer and *syn*-isomer) (Bacher R., 2008). Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.0025 mg/kg for each isomer) and at a higher level (0.025 mg/kg). Control samples were analysed in duplicate. The matrices are representative of the six types specified in the EU guidance (*SANCO 825/00 rev.7, 17/03/2004*). Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions monitored for both isomers. Significant matrix effects were observed and matrix-matched calibration standards were used for quantification. The recoveries obtained are detailed in Table 81 toTable 84.

Table 81 Recovery Results Obtained During Validation of Method S19 for *anti*-isomer in Foodstuffs of Animal Origin. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	85, 71, 80, 90, 71	5	79	11	71–90
	0.025	76, 82, 76, 79, 81	5	79	4	76–82
	Overall		10	79	8	71–90
Egg	0.0025*	95, 89, 98, 91, 102	5	95	5	89–102
	0.025	88, 93, 91, 98, 102	5	95	6	88–102
	Overall		10	95	5	88–102
Bovine Muscle	0.0025*	103, 105, 102, 100, 109	5	104	4	100–109
	0.025	99, 106, 106, 106, 112	5	106	4	99–112
	Overall		10	105	4	99–112
Bovine Liver	0.0025*	75, 85, 70, 78, 80	5	78	7	70–85
	0.025	76, 84, 83, 87, 90	5	84	6	83–90

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	Overall		10	81	8	70–90
Bovine Kidney	0.0025*	93, 91, 92, 102, 104	5	96	7	91–104
	0.025	97, 102, 103, 108, 109	5	104	5	97–109
	Overall		10	100	7	91–109
Bovine Fat	0.0025*	106, 104, 100, 110, 106	5	105	4	104–110
	0.025	107, 94, 69, 84, 88	5	88	16	69–107
	Overall		10	97	13	69–110

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 82 Recovery Results Obtained During Validation of Method S19 for *anti*-isomer in Foodstuffs of Animal Origin. Confirmatory transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	85, 70, 73, 88, 75	5	78	10	70–88
	0.025	75, 75, 71, 75, 81	5	75	5	71–81
	Overall		10	77	8	70–88
Egg	0.0025*	95, 86, 101, 92, 102	5	95	7	86–102
	0.025	88, 96, 91, 101, 106	5	96	8	88–106
	Overall		10	96	7	86–106
Bovine Muscle	0.0025*	103, 109, 98, 111, 109	5	106	5	98–109
	0.025	99, 106, 103, 106, 103	5	103	3	99–106
	Overall		10	105	4	98–109
Bovine Liver	0.0025*	76, 88, 69, 79, 86	5	80	9	69–88
	0.025	80, 89, 83, 92, 89	5	87	6	80–92
	Overall		10	83	9	69–92
Bovine Kidney	0.0025*	100, 100, 99, 107, 105	5	102	4	99–105
	0.025	96, 99, 104, 105, 103	5	101	4	96–105
	Overall		10	102	3	96–105
Bovine Fat	0.0025*	105, 108, 105, 109, 99	5	105	4	99–109
	0.025	106, 96, 69, 91, 93	5	91	15	69–106
	Overall		10	98	12	69–109

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 83 Recovery Results Obtained During Validation of Method S19 for *syn*-isomer in Foodstuffs of Animal Origin. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	74, 67, 78, 90, 73	5	77	11	67–90
	0.025	74, 78, 70, 79, 80	5	76	5	70–80
	Overall		10	76	8	67–90
Egg	0.0025*	90, 104, 89, 91, 91	5	93	7	89–104
	0.025	86, 93, 92, 96, 105	5	94	7	86–105
	Overall		10	94	7	86–105
Bovine Muscle	0.0025*	100, 109, 96, 108, 107	5	104	6	96–109
	0.025	97, 103, 102, 104, 107	5	102	4	97–107
	Overall		10	103	4	96 -109
Bovine Liver	0.0025*	83, 98, 83, 90, 92	5	89	7	83–98
	0.025	84, 93, 91, 100, 94	5	93	6	84–100
	Overall		10	91	7	83–100
Bovine Kidney	0.0025*	106, 103, 96, 108, 106	5	104	4	96–108
	0.025	97, 96, 99, 103, 99	5	99	3	96–103
	Overall		10	101	4	96–108
Bovine Fat	0.0025*	98, 96, 94, 94, 93	5	95	2	93–98
	0.025	103, 98, 73, 95, 94	5	93	12	73–103
	Overall		10	94	8	73–103

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 84 Recovery Results Obtained During Validation of Method S19 for *syn*-isomer in Foodstuffs of Animal Origin. Confirmatory transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	84, 70, 80, 89, 71	5	79	10	70–89
	0.025	76, 76, 76, 76, 81	5	77	3	76–81
	Overall		10	78	7	70–89
Egg	0.0025*	89, 92, 91, 87, 98	5	91	4	87–98
	0.025	87, 90, 90, 95, 101	5	93	6	87–101
	Overall		10	92	5	87–101
Bovine Muscle	0.0025*	101, 107, 101, 110, 106	5	105	4	101–110
	0.025	98, 104, 104, 105, 105	5	103	3	98–105
	Overall		10	104	3	98–110
Bovine Liver	0.0025*	80, 92, 76, 89, 90	5	85	8	80–92
	0.025	85, 95, 88, 104, 98	5	94	8	85–104
	Overall		10	90	9	80–104
Bovine Kidney	0.0025*	102, 107, 98, 109, 106	5	104	4	98–107
	0.025	96, 97, 100, 104, 104	5	100	4	96–104
	Overall		10	102	4	96 -107
Bovine Fat	0.0025*	105, 106, 101, 105, 95	5	102	4	95–106
	0.025	106, 100, 73, 93, 100	5	94	14	73–106
	Overall		10	98	10	73–106

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

During independent laboratory validation of method S19, bovine liver and milk were fortified with isopyrazam (as the isomers *anti*-isomer and *syn*-isomer)(Lakaschus S., Gitzler A.S., 2008b). Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.0025 mg/kg for each isomer) and in quintuplet at a higher level (0.025 mg/kg). Control samples were analysed in duplicate. Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of < 20% were found for both transitions monitored for both isomers. The recoveries obtained are detailed in Table 85 to Table 88.

Table 85 Recovery Results Obtained During Independent Validation of Method S19 for *anti*-isomer in Foodstuffs of Animal Origin. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	102, 103, 107, 106, 94	5	102	5	94–107
	0.025	93, 82, 81, 84, 88	5	86	6	81–93
	Overall		10	94	11	81–107
Bovine Liver	0.0025*	92, 86, 89, 62, 69	5	80	17	62–92
	0.025	74, 74, 79, 85, 90	5	80	9	74–90
	Overall		10	80	13	62–92

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 86 Recovery Results Obtained During Independent Validation of Method S19 for *anti*-isomer in Foodstuffs of Animal Origin. Confirmatory transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	104, 104, 107, 103, 96	5	103	4	96–107
	0.025	97, 81, 80, 85, 89	5	86	8	80–97
	Overall		10	95	11	80–107

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Bovine Liver	0.0025*	85, 85, 90, 62, 71	5	79	15	62–90
	0.025	70, 71, 78, 82, 88	5	78	10	70–88
	Overall		10	78	12	62–90

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 87 Recovery Results Obtained During Independent Validation of Method S19 for *syn*-isomer in Foodstuffs of Animal Origin. Primary transition *m/z* 360 → 320

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	90, 89, 96, 90, 96	5	92	4	89–96
	0.025	88, 76, 76, 79, 80	5	80	6	76–88
	Overall		10	86	9	76–96
Bovine Liver	0.0025*	88, 84, 85, 60, 66	5	77	17	60–88
	0.025	71, 78, 81, 85, 88	5	81	8	71–88
	Overall		10	79	12	60–88

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

Table 88 Recovery Results Obtained During Independent Validation of Method S19 for *syn*-isomer in Foodstuffs of Animal Origin. Confirmatory transition *m/z* 360 → 244

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Milk	0.0025*	94, 92, 90, 94, 90	5	92	2	90–94
	0.025	86, 75, 77, 79, 80	5	79	4	75–86
	Overall		10	86	9	75–94
Bovine Liver	0.0025*	80, 85, 83, 65, 68	5	76	12	65–85
	0.025	72, 79, 81, 83, 90	5	81	8	72–90
	Overall		10	79	10	65–90

*–Limit of quantification, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.

During validation of the DFG S19 method, the linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range 0.025 ng/mL to 2.5 ng/mL (equivalent to 0.00125 ng to 0.125 ng for a 50 µL injection volume) for milk and fat matrices and over the range 0.05 ng/mL to 5.0 ng/mL (equivalent to 0.0025 ng to 0.25 ng for a 50 µL injection volume) for all other matrices. The correlation coefficients (R^2) of the calibration curves were > 0.998.

During independent laboratory validation, the linearity of the LC-MS/MS detector response for *anti*-isomer and *syn*-isomer was tested over the range 0.25 ng/mL to 25 ng/mL (equivalent to 0.00375 ng to 0.375 ng for a 15 µL injection volume). The lower level of the linearity test was lower by at least 50 % of the LOQ. The upper level was higher by at least 20 % of the highest procedural recovery for milk and liver matrices. The correlation coefficients (R^2) of the calibration curves were > 0.996.

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.0025 mg/kg for each isomer) and in quintuplet at a higher fortification level (0.025 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions for both isomers on all matrices tested.

The relative standard deviations (RSDs) of recoveries of each isomer at each fortification level and overall for each matrix tested during method validation using LC-MS/MS final determination were < 20%.

DFG Method S19 was successfully validated and independently validated for the analysis of isopyrazam residues (analysed as the isomers *anti*-isomer and *syn*-isomer) in foodstuffs of animal origin and an LOQ of 0.0025 mg/kg for each isomer was established, corresponding to 0.005 mg/kg for isopyrazam. Therefore, DFG method S19 may reliably be used for post registration control and monitoring of isopyrazam residues in foodstuffs of animal origin.

Stability of Pesticide Residues in Stored Analytical Samples

The Meeting received frozen storage stability of residues of isopyrazam and its metabolites in crops and animal products.

Plant commodities

Storage stability of all these compounds in crop commodities stored frozen was examined in four separate storage stability studies. In all cases, residues were judged to be stable so long as losses during storage were not significant (more than 70% remaining).

A study to demonstrate the stability of residues of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) in plant commodities was conducted by Marshall, L. (2010) between 2006 and 2008. Stability was tested in raw agricultural commodities: tomato fruit, rape seeds, lentil seeds, potato tubers, barley grain, barley straw, ryegrass forage and spinach leaves. .

Crop samples (5–10 g) were fortified with known amounts of *anti*-isomer and *syn*-isomer in acetonitrile at a rate of 0.5 mg/kg. The solvent was allowed to evaporate and the samples sealed and stored in a freezer at a nominal temperature of ≤-18 °C. The initial concentrations were determined by analysis of five freshly-prepared fortified samples. At subsequent storage intervals of approximately 1, 3, 6, 12 and 24 months, a sample set of each substrate, consisting of a control sample, two freshly fortified samples and three freezer-stored fortified samples, were analysed for residues of *anti*-isomer and *syn*-isomer using either method GRM006.01A (for sample intervals up to 6 months) or method GRM006.01B (for 12 and 24-month samples).

There were no significant changes in the concentrations of *anti*-isomer or *syn*-isomer in potato, tomato, lentils, barley grain, barley straw, ryegrass forage and rape seed during the 24 months of storage (i.e., losses were less than 30%). No data are available for spinach leaves after 6 months because the stored samples had not been fortified, in error, at the start of the study. Residues in spinach leaves were stable at the 6-month interval, the last occasion for which data could be obtained.The residues found in the crop commodities tested at each interval are detailed in Tables 89 and 90.

Table 89 Stability of *anti*-isomer Residues in Crop Commodities Following Storage at ≤-18 °C

Commodity	Storage Period (months)	*anti*-isomer Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Tomato–Fruit	0	0.49	100	105
	3	0.48	98	103
	6	0.46	94	98
	12	0.52	106	111
	24	0.50	102	99
Oilseed rape –Seed	0	0.50	100	96
	3	0.38	76	88
	6	0.45	90	92
	12	0.43	86	86
	24	0.48	96	93
Lentil–Seed	0	0.46	100	102
	3	0.49	107	101
	6	0.50	109	100
	12	0.48	104	107
	24	0.53	115	96

Commodity	Storage Period (months)	*anti*-isomer Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Potato–Tuber	0	0.47	100	98
	3	0.47	100	96
	6	0.44	94	90
	12	0.53	113	114
	24	0.48	102	101
Barley–Grain	0	0.49	100	99
	1	0.45	92	89
	3	0.50	102	106
	6	0.48	98	101
	12	0.50	102	114
	24	0.54	110	106
Barley–Straw	0	0.47	100	93
	1	0.38	81	74
	3	0.41	87	82
	6	0.44	94	91
	12	0.45	96	83
	24	0.49	104	95
Ryegrass–Forage	0	0.47	100	96
	1	0.45	96	95
	3	0.50	106	104
	6	0.46	98	93
	12	0.52	111	108
	24	0.49	104	101
Spinach–Leaves	0	0.50	100	93
	3	0.49	98	101
	6	0.51	102	95

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Table 90 Stability of *syn*-isomer Residues in Crop Commodities Following Storage at ≤-18 °C

Commodity	Storage Period (months)	*syn*-isomer Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Tomato–Fruit	0	0.49	100	104
	3	0.48	98	104
	6	0.52	106	103
	12	0.53	108	111
	24	0.49	100	102
Oilseed rape –Seed	0	0.49	100	99
	3	0.39	80	90
	6	0.43	88	89
	12	0.38	78	74
	24	0.48	98	92
Lentil–Seed	0	0.46	100	101
	3	0.50	109	101
	6	0.45	98	92
	12	0.49	107	105
	24	0.50	109	84
Potato–Tuber	0	0.50	100	104
	3	0.49	98	99
	6	0.50	100	97
	12	0.55	110	114
	24	0.46	92	102
Barley–Grain	0	0.48	100	100
	1	0.47	98	91
	3	0.49	102	103
	6	0.47	98	96
	12	0.46	96	106

Commodity	Storage Period (months)	*syn*-isomer Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
	24	0.54	113	105
Barley–Straw	0	0.49	100	101
	1	0.40	82	76
	3	0.43	88	87
	6	0.43	88	82
	12	0.45	92	81
	24	0.51	104	86
Ryegrass–Forage	0	0.47	100	93
	1	0.48	102	99
	3	0.50	106	100
	6	0.44	94	92
	12	0.50	106	108
	24	0.46	98	94
Spinach–Leaves	0	0.51	100	90
	3	0.49	96	102
	6	0.48	94	100

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Residues of *anti*-isomer and *syn*-isomer were shown to be stable in potato, tomato, lentils, barley grain, barley straw, ryegrass forage and rape seed when stored at ≤-18 °C for 24 months, and for at least 6 months in spinach. Residues of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) are, therefore, expected to be stable for at least 24 months in all crop commodities stored under these conditions.

Another study was conducted by Heillaut, C. (2008a) between 2007 and 2008 to demonstrate the stability of residues of CSCD459488 in crop commodities. The intended duration of the study is two years. The stability was tested in raw agricultural commodities wheat grain and straw, oilseed rape seed, apple fruit, lentils seed, orange fruit, spinach leaves and carrot root.

Crop samples (10 g) were fortified with a known amount of CSCD459488 in acetonitrile at a rate of 0.5 mg/kg. The solvent was allowed to evaporate and the samples sealed and stored in a freezer at a nominal temperature of -20 ± 5 °C. The initial concentration was determined by analysis of three freshly-prepared fortified samples. At subsequent storage intervals of approximately 3, 6 and 11 months a sample set of each substrate, consisting of a control sample, two freshly fortified samples and two freezer-stored fortified samples, were analysed for residues of CSCD459488 using method GRM006.03A.

The residues found in the crop commodities tested at each interval are detailed in Table 91. There was no significant change in the residue level of CSCD459488 in any commodity during the 11 months of storage period. Therefore, residues of CSCD459488 are expected to be stable in all crop commodities when stored at -20 ± 5 °C for at least 11 months.

Table 91 Stability of CSCD459488 Residues in Crop Commodities Following Storage at -20 ± 5 °C

Commodity	Storage Period (months)	CSCD459488 Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Wheat–Grain	0	0.40	100	81
	3	0.52	130	103
	6	0.37	93	80
	11	0.40	100	98
Wheat–Straw	0	0.44	100	88
	3	0.47	107	84
	6	0.43	98	83
	11	0.41	93	87

Commodity	Storage Period (months)	CSCD459488 Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Oilseed rape –Seed	0	0.37	100	77
	3	0.47	127	87
	6	0.41	111	83
	11	0.47	127	90
Apple–Fruit	0	0.40	100	81
	3	0.53	133	100
	6	0.44	110	83
	11	0.49	123	101
Lentils–Seed	0	0.38	100	74
	3	0.43	113	93
	6	0.45	118	87
	11	0.43	113	87
Orange–Fruit	0	0.43	100	85
	3	0.46	107	98
	6	0.39	91	92
	11	0.40	93	86
Spinach–Leaves	0	0.40	100	78
	3	0.45	113	88
	6	0.43	108	78
	11	0.45	113	82
Carrot–Roots	0	0.40	100	81
	3	0.52	130	98
	6	0.46	115	89
	11	0.48	120	85

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Gemrot, F. (2010) also conducted a study to demonstrate the stability of residues of CSCD459489 in crop commodities between 2007 and 2010. The intended duration of the study was two years but extended to 28 months. The stability was tested in raw agricultural commodities wheat grain, barley straw, oilseed rape seed, apple fruit, lentils seed, orange fruit, spinach leaves and carrot root.

Crop samples (10 g) were fortified with a known amount of CSCD459489 in acetonitrile at a rate of 0.5 mg/kg. The solvent was allowed to evaporate and the samples sealed and stored in a freezer at a nominal temperature of -20 ± 5 °C.

The initial concentration was determined by analysis of three freshly-prepared fortified samples. At subsequent storage intervals of approximately 3, 6, 11, 23 and 28 months a sample set of each substrate, consisting of a control sample, two freshly-fortified samples and two freezer-stored fortified samples, were analysed for residues of CSCD459489 using method GRM006.03A.

The residues found in the crop commodities tested at each interval are detailed in Table 92. At the end of 28 month storage period, CSCD459489 concentrations were > 86% of the initial concentration. Therefore residues of CSCD459489 are expected to be stable in all crop commodities when stored at -20 ± 5 °C for at least 28 months.

Table 92 Stability of CSCD459489 Residues in Crop Commodities Following Storage at -20 ± 5 °C

Commodity	Storage Period (months)	CSCD459489 Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Wheat–Grain	0	0.43	100	85
	3	0.47	109	99
	6	0.43	100	88
	11	0.59	137	104
	23	0.44	102	83
	28	0.37	86	91
Barley–Straw	0	0.44	100	92
	3	0.41	93	86
	6	0.45	102	96
	11	0.45	102	100
	23	0.41	93	82
	28	0.46	105	93
Oilseed rape –Seed	0	0.45	100	87
	3	0.41	91	90
	6	0.41	91	83
	11	0.52	116	97
	23	0.45	100	93
	28	0.48	117	87
Apple–Fruit	0	0.41	100	78
	3	0.49	120	92
	6	0.44	107	94
	11	0.48	117	101
	23	0.51	124	91
	28	0.49	120	96
Lentils–Seed	0	0.43	100	82
	3	0.45	105	98
	6	0.47	109	89
	11	0.57	133	109
	23	0.41	95	85
	28	0.48	112	87
Orange–Fruit	0	0.45	100	90
	3	0.47	104	94
	6	0.36	80	79
	11	0.43	96	93
	23	0.51	113	89
	28	0.50	111	77
Spinach–Leaves	0	0.43	100	79
	3	0.47	109	103
	6	0.44	102	81
	11	0.46	107	92
	23	0.46	105	102
	28	0.46	107	91
Carrot–Roots	0	0.40	100	82
	3	0.48	120	97
	6	0.43	108	85
	11	0.47	118	80
	23	0.48	120	100
	28	0.49	123	100

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Lakaschus, S. and Gizler, A. (2009b) conducted a study to demonstrate the stability of residues of CSCD465008 and CSAA798670 in crop commodities in 2008. The duration of the study

was one year. The stability was tested in raw agricultural commodities: wheat grain, wheat straw, barley forage, spinach leaves, carrot leaves and carrot roots. These commodities are representative of the crop types (cereal, leafy and root crop) included in the field rotational crop studies, the only studies in which these two compounds were analysed.

Crop samples (5 g for straw, 10 g for the remaining crops) were fortified with known amounts of CSCD465008 and CSAA798670 in acetonitrile at a rate of 0.5 mg/kg. The solvent was allowed to evaporate and the samples sealed and stored in a freezer at a nominal temperature of ≤-18 °C. The initial concentrations were determined by analysis of five freshly-prepared fortified samples. At subsequent storage intervals of approximately 3, 6 and 12 months, a sample set of each substrate, consisting of a control sample, two freshly-fortified samples and two freezer-stored fortified samples, was analysed for residues of CSCD465008 and CSAA798670 using method GRM006.08A.

The residues found in the crop commodities tested at each interval are detailed in Tables 93 and 94. There was no significant change in the residue level of CSCD465008 and CSAA798670 in any commodity during the 12 months of storage period (i.e., any losses were less than 30%). Therefore residues of CSCD465008 and CSAA798670 are expected to be stable in all crop commodities when stored at ≤-18 °C for at least 12 months.

Table 93 Stability of CSCD465008 Residues in Crop Commodities Following Storage at ≤-18 °C

Commodity	Storage Period (months)	CSCD465008 Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Wheat–Grain	0	0.43	100	86
	3	0.42	102	83
	6	0.42	100	85
	12	0.42	104	81
Wheat–Straw	0	0.40	100	79
	3	0.49	119	82
	6	0.38	110	70
	12	0.37	94	79
Barley–Forage	0	0.42	100	84
	3	0.42	100	84
	6	0.41	102	81
	12	0.41	105	79
Spinach–Leaves	0	0.51	100	101
	3	0.40	85	95
	6	0.52	103	101
	12	0.54	102	106
Carrot–Leaves	0	0.46	100	91
	3	0.45	97	93
	6	0.47	98	96
	12	0.47	96	98
Carrot–Roots	0	0.49	100	99
	3	0.46	97	96
	6	0.52	101	104
	12	0.51	113	91

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Table 94 Stability of CSAA798670 Residues in Crop Commodities Following Storage at ≤-18 °C

Commodity	Storage Period (months)	CSAA798670 Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Wheat–Grain	0	0.47	100	94
	3	0.46	98	92
	6	0.46	98	94

Commodity	Storage Period (months)	CSAA798670 Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
	12	0.38	81	78
Wheat–Straw	0	0.40	100	79
	3	0.52	130	97
	6	0.43	108	84
	12	0.52	130	106
Barley–Forage	0	0.44	100	87
	3	0.48	109	85
	6	0.45	102	88
	12	0.47	107	93
Spinach–Leaves	0	0.47	100	95
	3	0.48	102	98
	6	0.51	109	99
	12	0.52	111	105
Carrot–Leaves	0	0.46	100	91
	3	0.49	107	95
	6	0.50	109	98
	12	0.51	111	104
Carrot–Roots	0	0.47	100	95
	3	0.49	104	99
	6	0.51	109	102
	12	0.51	109	98

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Animal products

A study to demonstrate the stability of residues of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) in milk, eggs and animal tissues was conducted by Heillaut, C. (2009a) between 2007 and 2008. The intended duration of the study is 14 months. The stability of isopyrazam was examined in milk, eggs, liver, muscle, fat and kidney.

Samples (10 g) of milk, eggs, liver, muscle, fat and kidney were fortified with known amounts of *anti*-isomer and SYN543969 in acetonitrile at a rate of 0.5 mg/kg. The solvent was allowed to evaporate and the samples sealed and stored in a freezer at a nominal temperature of -20 ± 5 °C. The initial concentration was determined by analysis of three freshly-prepared fortified samples. At subsequent storage intervals of approximately 3, 6 and 14 months a sample set of each substrate, consisting of a control sample, two freshly-fortified samples and two freezer-stored fortified samples, were analysed for residues of *anti*-isomer and *syn*-isomer using method GRM006.09A.

The residues found in the animal commodities tested at each interval are detailed in Tables 95 and 96. After six-months storage, recovered residues of *anti*-isomer and *syn*-isomer in eggs were only 54% and 59%, respectively, of the initial residue. Further samples were analysed after eight months storage to investigate these results. The eight-month analyses gave recovered residues of 96% and 86% of the initial residue, demonstrating no significant loss in storage. It is unclear why the results at six months were apparently low.

Therefore residues of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) are stable in all animal commodities when stored at -20 ± 5 °C for at least 14months.

Table 95 Stability of *anti*-isomer and *syn*-isomer in Animal Commodities Following Storage at -20 ± 5 °C

Commodity	Storage Period (months)	*anti*-isomer Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Milk	0	0.49	100	97
	3	0.44	90	80
	6	0.47	96	95
	14	0.47	96	97
Eggs	0	0.47	100	95
	3	0.44	94	99
	6	0.25	53	94
	8	0.48	102	102
	14	0.46	98	93
Liver	0	0.52	100	100
	3	0.53	102	112
	6	0.47	90	88
	14	0.45	87	100
Muscle	0	0.42	100	79
	3	0.47	112	93
	6	0.49	117	99
	14	0.52	124	109
Fat	0	0.42	100	91
	3	0.45	107	92
	6	0.51	121	101
	14	0.43	102	100
Kidney	0	0.44	100	94
	3	0.47	107	113
	6	0.38	86	108
	14	0.49	111	108
Milk	0	0.48	100	100
	3	0.46	96	87
	6	0.50	104	95
	14	0.51	106	108
Eggs	0	0.47	100	98
	3	0.42	89	99
	6	0.27	57	94
	8	0.46	98	109
	14	0.50	106	99
Liver	0	0.50	100	97
	3	0.49	98	99
	6	0.45	90	90
	14	0.43	86	96
Muscle	0	0.45	100	85
	3	0.44	98	88
	6	0.46	102	97
	14	0.52	116	110

Commodity	Storage Period (months)	*anti*-isomer Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Fat	0	0.43	100	92
	3	0.46	107	92
	6	0.49	114	96
	14	0.40	93	92
Kidney	0	0.46	100	95
	3	0.46	100	110
	6	0.40	87	103
	14	0.45	98	104

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

Another study to demonstrate the stability of residues of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) and its metabolites CSCD610195, CSCD610196 and CSCD591489 in milk, eggs and animal tissues was initiated in 2008 by Heillaut. C. (2009b). The intended duration of the study is one year. The stability was tested in milk, eggs, liver, muscle, fat and kidney.

Milk, eggs, liver, muscle, fat and kidney samples (10 g) were fortified with known amounts of *anti*-isomer, *syn*-isomer, CSCD610195, CSCD610196 and CSCD591489 in acetonitrile at a rate of 0.2 mg/kg per analyte, corresponding to a total concentration of 0.474 mg/kg of the common moiety CSAA798670. The solvent was allowed to evaporate and the samples sealed and stored in a freezer at a nominal temperature of -20 ± 5 °C.

The initial concentration was determined by analysis of three freshly-prepared fortified samples. At subsequent storage intervals of approximately 3, 4 and 12 months a sample set of each substrate, consisting of a control sample, two freshly fortified samples and two freezer-stored fortified samples, were analysed for residues determined as the common moiety CSAA798670 using method GRM006.10A.

The residues found in the animal commodities tested at each interval are detailed in Table 97. There was no significant change in the residue levels of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) and its metabolites CSCD610195, CSCD610196 and CSCD591489, determined as the common moiety CSAA798670, found in milk, eggs and animal tissues after storage deep frozen for at least four months.

Therefore, residues of isopyrazam (as isomers *anti*-isomer and *syn*-isomer) and its metabolites CSCD610195, CSCD610196 and CSCD591489 are stable, when stored at -20 ± 5 °C, in all animal commodities for at least 12 months.

Table 97 Stability of *anti*-isomer, *syn*-isomer, CSCD610195, CSCD610196 and CSCD591489 Residues in Animal Commodities Following Storage at -20 ± 5 ºC

Commodity	Storage Period (months)	CSAA798670* Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
Milk	0	0.42	100	83
	3	0.38	90	96
	4	0.43	102	91
	12	0.42	100	87
Eggs	0	0.46	100	101
	3	0.28	61	77
	4	0.46	100	93
	12	0.43	93	90
Liver	0	0.44	100	94
	3	0.32	73	72
	4	0.44	100	92

Commodity	Storage Period (months)	CSAA798670* Concentration (mg/kg)[a]	% Remaining	Mean Procedural Recovery (%)[b]
	12	0.43	98	86
Muscle	0	0.41	100	95
	3	0.34	83	76
	4	0.43	105	91
	12	0.43	105	89
Fat	0	0.45	100	92
	3	0.37	82	98
	4	0.44	98	94
	12	0.44	98	94
Kidney	0	0.41	100	86
	3	0.39	95	89
	4	0.44	107	90
	12	0.33	80	85

[a] Mean of three samples (five at 0 months), not corrected for procedural recovery

[b] Mean of two recoveries

*–Combined residues of *anti*-isomer, *syn*-isomer, CSCD610195, CSCD610196 and CSCD591489 were determined as the common moiety CSAA798670.

The stability of residues during storage of samples frozen at approximately -15 to -20 ºC has been investigated in a range of crop and animal matrices covering the representative commodities specified in EU guidance. Studies have included all the compounds relevant to the proposed definition of the residue in both plant and animal commodities, as well as compounds which had been included in the field residue trials and/or field succeeding crop studies. Storage intervals reported to date from the on-going studies covered periods from 4 to 12 months. In every case, residues of the tested analytes were stable under the storage conditions. The results of the storage stability studies to date are summarised in Table 98.

Table 98 Stability of residues during storage of samples

Analyte	Commodity Type	Period (Months)	Outcome
anti-isomer	Crop	24	Stable
syn-isomer	Crop	24	Stable
CSCD459488	Crop	11	Stable
CSCD459489	Crop	28	Stable
CSCD465008	Crop	12	Stable
CSAA798670	Crop	12	Stable
anti-isomer	Animal	14	Stable
syn-isomer	Animal	14	Stable
anti-isomer*, *syn*-isomer*, CSCD610195*, CSCD610196*, CSCD591489*	Animal	12	Stable

*–Determined by hydrolysis to the common moiety CSAA798670

USE PATTERNS

The meeting received labels approved in Columbia, New Zealand and the UK. Formulations containing isopyrazam, alone or in combination with other compounds are registered for use on a wide variety of crops. Labels including the details on the Good Agricultural Practices (GAP) are provided.

Isopyrazam is efficient at controlling a wide range of fungal pathogens. Isopyrazam contains two diastereomers designated *syn* and *anti*-isomers. Both of the isomers are biologically active and the

specification for technical isopyrazam covers the range of *syn*:*anti* isomer ratios from 70:30 to 100:0. A summary of the target pests as given in the labels provided is given in Table 99.

Table 99 Pests controlled by isopyrazam

Crop	Pest/diseases controlled	Type and timing of application(s)
FRUITS		
Assorted tropical and subtropical fruits–inedible peel		
Banana	Mycosphaerella fijiensis/ Black sigatoka	Start application at first occurrence of disease. Make a maximum of 5 applications per year, leave an interval of at least 8 weeks between applications. It is useful to mix the product with protective fungicides or with fungicides of a different mechanism of action as an anti-resistance strategy.
GRASSES		
Cereal grains		
Wheat	Puccinia recondita/ Brown rust Puccinia striiformis/ Yellow rust Septoria tritici/ Leaf spot Septoria nodorum/ Glume blotch Mycosphaerellagraminicola/Speckled leaf blotch	Apply as preventative spray between growth stages BBCH 30-69. Do not apply more than two applications per season. Apply in mixture with another approved, non-cross resistant fungicide recommended for the control of the same target diseases in cereals.
Rye	Pucciniare condita/ Brown rust Rhynchosporium secalis/ Leaf blotch	See wheat.
Triticale	Puccinia recondita/ Brown rust *Septoria* spp/ Leaf blotch	See wheat.
Barley	Puccinia hordei/ Brown rust Pyrenophora teres/ Net blotch Ramularia collo-cygni/Ramularia Rhynchosporium secalis/ Leaf blotch Erysiphe graminis hordei/Powdery mildew	Apply as preventative spray between growth stages BBCH 30-59. Do not apply more than two applications per season. Apply in mixture with another approved, non-cross resistant fungicide recommended for the control of the same target diseases in cereals.
Oats	*Pyrenophora avenae*/Red-brown leaf spot	See barley.

A summary of the use patterns relevant to the current evaluation is presented in Table 100.

Table 100 Summary of currently registered uses of isopyrazam in the individual countries

Crop	Country/ Region	Formulation[a]	F/G/P[b]	Application					PHI (days)
				Method	Rate (kg ai/ha)	Spray conc. (kg ai/hL)	Spray Interval (days)	Number	
FRUITS									
Assorted tropical and subtropical fruits–inedible peel									
Banana	Columbia	EC (12.5% isopyrazam)	F	foliar spray	0.075	0.30-0.40	56	5	0
GRASSES									
Cereal grains									
Barley	New Zealand	EC (12.5% isopyrazam)	F	foliar spray	0.075	0.025-0.0325	-[d]	2	42 28[e]
Barley	UK	EC (6.25% isopyrazam/18.8% cyprodinil)	F	foliar spray	0.125	0.0313-0.125	-[f]	2	-
Barley	UK	EC (12.5% isopyrazam/9.0% epoxiconazole)	F	foliar spray	0.125	0.0313-0.125	-[f]	2	-
Rye	UK	EC (12.5% isopyrazam/9.0% epoxiconazole)	F	foliar spray	0.125	0.0313-0.125	-[g]	2	-
Triticale	UK	EC (12.5% isopyrazam/9.0% epoxiconazole)	F	foliar spray	0.125	0.0313-0.125	-[g]	2	-

Crop	Country/ Region	Formulation[a]	F/G/P[b]	Application					PHI (days)
				Method	Rate (kg ai/ha)	Spray conc. (kg ai/hL)	Spray Interval (days)	Number	
Wheat	New Zealand	EC (12.5% isopyrazam)	F	foliar spray	0.075 or 0.125[c]	0.025-0.0625	-[h]	2	42 28[e]
Wheat	UK	EC (12.5% isopyrazam/9.0% epoxiconazole)	F	foliar spray	0.125	0.0313-0.125	-[g]	2	-

[a] % w/v.

[b] F=outdoor or field use, G=glasshouse, P=protected, I=indoor application

[c] 0.075 kg ai/ha is recommended against leaf rust and stripe rust, 0.125 kg ai/ha is recommended against speckled leaf blotch.

[d] The product must NOT be applied later than BBCH 59 (end of heading, influorescence fully emerged).

[e] For grain crops, 42 days; and for green feed and silage, 28 days.

[f] The product is applied between growth stages 30-61(beginning of flowering: first anthers visible), and must NOT be applied later than BBCH growth stage 61.A minimum of 21 days must be observed between applications.

[g] The product is applied between growth stages 30-71(watery ripe stage), and must NOT be applied later than BBCH growth stage 71. A minimum of 21 days must be observed between applications.

[h] The product must NOT be applied later than BBCH 69 (end of flowering). The application interval is determined by growth stage.

RESIDUES RESULTING FROM SUPERVISED/TRIALS ON CROPS

The Meeting received information on supervised field trials of isopyrazam on the following crops conducted in Central and South America, Europe and New Zealand.

Crop	Country/Region:	
Banana	Central and South America	Table 101
Barley grain	New Zealand	Table 102
Barley grain	Europe	Table 103
Wheat grain	New Zealand	Table 104
Wheat grain	Europe	Table 105
Barley forage and straw	New Zealand	Table 106
Barley whole plant and straw	Europe	Table 107
Wheat forage and straw	New Zealand	Table 108
Wheat whole plant and straw	Europe	Table 109

Trials were generally well documented with full laboratory and field reports. Laboratory reports included the dates of spray applications, sampling dates, method validation, with procedural recovery data. Dates of analyses or duration of residue sample storage were also provided. In general, data on procedural recoveries were within the acceptable range 70–120%, with % relative standard deviation of < 20%.

All trials were conducted outdoors. Application rates were reported as isopyrazam equivalents. Residue concentrations were reported for isopyrazam (as summation of separately determined *anti*-isomer and *syn*-isomer), CSCD459488 and CSCD459489. Since no residues of CSCD459489 were detected in any of the samples, CSCD459489 residue data are not included in the tables. Where samples were analysed in replicate, the mean residue has been reported. Residue concentrations are recorded unadjusted for recoveries or for residue values in control samples. Where trials were conducted in the same location, with the same varieties, similar formulations, and at the same or similar timing, they are not regarded as independent and the highest residues from these trials was recorded. Although trials included control plots, no control data are recorded in the tables below unless residues in control samples significantly exceeded the LOQ.

Total residues were calculated by summing up the concentrations of isopyrazam and CSCD459488. In the trials, residues found to be below the limit of quantitation (LOQ) were reported as < LOQ.

Residues from the trials used for the estimation of maximum residue levels are underlined.

Abbreviations contained in the tables are indicated in the legend.

Assorted tropical and subtropical fruits–inedible peel

Banana

The registered use on banana is for five applications per year with minimum application intervals of 8 weeks, at rates of 75 g ai/ha and a PHI of 0 day.

A total of twelve supervised residue trials were conducted on bananas in 2008 in Columbia, Costa Rica, Ecuador, Guatemala and Honduras. In these trials, isopyrazam was formulated as an emulsifiable concentrate (EC) containing 125 g isopyrazam per litre. This formulation contained *syn*-isomer (the *syn*-isomer of isopyrazam) and *anti*-isomer (the *anti*-isomer of isopyrazam) with a nominal ratio of 70:30.

The formulation was applied five (or, in one case, six) times at a rate of 75 g ai/ha, with an interval between applications of 10 days. Water volumes were 25 L/ha. Applications were made to both bagged and unbagged bananas. Generally, in commercial banana production, the fruit bunches are covered with polythene or similar bags, which are removed at harvest. Under these conditions, there is negligible exposure to direct spray and residues would be expected to be low or none existent. However, in practice, some of the bags may be torn, blown off or otherwise holed or removed. Therefore, the analysis of unbagged fruits represents a worst-case situation for potential residues. Although one trial in Honduras received six applications, this is within 25% of the desired GAP.

Samples of banana fruit were collected immediately after the final spray application had dried on the plants.

All samples from residue trials were analysed for isopyrazam (as separate *anti*-isomer and *syn*-isomer), CSCD459488 and CSCD459489. *anti*-isomer and *syn*-isomer were analysed using method GRM006.01B, and isopyrazam residues were determined by summation. Metabolites CSCD459488 and CSCD459489 were determined using method GRM006.03A. Since no residues of CSCD459489 were detected in any of the samples, CSCD459489 residue data are not tabulated. Where samples were analysed in replicate, the mean residue has been reported. The longest period of frozen storage of samples was 53 days.

For trials conducted in 2008, the mean *anti*-isomer recovery in banana fruit, peel and pulp were 99 ± 6.5% (n = 26), 98 ± 6.4% (n = 26) and 100 ± 5.6% (n = 18) for all fortification levels (0.005 mg/kg and 0.05 mg/kg, respectively. The mean *syn*-isomer recovery in banana fruit, peel and pulp were 96 ± 6.3% (n = 26), 97 ± 5.8% (n = 26) and 97 ± 4.1% (n = 18) for all fortification levels (0.005 mg/kg and 0.05 mg/kg, respectively. The mean CSCD459488 recovery in banana fruit, peel and pulp were 97 ±13% (n = 28), 94±7.8% (n = 24) and 95±7.0% (n = 20) for all fortification levels (0.005 mg/kg and 0.05 mg/kg, respectively.

The results of the supervised field trials with isopyrazam on banana are summarised in Table 101.

Table 101 Residues of isopyrazam and CSCD459488 following foliar applications from supervised trials on banana in Columbia, Ecuador, Guatemala and Honduras

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam *	CSCD 459488	Total**	
GAP in Columbia	125EC	0.075		5	0							
Banana Columbia, 2008 (Grand Nain) W38CL081740	125EC	0.073 0.073 0.074 0.073 0.073	70 72 79 79 82	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Peel, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Columbia, 2008 (Grand Nain) W38CL081741	125EC	0.073 0.073 0.074 0.073 0.072	70 72 79 79 82	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Peel, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Costa Rica, 2008 (Grand Nain) W38CR081742	125EC	0.075 0.078 0.076 0.076 0.076	72 72 72 79 79	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	0.008[a]	0.005[a]	0.013	< 0.005[a]	0.018	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.010[a]	< 0.005[a]	0.015	0.008[b]	0.023	
				5	0	Peel, unbagged	0.026[a]	0.011[a]	0.037	0.013[a]	0.050	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Peel, bagged	0.006[a]	< 0.005[a]	0.011	< 0.005[a]	0.016	
				5	1	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Fruit, unbagged	0.009[b]	< 0.005[b]	0.014	0.009[b]	0.023	
				5	1	Peel, unbagged	0.015[a]	0.006[a]	0.021	0.010[a]	0.031	
				5	1	Pulp, unbagged	< 0.005	< 0.005	< 0.010	0.005[a]	< 0.015	
				5	3	Fruit, bagged	< 0.005[a]	< 0.005[a]	< 0.010	< 0.005[b]	< 0.015	
				5	3	Peel, bagged	0.008[a]	0.005[a]	0.013	< 0.005[a]	0.018	
				5	3	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	3	Fruit, unbagged	0.022[b]	0.012[b]	0.034	0.015[a]	0.049	
				5	3	Peel, unbagged	0.039[a]	0.019[a]	0.058	0.015[a]	0.073	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam *	CSCD 459488	Total**	
				5	3	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Costa Rica 2008 (Grand Nain) W38CR081743	125EC	0.077 0.077 0.077 0.077 0.077	72 72 72 79 79	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.012	< 0.005	0.017	0.009[a]	0.026	
				5	0	Peel, unbagged	0.020	0.011	0.031	0.012[a]	0.043	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Costa Rica 2008 (Grand Nain) W38CR081744	125EC	0.076 0.076 0.076 0.076 0.076	72 72 72 79 79	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	0.005	< 0.005	0.010	< 0.005	0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.010	< 0.005	0.015	0.011[2]	0.026	
				5	0	Peel, unbagged	0.031	0.014	0.045	0.015[2]	0.060	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Costa Rica 2008 (Grand Nain) W38CR081745	125EC	0.075 0.076 0.075 0.075 0.075	72 72 72 79 79	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.007	< 0.005	0.012	0.008	0.020	
				5	0	Peel, unbagged	0.019	0.010	0.029	0.010	0.039	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Ecuador 2008 (Williams) W38EC081746	125EC	0.075 0.075 0.075 0.075 0.075	74 75 77 79 80	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	< 0.005[a]	< 0.005[a]	< 0.010	< 0.005[a]	< 0.015	
				5	0	Peel, unbagged	0.010[a]	0.006[a]	0.016	0.005[a]	0.021	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	1	Fruit, unbagged	0.006[a]	< 0.005[a]	0.013	< 0.005[a]	0.018	
				5	1	Peel, unbagged	0.024[a]	0.012[a]	0.036	0.008[a]	0.044	
				5	1	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	3	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam *	CSCD 459488	Total**	
				5	3	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	3	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	3	Fruit, unbagged	< 0.005[a]	< 0.005[a]	< 0.010	< 0.005[a]	< 0.015	
				5	3	Peel, unbagged	0.014[a]	0.007[a]	0.021	0.006[a]	0.027	
				5	3	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Ecuador 2008 (Giant Cavendish) W38EC081747	125EC	0.075 0.075 0.075 0.075 0.075	74 75 77 79 80	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.010	0.006	0.016	< 0.005	0.021	
				5	0	Peel, unbagged	0.010	0.006	0.016	< 0.005	0.021	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Ecuador 2008 (Giant Cavendish) W38EC081748	125EC	0.075 0.075 0.075 0.075 0.075	75 77 79 80 74	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.006[a]	< 0.005[a]	0.011	< 0.005	0.016	
				5	0	Peel, unbagged	< 0.005[a]	< 0.005[a]	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Guatemala 2008 (Seda) W38GT081752	125EC	0.074 0.079 0.072 0.079 0.075	72 72 72 72 72	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	0.014	0.007	0.021	< 0.005	0.026	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.026[a]	0.014[a]	0.040	0.013[a]	0.053	
				5	0	Peel, unbagged	0.022[b]	0.011[b]	0.033	0.009[a]	0.042	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Guatemala 2008 (Seda) W38GT081753	125EC	0.077 0.078 0.076 0.076 0.074	72 72 72 72 72	5	0	Fruit, bagged	< 0.005[a]	< 0.005[a]	< 0.010	< 0.005[a]	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				5	0	Peel, bagged	0.038[b]	0.017[b]	0.055	0.009[a]	0.064	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	0.014	0.008	0.022	0.011[c]	0.033	
				5	0	Peel, unbagged	0.024	0.013	0.037	0.006[a]	0.043	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Banana Honduras 2008 (Grand Nain) W38HO081751	125EC	0.075 0.075 0.074 0.074 0.075 0.075	72 72 79 79 81 81	5	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Mäyer, T, T001542-07 2009 T001542-07 Isopyrazam/11420
				6	0	Fruit, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam *	CSCD 459488	Total**	
				6	0	Peel, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				6	0	Pulp, bagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Fruit, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				6	0	Fruit, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				5	0	Peel, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				6	0	Peel, unbagged	0.005	< 0.005	0.010	< 0.005	0.015	
				5	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				6	0	Pulp, unbagged	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	

* - Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

** - Total residue for risk assessment purposes (calculated; sum of *anti*-isomer, *syn*-isomer and CSCD459488)

na - Not applicable

[a] - Mean of two replicate analyses

[b] - Mean of three replicate analyses

[c] - Mean of four replicate analyses

Cereal grains

Barley

The registered use on barley in NewZealand is for two foliar applications per season prior to growth stage 59 (ear emergence) at a rate of 75 g ai/ha and a PHI of not shorter than 42 days.

A total of three supervised residue trials were conducted on barley during 2008 in New Zealand.

The trials conducted in New Zealand are summarised in Table 102. In these trials isopyrazam was formulated as emulsifiable concentrate (EC) containing 125 g isopyrazam per litre. This formulation contained *syn*-isomer and *anti*-isomer with a nominal ratio of 85:15. The formulation was applied 2 times at a rate of nominally 75, 125 and 250 g ai/ha. The applications were made around growth stages Zadoks 39 and 69. Water volumes were 192 to 323 L/ha.

Barley grain and straw were sampled at normal commercial harvest and analysed for residues using methods Draft GRM006.01A, GRM006.01A or GRM006.01B, determining *anti*-isomer and *syn*-isomer separately and isopyrazam by summation. Metabolites CSCD459488 and CSCD459489 were determined using method GRM006.03A. Where samples were analysed in replicate, the mean residue has been reported. Samples were stored frozed for 21–61 days.

For trials conducted in 2009, the mean *syn*-isomer recovery in forage, grain and straw were 111 ± 8% (n = 19) for all fortification levels (0.004–2.53 mg/kg), 99 ± 12% (n = 11) for all fortification levels (0.004–0.25 mg/kg) and 81 ± 14% (n = 11) for all fortification levels (0.004–2.53 mg/kg), respectively. The mean *anti*-isomer recovery in forage, grain and straw were 109 ± 8% (n = 19) for all fortification levels (0.008–0.46 mg/kg), 91 ± 21% (n = 12)for all fortification levels (0.008–0.05 mg/kg) and 82 ± 13% (n = 10) for all fortification levels (0.008–0.46 mg/kg), respectively. The mean CSCD459488 recovery in forage, grain and straw were 93 ± 18% (n = 19) for

all fortification levels (0.01–2.50 mg/kg), 93 ± 7% (n = 12) for all fortification levels (0.01–0.25 mg/kg) and 99 ± 11% (n = 12) for all fortification levels (0.01–2.50 mg/kg), respectively.

Table 102 Residues of isopyrazam and CSCD459488 following foliar applications from supervised trials on barley in New Zealand

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in NewZealand	125EC	0.075	-*	2	42	Grain						
Barley NewZealand 2008 (Optic) SYN-0801/S2	125EC (15149W)	0.077 0.077	49 55	2	41	Grain	< 0.005[a]	< 0.005[a]	< 0.010	0.010[a]	0.020	Farrell P. SYN-0801 2009 SYN-0801 Isopyrazam/50008
		0.128 0.127	49 55	2	41	Grain	0.036	0.013	0.049	0.030	0.079	
		0.255 0.254	49 55	2	41	Grain	0.076	0.017	0.093	0.036	0.129	
Barley NewZealand 2008 (Sherwood) SYN-0801/S5	125EC (15149W)	0.078 0.078	45 59	2	42	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Farrell P. SYN-0801 2009 SYN-0801 Isopyrazam/50008
		0.130 0.130	45 59	2	42	Grain	0.012	< 0.005	0.017	0.008	0.025	
		0.259 0.259	45 59	2	42	Grain	0.018	< 0.005	0.023	0.011	0.034	
Barley NewZealand 2008 (County) SYN-0801/S6	125EC (15149W)	0.078 0.078	45 59	2	42	Grain	0.013	< 0.005	0.018	0.006	0.024	Farrell P. SYN-0801 2009 SYN-0801 Isopyrazam/50008
		0.130 0.130	45 59	2	42	Grain	0.029	< 0.005	0.034	0.011	0.045	
		0.259 0.259	45 59	2	42	Grain	0.053[a]	0.009[a]	0.062	0.022[a]	0.084	

* -The product must NOT be applied later than BBCH 59 (ear emergence).

** - Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

na - Not applicable

[a] - Mean of two replicate analyses

The registered use of isopyrazam on barley in the United Kingdom is for two applications per season prior to growth stage 61 (before beginning of flowering), each at a rate of 125 g ai/ha isopyrazam.

A total of eighteen supervised residue trials were conducted on barley during 2006 and 2007, nine in Northern Europe (Germany, Switherland, UK and France) and nine in Southern Europe (France, Italy and Spain).

The trials conducted in Europe are summarised in Table 103. In these trials isopyrazam was formulated as two emulsifiable concentrates (EC), both containing 125 g isopyrazam per litre. The first contained *syn*-isomer and *anti*-isomer with a nominal ratio of 95:5, and the second contained *syn*-isomer and *anti*-isomer with a nominal ratio of 70:30. The eight trials conducted in 2006 used the first formulation; of these, the four trials in northern Europe also contained plots treated with the second formulation to allow comparison of the total isopyrazam residues found when isopyrazam was applied at different *syn/anti* ratios. The ten trials conducted in 2007 used only the second formulation. Both formulations were applied three times at a rate of 125 g ai/ha. The first and second applications were made around growth stages BBCH 31 and 59, respectively, corresponding to the critical GAP. Water volumes were 189 to 307 L/ha. The 2006 trials also contained plots treated with the first formulation at the same rate but with the first and second applications at around growth stages BBCH 31 and 49, respectively. Data from these plots are included in Table 103 for completeness but are not discussed further.

Barley grain and straw were sampled at normal commercial harvest and analysed for residues using methods Draft GRM006.01A, GRM006.01A or GRM006.01B, determining *anti*-isomer and *syn*-isomer separately and isopyrazam by summation. Metabolites CSCD459488 and CSCD459489 were determined using method GRM006.03A. Where samples were analysed in replicate, the mean residue has been reported. Samples were stored frozen for a maximum of 25 months.

For trials conducted in 2006 in Switzerland, the mean *syn*-isomer recovery in plant, grain and straw were 98 ± 10% (n = 9) for all fortification levels (0.005–1.0 mg/kg), 100 ± 3% (n = 3) for all fortification levels (0.005–0.10 mg/kg) and 82 ± 17% (n = 5) for all fortification levels (0.005–1.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 95±8% (n = 9) for all fortification levels (0.005–0.20 mg/kg), 103 ± 3% (n = 5)for all fortification levels (0.005–0.10 mg/kg) and 83 ± 14% (n = 7) for all fortification levels (0.005–0.40 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 104 ± 5% (n = 4) for all fortification levels (0.005–0.05 mg/kg), 100% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 103 ± 9% (n = 4) for all fortification levels (0.005–0.05 mg/kg), respectively.

For trials conducted in 2006 in Germany, the mean *syn*-isomer recovery in grain and straw were 103% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 105% (n = 2) for all fortification levels (0.005–2.0 mg/kg), respectively. The mean *anti*-isomer recovery in grain and straw were 102% (n = 2)for all fortification levels (0.005–0.05 mg/kg) and 92% (n = 2) for all fortification levels (0.005–0.2 mg/kg), respectively. The mean CSCD459488 recovery in grain and straw were 102% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 102% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively.

For trials conducted in 2007 in Germany, the mean *syn*-isomer recovery in plant, grain and straw were 102 ± 3% (n = 3) for all fortification levels (0.005–1.0 mg/kg), 96% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 86 ± 5% (n = 3) for all fortification levels (0.005–1.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 106 ± 4% (n = 3) for all fortification levels (0.005–1.0 mg/kg), 103% (n = 2)for all fortification levels (0.005–0.05 mg/kg) and 87 ± 6% (n = 3) for all fortification levels (0.005–1.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 101% (n = 2) for all fortification levels (0.005–1.0 mg/kg), 109% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 90% (n = 2) for all fortification levels (0.005 -1.0 mg/kg), respectively.

For trials conducted in 2006 in Italy, the mean *syn*-isomer recovery in plant, grain and straw were 114 ± 8% (n = 4) for all fortification levels (0.005–1.0 mg/kg), 88 ± 14% (n = 6) for all fortification levels (0.005–0.01 mg/kg) and 101± 12% (n = 4) for all fortification levels (0.005–1.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 110 ± 17% (n = 4) for all fortification levels (0.005–1.0 mg/kg), 89 ± 13% (n = 6)for all fortification levels (0.005–0.01 mg/kg) and 80 ± 7% (n = 4) for all fortification levels (0.005–1.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 87% (n = 2) for all fortification levels (0.005–0.05 mg/kg), 108% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 92% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively.

For trials conducted in 2007 in Spain, the mean *syn*-isomer recovery in plant, grain and straw were 106 ± 4% (n = 6) for all fortification levels (0.005–1.0 mg/kg), 89% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 91 ± 17% (n = 3) for all fortification levels (0.01–1.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 107 ± 3% (n = 6) for all fortification levels (0.005–1.0 mg/kg), 96% (n = 2)for all fortification levels (0.005–0.05 mg/kg) and 99 ± 15% (n = 3) for all fortification levels (0.01–1.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 95% (n = 2) for all fortification levels (0.005–0.05 mg/kg), 96% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 95% (n = 2) for all fortification levels (0.005–1.0 mg/kg), respectively.

Examination of the trials data for parent isopyrazam in Table 103 shows that the ratio of *syn*-isomer to *anti*-isomer was essentially unchanged over time as in the case of wheat (see page 826). Thus, the decline rates of *syn*-isomer and *anti*-isomer in barley field trials are approximately equal.

Where side-by-side plots in the same trials were treated with different formulations, containing isopyrazam at different *syn/anti* ratios, the different ratios resuled in similar total residues and decline rates.

Table 103 Residues of isopyrazam and CSCD459488 following foliar applications from supervised trials on barley in Europe

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in UK	125EC	0.125	-*	2	-							
Northern Europe												
Barley Switzerland 2006 (Landi) CH-FR-06-0023	125EC (A15149G)	0.127 0.126	31 47–49	2	54	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Marshall L. CEMR-3393-REG 2008b T000674-06 A15149K/11192
	125EC (A15149G)	0.127 0.126	31 59	2	48	Grain	0.019	< 0.005	0.024	0.019	0.043	
	125EC (A15149K)	0.126 0.126	31 59	2	48	Grain	0.010	0.005	0.015	0.011	0.026	
Barley Switzerland 2006 (Merlot) CH-FR-06-0024	125EC (A15149G)	0.126 0.127	31 47–49	2	54	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Marshall L. CEMR-3393-REG 2008b T000674-06 A15149/11192
	125EC (A15149G)	0.126 0.125	31 57–59	2	48	Grain	0.009	< 0.005	0.014	0.006	0.020	
	125EC (A15149K)	0.125 0.128	31 57–59	2	48	Grain	0.008	0.006	0.014	0.006	0.020	
Barley France 2006 (Esterel) FR-FR-06-0026	125EC (A15149G)	0.127 0.129	31 49	2	60	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Marshall L. CEMR-3393-REG 2008b T000674-06 A15149/11192
	125EC (A15149G)	0.124 0.126	31 59	2	43	Ear	0.025	< 0.005	0.030	0.046	0.076	
				2	48	Grain	0.023	< 0.005	0.028	0.020	0.048	
	125EC (A15149K)	0.124 0.131	37 59	2	43	Ear	0.029	0.024	0.053	0.068	0.121	
				2	54	Grain	0.020	0.015	0.035	0.023	0.058	
Barley Germany 2006 (Carat) DE-FR-06-4422	125EC (A15149G)	0.124 0.124	31–32 49	2	52	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Simon P. T001788-06-REG 2008b T001788-06 A15149G/10000
		0.124 0.124	31–32 59	2	45	Grain	0.021	< 0.005	0.026	0.022	0.048	
	125EC (A15149K)	0.124 0.124	31–32 59	2	45	Grain	0.014	0.008	0.022	0.020	0.042	
Barley Germany 2007 (Braemar) AF/11518/SY/1	125EC (A15149K)	0.120 0.124	31 57- 59	2	45	Grain	0.012	0.008	0.020	0.012	0.032	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
Barley Germany 2007 (Braemar) AF/11518/SY/2	125EC (A15149K)	0.124 0.124	32 69	2	30	Immature Grain	0.026	0.027	0.053	0.049	0.102	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
				2	38	Grain	0.009	0.007	0.016	0.013	0.029	
Barley UK 2007 (Westmi-nster) AF/11518/SY/3	125EC (A15149K)	0.124 0.123	32 57–59	2	42	Grain	0.011	0.005	0.016	0.006	0.022	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
Barley France 2007 (Colibri) AF/11518/SY/4	125EC (A15149K)	0.124 0.124	32 57–59	2	44	Immature Grain	0.011	0.008	0.019	0.015	0.034	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
				2	61	Grain	0.010	0.007	0.017	0.012	0.029	
Barley France 2007 (Platine)	125EC (A15149K)	0.124 0.120	31 58	2	30	Immature Grain	0.022	0.019	0.041	0.026	0.067	Bell A. CEMR-3367-REG 2008c
				2	42	Grain	0.015	0.011	0.026	0.020	0.046	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
AF/11518/SY/5												CEMS-3367 A15149K/10525
Southern Europe												
Barley Italy 2006 (Boreale) IT-FR-06-0027	125EC (A15149G)	0.130 0.125	30 47–49	2	53	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149/10525
	125EC (A15149G)	0.133 0.132	30 57–59	2	31	Grain	0.007	< 0.005	0.012	< 0.005	0.017	
				2	45	Grain	0.011	< 0.005	0.016	0.016	0.032	
Barley Spain 2006 (Verticale) ES-FR-06-0028	125EC (A15149G)	0.124 0.131	31 47–49	2	57	Grain	0.009	< 0.005	0.014	< 0.005	0.019	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149/11030
		0.127 0.133	31 58–61	2	30	Immature Grain	0.117	0.008	0.125	0.016	0.141	
				2	42	Grain	0.154	0.016	0.170	0.041	0.211	
				2	50	Grain	0.133	0.012	0.145	0.052	0.197	
Barley France 2006 (Baraka) FR-FR-06-0029	125EC (A15149G)	0.131 0.129	30 49	2	52	Grain	0.006	< 0.005	0.011	< 0.005	0.016	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149K/11030
		0.130 0.131	30 59	2	41	Grain	0.168	< 0.005	0.173	0.046	0.219	
Barley France 2006 (Nevada) FR-FR-06-0030	125EC (A15149G)	0.126 0.127	31 47	2	56	Grain	0.010	< 0.005	0.015	< 0.005	0.020	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149K/11030
		0.127 0.127	31 57	2	42	Immature Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				2	50	Grain	< 0.005	< 0.005	< 0.010	0.006	0.016	
Barley Spain 2007 (Chamorro) AF/11519/SY/1	125EC (A15149K)	0.130 0.125	31 58	2	30	Grain	0.338	0.166	0.504	0.030	0.534	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
				2	42	Grain	0.268	0.140	0.408	0.023	0.431	
Barley Italy 2007 (Sonora) AF/11519/SY/2	125EC (A15149K)	0.126 0.122	32 59	2	42	Grain	0.190	0.080	0.233	0.090	0.360	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
Barley France 2007 (Scarlett) AF/11519/SY/3	125EC (A15149K)	0.124 0.124	30 58	2	30	Immature Grain	0.013	0.008	0.021	0.008	0.029	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
				2	43	Grain	0.030	0.016	0.046	0.016	0.062	
Barley France 2007 (Prestige) AF/11519/SY/4	125EC (A15149K)	0.122 0.124	32 57–59	2	45	Grain	0.014	0.010	0.024	0.028	0.052	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
Barley France 2007 (Platine) AF/11519/SY/5	125EC (A15149K)	0.118 0.124	31 59	2	45	Ear	< 0.005	< 0.005	< 0.010	0.017	0.027	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149K/10524
				2	63	Grain	< 0.005	< 0.005	< 0.010	0.008	0.018	

* - The product is applied between growth stage 30–61, and must NOT be applied later than BBCH 61 (before beginning of flowering).A minimum of 21 days must be observed between applications.

** –Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

Wheat

The registered use of isopyrazam on wheat in New Zealand is for two applications per season prior to growth stage 69 (end of flowering) at rates of 75-125 g ai/ha and a PHI of not shorter than 42 days.

A total of three supervised residue trials were conducted on wheat during 2008 in New Zealand which are summarised in Table 104. In these trials isopyrazam was formulated as emulsifiable concentrate (EC) containing 125 g isopyrazam per litre. This formulation contained *syn*-isomer (the *syn*-isomer of isopyrazam) and *anti*-isomer (the *anti*-isomer of isopyrazam) with a nominal ratio of 85:15. The formulation was applied 2 times at a rate of nominally 75, 125 and 250 g ai/ha. The applications were made around growth stages Zadoks 39 and 69. Water volumes were 192 to 323 L/ha.

Wheat grain and straw were sampled at normal commercial harvest and analysed for residues using methods Draft GRM006.01A (quantification using GC-MS/MS rather than LC-MS/MS of GRM006.01A), GRM006.01A or GRM006.01B, determining *anti*-isomer and *syn*-isomer separately and isopyrazam by summation. Metabolites CSCD459488 and CSCD459489 were determined using method GRM006.03A. Since no residues of CSCD459489 were detected in any of the samples, CSCD459489 residue data are not tabulated. Where samples were analysed in replicate, the mean residue has been reported. Samples were stored frozen for 21–61 days.

For trials conducted in 2008, the mean *syn*-isomer recovery in forage, grain and straw were 111 ± 8% (n = 19) for all fortification levels (0.004–2.53 mg/kg), 99 ± 12% (n = 11) for all fortification levels (0.004–0.25 mg/kg) and 81 ± 14% (n = 11) for all fortification levels (0.004–2.53 mg/kg), respectively. The mean *anti*-isomer recovery in forage, grain and straw were 109 ± 8% (n = 19) for all fortification levels (0.008–0.46 mg/kg), 91 ± 21% (n = 12)for all fortification levels (0.008–0.05 mg/kg) and 82 ± 13% (n = 10) for all fortification levels (0.008–0.46 mg/kg), respectively. The mean CSCD459488 recovery in forage, grain and straw were 93 ± 18% (n = 19) for all fortification levels (0.01–2.50 mg/kg), 93 ± 7% (n = 12) for all fortification levels (0.01–0.25 mg/kg) and 99 ± 11% (n = 12) for all fortification levels (0.01–2.50 mg/kg), respectively.

Table 104 Residues of isopyrazam and CSCD459488 following foliar applications from supervised trials on wheat in New Zealand

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in NewZealand	125EC	0.075-0.125	-*	2	42	Grain						
Wheat NewZealand 2008 (variety not re-ported) SYN-0801/S1	125EC (A15149W)	0.077 0.076	49 59	2	42	Grain	0.011	< 0.005	0.016	0.012	0.028	Farrell P. SYN-0801 2009 Isopyrazam/11457
		0.128 0.127	49 59	2	42	Grain	0.015	< 0.005	0.020	0.014	0.034	
		0.255 0.254	49 59	2	42	Grain	0.084	0.013	0.097	0.040	0.137	
Wheat NewZealand 2008 (Phoenix) SYN-0801/S3	125EC (A15149W)	0.078 0.078	49 63	2	42	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Farrell P. SYN-0801 2009 Isopyrazam/11457
		0.130 0.130	49 63	2	42	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
		0.259 0.259	49 63	2	42	Grain	< 0.005	< 0.005	0.100	< 0.005	0.015	
Wheat NewZealand 2008 (Raffles) SYN-0801/S4	125EC (A15149W)	0.078 0.078	39 69	2	42	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Farrell P. SYN-0801 2009 Isopyrazam/11457
		0.130 0.130	39 69	2	42	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
		0.259 0.259	39 69	2	42	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	

* - The product must NOT be applied later than BBCH 69 (end of flowering).

** –Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

The Zadoks growth scale for cereals is numerically identical to the BBCH scale

The registered use of isopyrazam on wheat, rye and triticale in the United Kingdom is for two applications per season prior to growth stage 71 (before grain watery ripe stage), each at a rate of 125 g ai/ha isopyrazam.

A total of 22 supervised residue trials were conducted on wheat during 2006 and 2007, 13 in northern Europe (France, Germany and the UK) and nine in southern Europe (France, Italy and Spain).

The trials conducted in Europe are summarised in Table 105. In these trials isopyrazam was formulated as two emulsifiable concentrates (EC), both containing 125 g isopyrazam per litre. The first formulation contained *syn*-isomer and *anti*-isomer with a nominal ratio of 95:5, and the second contained *syn*-isomer and *anti*-isomer with a nominal ratio of 70:30. The eight trials conducted in 2006 used the first formulation; of these, the four trials in northern Europe also contained plots treated with the second formulation to allow comparison of the total isopyrazam residues found when isopyrazam was applied at different *syn/anti* ratios. The ten trials conducted in 2007 used only the second formulation. Both formulations were applied three times at a rate of 125 g ai/ha. The applications were made around growth stages BBCH 31, 39 and 69. Water volumes were 192 to 323 L/ha. The 2006 trials also contained plots treated with the first formulation at the same rate but with only two applications, made at around growth stages BBCH 31 and 49–55. Data from these plots are included in Table 113 for completeness.

Wheat grain and straw were sampled at normal commercial harvest and analysed for residues using methods Draft GRM006.01A, GRM006.01A or GRM006.01B, determining *anti*-isomer and *syn*-isomer separately and isopyrazam by summation. Metabolites CSCD459488 and CSCD459489 were determined using method GRM006.03A. Since no residues of CSCD459489 were detected in any of the samples, CSCD459489 residue data are not tabulated. Where samples were analysed in replicate, the mean residue has been reported. Samples were stored frozen for a maximum of 23 months.

For trials conducted in 2006 in France, the mean *syn*-isomer recovery in plant, grain and straw were 96 ± 10% (n = 5) for all fortification levels (0.005–1.0 mg/kg), 106 ± 2% (n = 4) for all fortification levels (0.005–0.20 mg/kg) and 84 ± 15% (n = 6) for all fortification levels (0.005–5.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 104 ± 5% (n = 5) for all fortification levels (0.005–1.0 mg/kg), 105 ± 2% (n = 4)for all fortification levels (0.005–0.20 mg/kg) and 86± 12% (n = 6) for all fortification levels (0.005–5.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 102% (n = 2) for all fortification levels (0.005–0.05 mg/kg), 108±4% (n = 4) for all fortification levels (0.005–0.05 mg/kg) and 92% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively.

For trials conducted in 2006 in Germany, the mean *syn*-isomer recovery in plant, grain and straw were 102 ± 1% (n = 3) for all fortification levels (0.005–0.125 mg/kg), 105 ± 5% (n = 3) for all fortification levels (0.005–0.125 mg/kg) and 99% (n = 2) for all fortification levels (0.005–2.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 103 ± 6% (n = 3) fora fortification level (0.10 mg/kg), 101 ± 2% (n = 3)for all fortification levels (0.005–0.125 mg/kg) and 93% (n = 2) for all fortification levels (0.005–2.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 100% (n = 2) for all fortification levels (0.005–0.05 mg/kg), 102% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 102% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively.

For trials conducted in 2007 in Germany, the mean *syn*-isomer recovery in plant, grain and straw were 93 ± 8% (n = 9) for all fortification levels (0.005–1.0 mg/kg), 94% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 82 ± 10% (n = 5) for all fortification levels (0.005–0.50 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 97 ± 9% (n = 9) for all fortification levels (0.005–1.0 mg/kg), 102% (n = 2) for all fortification levels (0.005–

0.05 mg/kg) and 82 ± 10% (n = 5) for all fortification levels (0.005–5.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 87 ± 17% (n = 4) for all fortification levels (0.005–0.05 mg/kg), 86 ± 11% (n = 7) for all fortification levels (0.005–0.05 mg/kg) and 93 ± 15% (n = 6) for all fortification levels (0.005–0.05 mg/kg), respectively.

For trials conducted in 2006 in Spain, the mean *syn*-isomer recovery in plant, grain and straw were 84 ± 21% (n = 4) for all fortification levels (0.005–1.0 mg/kg), 106 ± 6% (n = 6) for all fortification levels (0.005–0.02 mg/kg) and 83 ± 17% (n = 6) for all fortification levels (0.005–2.0 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 84 ± 6% (n = 4) for all fortification levels (0.005–1.0 mg/kg), 102 ± 7% (n = 6)for all fortification levels (0.005–0.02 mg/kg) and 79 ± 14% (n = 6) for all fortification levels (0.005 -2.0 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 103% (n = 2) for all fortification levels (0.005–0.05 mg/kg), 97% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 101 ± 8% (n = 4) for all fortification levels (0.005–0.20 mg/kg), respectively.

For trials conducted in 2007 in Spain, the mean *syn*-isomer recovery in plant, grain and straw were 95 ± 3% (n = 3) for all fortification levels (0.005–1.0 mg/kg), 82% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 95% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively. The mean *anti*-isomer recovery in plant, grain and straw were 98 ± 2% (n = 3) for all fortification levels (0.005–1.0 mg/kg), 82% (n = 2)for all fortification levels (0.005–0.05 mg/kg) and 99% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively. The mean CSCD459488 recovery in plant, grain and straw were 107% (n = 2) for all fortification levels (0.005–0.05 mg/kg), 113% (n = 2) for all fortification levels (0.005–0.05 mg/kg) and 106% (n = 2) for all fortification levels (0.005–0.05 mg/kg), respectively.

Examination of the trials data for parent isopyrazam in Table 105 shows that the ratio of *syn*-isomer to *anti*-isomer was essentially unchanged over time (i.e., where the formulation with a nominal isopyrazam *syn/anti* ratio of 95:5 was applied, measured residues of *syn*-isomer and *anti*-isomer at harvest were still approximately in the ratio of 95:5; where another with a nominal isopyrazam *syn/anti* ratio of 85:15 was applied, measured residues of *syn*-isomer and *anti*-isomer at harvest were still approximately in the ratio of 85:15; where the other with a nominal isopyrazam *syn/anti* ratio of 70:30 was applied, measured residues of *syn*-isomer and *anti*-isomer at harvest were still approximately in the ratio of 70:30). Thus, the decline rates of *syn*-isomer and *anti*-isomer in wheat field trials are approximately equal.

Where side-by-side plots in the same trials were treated with two different formulations containing isopyrazam at different *syn/anti* ratios, the different ratios in the formulation give similar total residues and decline rates.

Table 105 Residues of isopyrazam and CSCD459488 following foliar applications from supervised trials on wheat in Europe

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in UK	125EC	0.125	-*	2	-							
Northern Europe												
Wheat France 2006 (Lancelot) FR-FR-06-0032	125EC (A15149G)	0.127 0.121	32 55	2	61	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
Wheat France 2006 (Lancelot)	125EC (A15149G)	0.129 0.125 0.127	32 41 67	3	31	Immature Grain	0.347	0.027	0.374	0.254	0.628	Oliver-Kang J. CEMR-3391-REG 2008aT000672-

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
FR-FR-06-0032				3	51	Grain	0.007	< 0.005	0.012	0.006	0.018	06 A15149K/11031
Wheat France 2006 (Lancelot) FR-FR-06-0032	125EC (A15149K)	0.122 0.130 0.126	33 41 67	3	31	Immature Grain	0.079	0.038	0.117	0.070	0.187	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
				3	51	Grain	0.007	< 0.005	0.012	0.007	0.019	
Wheat France 2006 (Limes) FR-FR-06-0033	125EC (A15149G)	0.124 0.122	32 55	2	62	Grain	0.008	< 0.005	0.013	0.005	0.018	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
		0.125 0.124 0.128	32 41 67	3	51	Grain	0.012	< 0.005	0.017	0.009	0.026	
	125EC (A15149K)	0.122 0.125 0.125	33 41 67	3	51	Grain	0.008	< 0.005	0.013	0.006	0.019	
Wheat France 2006 (Charger) FR-FR-06-0034	125EC (A15149G)	0.131 0.124	31 49	2	61	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
		0.120 0.120 0.125	31 39 68	3	41	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
	125EC (A15149K)	0.119 0.123 0.129	31 39 68	3	41	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Wheat Germany 2006 (Dekan) DE-FR-06-4421	125EC (A15149G)	0.124 0.124	31 49–51	2	51	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Simon P. T001787-06-REG 2008a T001787-06 A15149/10595
Wheat Germany 2006 (Dekan) DE-FR-06-4421	125EC (A15149G)	0.124 0.124 0.124	31 39 69	3	29	Grain	0.005	< 0.005	0.010	< 0.005	0.015	Simon P. T001787-06-REG 2008a T001787-06 A15149/10595
				3	35	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Wheat Germany 2006 (Dekan) DE-FR-06-4421	125EC (A15149K)	0.124 0.124 0.124	31 39 69	3	29	Grain	0.006	< 0.005	0.011	< 0.005	0.016	Simon P. T001787-06-REG 2008a T001787-06 A15149/10595
				3	35	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Wheat Germany 2007 (Herman) AF/11520/SY/1	125EC (A15149K)	0.122 0.125 0.127	30–31 41 67	3	43	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
Wheat Germany 2007 (Atlantis) AF/11520/SY/2	125EC (A15149K)	0.125 0.126 0.125	30–31 39 69	3	43	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
Wheat United Kingdom 2007 (Malacca) AF/11520/SY/3	125EC (A15149K)	0.125 0.125 0.123	32 39 69	3	42	Grain	0.009	< 0.005	0.014	< 0.005	0.019	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
Wheat France 2007 (Courtot) AF/11520/SY/4	125EC (A15149K)	0.127 0.127 0.129	31 41 69	3	30	Immature Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
				3	44	Immature Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
				3	57	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Wheat France 2007 (Soissons) AF/11520/SY/5	125EC (A15149K)	0.123 0.124 0.125	31 39 69	3	30	Immature Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149G/10595
				3	44	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	
Southern Europe												
Wheat Spain 2006 (Fiufa) ES-FR-06-0035	125EC (A15149G)	0.134 0.133	31 49–53	2	52	Grain	0.009	< 0.005	0.014	< 0.005	0.019	Marshall L. CEMR-3392-REG 2008a T000673-06 A15149K/11279
Wheat Spain 2006 (Fiufa) ES-FR-06-0035	125EC (A15149G)	0.120 0.133 0.133	31 39 69	3	30	Immature Grain	0.021	< 0.005	0.026	0.017	0.043	Marshall L. CEMR-3392-REG 2008a T000673-06 A15149K/11279
				3	41	Grain	0.025	< 0.005	0.030	0.006	0.036	
Wheat Spain 2006 (Soissons) ES-FR-06-0036	125EC (A15149G)	0.133 0.135	30 47–49	2	55	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Marshall L. CEMR-3392-REG 2008a T000673-06
		0.135	30	3	29	Grain	0.018	< 0.005	0.023	< 0.005	0.028	
		0.128 0.124	37–39 69–71	3	35	Grain	0.023	< 0.005	0.028	0.008	0.036	
Wheat France 2006 (Aztec) FR-FR-06-0037	125EC (A15149G)	0.129 0.127	32 49	2	67	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Marshall L. CEMR-3392-REG 2008a T000673-06
		0.131 0.117 0.128	32 39 69	3	43	Grain	0.014	< 0.005	0.019	0.006	0.025	
		0.123 0.127	31 37	2	55	Grain	0.005	< 0.005	0.010	< 0.005	0.015	
		0.123 0.126 0.124	31 41 68	3	46	Grain	0.013	< 0.005	0.018	< 0.005	0.023	
Wheat Spain 2007 (Otira) AF/11521/SY/1	125EC (A15149K)	0.123 0.128 0.124	32 41 69	3	30	Grain	0.059	0.027	0.086	0.005	0.091	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	42	Grain	0.034	0.016	0.050	< 0.005	0.055	
Wheat Italy 2007 (Bologna) AF/11521/SY/2	125EC (A15149K)	0.129 0.124 0.126	32 41 69	3	42	Grain	0.080	0.036	0.116	0.038	0.154	Bell A. CEMR-3364-REG 2008b CEMS-3364
Wheat Italy 2007 (Duilio) AF/11521/SY/3	125EC (A15149K)	0.127 0.125 0.127	32 41 69	3	41	Grain	0.014	0.007	0.021	0.007	0.028	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	53	Grain	0.027	0.014	0.041	0.021	0.062	
Wheat France 2007	125EC (A15149K)	0.120 0.122 0.120	30–32 39 67–69	3	41	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	Bell A. CEMR-3364-REG

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
(Quality) AF/11521/SY/4												2008b CEMS-3364
Wheat France 2007 (Pr22r58) AF/11521/SY/5	125EC (A15149K)	0.125 0.126 0/123	32 39 67	3	45	Immature Grain	0.025	0.016	0.041	0.056	0.097	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	60	Grain	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	

* - the product is applied between growth stage 30-71, and must NOT be applied later than BBCH 71 (before grain watery ripe stage). A minimum of 21 days must be observed between applications.

** –Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

Animal food stuffs

Barley forage and straw

Table 106 Residues of isopyrazam and CSCD459488 in barley forage and straw following foliar applications from supervised trials on barley in New Zealand

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in NewZealand	125EC	0.075	-*	2	28	Forage						
Barley NewZealand 2008 (Optic) SYN-0801/S2	125EC (15149W)	0.077 0.077	49 55	2	0	Forage	2.380	0.301	2.681	0.125	2.806	Farrell P. SYN-0801 2009 SYN-0801 Isopyrazam/50008
				2	14	Forage	0.581[a]	0.147[a]	0.728	0.316[a]	1.044	
				2	28	Forage	0.523	0.132	0.655	0.376	1.031	
				2	41	Straw	0.747	0.178	0.925	0.187	1.112	
		0.128 0.127	49 55	2	0	Forage	7.130	0.990	8.120	0.187	8.307	
				2	14	Forage	1.420	0.300	1.720	0.470	2.190	
				2	28	Forage	0.841	0.217	1.058	0.460	1.518	
				2	41	Straw	1.350	0.393	1.743	0.182	1.925	
		0.255 0.254	49 55	2	0	Forage	12.40	1.77	14.17	0.364	14.534	
				2	14	Forage	2.280	0.383	2.663	0.550	3.213	
				2	28	Forage	1.350	0.316	1.666	0.690	2.356	
				2	41	Straw	3.140	0.708	3.848	0.518	4.366	
Barley NewZealand 2008 (Sherwood) SYN-0801/S5	125EC (15149W)	0.078 0.078	45 59	2	0	Forage	7.420	0.344	7.764	0.064	7.828	Farrell P. SYN-0801 2009 SYN-0801 Isopyrazam/50008
				2	14	Forage	0.197	0.0370	0.234	0.057	0.291	
				2	28	Forage	0.103[a]	0.027[a]	0.130	0.074[a]	0.204	
				2	42	Straw	< 0.005	< 0.005	< 0.010	0.174	0.184	
		0.130 0.130	45 59	2	0	Forage	2.830	0.350	3.180	0.146	3.326	
				2	14	Forage	0.862	0.165	1.027	0.237	1.264	
				2	28	Forage	0.289	0.069	0.358	0.187	0.545	
				2	42	Straw	0.500	0.122	0.622	0.401	1.023	
		0.259 0.259	Zadoks 45 Zadoks 59	2	0	Forage	11.000	1.470	12.470	0.226	12.696	
				2	14	Forage	1.090	0.199	1.280	0.295	1.584	
				2	28	Forage	0.536	0.126	0.662	0.342	1.004	
				2	42	Straw	1.300	0.293	1.593	0.776	2.369	
Barley NewZealand 2008 (County) SYN-0801/S6	125EC (15149W)	0.078 0.078	45 59	2	0	Forage	2.260	0.317	2.577	0.099	2.676	Farrell P. SYN-0801 2009 SYN-0801 Isopyrazam/50008
				2	14	Forage	0.573	0.110	0.683	0.172	0.855	
				2	28	Forage	0.248	0.056	0.304	0.198	0.502	
				2	42	Straw	1.130	0.242	1.372	0.796	2.168	
		0.130 0.130	45 59	2	0	Forage	4.710	0.660	5.370	0.284	5.654	
				2	14	Forage	1.090	0.205	1.295	0.252	1.547	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
				2	28	Forage	0.798	0.171	0.969	0.373	1.342	
				2	42	Straw	2.290	0.510	2.800	2.970	5.77	
		0.259 0.259	45 59	2	0	Forage	9.860	1.370	11.230	0.487	11.717	
				2	14	Forage	1.820	0.350	2.170	0.456	2.626	
				2	28	Forage	3.900	0.770	4.670	0.919	5.589	
				2	42	Straw	6.600[a]	1.250[a]	7.850	3.460[a]	11.310	

* -The product must NOT be applied later than BBCH 59 (ear emergence).

** - Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

na - Not applicable

[a] Mean of two replicate analyses

Table 107 Residues of isopyrazam and CSCD459488 in barley whole plant and straw following foliar applications from supervised trials on barley in Europe

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in UK	125EC	0.125	-*	2	-							
Northern Europe												
Barley Switzerland 2006 (Landi) CH-FR-06-0023	125EC (A15149G)	0.127 0.126	31 47–49	1	-0	Whole plant	0.033	< 0.005	0.038	< 0.005	0.043	Marshall L. CEMR-3393-REG 2008b T000674-06 A15149K/11192
				2	0	Whole plant	1.901	0.115	2.016	0.007	2.023	
				2	7	Whole plant	0.764	0.043	0.807	0.067	0.874	
				2	13	Whole plant	0.240	0.013	0.253	0.058	0.311	
				2	21	Whole plant	0.115	0.012	0.127	0.053	0.180	
				2	34	Whole plant	0.222	0.014	0.236	0.089	0.325	
				2	54	Straw	0.919	0.038	0.957	0.111	1.068	
	125EC (A15149G)	0.127 0.126	31 59	1	-0	Whole plant	0.046	< 0.005	0.051	< 0.005	0.056	
				2	0	Whole plant	2.062	0.081	2.143	0.007	2.150	
				2	10	Whole plant	0.302	0.014	0.316	0.040	0.356	
				2	20	Whole plant	0.169	0.007	0.176	0.032	0.208	
				2	30	Whole plant	0.333	0.020	0.353	0.049	0.402	
				2	42	Whole plant	0.375	0.025	0.400	0.053	0.453	
				2	48	Straw	1.033	0.023	1.056	0.073	1.129	
	125EC (A15149K)	0.126 0.126	31 59	1	-0	Whole plant	0.027	< 0.005	0.032	< 0.005	0.037	
				2	0	Whole plant	1.641	0.064	1.705	< 0.005	1.710	
				2	10	Whole plant	0.278	0.123	0.401	0.032	0.433	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
				2	20	Whole plant	0.165	0.071	0.236	0.030	0.266	
				2	30	Whole plant	0.166	0.081	0.247	0.040	0.287	
				2	42	Whole plant	0.507	0.166	0.673	0.052	0.725	
				2	48	Straw	0.379[b]	0.147[b]	0.526	0.066	0.592	
Barley Switzerland 2006 (Merlot) CH-FR-06-0024	125EC (A15149G)	0.126 0.127	31 47–49	1	-0	Whole plant	0.032	< 0.005	0.037	< 0.005	0.042	Marshall L. CEMR-3393-REG 2008b T000674-06 A15149/11192
				2	0	Whole plant	2.170	0.110	2.280	0.006	2.286	
				2	7	Whole plant	0.959	0.049	1.008	0.041	1.049	
				2	13	Whole plant	0.494	0.024	0.518	0.043	0.561	
				2	21	Whole plant	0.345	0.019	0.364	0.064	0.428	
				2	34	Whole plant	0.240[a]	0.012[a]	0.252	0.052	0.304	
				2	54	Straw	0.332	0.017	0.349	0.140	0.489	
	125EC (A15149G)	0.126 0.125	31 57–59	1	-0	Whole plant	0.022	< 0.005	0.027	< 0.005	0.032	
				2	0	Whole plant	1.662	0.051	1.713	0.005	1.718	
				2	10	Whole plant	0.223	0.016	0.239	0.034	0.273	
				2	20	Whole plant	0.133	0.010	0.143	0.039	0.182	
				2	30	Whole plant	0.233	0.020	0.253	0.058	0.311	
				2	42	Whole plant	0.163	0.013	0.176	0.040	0.216	
				2	48	Straw	0.165	0.011	0.176	0.078	0.254	
	125EC (A15149K)	0.125 0.128	31 57–59	1	-0	Whole plant	0.015	< 0.005	0.020	< 0.005	0.025	
				2	0	Whole plant	1.713	0.551	2.264	< 0.005	2.269	
				2	10	Whole plant	0.252	0.109	0.361	0.030	0.391	
				2	20	Whole plant	0.103[a]	0.058[a]	0.161	0.026	0.187	
				2	30	Whole plant	0.137	0.096	0.233	0.039	0.272	
				2	42	Whole plant	0.209	0.108	0.317	0.043	0.360	
				2	48	Straw	0.208	0.123	0.331	0.081	0.412	
Barley France 2006 (Esterel) FR-FR-06-0026	125EC (A15149G)	0.127 0.129	31 49	2	60	Straw	0.364	0.027	0.391	0.540	0.931	Marshall L. CEMR-3393-REG 2008b T000674-06 A15149/11192
	125EC (A15149G)	0.124 0.126	31 59	2	43	Immature Straw	0.221	0.018	0.239	0.238	0.477	
				2	54	Straw	0.499[b]	0.339[b]	0.838	0.470	1.308	
	125EC (A15149K)	0.124 0.131	37 59	2	43	Immature Straw	0.350	0.228	0.578	0.259	0.837	
				2	54	Straw	0.673[b]	0.050[b]	0.723	0.419	1.142	
Barley Germany 2006 (Carat) DE-FR-06-4422	125EC (A15149G)	0.124 0.124	31–32 49	2	52	Straw	0.108	0.009	0.117	0.146	0.263	Simon P. T001788-06-REG 2008b T001788-06 A15149G/10000
		0.124 0.124	31–32 59	2	45	Straw	0.107	0.008	0.115	0.194	0.309	
	125EC (A15149K)	0.124 0.124	31–32 59	2	45	Straw	0.084	0.045	0.129	0.126	0.255	
Barley Germany 2007 (Braemar)	125EC (A15149K)	0.120 0.124	31 57- 59	1	-0	Whole plant	0.042	0.054	0.096	0.051	0.147	Bell A. CEMR-3367-REG 2008c
				2	0	Whole plant	2.252	1.007	3.259	0.056	3.315	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
AF/11518/SY/1				2	10	Whole plant	0.278	0.166	0.444	0.072	0.516	CEMS-3367 A15149K/10525
				2	20	Whole plant	0.229	0.150	0.379	0.089	0.468	
				2	30	Whole plant	0.196	0.138	0.334	0.079	0.413	
				2	45	Straw	0.264	0.202	0.362	0.133	0.599	
Barley Germany 2007 (Braemar) AF/11518/SY/2	125EC (A15149K)	0.124 0.124	32 69	1	-0	Whole plant	0.219	0.172	0.391	0.097	0.488	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
				2	0	Whole plant	2.422	1.205	3.627	0.098	3.725	
				2	10	Whole plant	0.279	0.223	0.502	0.126	0.628	
				2	20	Whole plant	0.060	0.064	0.124	0.092	0.216	
				2	30	Remaining Plant	0.054	0.063	0.117	0.120	0.237	
				2	38	Straw	0.094	0.112	0.206	0.110	0.316	
Barley UK 2007 (Westmi-nster) AF/11518/SY/3	125EC (A15149K)	0.124 0.123	32 57–59	1	-0	Whole plant	0.062	0.057	0.119	0.052	0.171	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
				2	0	Whole plant	2.178	0.747	2.925	0.057	2.982	
				2	10	Whole plant	0.114	0.080	0.194	0.055	0.249	
				2	20	Whole plant	0.094	0.061	0.155	0.057	0.212	
				2	30	Whole plant	0.052	0.038	0.090	0.032	0.122	
				2	42	Straw	0.044	0.032	0.076	0.021	0.097	
Barley France 2007 (Colibri) AF/11518/SY/4	125EC (A15149K)	0.124 0.124	32 57–59	1	0	Whole plant	1.592	0.711	2.303	0.031	2.334	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
				2	10	Whole plant	0.733	0.374	1.107	0.049	1.156	
				2	20	Whole plant	0.254	0.155	0.409	0.043	0.452	
				2	30	Whole plant	0.207	0.119	0.326	0.033	0.359	
				2	44	Remaining Plant	0.429	0.233	0.662	0.073	0.735	
				2	61	Straw	0.449	0.230	0.679	0.033	0.712	
Barley France 2007 (Platine) AF/11518/SY/5	125EC (A15149K)	0.124 0.120	31 58	1	-0	Whole plant	0.083	0.054	0.137	0.024	0.161	Bell A. CEMR-3367-REG 2008c CEMS-3367 A15149K/10525
				2	0	Whole plant	1.670	0.778	2.448	0.029	2.477	
				2	10	Whole plant	0.861	0.460	1.321	0.073	1.394	
				2	20	Whole plant	0.213	0.146	0.359	0.061	0.420	
				2	30	Remaining Plant	0.492	0.322	0.814	0.120	0.934	
				2	42	Straw	0.306	0.189	0.495	0.096	0.591	
Southern Europe												
Barley Italy 2006 (Boreale) IT-FR-06-0027	125EC (A15149G)	0.130 0.125	30 47–49	1	-0	Whole plant	1.230	0.062	1.292	0.049	1.341	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149/10525
				2	0	Whole plant	1.489	0.085	1.574	0.044	1.618	
				2	7	Whole plant	0.522	0.033	0.555	0.058	0.613	
				2	14	Whole plant	0.225	0.016	0.241	0.051	0.292	
				2	21	Whole plant	0.113	0.007	0.120	0.050	0.170	
				2	35	Whole plant	0.085	0.007	0.092	0.034	0.126	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
				2	53	Straw	0.110	0.010	0.120	0.190	0.310	
	125EC (A15149G)	0.133 0.132	30 57–59	1	-0	Whole plant	0.154	0.012	0.166	0.026	0.192	
				2	0	Whole plant	2.787	0.157	2.944	0.037	2.981	
				2	10	Whole plant	0.287	0.024	0.311	0.069	0.380	
				2	20	Whole plant	0.144	0.010	0.154	0.073	0.227	
				2	31	Remaining Plant	0.081	0.007	0.088	0.093	0.181	
				2	45	Straw	0.138	0.007	0.145	0.188	0.333	
Barley Spain 2006 (Verticale) ES-FR-06-0028	125EC (A15149G)	0.124 0.131	31 47–49	1	-0	Whole plant	0.773	0.046	0.819	0.046	0.865	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149/11030
				2	0	Whole plant	3.150	0.189	3.339	0.044	3.383	
				2	7	Whole plant	1.155	0.082	1.237	0.077	1.314	
				2	14	Whole plant	1.104	0.082	1.186	0.127	1.313	
				2	21	Whole plant	0.873	0.066	0.939	0.121	1.060	
				2	35	Whole plant	0.832	0.062	0.894	0.105	0.999	
				2	57	Straw	2.367	0.145	2.512	1.159	3.671	
		0.127 0.133	31 58–61	1	-0	Whole plant	1.006	0.063	1.069	0.063	1.132	
				2	0	Whole plant	3.282	0.212	3.494	0.069	3.563	
				2	10	Whole plant	2.288	0.153	2.441	0.142	2.583	
				2	20	Whole plant	1.559	0.094	1.653	0.128	1.781	
				2	30	Remaining Plant	3.000	0.113	3.113	0.250	3.363	
				2	42	Straw	2.868	0.107	2.975	0.329	3.304	
				2	50	Straw	3.360	0.135	3.495	0.538	4.033	
Barley France 2006 (Baraka) FR-FR-06-0029	125EC (A15149G)	0.131 0.129	30 49	2	52	Straw	1.714	0.121	1.835	0.520	2.355	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149K/11030
		0.130 0.131	30 59	2	41	Straw	5.548	0.314	5.862	0.874	6.736	
Barley France 2006 (Nevada) FR-FR-06-0030	125EC (A15149G)	0.126 0.127	31 47	2	56	Straw	1.375	0.048	1.423	0.551	1.974	Oliver-Kang J. CEMR-3394-REG 2008b T000675-06 A15149K/11030
		0.127 0.127	31 57	2	42	Immature Straw	1.299	0.057	1.356	0.409	1.765	
				2	50	Straw	1.034	0.075	1.109	0.644	1.753	
Barley Spain 2007 (Chamorro) AF/11519/SY/1	125EC (A15149K)	0.130 0.125	31 58	2	-0	Whole plant	0.136	0.120	0.256	0.046	0.302	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
				2	0	Whole plant	3.263	1.467	4.730	0.031	4.761	
				2	10	Whole plant	2.800	1.388	4.188	0.086	4.274	
				2	20	Whole plant	3.741	1.753	5.494	0.110	5.604	
				2	30	Straw	4.125	2.003	6.128	0.269	6.397	
				2	42	Straw	4.582	2.318	6.900	0.256	7.156	
Barley Italy 2007 (Sonora) AF/11519/SY/2	125EC (A15149K)	0.126 0.122	32 59	2	-0	Whole plant	0.012	0.007	0.019	< 0.005	0.024	Bell A. CEMR-3365-REG 2008d CEMS-3365
				2	0	Whole plant	3.468	1.559	5.027	< 0.005	5.032	
				2	10	Whole	1.429	0.208	2.073	0.078	1.715	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
						plant						A15149/10524
				2	20	Whole plant	2.174	1.122	3.296	0.274	3.570	
				2	30	Whole plant	0.968	0.469	1.437	0.148	1.585	
				2	42	Straw	1.988	0.504	2.492	0.483	2.975	
Barley France 2007 (Scarlett) AF/11519/SY/3	125EC (A15149K)	0.124 0.124	30 58	2	-0	Whole plant	0.007	0.006	0.013	0.009	0.022	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
				2	0	Whole plant	2.407	1.114	3.521	0.016	3.537	
				2	10	Whole plant	0.354	0.209	0.563	0.065	0.628	
				2	20	Whole plant	0.318	0.191	0.509	0.083	0.592	
				2	30	Remaining Plant	0.316	0.173	0.489	0.065	0.554	
				2	43	Straw	0.157	0.105	0.262	0.047	0.309	
Barley France 2007 (Prestige) AF/11519/SY/4	125EC (A15149K)	0.122 0.124	32 57–59	2	-0	Whole plant	0.104	0.056	0.160	0.014	0.174	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149/10524
				2	0	Whole plant	2.698	1.239	3.937	0.013	3.950	
				2	10	Whole plant	0.257	0.152	0.409	0.051	0.460	
				2	20	Whole plant	0.127	0.073	0.200	0.043	0.243	
				2	30	Whole plant	0.105	0.068	0.173	0.045	0.218	
				2	45	Straw	0.285	0.161	0.446	0.145	0.591	
Barley France 2007 (Platine) AF/11519/SY/5	125EC (A15149K)	0.118 0.124	31 59	2	-0	Whole plant	0.043	0.030	0.073	0.014	0.087	Bell A. CEMR-3365-REG 2008d CEMS-3365 A15149K/10524
				2	0	Whole plant	2.194	0.932	3.126	0.009	3.135	
				2	10	Whole plant	0.269	0.158	0.427	0.033	0.460	
				2	20	Whole plant	0.103	0.080	0.183	0.038	0.221	
				2	30	Whole plant	0.104	0.070	0.174	0.040	0.214	
				2	45	Remaining Plant	0.045	0.031	0.076	0.035	0.111	
				2	63	Straw	0.070	0.055	0.125	0.066	0.191	

* - The product is applied between growth stage 30 -61, and must NOT be applied later than BBCH 61 (before beginning of flowering period).A minimum of 21 days must be observed between applications.

** -Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

-0 - Indicates sample taken prior to last application

na - Not applicable

[a] - Mean of two replicate analyses

[b] - Mean of three replicate analyses.

Wheat forage and straw

Table 108 Residues of isopyrazam and CSCD459488 in wheat forage and straw following foliar applications from supervised trials on wheat in New Zealand

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in NewZealand	125EC	0.075-0.125	-*	2	28	Forage						
Wheat NewZealand 2008 (variety not re-ported) SYN-0801/S1	125EC (A15149W)	0.077 0.076	49 59	2	0	Forage	4.750	0.660	5.410	0.152	5.562	Farrell P. SYN-0801 2009 Isopyrazam/11457
				2	14	Forage	0.670	0.121	0.791	0.123	0.914	
				2	28	Forage	0.108	0.025	0.133	0.102	0.235	
				2	42	Straw	0.548	0.101	0.649	0.448	1.097	
		0.128 0.127	49 59	2	0	Forage	6.260	0.880	7.140	0.223	7.363	
				2	14	Forage	0.544	0.115	0.659	0.152	0.811	
				2	28	Forage	0.334[a]	0.063[a]	0.397	0.157[a]	0.555	
				2	42	Straw	0.853[a]	0.140[a]	0.993	0.796[a]	1.790	
		0.255 0.254	49 59	2	0	Forage	12.100	1.720	13.820	0.372	14.192	
				2	14	Forage	1.390	0.231	1.621	0.221	1.842	
				2	28	Forage	1.030	0.171	1.201	0.286	1.487	
				2	42	Straw	3.540	0.546	4.086	2.140	6.226	
Wheat NewZealand 2008 (Phoenix) SYN-0801/S3	125EC (A15149W)	0.078 0.078	49 63	2	0	Forage	2.220	0.286	2.506	0.096	2.602	Farrell P. SYN-0801 2009 Isopyrazam/11457
				2	14	Forage	0.870	0.132	1.002	0.101	1.103	
				2	28	Forage	0.326	0.056	0.382	0.074	0.456	
				2	42	Straw	0.908	0.144	1.052	0.308	1.360	
		0.130 0.130	49 63	2	0	Forage	4.610	0.610	5.220	0.164	5.384	
				2	14	Forage	1.070	0.169	1.239	0.196	1.435	
				2	28	Forage	0.721	0.114	0.835	0.159	0.994	
				2	42	Straw	1.540	0.248	1.788	0.623	2.411	
		0.259 0.259	49 63	2	0	Forage	8.720	1.210	9.930	0.338	10.268	
				2	14	Forage	2.560[a]	0.398[a]	2.958	0.438[a]	3.397	
				2	28	Forage	1.92	0.288	2.208	0.363	2.571	
				2	42	Straw	3.950	0.590	4.540	2.390	6.930	
Wheat NewZealand 2008 (Raffles) SYN-0801/S4	125EC (A15149W)	0.078 0.078	39 69	2	0	Forage	2.260	0.300	2.560	0.079	2.639	Farrell P. SYN-0801 2009 Isopyrazam/11457
				2	14	Forage	0.134	0.034	0.168	0.069	0.237	
				2	28	Forage	0.044	0.010	0.054	0.036	0.090	
				2	42	Straw	0.138	0.033	0.171	0.144	0.315	
		0.130 0.130	39 69	2	0	Forage	3.840	0.520	4.360	0.154	4.514	
				2	14	Forage	0.231	0.053	0.284	0.085	0.369	
				2	28	Forage	0.128	0.031	0.159	0.100	0.259	
				2	42	Straw	0.233[a]	0.051[a]	0.284	0.172[a]	0.457	
		0.259 0.259	39 69	2	0	Forage	9.370	1.290	10.660	0.303	10.963	
				2	14	Forage	1.088[a]	0.209[a]	1.297	0.254[a]	1.552	
				2	28	Forage	0.597	0.115	0.712	0.165	0.877	
				2	42	Straw	1.390	0.252	1.642	0.575	2.217	

* - The product must NOT be applied later than BBCH 69 (end of flowering).

** -Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

na - Not applicable

[a] - Mean of two replicate analyses

The Zadoks growth scale for cereals is numerically identical to the BBCH scale

Table 109 Residues of isopyrazam and CSCD459488 in wheat whole plant and straw following foliar applications from supervised trials on wheat in Europe

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
GAP in UK	125EC	0.125	-*	2	-							
Northern Europe												
Wheat France 2006 (Lancelot) FR-FR-06-0032	125EC (A15149G)	0.127 0.121	32 55	1	-0	Whole plant	0.063	0.011	0.074	0.023	0.097	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
				2	0	Whole plant	1.951	0.106	2.057	0.027	2.084	
				2	7	Whole plant	1.664	0.099	1.763	0.103	1.866	
				2	14	Whole plant	1.009	0.072	1.081	0.154	1.235	
				2	21	Whole plant	0.823	0.057	0.880	0.182	1.062	
				2	35	Whole plant	0.356	0.029	0.385	0.165	0.550	
				2	61	Straw	0.904	0.061	0.965	1.521	2.486	
Wheat France 2006 (Lancelot) FR-FR-06-0032	125EC (A15149G)	0.129 0.125 0.127	32 41 67	2	-0	Whole plant	0.520	0.041	0.561	0.105	0.666	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
				3	0	Whole plant	2.088	0.136	2.224	0.102	2.326	
				3	11	Whole plant	0.993	0.073	1.066	0.236	1.302	
				3	21	Whole plant	0.473	0.036	0.509	0.194	0.703	
				3	31	Remaining Plant	1.061	0.076	1.137	0.459	1.596	
				3	51	Straw	0.857	0.064	0.921	1.647	2.568	
Wheat France 2006 (Lancelot) FR-FR-06-0032	125EC (A15149K)	0.122 0.130 0.126	33 41 67	2	-0	Whole plant	0.469	0.231	0.700	0.096	0.796	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
				3	0	Whole plant	2.093	0.854	2.947	0.100	3.047	
				3	11	Whole plant	0.853	0.438	1.291	0.211	1.502	
				3	21	Whole plant	0.553	0.305	0.858	0.180	1.038	
				3	31	Remaining Plant	0.785	0.417	1.202	0.385	1.587	
				3	51	Straw	0.630[a]	0.347[a]	0.977	1.360	2.337	
Wheat France 2006 (Limes) FR-FR-06-0033	125EC (A15149G)	0.124 0.122	32 55	2	62	Straw	0.708	0.042	0.750	1.185	1.935	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
		0.125 0.124 0.128	32 41 67	3	51	Straw	0.780	0.051	0.831	1.307	2.138	
	125EC (A15149K)	0.122 0.125 0.125	33 41 67	3	51	Straw	0.652[a]	0.300[a]	0.952	1.175	2.127	
Wheat France 2006 (Charger) FR-FR-06-0034	125EC (A15149G)	0.131 0.124	31 49	2	61	Straw	0.046	0.005	0.051	0.262	0.313	Oliver-Kang J. CEMR-3391-REG 2008a T000672-06 A15149K/11031
		0.120 0.120 0.125	31 39 68	3	41	Straw	0.2400	0.02	0.260	0.586	0.846	
	125EC (A15149K)	0.119 0.123 0.129	31 39 68	3	41	Straw	0.098	0.059	0.157	0.331	0.488	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
Wheat Germany 2006 (Dekan) DE-FR-06-4421	125EC (A15149G)	0.124 0.124	31 49–51	1	-0	Whole plant	0.084	0.007	0.091	0.039	0.130	Simon P. T001787-06-REG 2008a T001787-06 A15149/10595
				2	0	Whole plant	1.786	0.094	1.880	0.041	1.921	
				2	6	Whole plant	1.132	0.070	1.202	0.071	1.273	
				2	13	Whole plant	0.650	0.041	0.691	0.122	0.813	
				2	20	Whole plant	0.379	0.022	0.401	0.134	0.535	
				2	34	Whole plant	0.154	0.010	0.164	0.100	0.264	
				2	51	Straw	0.268	0.025	0.293	0.703	0.996	
Wheat Germany 2006 (Dekan) DE-FR-06-4421	125EC (A15149G)	0.124 0.124 0.124	31 39 69	2	-0	Whole plant	0.075	0.006	0.081	0.061	0.142	Simon P. T001787-06-REG 2008a T001787-06 A15149/10595
				3	0	Whole plant	2.139	0.106	2.245	0.064	2.309	
				3	10	Whole plant	0.538	0.033	0.571	0.133	0.704	
				3	18	Whole plant	0.409	0.024	0.433	0.149	0.582	
				3	29	Remaining Plant	0.849	0.039	0.888	0.406	1.294	
				3	35	Straw	1.422	0.092	1.514	0.877	2.391	
Wheat Germany 2006 (Dekan) DE-FR-06-4421	125EC (A15149K)	0.124 0.124 0.124	31 39 69	2	-0	Whole plant	0.057	0.033	0.090	0.420	0.510	Simon P. T001787-06-REG 2008a T001787-06 A15149/10595
				3	0	Whole plant	1.533	0.562	2.095	0.055	2.150	
				3	10	Whole plant	0.619	0.270	0.889	0.128	1.017	
				3	18	Whole plant	0.493	0.186	0.679	0.152	0.831	
				3	29	Remaining Plant	0.596	0.190	0.786	0.473	1.259	
				3	35	Straw	0.700	0.362	1.062	0.709	1.771	
Wheat Germany 2007 (Herman) AF/11520/SY/1	125EC (A15149K)	0.122 0.125 0.127	30–31 41 67	2	-0	Whole plant	0.155	0.093	0.248	0.038	0.286	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
				3	0	Whole plant	0.765	0.409	1.174	0.052	1.226	
				3	10	Whole plant	0.269	0.177	0.446	0.062	0.508	
				3	20	Whole plant	0.167	0.102	0.269	0.043	0.312	
				3	30	Whole plant	0.095	0.048	0.143	0.033	0.176	
				3	43	Straw	0.186	0.102	0.288	0.037	0.325	
Wheat Germany 2007 (Atlantis) AF/11520/SY/2	125EC (A15149K)	0.125 0.126 0.125	30–31 39 69	2	-0	Whole plant	0.117	0.087	0.204	0.067	0.271	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
				3	0	Whole plant	0.973	0.556	1.529	0.071	1.600	
				3	10	Whole plant	0.126	0.132	0.258	0.075	0.333	
				3	20	Whole plant	0.134	0.081	0.215	0.071	0.286	
				3	30	Whole plant	0.046	0.029	0.075	0.049	0.124	
				3	43	Straw	0.068	0.045	0.113	0.059	0.172	
Wheat United Kingdom 2007 (Malacca) AF/11520/SY/3	125EC (A15149K)	0.125 0.125 0.123	32 39 69	2	-0	Whole plant	0.139	0.112	0.251	0.131	0.382	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
				3	0	Whole plant	0.875	0.459	1.334	0.181	1.515	
				3	10	Whole plant	0.267	0.149	0.416	0.116	0.532	
				3	20	Whole	0.152	0.147	0.299	0.189	0.488	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
						plant						
				3	30	Whole plant	0.198	0.123	0.321	0.117	0.438	
				3	42	Straw	0.722	0.386	1.108	0.110	1.218	
Wheat France 2007 (Courtot) AF/11520/SY/4	125EC (A15149K)	0.127 0.127 0.129	31 41 69	2	-0	Whole plant	0.201	0.141	0.342	0.065	0.407	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149K/10595
				3	0	Whole plant	1.618	0.837	2.455	0.110	2.565	
				3	10	Whole plant	0.464	0.294	0.758	0.086	0.844	
				3	20	Whole plant	0.341	0.242	0.583	0.097	0.680	
				3	30	Remaining Plant	0.501	0.233	0.734	0.090	0.824	
				3	44	Remaining Plant	0.754	0.330	1.084	0.081	1.165	
				3	57	Straw	0.655	0.292	0.947	0.036	0.983	
Wheat France 2007 (Soissons) AF/11520/SY/5	125EC (A15149K)	0.123 0.124 0.125	31 39 69	2	-0	Whole plant	0.228	0.142	0.370	0.087	0.457	Bell A. CEMR-3366-REG 2008a CEMS-3366 A15149G/10595
				3	0	Whole plant	0.254	0.106	0.360	0.100	0.460	
				3	10	Whole plant	0.720	0.319	1.039	0.126	1.165	
				3	20	Whole plant	0.332	0.169	0.501	0.135	0.636	
				3	30	Remaining Plant	1.137	0.627	1.764	0.249	2.013	
				3	44	Straw	0.915	0.490	1.405	0.178	1.583	
Southern Europe												
Wheat Spain 2006 (Fiufa) ES-FR-06-0035	125EC (A15149G)	0.134 0.133	31 49–53	1	-0	Whole plant	0.430	0.022	0.452	0.053	0.505	Marshall L. CEMR-3392-REG 2008a T000673-06 A15149K/11279
				2	0	Whole plant	1.817	0.103	1.920	0.044	1.964	
				2	7	Whole plant	2.203	0.123	2.326	0.127	2.453	
				2	14	Whole plant	1.589	0.087	1.676	0.121	1.797	
				2	21	Whole plant	1.382	0.068	1.450	0.118	1.568	
				2	35	Whole plant	2.058	0.116	2.174	0.245	2.419	
				2	52	Straw	5.236	0.272	5.508	0.513	6.021	
Wheat Spain 2006 (Fiufa) ES-FR-06-0035	125EC (A15149G)	0.120 0.133 0.133	31 39 69	2	-0	Whole plant	0.847	0.056	0.903	0.122	1.025	Marshall L. CEMR-3392-REG 2008a T000673-06 A15149K/11279
				3	0	Whole plant	4.711	0.252	4.963	0.170	5.133	
				3	10	Whole plant	2.112	0.118	2.230	0.129	2.359	
				3	23	Whole plant	2.024	0.121	2.145	0.318	2.463	
				3	30	Remaining Plant	4.042	0.224	4.266	0.641	4.907	
				3	41	Straw	7.914	0.452	8.366	0.889	9.255	
Wheat Spain 2006 (Soissons) ES-FR-06-0036	125EC (A15149G)	0.133 0.135	30 47–49	1	-0	Whole plant	0.108	0.009	0.117	0.030	0.147	Marshall L. CEMR-3392-REG 2008a T000673-06
				2	0	Whole plant	2.028	0.072	2.100	0.041	2.141	
				2	7	Whole plant	1.555	0.068	1.623	0.081	1.704	
				2	14	Whole plant	1.695	0.064	1.759	0.129	1.888	
				2	21	Whole plant	1.744	0.071	1.815	0.145	1.960	
				2	35	Whole	1.461	0.059	1.520	0.206	1.726	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
						plant						
				2	55	Straw	3.425	0.188	3.613	0.378	3.991	
		0.135 0.128 0.124	30 37–39 69–71	2	-0	Whole plant	< 0.005	< 0.005	< 0.010	0.115	0.125	
				3	0	Whole plant	3.935	0.145	4.080	0.118	4.198	
				3	11	Whole plant	2.697	0.100	2.797	0.224	3.021	
				3	20	Whole plant	2.982	0.118	3.100	0.230	3.330	
				3	29	Remaining Plant	3.492	0.191	3.683	0.400	4.083	
				3	35	Straw	6.711	0.366	7.077	0.500	7.577	
Wheat France 2006 (Aztec) FR-FR-06-0037	125EC (A15149G)	0.129 0.127	32 49	2	67	Straw	0.595	0.025	0.620	0.778[a]	1.398	Marshall L. CEMR-3392-REG 2008a T000673-06
		0.131 0.117 0.128	32 39 69	3	43	Straw	5.000	0.138	5.138	2.056[a]	7.194	
		0.123 0.127	31 37	2	55	Straw	3.941	0.219	4.160	1.527[a]	5.687	
		0.123 0.126 0.124	31 41 68	3	46	Straw	3.439	0.177	3.616	1.449[a]	5.065	
Wheat Spain 2007 (Otira) AF/11521/SY/1	125EC (A15149K)	0.123 0.128 0.124	32 41 69	2	-0	Whole plant	1.120	0.660	1.780	0.210	1.990	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	0	Whole plant	3.479	1.563	5.042	0.186	5.228	
				3	10	Whole plant	4.892	2.510	7.402	0.347	7.749	
				3	20	Whole plant	2.545	1.437	3.982	0.387	4.369	
				3	30	Straw	4.421	2.255	6.676	0.520	7.196	
				3	42	Straw	3.667	1.975	5.642	0.469	6.111	
Wheat Italy 2007 (Bologna) AF/11521/SY/2	125EC (A15149K)	0.129 0.124 0.126	32 41 69	2	-0	Whole plant	0.040	0.024	0.064	0.035	0.099	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	0	Whole plant	2.544	0.995	3.539	0.031	3.570	
				3	20	Whole plant	1.629	0.736	2.365	0.136	2.501	
				3	30	Whole plant	1.015	0.434	1.449	0.214	1.663	
				3	42	Straw	2.056	0.776	2.832	0.791	3.623	
Wheat Italy 2007 (Duilio) AF/11521/SY/3	125EC (A15149K)	0.127 0.125 0.127	32 41 69	2	-0	Whole plant	0.038	0.032	0.070	0.033	0.103	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	0	Whole plant	2.617	1.082	3.699	0.031	3.730	
				3	10	Whole plant	1.179	0.609	1.788	0.123	1.911	
				3	20	Whole plant	0.345	0.181	0.526	0.108	0.634	
				3	30	Whole plant	0.516	0.280	0.796	0.193	0.989	
				3	41	Straw	0.872	0.446	1.318	0.408	1.726	
				3	53	Straw	0.867	0.328	1.195	0.511	1.706	
Wheat France 2007 (Quality) AF/11521/SY/4	125EC (A15149K)	0.120 0.122 0.120	30–32 39 67–69	2	-0	Whole plant	0.277	0.148	0.425	0.061	0.486	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	0	Whole plant	1.739	0.795	2.534	0.055	2.589	
				3	10	Whole plant	0.346	0.173	0.519	0.052	0.571	
				3	20	Whole plant	0.302	0.162	0.464	0.050	0.514	
				3	30	Whole plant	0.212	0.114	0.326	0.078	0.404	

Crop Country, Year (Variety) Trial No.	Application				PHI (days)	Portion analysed	Residues (mg/kg)					Author Report Year Study No. Doc ID
	Form.	Rate (kg ai/ha)	Growth Stage (BBCH)	No.			*Syn*-isomer	*Anti*-isomer	Isopyrazam**	CSCD 459488	Total	
				3	41	Straw	0.125	0.074	0.199	0.234	0.433	
Wheat France 2007 (Pr22r58) AF/11521/SY/5	125EC (A15149K)	0.125 0.126 0/123	32 39 67	2	-0	Whole plant	0.188	0.115	0.303	0.043	0.346	Bell A. CEMR-3364-REG 2008b CEMS-3364
				3	0	Whole plant	2.287	1.069	3.356	0.046	3.402	
				3	10	Whole plant	0.474	0.260	0.734	0.062	0.796	
				3	20	Whole plant	0.369	0.201	0.570	0.078	0.648	
				3	30	Whole plant	0.192	0.103	0.295	0.057	0.352	
				3	45	Remaining Plant	0.278	0.136	0.414	0.164	0.578	
				3	60	Straw	< 0.005	< 0.005	< 0.010	< 0.005	< 0.015	

* - the product is applied between growth stage 30-71, and must NOT be applied later than BBCH 71 (before grain watery ripe stage). A minimum of 21 days must be observed between applications.

** -Total parent residue (calculated; sum of *anti*-isomer and *syn*-isomer)

-0 - Indicates sample taken prior to last application

na- Not applicable

[a] - Mean of three replicate analyses

FATE OF RESIDUES IN STORAGE AND PROCESSING

The Meeting received information on a high-temperature hydrolysis and processing of barley and wheat.

High-temperature hydrolysis

A high-temperature aqueous hydrolysis study with isopyrazam was conducted in 2007 (MacDonald A., 2008). The purpose of the study was to determine the nature of any metabolites derived from isopyrazam in processed commodities or by-products, under conditions typical of industrial or household processing.

Individual aqueous solutions of [phenyl-U-^{14}C] isopyrazam (*syn*/*anti:* 96/4) and [pyrazole-5-^{14}C] isopyrazam (*syn*/*anti:* 69/31) (nominal concentration 0.7 µg/mL) were prepared in acetate buffers at pH 4, 5 and 6. The solutions were placed in reaction vessels before being heated to 90, 100 and 120 °C respectively. The pH4 and 6 solutions were heated for 20 minutes, while the pH5 solution was heated for 60 minutes. These conditions are representative of pasteurisation, baking/brewing/boiling and sterilisation, respectively.

Further individual aqueous solutions of [phenyl-U-^{14}C] isopyrazam and [pyrazole-5-^{14}C] isopyrazam (nominal concentration 0.7 µg/mL) were prepared for each buffer and maintained at ambient temperature for the same length of time as the test vessels to serve as control samples. The initial concentration of isopyrazam in all experiments ranged from 0.635 to 0.699 µg/mL.

The total recovery of radioactivity was ≥ 95.9% for all hydrolysed samples. The total recovery of radioactivity for the control samples was ≥ 94.7%. These results indicated that there were no significant losses of radioactivity during the experimental procedures. Characterisation and identification of the radiolabelled components in all samples was conducted by co-chromatography against reference standards using TLC. Confirmatory chromatography was conducted using HPLC.

In the control samples (both labels), the major component was determined to be isopyrazam, representing ≥ 96.3% of the radioactivity. Unidentified components were present at very low levels representing ≤ 1.0% of the radioactivity.

In the hydrolysed samples (both labels), the major component was determined to be isopyrazam, representing ≥ 95.8% of the radioactivity. Unidentified components were present at very low levels representing ≤ 1.1% of the radioactivity. No significant differences were therefore observed between the unheated controls and heated test samples.

Isopyrazam is concluded to be stable against hydrolysis under conditions representative of pasteurisation, baking/brewing/boiling and sterilisation. Therefore, processed commodities do not require analysis for any additional metabolites compared to raw agricultural commodities.

Barley

A barley processing study was conducted in 2007, in Northern France (Eversfield S., 2008a). This study, summarised in detail below, included two balance and two follow-up trials for brewing and production of pot barley. In order to allow the maximum likelihood of detecting products arising from the *anti* isomer *anti*-isomer, the processing studies were conducted with isopyrazam having a *syn*/*anti* ratio of 70:30.

A residue field study was conducted in Northern France during 2007, in which isopyrazam formulated as an emulsifiable concentrate (EC) containing 125 g isopyrazam per litre, was applied to winter barley. Two applications, at growth stage BBCH 32 and BBCH 68 and separated by intervals of 32 days, were made at 250 g ai/ha for each application. Forty-eight days after the final application, samples (~100 kg) of treated and untreated barley grain were harvested and transported at ambient temperature to the processing facility. In addition, grain samples (1 kg) were frozen for residue analysis.

Barley grain was processed into beer and pot barley (20–25% husk removal). Two balance studies and two follow-up studies were carried out on each process.

Samples were analysed for isopyrazam (as *syn*-isomer and *anti*-isomer) using method GRM006.01B and for CSCD459488 and CSCD459489 using method GRM006.03A.

Separate mass balances for isopyrazam and CSCD459488 residues were calculated. Transfer factors were calculated for isopyrazam and for the sum of isopyrazam and CSCD459488.

The isopyrazam and CSCD459488 residues in barley grain prior to processing and in beer and pot barley with intermediate processed fractions and the processing by-products are presented in Tables 110 and 111.

Residues of isopyrazam in the grain samples prior to processing were 0.077 mg/kg. Residues of CSCD459488 in the grain samples prior to processing were 0.048 mg/kg. Residues of CSCD459489 in the grain samples prior to processing and in the processed fractions were all below the LOQ; therefore no calculations of mass balance or transfer factors were possible.

The isopyrazam residues in beer were significantly lower (< 0.01 mg/kg) than in the grain samples prior to processing. A total of 97–98% of the isopyrazam on the barley grain was accounted for in the malting and brewing balance studies. The CSCD459488 residues in beer were also significantly lower (< 0.005 mg/kg) than in the grain samples prior to processing. A total of 61–62% of the CSCD459488 on the barley grain was accounted for in the malting and brewing balance studies.

The isopyrazam residues in pot barley were also significantly lower (mean 0.031 mg/kg) than in the grain samples prior to processing. A total of 77 and 74% of the isopyrazam on the barley grain were accounted for in the pot barley balance studies. The CSCD459488 residues in pot barley were also significantly lower (mean 0.014 mg/kg) than in the grain samples prior to processing. A total of 108–117% of the CSCD459488 on the barley grain was accounted for in the pot barley balance studies.

Processing factors were calculated for parent isopyrazam and for the sum of isopyrazam and CSCD459488. Processing factors for isopyrazam from barley grain were 0.55 into malt, 0.82 into spent grain, into < 0.13 spent yeast, into < 0.13 beer and 0.37 into pot barley.

Processing factors for total residues of isopyrazam and CSCD459488 from barley grain were 0.59 into malt, 0.54 into spent grain, 0.13 into spent yeast, < 0.12 into beer and 0.33 into pot barley.

Table 110 Total Isopyrazam Residues in Beer and Pot Barley, Intermediates and By-products

Process	Isopyrazam residue	Isopyrazam residue	Processing Factor
Balance 1			
Barley grain prior to processing	0.077		
Malt	0.077	0.034	0.558
Spent grain (brewers grain)	0.077	0.063	0.818
Spent yeast (brewer's yeast)	0.077	< 0.010	< 0.130
Beer	0.077	< 0.010	< 0.130
Pot barley	0.077	0.030	0.364
Balance 2			
Barley grain prior to processing	0.077		
Malt	0.077	0.032	0.532
Spent grain (brewers grain)	0.077	0.063	0.818
Spent yeast (brewer's yeast)	0.077	0.010	0.130
Beer	0.077	< 0.010	< 0.130
Pot barley	0.077	0.028	0.338
Follow-up 1			
Barley grain prior to processing	0.077		
Malt	0.077	0.032	0.519
Beer	0.077	< 0.010	< 0.130
Pot barley	0.077	0.031	0.377
Follow-up 2			
Barley grain prior to processing	0.077		
Malt	0.077	0.037	0.597
Beer	0.077	< 0.010	< 0.130
Pot barley	0.077	0.033	0.403
Median Processing Factors			
Malt			0.545
Spent grain (brewers grain)			0.818
Spent yeast (brewer's yeast)			< 0.130
Beer			< 0.130
Pot barley			0.370

Table 111 Total Isopyrazam and CSCD459488 Residues in Beer and Pot Barley, Intermediates and By-products

Process	Isopyrazam +	Isopyrazam +	Processing
Balance 1			
Barley grain prior to processing	0.125		
Malt	0.125	0.076	0.608
Spent grain (brewers grain)	0.125	0.068	0.544
Spent yeast (brewer's yeast)	0.125	0.017	0.136
Beer	0.125	< 0.015	< 0.120
Pot barley	0.125	0.041	0.328
Balance 2			
Barley grain prior to processing	0.125		
Malt	0.125	0.072	0.576
Spent grain (brewers grain)	0.125	0.068	0.544
Spent yeast (brewer's yeast)	0.125	0.016	0.128
Beer	0.125	< 0.015	< 0.120
Pot barley	0.125	0.041	0.328
Follow-up 1			
Barley grain prior to processing	0.125		

Process	Isopyrazam +	Isopyrazam +	Processing
Malt	0.125	0.072	0.576
Beer	0.125	< 0.015	< 0.120
Pot barley	0.125	0.043	0.344
Follow-up 2			
Barley grain prior to processing	0.125		
Malt	0.125	0.081	0.648
Beer	0.125	< 0.015	< 0.120
Pot barley	0.125	0.044	0.352
Median Processing Factors			
Malt			0.592
Spent grain (brewers grain)			0.544
Spent yeast (brewer's yeast)			0.132
Beer			< 0.120
Pot barley			0.328

Wheat

A wheat processing study was conducted in 2007, in Northern France (Eversfield S., 2008b). This study, summarised in detail below, included two balance and two follow-up trials to produce flour type 550, wholemeal flour and wholemeal bread, and wheat germ.

A residue field trial was conducted in Northern France during 2007, in which isopyrazam formulated as an emulsifiable concentrate (EC) containing 125 g isopyrazam per litre, was applied to winter wheat. In order to allow the maximum likelihood of detecting products arising from the *anti* isomer *anti*-isomer, the processing studies were conducted with isopyrazam having a *syn*/*anti* ratio of 70:30.Three applications, at growth stage 30–32 BBCH, 39–41 BBCH and at 67–69 BBCH, were made at 250 g ai/ha for each application. Forty-three days after the final application, samples (~100 kg) of treated and untreated wheat grain were harvested and transported at ambient temperature to the processing facility. In addition, grain samples (1 kg) were frozen for residue analysis.

Wheat grain was processed into flour type 550, wholemeal flour, wholemeal bread and wheat-germ. Two balance studies and two follow-up studies were carried out on each process.

Samples were analysed for isopyrazam (as *syn*-isomer and *anti*-isomer) using method GRM006.01B and for CSCD459488 and CSCD459489 using method GRM006.03A.

Separate mass balances for isopyrazam and CSCD459488 residues were calculated. Transfer factors were calculated for isopyrazam and for the sum of isopyrazam and CSCD459488.

The residues of the sum of isopyrazam and CSCD459488 in wheat grain prior to processing and in flour type 550, wholemeal flour, wholemeal bread and wheat germ as well as in the intermediate processed fractions and the processing by-products are presented in Tables 112 and 113.

Residues of isopyrazam in the wheat grain prior to processing were 0.054 mg/kg. Residues of CSCD459488 in the grain prior to processing were 0.015 mg/kg. Residues of CSCD459489 in the wheat grain prior to processing and in the processed fractions were below the LOQ; therefore no calculations of mass balance and transfer factors were possible.

The isopyrazam residues in flour type 550 were significantly lower (0.012–0.013 mg/kg) than in the wheat grain prior to processing. A total of 80–85% of the isopyrazam on the wheat grain was accounted for in the flour 550 balance studies. The CSCD459488 residues in flour type 550 were significantly lower (< 0.005 mg/kg) than in the wheat grain prior to processing. A total of 96–100% of the CSCD459488 on the wheat grain was accounted for in the flour type 550 balance studies.

The isopyrazam residues in wholemeal flour were essentially the same (mean 0.041 mg/kg) as in the wheat grain prior to processing. The CSCD459488 residues in wholemeal flour were also essentially the same (mean 0.016 mg/kg) as in the grain prior to processing.

The isopyrazam residues in wholemeal bread were lower (mean 0.027 mg/kg) than in the grain prior to processing. A total of 78–80% of the isopyrazam on the grain prior to processing was accounted for in wholemeal bread balance studies. The CSCD459488 residues in wholemeal bread were also lower (mean 0.011 mg/kg) and a total of 105–113% of the CSCD459488 on the wheat grain prior to processing was accounted for in the baking of wholemeal bread balance studies.

Processing factors were calculated for parent isopyrazam and for the sum of isopyrazam and CSCD459488. Processing factors for isopyrazam from wheat grain were 1.46 into middlings, 4.07 into total bran, 1.53 into toppings, 0.20 into type 550 flour, 0.73 into wholemeal flour, 0.50 into wholemeal bread and < 0.19 into wheat germ.

Processing factors for total residues of isopyrazam and CSCD459488 from wheat grain were 1.99 into middlings, 4.39 into total bran, 1.62 into toppings, 0.23 into type 550 flour, 0.81 into wholemeal flour, 0.55 into wholemeal bread and 0.25 into wheat germ.

Table 112 Total Isopyrazam Residues in Wholemeal Flour, Wholemeal Bread and Wheat Germ, Intermediates and By-products

Process	Isopyrazam residue before processing (mg/kg)	Isopyrazam residue in processed commodity (mg/kg)	Processing Factor
Balance B1			
Wheat grain prior to processing	0.054		
Middlings	0.054	0.097	1.796
Total bran	0.054	0.208	3.852
Toppings	0.054	0.068	1.259
Flour Type 550	0.054	0.013	0.241
Wholemeal flour	0.054	0.042	0.778
Wholemeal bread	0.054	0.026	0.481
Germs	0.054	0.013	0.241
Balance B2			
Wheat grain prior to processing	0.054		
Middlings	0.054	0.061	1.130
Total bran	0.054	0.231	4.278
Toppings	0.054	0.097	1.796
Flour Type 550	0.054	0.012	0.222
Wholemeal flour	0.054	0.040	0.741
Wholemeal bread	0.054	0.028	0.519
Germs	0.054	< 0.010	< 0.185
Follow-up F1			
Wheat grain prior to processing	0.054		
Flour Type 550	0.054	0.010	0.185
Wholemeal flour	0.054	0.039	0.722
Wholemeal bread	0.054	0.037	0.685
Germs	0.054	< 0.010	< 0.185
Follow-up F2			
Wheat grain prior to processing	0.054		
Flour Type 550	0.054	< 0.010	< 0.185
Wholemeal flour	0.054	0.037	0.685
Wholemeal bread	0.054	0.026	0.481
Germs	0.054	< 0.010	< 0.185
Median Processing Factors			
Middlings			1.463
Total bran			4.065
Toppings			1.528
Flour Type 550			0.204
Wholemeal flour			0.732
Wholemeal bread			0.500
Germ *			0.185

*–To calculate mean transfer factor for germs, the LOQ value was used in the 3 studies where residues below the LOQ were determined

Table 113 Total Isopyrazam and CSCD459488 Residues in Wholemeal Flour, Wholemeal Bread and Wheat Germ, Intermediates and By-products

Process	Isopyrazam + CSCD459488 residue before processing (mg/kg)	Isopyrazam + CSCD459488 residue in processed commodity (mg/kg)	Processing Factor
Balance B1			
Wheat grain prior to processing	0.069		
Middlings	0.069	0.153	2.217
Total bran	0.069	0.293	4.246
Toppings	0.069	0.095	1.377
Flour Type 550	0.069	0.018	0.261
Wholemeal flour	0.069	0.057	0.826
Wholemeal bread	0.069	0.036	0.522
Germs	0.069	0.022	0.319
Balance B2			
Wheat grain prior to processing	0.069		
Middlings	0.069	0.122	1.768
Total bran	0.069	0.313	4.536
Toppings	0.069	0.128	1.855
Flour Type 550	0.069	0.017	0.246
Wholemeal flour	0.069	0.056	0.812
Wholemeal bread	0.069	0.040	0.580
Germs	0.069	0.017	0.246
Follow-up F1			
Wheat grain prior to processing	0.069		
Flour Type 550	0.069	< 0.015	< 0.217
Wholemeal flour	0.069	0.055	0.797
Wholemeal bread	0.069	0.049	0.710
Germs	0.069	0.018	0.261
Follow-up F2			
Wheat grain prior to processing	0.069		
Flour Type 550	0.069	< 0.015	< 0.217
Wholemeal flour	0.069	0.051	0.739
Wholemeal bread	0.069	0.036	0.522
Germs	0.069	0.017	0.246
Median Transfer Factors			
Middlings			1.993
Total bran			4.391
Toppings			1.616
Flour Type 550			0.232
Wholemeal flour			0.805
Wholemeal bread			0.551
Germs			0.254

Summary of residue transfer (processing/concentration) factors

Isopyrazam is hydrolytically stable under conditions representative of pasteurisation, baking, brewing, boiling and sterilisation.

Two balance and two follow-up studies have been conducted on barley (processed to beer and pot barley) and wheat (processed to flour and bread). Mass balances were acceptable; 72 to 98% of the initial isopyrazam and 61 to 117% of the initial CSCD459488 in the starting crops was accounted for in the processed products and by-products.

Median processing factors from all the above processing studies on isopyrazam are summarised in Table 114.

Table 114 Median Processing Factors

Crop	Commodity	Median Processing Factors	
		Isopyrazam*	Isopyrazam and CSCD459488**
Barley	Malt	0.55	0.59
	Spent grain	0.82	0.54
	Spent yeast	< 0.13	0.13
	Beer	< 0.13	< 0.12
	Pot barley	0.37	0.33
Wheat	Middlings	1.46	1.99
	Bran (total)	4.07	4.39
	Toppings	1.53	1.62
	Type 550 flour	0.20	0.23
	Wholemeal flour	0.73	0.81
	Wholemeal bread	0.50	0.55
	Wheatgerm	0.19	0.25

*–According to the definition of the residue for MRL-setting (*anti*-isomer + *syn*-isomer)

**–According to the definition of the residue for risk-assessment (*anti*-isomer + *syn*-isomer + CSCD459488)

RESIDUES IN ANIMAL COMMODITIES

Farm animal feeding studies

Lactating cows

A residue transfer study was conducted with isopyrazam in dairy cows in the United Kingdom during 2007 and 2008 (Ferguson L., 2008). The purpose of this study was to assess the magnitude of the residues in ruminant products following exposure of cows to isopyrazam in the diet over a 28-day period.

Twelve lactating Holstein/Friesian cross-breed cows were split into four groups (three cows per group) for use in the study. Three control cows received empty gelatine capsules. Isopyrazam was administered orally in gelatin capsules to the remaining three groups at levels based upon the average daily dietary intake. The test material had a *syn/anti* ratio of approximately 70:30. The intended dose levels were 12 (1×), 36 (3×) and 120 ppm (10×) in the animal diet on a dry weight basis assuming a mean consumption of 20 kg of dry weight matter per day. During the study it became evident that the animals were eating less than the theoretical 20 kg per day on which the EU guideline calculations are based, so that the dietary concentrations of isopyrazam actually achieved in the cow feeding study were 15, 42 and 137 ppm.

Milk was collected twice daily, in the morning before administration of the daily dose and again in the evening. Samples from each morning milking were combined with the samples from the previous evening. Milk samples were collected and analysed from individual cows on days -1, 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28. Skimmed milk and cream samples were prepared by centrifugation from milk collected on days 21 and 28 and analysed.

Treated cows and one cow from the control group were sacrificed approximately 22-24 hours after the last dose by captive bolt followed by pithing and exsanguination. Samples of liver, kidney, muscle (round, tenderloin and diaphragm) and fat (renal, mesenterial and subcutaneous) were collected from all the sacrificed animals.

Samples were analysed for isopyrazam (as *anti*-isomer and *syn*-isomer). Samples were also analysed using a common moiety residue method designed to measure any structurally-related

compounds hydrolysable to the common moiety, CSAA798670 (including both isomers of isopyrazam). The results are shown in Tables 115 through 118.

Milk and tissue samples were analysed for isopyrazam (as *anti*-isomer and *syn*-isomer) using analytical method GRM006.09A. The individual isomer residues are reported separately and summed up to give a total residue of isopyrazam. In cases where one of the individual isomer residues was below the limit of quantification (LOQ, < 0.005 mg/kg), residues below the LOQ were taken to be at the LOQ (0.005 mg/kg). In cases where both the individual isomer residues were below the LOQ, the total residue was expressed as being less than twice the LOQ (i.e., < 0.01 mg/kg).

Milk and tissue samples were also analysed for the common moiety (CSAA798670) using analytical method GRM006.10A. The method determines isopyrazam and any structurally-related metabolites as the common moiety CSAA798670 in animal commodities. The method employs concentrated (12M) potassium hydroxide solution to hydrolyse isopyrazam and any structurally-related metabolites to CSAA798670. The resultant common moiety product CSAA798670 is then quantified by LC-MS/MS relative to a matrix-matched CSAA798670 standard.

There were no detectable residues of isopyrazam in whole milk samples from the 1× and 3× dose groups. Residues of isopyrazam were found in milk from the 10× dose group. These residues reached a plateau after 5 days of dosing; the highest individual residue found was 0.017 mg/kg. There were no residues of isopyrazam above the limit of quantification in any of the skimmed milk samples. Highest residues of isopyrazam in cream were 0.010, 0.040 and 0.141 mg/kg from the 1×, 3× and 10× groups, respectively.

The mean isopyrazam residues in liver were 0.010, 0.031 and 0.134 mg/kg from the 1×, 3× and 10× groups, respectively, and the mean isopyrazam residues in kidney were < 0.01, 0.011 and 0.028 mg/kg from the 1×, 3× and 10× groups, respectively.

Samples of round, tenderloin and diaphragm muscle were analysed. The highest mean residues of isopyrazam occurred in diaphragm muscle, where residues were < 0.01, 0.010 and 0.024 mg/kg from the 1×, 3× and 10× groups, respectively. Mean isopyrazam residues in the other types of muscle analysed were < 0.01, < 0.01, 0.016 mg/kg (round) and < 0.01, < 0.01, 0.014 mg/kg (tenderloin) from the 1×, 3× and 10× groups, respectively.

Samples of renal, mesenterial and subcutaneous fat were analysed. The highest mean residues of isopyrazam were detected in renal fat, where residues were < 0.01, 0.034 and 0.120 mg/kg from the 1×, 3× and 10× groups, respectively. Mean isopyrazam residues in other types of fat analysed were < 0.01, 0.032, 0.107 mg/kg (mesenterial) and < 0.01, 0.020, 0.053 mg/kg (subcutaneous) from the 1×, 3× and 10× groups, respectively.

Residues of CSAA798670, resulting from the hydrolysis of isopyrazam and structurally-related metabolites were present in all milk and tissue samples from treated cows and were generally dose dependent, with CSAA798670 residues increasing in direct proportion to increasing dose. No residues of this common moiety above the limit of quantification of the method (0.005 mg/kg) were seen in any samples of milk or tissues from the control animal.

Residues of CSAA798670 in whole milk , expressed as isopyrazam equivalents, reached a maximum after 3 days of dosing in all 3 dose groups with mean CSAA798670 residues of 0.039, 0.120 and 0.340 mg/kg from the 1×, 3× and 10× groups, respectively. The mean residues decreased by day 5 to 0.026, 0.067 and 0.184 mg/kg from the 1×, 3× and 10× groups, respectively, and remained approximately at that level during the remainder of the dosing period.

Residue levels in cream were higher than in skimmed milk. The mean residues of CSAA798670 in skimmed milk, expressed as isopyrazam equivalents, were 0.022, 0.069 and 0.189 mg/kg, from the 1×, 3× and 10× groups, respectively. The mean residues in cream were 0.024, 0.081 and 0.262 mg/kg from the 1×, 3× and 10× groups, respectively.

The mean CSAA798670 residues in liver, expressed as isopyrazam equivalents, were 0.219, 0.597 and 1.907 mg/kg from the 1×, 3× and 10× groups, respectively, and the mean residues in kidney were 0.060, 0.162 and 0.658 mg/kg from the 1×, 3× and 10× groups, respectively.

Samples of round, tenderloin and diaphragm muscle were analysed. The highest mean residues of CSAA798670 expressed as isopyrazam equivalents were detected in diaphragm muscle, where residues were 0.022, 0.052 and 0.174 mg/kg from the 1×, 3× and 10× groups, respectively. Mean residues in the other types of muscle analysed were 0.013, 0.037, 0.124 mg/kg (round) and 0.012, 0.035, 0.120 mg/kg (tenderloin) from the 1×, 3× and 10× groups, respectively.

Samples of renal, mesenterial and subcutaneous fat were analysed. The highest mean residues of CSAA798670 expressed as isopyrazam equivalents were detected in renal fat, where residues were 0.028, 0.089 and 0.346 mg/kg from the 1×, 3× and 10× groups, respectively. Mean residues in other types of fat analysed were 0.019, 0.074, 0.228 mg/kg (mesenterial) and 0.019, 0.059, 0.254 mg/kg (subcutaneous) from the 1×, 3× and 10× groups, respectively.

Table 115 Isopyrazam Residues in Bovine Milk

Study Day	Milk Residue (mg/kg)**								
	anti-isomer			*syn*-isomer			Isopyrazam*		
	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm
-1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
3	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.011
5	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.013
7	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.012
10	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.012
14	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.012
17	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.012
21	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.012
24	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.013
28	< 0.005	< 0.005		< 0.005	< 0.005		< 0.01	< 0.01	0.012
21 (Skimmed milk)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
28 (Skimmed milk)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.006	< 0.01	< 0.01	< 0.01
21 (Cream)	0.005	0.012	0.047	< 0.005	0.015	0.056	0.01	0.027	0.103
28 (Cream)	< 0.005	0.011	0.046	< 0.005	0.013	0.053	< 0.01	0.023	0.099

* Sum of *anti*-isomer and *syn*-isomer

** Mean of three cows

Table 116 CSAA798670 Residues in Bovine Milk

Study Day	Milk Residue (mg/kg)**					
	CSAA798670			CSAA798670 (as Isopyrazam equivalents)		
	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm
-1	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
1	0.009	0.030	0.073	0.018	0.055	0.146
3	0.020	0.056	0.170	0.039	0.120	0.340
5	0.013	0.032	0.092	0.026	0.067	0.184
7	0.017	0.024	0.081	0.033	0.058	0.162
10	0.011	0.025	0.084	0.021	0.051	0.168
14	0.012	0.036	0.097	0.025	0.072	0.194
17	0.012	0.038	0.107	0.024	0.079	0.214
21	0.010	0.033	0.091	0.021	0.063	0.183
24	0.012	0.033	0.089	0.024	0.063	0.179
28	0.009	0.029	0.085	0.018	0.060	0.170
Mean milk residue*	0.012	0.034	0.097	0.025	0.069	0.194
21 (Skimmed milk)	0.012	0.037	0.093	0.023	0.075	0.186
28 (Skimmed milk)	0.011	0.029	0.096	0.022	0.063	0.192
Mean skimmed milk residue	0.011	0.034	0.095	0.022	0.068	0.189
21 (Cream)	0.013	0.053	0.137	0.025	0.090	0.273
28 (Cream)	0.011	0.035	0.126	0.023	0.072	0.251
Mean cream residue	0.012	0.041	0.131	0.024	0.081	0.262

* Mean residue in milk from day 1 to day 28

** Mean of three cows

Table 117 Isopyrazam Residues in Bovine Tissues

Tissue	Tissue Residue (mg/kg)**								
	anti-isomer			*syn*-isomer			Isopyrazam*		
	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm
Liver	0.005	< 0.005	0.005	< 0.005	< 0.005	< 0.005	0.010	< 0.01	0.010
Kidney	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
Muscle–Round	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
Muscle -Tenderloin	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
Muscle -Diaphragm	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
Fat–Renal	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
Fat–Mesenterial	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
Fat–Subcutaneous	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01

* Sum of *anti*-isomer and *syn*-isomer

** Mean of three cows

Table 118 CSAA798670 Residues in Bovine Tissues

Tissue	Tissue Residue (mg/kg)					
	CSAA798670			CSAA798670 (as Isopyrazam equivalents*)		
	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm	(1×) 15 ppm	(3×) 42 ppm	(10×) 137 ppm
Liver	0.109	0.298	0.954	0.219	0.597	1.907
Kidney	0.030	0.081	0.329	0.060	0.162	0.658
Muscle–Round	0.007	0.019	0.062	0.013	0.037	0.124
Muscle -Tenderloin	0.006	0.018	0.060	0.012	0.035	0.120
Muscle -Diaphragm	0.011	0.026	0.087	0.022	0.052	0.174
Fat–Renal	0.014	0.044	0.173	0.028	0.089	0.346
Fat–Mesenterial	0.009	0.037	0.114	0.019	0.074	0.228
Fat–Subcutaneous	0.010	0.030	0.127	0.019	0.059	0.254

* To convert the CSAA798670 residue to Isopyrazam equivalents, results were multiplied by two

** Mean of three cows

Residues of isopyrazam in all the commodities (milk, cream, tissues) from cows treated at the 1× dose (15 mg/kg DM diet) were all at or below the limit of quantification (LOQ) of the method (0.01 mg/kg). Only in cream and liver residues were found at the LOQ. Residues of isopyrazam were higher at higher dose levels, and the increase in residues was approximately linear with increasing dose. The highest residues of isopyrazam found at the 10× dose (137 ppm DM diet) were 0.174 mg/kg in liver; the highest residue in milk at this dose level was 0.017 mg/kg.

Residues of CSAA798670, the common moiety from hydrolysis, were higher than isopyrazam residues in the corresponding commodities. The highest residue found in milk from the 1× dose group was 0.046 mg/kg. The highest residue found in any tissue from this group was 0.240 mg/kg in liver. Residues of CSAA798670 were higher at higher dose levels, and the increase in residues was again approximately linear with increasing dose. The highest residues of CSAA798670 found at the 10× dose were 1.958 mg/kg in liver; the highest residue in milk at this dose level was 0.378 mg/kg.

The highest residues of isopyrazam and of CSAA798670 found in individual animals from the three dose groups are summarised in Tables 119 and 120.

Table 119 Mean and Highest Residues of Isopyrazam in Bovine Commodities

Matrix	Highest Isopyrazam residue (mg/kg)					
	1×, 15 ppm		3×, 42 ppm		10×, 137 ppm	
	Mean	Highest	Mean	Highest	Mean	Highest
Whole Milk	< 0.01	< 0.01	< 0.01	< 0.01	0.012	0.017
Skimmed Milk	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cream	0.005	0.010	0.025	0.040	0.101	0.141
Liver	< 0.01	0.010	0.030	0.036	0.134	0.174
Kidney	< 0.01	< 0.01	0.011	0.012	0.029	0.042
Muscle–Round	< 0.01	< 0.01	< 0.01	< 0.01	0.016	0.020
Muscle–Tenderloin	< 0.01	< 0.01	< 0.01	< 0.01	0.014	0.016
Muscle–Diaphragm	< 0.01	< 0.01	0.01	0.010	0.025	0.030
Fat–Renal	< 0.01	< 0.01	0.034	0.053	0.120	0.152
Fat–Mesenterial	< 0.01	< 0.01	0.032	0.053	0.107	0.134
Fat–Subcutaneous	< 0.01	< 0.01	0.017	0.031	0.053	0.061

Table 120 Mean and Highest Residues of CSAA798670 in Bovine Commodities

Matrix	CSAA798670 residue (mg/kg Isopyrazam equivalents)*					
	1×, 15 ppm		3×, 42 ppm		10×, 137 ppm	
	Mean	Highest	Mean	Highest	Mean	Highest
Whole Milk	0.025	0.046	0.069	0.144	0.194	0.378
Skimmed Milk	0.022	0.026	0.068	0.080	0.189	0.216
Cream	0.024	0.030	0.081	0.106	0.262	0.326
Liver	0.219	0.240	0.597	0.656	1.907	1.958
Kidney	0.060	0.073	0.162	0.174	0.658	0.678
Muscle–Round	0.013	0.014	0.037	0.041	0.124	0.140
Muscle–Tenderloin	0.012	0.013	0.035	0.039	0.120	0.139
Muscle–Diaphragm	0.022	0.026	0.052	0.057	0.174	0.206
Fat–Renal	0.028	0.045	0.089	0.099	0.346	0.580
Fat–Mesenterial	0.019	0.021	0.074	0.097	0.228	0.318
Fat–Subcutaneous	0.019	0.021	0.059	0.075	0.254	0.330

* CSAA798670 expressed as Isopyrazam equivalents is calculated by multiplying the measured CSAA798670 residue by two (the CSAA798670 residue includes parent isopyrazam residues which are captured during hydrolysis to the common moiety).

Laying hens

No feeding study on laying hens was conducted as expected dietary burdens for hen were low.

In the hen metabolism study conducted at an actual dose of 11 ppm dry matter in feed, the highest residue (as total radioactive residue, TRR) found in any commodity from the metabolism study was 0.164 mg/kg in liver, corresponding to a transfer factor of 0.015.

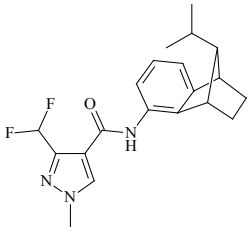
APPRAISAL

Isopyrazam is a broad-spectrum foliar fungicide belonging to the chemical class of ortho-substituted phenyl amides. It controls a wide range of fungal pathogens.

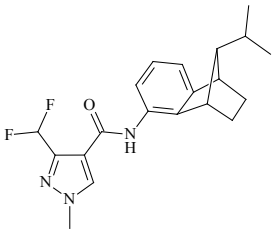
The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, processing and farm animal feeding.

Isopyrazam contains two diastereoisomers designated syn- and anti-isomers. Both of these isomers are biologically active and the specification for technical isopyrazam covers the range of syn:anti isomer ratios from 70:30 to 100:0.

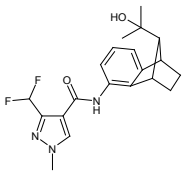
syn-isomer



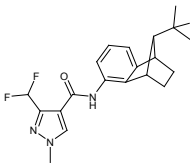
anti-isomer



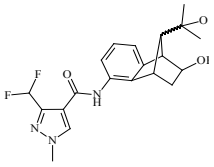
In this Appraisal, the following abbreviated names were used.



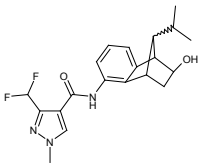
CSCD459488 Hydroxylated syn-isomer (tertiary alcohol)



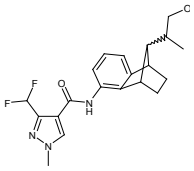
CSCD459489 Hydroxylated anti-isomer (tertiary alcohol)



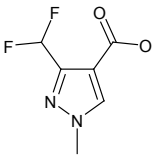
CSCD656800 Dihydroxylated isopyrazam



CSCD563692 Hydroxylated isopyrazam (secondary alcohol)



CSCD563691 Hydroxylated isopyrazam (primary alcohol)



CSAA798670 3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid

Animal metabolism

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

For the animal metabolism studies, three types of radioactive isopyrazam were used: isopyrazam uniformly labelled with ^{14}C on phenyl ring and with syn/anti ratio of 70:30 or 95:5; and one with ^{14}C at position 5 of pyrazole ring with syn/anti ratio of approximately 95:5. In addition, in a goat metabolism study, CSCD 459488 labelled at position 5 of pyrazole ring with ^{14}C was used.

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

Metabolism of isopyrazam in rats

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

When radio-labelled isopyrazam was administered once at 1 or 75 mg/kg bw to rats, approximately 70% of the dose was absorbed. Most of the absorbed dose was excreted within 24 hours after administration, 65–90% via bile and the rest via urine. Highest residues were identified in the liver, kidney, thyroid and adrenals. After repeated dosing, no accumulation of radioactivity was observed in rats. There were no significant differences between the toxicokinetic parameters of syn- and anti isomers. The predominant metabolic pathway for isopyrazam or its N-demethylated metabolite is hydroxylation in the bicyclic-isopropyl moiety, followed by further oxidation to form carboxylic acid and/or to give rise to multiple hydroxylated metabolites with subsequent formation of glucuronic acid or sulphate conjugates.

Metabolism of isopyrazam in lactating goats

The three types of [^{14}C]isopyrazam were administered orally to three groups of lactating goats (each type of radio-labelled isopyrazam to each different group) at a dose equivalent to a dietary concentration of 30 ppm (in dry matter) daily for seven consecutive days. Of the total administered radioactivity (TAR), 60–63% and 12–13% was eliminated via faeces and urine, respectively. Administration of radio-labelled isopyrazam with different ^{14}C position or syn/anti ratio did not reveal any significant difference in the excretion. Total recovered radioactivity was 80–84% of the TAR.

Radioactivity in the Day 5 am milk was 0.055–0.076 mg/kg from the use of three different types of radioactive isopyrazam.

Total radioactive residues (TRR) in tissues except liver after sacrifice (16 hours after the last dose) were similar regardless of the label position or the syn/anti ratio. In the liver, TRR (0.331 mg/kg) after administration of phenyl-labelled isopyrazam of syn/anti ratio of 70:30 was about one half of the TRR (0.604 and 0.612 mg/kg) after administration of pheyl-labelled or pyrazole-labelled isopyrazam of syn/anti ratio of 95:5. In other tissues, TRR were 0.143–0.189 mg/kg in the kidney, 0.022–0.032 mg/kg in the muscle, and 0.012–0.020 mg/kg in the fat.

In the milk, liver, kidney and muscle extracts, parent compound was a minor component with the maximum of 0.0063 mg/kg (1.9% TRR) in the liver or 8.6% of TRR (0.0019 mg/kg) in the muscle from the administration of three different types of radio-labelled isopyrazam.

In the fat, the parent compound was predominant at 40–51% of TRR but the concentration was very low at 0.005–0.010 mg/kg.

The major residue was CSCD656800, dihydroxylated isopyrazam, in the extracts of milk (15–32% of TRR; 0.008–0.019 mg/kg), liver (6–17% of TRR; 0.020–0.104 mg/kg), kidney (13–25% of TRR; 0.023–0.038 mg/kg) and muscle (29–44% of TRR; 0.007–0.013 mg/kg). CSCD656800 was not detected in the fat extracts. In the liver, CSCD656800 existed as glucuronide or sulphate conjugates.

No other identified metabolites existed at quantifiable concentrations. All identified metabolites contained both the pyrazole and phenyl moieties indicating that there was no or little cleavage of amide in metabolism.

Treatment of the liver extraction debris with protease released a significant portion of radioactivity which was composed of multiple minor metabolites.

Metabolism of CSCD459488 in lactating goats

Metabolism of CSCD459488, hydroxylated syn-isomer of isopyrazam and major metabolite in wheat, grapes and lettuce, was studied by orally administering pyrazole-labelled CSCD459488 at a dose equivalent to dietary concentration of 12 ppm to lactating goats daily for seven consecutive days. The major portion of administered radioactivity was excreted via faeces (57% of TAR) and urine (30% of TAR).

Radioactivity in the Day 6 pm milk was 0.123 mg/kg in CSCD459488 equivalents. After sacrifice, the highest radioactivity was found in the liver at 0.457 mg/kg followed by kidney at 0.246 mg/kg, muscle at 0.038 mg/kg, and fat at 0.007 mg/kg.

In this study, the predominant metabolite was CSCD656800 in the milk and all the tissues tested (33–56% of TRR). The highest concentration was found in the liver at 0.159 mg/kg (36% of TRR) where the majority of CSCD656800 existed as conjugates.

CSCD459488 was detected in the milk and all the tissues tested but at only very low levels (< 0.001–0.007 mg/kg; 0.1–6.2% of TRR) and in conjugated forms.

Metabolism of isopyrazam in laying hens

The three types of [^{14}C]isopyrazam were administered orally to three groups of laying hens (each type of radio-labelled isopyrazam to each different group) at a dose equivalent to a dietary concentration of 11 ppm (in dry matter) daily for 14 consecutive days. Of the total administered radioactivity (TAR),

88–93% was eliminated in excreta. Administration of radio-labelled isopyrazam with different ^{14}C position or syn/anti ratio did not reveal any significant difference in the excretion. Total recovered radioactivity was 91–95% of the TAR.

TRR in the composite egg white and egg yolk samples obtained in Days 7–14 from hens dosed with radio-labelled isopyrazam with syn/anti ratio of 95:5 were 0.017 and 0.039–0.042 mg/kg, respectively. On the other hand, when phenyl-labelled isopyrazam with syn/anti ratio of 70:30 was administered, TRR in the composite egg white and egg yolk samples were higher at 0.024 and 0.080 mg/kg respectively.

The TRR in tissues after sacrifice (16 hours after the last dose) were 0.119–0.143 mg/kg, 0.004–0.006 mg/kg, 0.008–0.020 mg/kg and 0.011–0.020 mg/kg in liver, muscle, skin and attached fat, and peritoneal fat, respectively.

Parent isopyrazam was detected in all fat samples and in egg yolk samples (from administration of pyrazole-labeled isopyrazam with the syn/anti ratio of 95:5 and phenyl-labelled isopyrazam with the syn/anti ratio of 70:30) and in liver (from administration of pyrazole-labelled isopyrazam with the syn/anti ratio of 95:5) but the concentrations were < 0.01 mg/kg (< 5% of TRR in egg yolk and liver; up to 21% TRR in fat).

From the egg and liver samples, three metabolites were identified: CSCD656800 (dihydroxylated isopyrazam), hydroxy CSCD459489 and unsaturated carboxylic acid. None of them existed in a concentration higher than 0.012 mg/kg but CSCD656800 contributed up to 29% of TRR in egg white. Detection of hydroxyl CSCD459489 (anti configuration) in egg and liver samples from treatment with the syn/anti ratio of 70:30 was consistent with the larger proportion of anti-isomer administered in the experiment.

Treatment of the liver extraction debris with protease followed by 0.1 NHCl released a significant portion of radioactivity which was composed of multiple minor metabolites.

No other identified metabolites existed at quantifiable concentrations. All identified metabolites contained both the pyrazole and phenyl moieties indicating that there was no or little cleavage of amide in metabolism.

The metabolic pathway in lactating goats and laying hens was similar to the one in rats. The primary metabolism of isopyrazam in these animals, in relation to edible tissues, milk and eggs, proceeded through hydroxylation of the isopropyl group and bicyclic portion of the molecule; and then oxidation of primary alcohols to form carboxylic acids and/or multiple hydroxyl moieties which subsequently converted into glucuronic acid or sulphate conjugates.

No significant inter-conversion of the two isomers of isopyrazam occurred in metabolism.

Plant metabolism

The Meeting received information on the fate of isopyrazam after foliar applications on wheat, grape and lettuce.

For the plant metabolism studies, three types of radioactive isopyrazam were used: isopyrazam uniformly labelled with ^{14}C on phenyl ring and with syn/anti ratio of 70:30 or 96:4; and one with ^{14}C at position 5 of pyrazole ring with syn/anti ratio of 96:4.

Wheat

Wheat grown in pots in glasshouse was treated three times (approximating BBCH 31, BBCH 39 and BBCH 69) with foliar spray application of the three types of isopyrazam separately at a rate of 125 g ai/ha.

Total radioactive residues (TRR) in forage collected 13 days after the second application were 4.75–7.09 mg/kg, and in grain and straw collected 4–48 days after the last application were 0.031–0.059 mg/kg and 14.1–20.8 mg/kg respectively. This indicates that translocation to grains is very small.

Application of radio-labelled isopyrazam with different ^{14}C position did not result in significant difference in the TRR while the application of isopyrazam with the syn/anti ratio of 70:30 resulted in significantly lower TRR.

Parent isopyrazam was the major residue: 4.50–5.81 mg/kg (79–91% of TRR) in forage, 8.56–15.5 mg/kg (61–69% of TRR) in straw, and 0.021–0.037 mg/kg (53–66% of TRR) in grain.

In grain, no identified metabolites existed in excess of 0.0013 mg/kg (< 5% of TRR).

In straw, the most significant metabolite was CSCD459488 (tertiary alcohol) at 1.01–1.94 mg/kg (7.3–9.7% of TRR). This compound was also found in forage and grain but at lower concentrations: 0.020–0.15 mg/kg (0.4–2.4% of TRR) in forage and 0.0004–0.0008 mg/kg (1.2–1.4% of TRR) in grain.

In straw, CSCD563692 (secondary alcohol) and CSCD563691 (primary alcohol) were also identified but both of them were less than 5% of TRR (up to 0.760 and 0.540 mg/kg respectively). These compounds were also found in forage at lower levels.

A dihydroxylated compound was also identified but accounted for less than 5% of TRR in forage, straw and grain. A number of additional components were identified as the N-demethylated isopyrazam and unsaturated products (< 5% of TRR).

Major portions of the above mentioned metabolites were released after pectinase treatment indicating their presence in conjugated form.

Grapes

Grape vines in the field were given a single foliar application of either of phenyl-labelled or pyrazole-labelled isopyrazam (syn/anti ratio of 70:30) at a nominal rate of 400 g ai/ha. Grape berries were harvested 21 days after the application.

Total radioactive residues (TRR) in unwashed grape berries were 0.156 and 0.147 mg/kg for the pheny-labelled and pyrazole-labelled isopyrazam application respectively. The TRR in the vine leaves were 11.0 and 3.77 mg/kg for the pheny-labelled and pyrazole-labelled isopyrazam application respectively. Residue concentrations were much lower in berries than in leaves.

Parent isopyrazam is the major component of the residues from the both treatments at 0.116–0.131 mg/kg (89–90% of TRR) in grape berries. In leaf samples, parent compound was present at 10.1 mg/kg (91% of TRR) from treatment with phenyl-labelled isopyrazam and 3.25 mg/kg (86% of TRR) from pyrazole-labelled isopyrazam. The syn/anti ratio of isopyrazam in fruit and leaf fractions was approximately 70:30 in the HPLC analysis indicating no significant change in ratio from the applied isopyrazam.

The metabolites CSCD563692 (secondary alcohol), CSCD610195 (primary alcohol) and CSCD459488 (syn-form tertiary alcohol) were found in berries. Corresponding anti-form CSCD459489, dihydroxylated metabolite CSCD656800 and N-demethylated metabolites were found in leaves only. None of them accounted for more than 5% of TRR (individually up to 0.110 mg/kg in leaf).

Lettuce

Lettuce grown outdoors was treated three times (BBCH < 40, 42 and 46) with phenyl-labelled or pyrazole-labelled isopyrazam at a nominal application rate of 125 g ai/ha. Lettuce was harvested 3 (early harvest) or 14 days (normal harvest) after the last application.

The total radioactive residues in lettuce were 1.54–1.56 mg/kg 3 days after the last application but decreased to 0.22–0.31 mg/kg at full maturity, 14 days after the last application.

Also in lettuce, parent isopyrazam was the major component of residues: 1.03–1.09 mg/kg (66–71% of TRR) in early harvest and 0.100–0.108 mg/kg (35–45% of TRR) in normal harvest.

CSCD459488 (tertiary alcohol), mostly in a conjugated form, was the most significant metabolite: 0.009–0.022 mg/kg (0.6–1.4% of TRR) in early harvest and 0.031–0.053 mg/kg (14–17% of TRR) in normal harvest. This indicates biotransformation of isopyrazam into CSCD459488.

There were a number of minor metabolites characterized in lettuce from both harvest timings from the both treatments. They were hydrolyzed, N-demethylated or cleaved compounds and existed at very low levels.

The studies on wheat, grape and lettuce indicate that the metabolism of isopyrazam in these plants was qualitatively the same. The position of radiolabel or the syn/anti ratio of isopyrazam did not reveal significant difference in metabolic profiles.

In all plants tested, parent compound was the major residue component with a number of hydrolyzed or N-demethylated metabolites identified or characterized.

In plants, isopyrazam undergoes hydroxylation of the isopropyl group to produce a variety of alcohol products with CSCD459488 as the major metabolite; conjugation of these metabolites with natural carbohydrates; or N-demethylation of the pyrazole ring. None of the metabolites, except CSCD459488 in lettuce leaf (14–17% of TRR) and wheat straw (7.3–9.7% of TRR), accounted for more than 5% of TRR.

Environmental fate in soil

Since isopyrazam is a fungicide with foliar applications and its uses are currently limited to cereals and bananas, the Meeting reviewed hydrolysis and succeeding crop studies.

Hydrolysis

Isopyrazam was stable for 30 days at 25 °C at pH 5, 7 and 9 and for 5 days at 50 °C at pH 4, 5, 7 and 9.

Residues in succeeding crops

A confined study was conducted to examine the nature and levels of residues of isopyrazam in succeeding crops. A single application of either pyrazole-labelled or pheyl-labelled isopyrazam (syn/anti ratio of 96:4) was made to sandy loam soil in containers at a nominal rate of 375 g ai/ha, higher than the proposed maximum annual application rate of 250 g ai/ha.

At each rotational interval of 30, 90 and 300 days after treatment (DAT), spring wheat, lettuce and turnips were sown into the treated soil, grown in glasshouses, and harvested at maturity.

Following application of isopyrazam to soil and aging of the treated soil for up to 300 days, uptake of radioactivity into rotational crops was most significant in straw, hay and forage of wheat (0.80–1.02 mg/kg for 30-day plant back interval, 0.35–0.40 mg/kg for 90-day plant back interval (no harvest for 30-day plant back interval), and 0.073–0.154 mg/kg for 30-day plant back interval, respectively). The uptake gradually decreased in samples as plant back interval increased. In wheat grain, turnip roots and leaves, and lettuce, uptake of radioactivity was much smaller (0.012–0.023 mg/kg, 0.016–0.018 mg/kg, 0.026–0.051 mg/kg, and 0.010–0.023 mg/kg respectively for 30-day plant back interval).

In general, the metabolism of isopyrazam in succeeding crops was similar to that in primary crops but parent compound accounted for a much lower percentage of the residue in the confined study (< 10% of TRR) and was not predominant residue. The only exception was turnip roots, where unchanged parent accounted for up to 34% TRR, but this represented a residue of only 0.0055 mg/kg. CSCD459488 was detected in all rotational crop commodities and reached 22–25% TRR in wheat straw and hay (0.17 mg/kg in straw and 0.090 mg/kg in hay). Pyrazole-specific half-molecule metabolites (CSAA798670 and its N-demethylated compound) were relatively more abundant in the confined succeeding crop study (CSAA798679 up to 48% TRR and 0.025 mg/kg in turnip foliage; and CSCD465008 up to 14% TRR and 0.003 mg/kg in turnip root) indicating that cleavage of the

amide bond of isopyrazam seem to play some role in succeeding crops while they were negligible in the primary crops receiving foliar applications.

Four field trials were conducted to investigate the magnitude of the residues of isopyrazam and its metabolites in succeeding or rotational crops. Isopyrazam was applied three times to primary crops of wheat at a nominal rate of 125 g ai/ha, giving a total application rate of 375 g ai/ha, i.e., higher than the proposed maximum annual application rate of 250 g ai/ha.

Neither syn-isomer nor anti-isomer of isopyrazam was detected at or above the LOQ of 0.005 mg/kg in any of the succeeding crops (barley, carrots and spinach) from all plant-back intervals up to about one year, except in one trial where residues of syn-isomer were found at very low levels (0.005–0.006 mg/kg) in the carrot roots, barley forage and whole barley plant samples from the first (28-day) plant-back interval. CSCD459488 (hydroxylated syn-isomer) was found at low levels (up to 0.054 mg/kg in barley straw) while CSCD459489 (hydroxylated anti-isomer) or CSAA798670 was not found in any of the rotational crops.

CSCD465008 was found in all crops and at slightly higher levels (up to 0.15 mg/kg in carrot leaves).

The metabolic pathway in rotational crops is similar to that in primary crops but parent compound represented a much lower percentage of the residue and pyrazole-specific metabolites account for a higher proportion of the residue in rotational crops. Of these, CSAA798670 was not found in any of the field rotational crop studies.

The Meeting concluded that isopyrazam residue was not expected to be found above the LOQ in barley grains, carrot roots and spinach leaves. CSCD459488 or CSCD465008 were not detected more than 0.01 mg/kg in barley grains and carrot roots while these were found in spinach at 0.015 and 0.06 mg/kg respectively. These residues were found at higher concentrations in barley forage, hay and straw and carrot leaves.

Analytical methods

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

In general, the methods for data generation employ extraction by homogenization with a mixture of acetonitrile and water (mostly 80:20 v/v), clean-up with solid phase extraction or a process of centrifugation and dilution, and determination of analytes using LC-MS/MS or, in one method, GC-MS/MS.

A number of methods for plant matrices were successfully validated for each isomer of isopyrazam at LOQ (0.005 mg/kg for each analyte) and higher concentrations in barley grain, forage and straw, ryegrass, apples, carrots, spinach, tomatoes, oranges, potatoes, lentils, sunflower seeds, rapeseed, bran bread and beer.

One method was successfully validated for determination of monohydroxylated metabolites (CSCD459488 and CSCD459489) of isopyrazam at LOQ of 0.005 mg/kg and above for barley grain, forage, straw, apples, carrots, spinach, rapeseed, lentils, bran, bread and beer. This method involves hydrolysis with 0.1 M HCl at 60 °C for 3 hours.

Another method was successfully validated for CSCD465008 and CSAA798670 at LOQ of 0.01 mg/kg and above in barley grain, forage and straw, carrot leaves and roots, and spinach. This method involves hydrolysis with pectinase at pH 5 at 37 °C for 16–20 hours and partition with hexane.

For commodities of animal origin, one method was successfully validated for the determination of each isomer of isopyrazam at 0.005 mg/kg and above in eggs, milk, muscle, liver, kidney and fat. Another method determines isopyrazam and its metabolites in commodities of animal origin as the common moiety CSAA798670; i.e., this method determines not only isopyrazam but any metabolites hydrolysable to CSAA798670. The analytical procedure involves hydrolysis of acetonitrile + water extract with 12 M potassium hydroxide solution at 100 °C for 3 hours. The

method was successfully validated for the determination of isopyrazam and its metabolites hydrolysable to CSAA798670 at 0.005 mg/kg in CSAA798670 equivalents and above in eggs, milk, muscle, liver, kidney and fat.

For enforcement, a multi-residue method DFG-S19 was investigated for monitoring of isopyrazam in plant and animal commodities using GC-MSD (selected ion monitoring) or LC-MS/MS (positive or negative multiple reaction monitoring). DFG-S19 using negative multiple reaction monitoring LC-MS/MS was successfully validated in-house and independently for the determination of isopyrazam (determined as syn- and anti-isomer separately) at 0.005 mg/kg and above (plant commodities) or 0.0025 mg/kg and above (animal commodities) for each isomer, In the case of wheat grain, the method could only be validated at 0.05 mg/kg when ion m/z 316 was monitored with GC-MSD in an independent laboratory validation.

Both GC-MSD and LC-MS/MS are specific and either of them can be used for quantification of residues. However, only the negative multiple reaction monitoring LC-MS/MS received full validation and independent laboratory validation.

Stability of pesticide residues in stored analytical samples

The stability of isopyrazam residues during storage of samples frozen at approximately -15 to -20 ºC was investigated in a range of plant and animal matrices: tomato fruit, rape seeds, lentil seeds, potato tubers, barley grain, barley straw, ryegrass forage and spinach leaves; and milk, eggs, liver, kidney, muscle and fat.

Compounds tested were: both isomers of isopyrazam, CSCD459488, CSCD459489, CSCD465008 and CSAA798670. Each compound was spiked to matrices at 0.5 mg/kg.

All of the compounds tested were found stable (> 70% remaining) at least for the following storage periods tested: in plant commodities, both isomers of isopyrazam, 24 months; CSCD459488, 11 months; CSCD459489, 28 months; and CSCD45008 and CSAA798670, 12 months; and in animal commodities, both isomers of isopyrazam, 14 months; and isopyrazam and metabolites hydrolysable to CSAA798670, 12 months.

The storage durations of samples from the supervised field trials were within the above storage periods.

Definition of the residue

In animal metabolism studies, parent isopyrazam was detected in all the tissues tested, milk and eggs at concentrations < 0.01 mg/kg. It was metabolized extensively and accounted for < 9% of TRR in milk, eggs and tissues other than fat, and up to 51% of TRR in fat.

Sufficiently validated multi-residue LC-MS/MS or GC-MSD method was available for determining the parent compound as the two separate isomers in animal commodities for enforcement. A number of LC-MS/MS methods were validated for analysing isopyrazam in animal commodities.

CSCD656800 (dihydroxylated metabolite) was found in all tissues (except fat), milk and eggs as a major metabolite at significant concentrations (up to 0.104 mg/kg in goat liver) and accounted for 6–44% of TRR in goats and 1.0–29% of TRR in hens. While CSCD656800 was the major metabolite in animals, it is difficult to obtain analytical standard material for this compound and CDCD656800 was not separately analysed in the animal feeding study due to the lack of validated specific analytical methods.

An LC-MS/MS method was validated for analysing parent compound and any metabolites (including CSCD656800) hydrolysable to the common moiety (CSAA798670) in animal commodities. However, the Meeting noted that CSAA798670 moiety is not specific to isopyrazam and may arise from the use of other pesticides containing this moiety, such as sedaxane.

The Meeting therefore concluded that the parent isopyrazam was suitable residue definition for enforcement.

For estimation of dietary intakes, the Meeting considered inclusion of CSCD656800 in the residue definition for animal commodities but it was not possible to include it as no specific analytical method was available. Its contribution to dietary exposure would be no more than 1% of the maximum ADI or ARfD even when uses were expanded.

In plant metabolism, parent isopyrazam was the predominant residue component (53–66% in wheat grain, 86–91% in grape berries and 35–45% in lettuce).

While CSCD459488 was the most significant metabolite in wheat foliage (< 10% of TRR), grapes (< 5% of TRR) and lettuce (14–17% of TRR), it was found at levels below 5% of TRR in wheat grain and grape berries. However, CSCD459488 was found in the supervised residue trials on bananas, barley and wheat, where, in some trials, CSCD459488 was found at levels approaching that of isopyrazam (e.g., 0.026 mg/kg of isopyrazam and 0.022 mg/kg of CSCD459488 in barley grain). CSCD459488 was considered to be of similar toxicity as the parent.

Sufficiently validated multi-residue LC-MS/MS or GC-MSD methods were available for determining the parent compound as the two separate isomers in plant commodities for enforcement.

A number of LC-MS/MS methods were successfully validated for analysing parent or CSCD459488 in plant commodities.

The Meeting therefore concluded that the parent isopyrazam was a suitable residue for enforcement.

For estimation of dietary intakes, the Meeting decided to include CSCD459488, the major metabolite, in the residue definition for plant commodities.

The syn-isomer of isopyrazam has log P_{ow} of 4.1 and the anti-isomer 4.4. In the goat metabolism studies, the isopyrazam concentrations in fat were ~ 5–9 times higher than those in muscle. In the cattle feeding study, the isopyrazam concentrations in cream were about eight times those in whole milk. In the hen metabolism study, isopyrazam was found only in egg yolk samples but not in egg white samples. The Meeting considered isopyrazam residues to be fat-soluble.

The Meeting recommended the following residue definition for plant and animal commodities:

Definition of the residue for plant commodities (for compliance with the MRL): *Isopyrazam (sum of syn-isomer and anti-isomer)*

Definition of the residue (for estimation of dietary intake) for plant commodities: *Sum of isopyrazam and 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid [9-(1-hydroxyl-1-methylethyl)-(1RS, 4RS, 9RS)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]amide expressed as isopyrazam*

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: *Isopyrazam (sum of syn-isomer and anti-isomer)*

The residue is considered fat-soluble.

Results of supervised trials on crops

The Meeting received supervised trial data for isopyrazam on bananas, barley, and wheat.

For all matrices, the LOQ was 0.005 mg/kg for each isomer of isopyrazam and CSCD459488. In the summing of the total residues, if syn- and anti-isomers and CSCD459488 were below the LOQ, the LOQ value of each was used for the calculation.

The OECD MRL calculator was used as a tool to assist in the estimation of maximum residue levels from the selected residue data set obtained from the supervised residue trials. As a first step, the Meeting reviewed trial conditions and other relevant factors related to each data set to arrive at a best estimate of the maximum residue level using expert judgement. Then, the OECD calculator was employed. If the statistical calculation spreadsheet suggested a different value, a brief explanation of the derivation was supplied.

Bananas

A total of 12 supervised trials were conducted on bananas in 2008 in Columbia, Costa Rica, Guatemala and Honduras. Isopyrazam was applied five times (or in one case, six) at a rate of 75 g ai/ha, with an interval between applications of 10 days. Applications were made to both bagged and unbagged bananas. Results from unbagged bananas in each trial were used to estimate a maximum residue level and STMR/HR as in all trials conducted, residue concentrations in bagged banana were never higher than those in unbagged bananas.

The GAP in Columbia allows five foliar applications at a rate of 75 g ai/ha with PHI of 0 days.

Residues of isopyrazam from trials matching he Columbian GAP in banana fruit were: < 0.01, < 0.01, < 0.01, 0.011, 0.012, 0.013, 0.015, 0.016, 0.017, 0.022, 0.034 and 0.040 mg/kg.

The Meeting estimated a maximum residue level of 0.06 mg/kg.

Corresponding total residues of isopyrazam and CSCD459488 in the pulp of unbagged banana were all < 0.015 mg/kg. After six applications instead of five applications, total residues in pulp were < 0.015 mg/kg regardless of whether banana fruit was bagged or unbagged.

The Meeting estimated an STMR and HR for banana pulp at 0.015 and 0.015 mg/kg respectively.

Barley

A total of 21 trials were conducted on barley: three in 2008 in New Zealand, two in 2006 in Switzerland, eight in 2006 and 2007 in France, three in 2006 and 2007 in Germany, one in 2007 in the United Kingdom, two in 2006 and 2007 in Italy, and two in 2006 and 2007 in Spain.

The registered use on barley in New Zealand allows two foliar applications per season prior to BBCH growth stage 59 (ear emergence) at a rate of 75 g ai/ha with a PHI of not shorter than 42 days.

In the three trials conducted in New Zealand, isopyrazam was applied twice at rates approximating 75, 125 and 250 g ai/ha with PHI of 41 or 42 days.

Residues of isopyrazam from trials matching GAP in New Zealand were: < 0.01, < 0.01 and 0.018 mg/kg.

The registered use of isopyrazam on barley in the United Kingdom allows two foliar applications per season between growth stages 30 and 61 (before beginning of flowering), each at a rate of 125 g ai/ha isopyrazam.

Residues of isopyrazam from the trials conducted in Northern France, Germany, Switzerland and the United Kingdom matching GAP in the UK were (n = 8): 0.014, 0.016, 0.017, 0.020, 0.024, 0.026, 0.026 and 0.035 mg/kg.

Based on trials conducted in Northern Europe and matching the GAP of the UK, the Meeting estimated a maximum residue level of 0.07 mg/kg for barley. The Meeting also estimated a median residue of 0.022 mg/kg for the purpose of calculating animal dietary burdens.

Corresponding total residues of isopyrazam and CSCD459488 in the trials conducted in northern Europe were: 0.020, 0.022, 0.029, 0.032, 0.043, 0.046, 0.048 and 0.058 mg/kg.

The Meeting estimated an STMR at 0.0375 mg/kg.

Wheat

A total of 25 trials were conducted on wheat: three in 2008 in New Zealand, ten in 2006 and 2007 in France, five in 2006 and 2007 in Germany, one in 2007 in the United Kingdom, two in 2006 and 2007 in Italy, and four in 2006 and 2007 in Spain.

The registered use of isopyrazam on wheat in New Zealand allows two foliar applications per season prior to BBCH growth stage 69 (end of flowering) at rates of 75–125 g ai/ha and a PHI of not shorter than 42 days.

In the three trials conducted in New Zealand, isopyrazam was applied twice at rates approximating 75, 125 and 250 g ai/ha with PHI of 42 days.

Residues of isopyrazam from trials matching GAP in New Zealand were: < 0.01, < 0.01 and 0.020 mg/kg.

The registered use of isopyrazam on wheat, rye and triticale in the United Kingdom allows two foliar applications per season between growth stages 30 and 71 (before grain watery ripe stage), each at a rate of 125 g ai/ha isopyrazam.

In most of the trials, isopyrazam was applied three times instead of twice. Therefore, the trials were not in compliance with the GAP of the UK. The isopyrazam concentrations in whole plants immediately before the third application were on average about 15% of those on the day of the third application. The Meeting decided to use data from these trials for estimating a maximum residue level in wheat if the contribution of isopyrazam from the second application was below 25% of residues after the third application.

Residues of isopyrazam from these trials conducted in Northern France, Germany and the United Kingdom were (n = 11): < 0.01 (7), 0.012, 0.012, 0.014 and 0.017 mg/kg.

The Meeting estimated a maximum residue level of 0.03 mg/kg for wheat on the basis of the trials conducted in northern Europe..

The Meeting estimated a median residue level of 0.01 mg/kg for the purpose of calculating animal dietary burdens.

Corresponding total residues of isopyrazam and CSCD459488 in the trials conducted in northern Europe were: < 0.015 (7), 0.018, 0.019, 0.019 and 0.026 mg/kg.

The Meeting estimated an STMR at 0.015 mg/kg.

As GAP in the UK covers not only wheat but also rye and triticale, the Meeting decided to extrapolate the maximum residue level, median residue and highest residue for wheat to rye and triticale.

Barley straw and fodder, dry, and forage

The registered use on barley in New Zealand allows two foliar applications per season prior to BBCH growth stage 59 (ear emergence) at a rate of 75 g ai/ha. The PHI is 28 days for forage and 42 days for straw.

Residues of isopyrazam in straw from trials matching GAP in New Zealand were: 0.010, 0.655 and 1.37 mg/kg.

Residues of isopyrazam in forage from trials matching GAP in New Zealand were: 0.130, 0.304 and 0.655 mg/kg.

Residues of isopyrazam in straw from appropriate trials conducted in northern Europe matching the GAP of the UK (two foliar applications per season between growth stage 30 and 61 , each at a rate of 125 g ai/ha isopyrazam) were (n = 8): 0.076, 0.079, 0.129, 0.349, 0.362, 0.495, 0.838 and 1.06 mg/kg.

The Meeting estimated a highest residue and median residue at 1.06 and 0.356 mg/kg, respectively, for the purpose of calculating animal dietary burdens.

Although a maximum residue level for barley straw and fodder would be 2 mg/kg, as barley and wheat straw are not distinguishable in trade, the Meeting recommended to use a maximum residue level for wheat straw and fodder at 3 mg/kg to cover barley straw and fodder, dry (see next section).

As for forage, since there is no description about PHI for forage, the Meeting selected the highest residue concentration from each trial conducted in Northern Europe in compliance with UK GAP. These residue concentrations were (n = 7): 2.14, 2.26, 2.30, 2.45, 2.93, 3.26 and 3.63 mg/kg.

The Meeting estimated a highest residue and median residue at 3.63 mg/kg and 2.45 mg/kg (as received) respectively for the purpose of calculating animal dietary burdens.

Wheat straw and fodder, dry, and forage

The registered use on barley in New Zealand allows two foliar applications per season prior to BBCH growth stage 69 (end of flowering) at rates of 75–125 g ai/ha. PHI is 28 days for forage and 42 days for straw.

Residues of isopyrazam in straw from trials matching GAP in New Zealand were: 0.284, 0.993 and 1.79 mg/kg.

Residues of isopyrazam in forage from trials matching GAP in New Zealand were: 0.9, 0.397 and 0.835 mg/kg.

Residues of isopyrazam in straw from trials conducted in Northern Europe approximating UK GAP (two foliar applications per season between growth stage 30 and 61 , each at a rate of 125 g ai/ha isopyrazam) were (n = 11): 0.113, 0.260, 0.288, 0.921, 0.947, 0.952, 0.977, 1.06, 1.11, 1.41 and 1.51 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg. The Meeting estimated a highest and median residue at 1.51 and 0.952 mg/kg for the purpose of calculating animal dietary burdens.

As for forage, since there is no description about PHI for forage, the Meeting selected the highest residue concentration from each trial conducted in compliance with GAP. These residue concentrations were (n = 9): 1.17, 1.33, 1.53, 1.88, 2.10, 2.22, 2.25, 2.46 and 2.95 mg/kg.

The Meeting estimated a highest residue and median residue at 2.95 mg/kg and 2.10 mg/kg (as received) respectively for the purpose of calculating animal dietary burdens.

As GAP in the UK covers not only wheat but also rye and triticale, the Meeting decided to extrapolate the maximum residue level for wheat straw and fodder, dry to rye straw and fodder, dry. The median and highest residues for wheat straw and fodder, dry, and for forage were extrapolated to straw and fodder, and forage of rye and triticale.

Fate of residues during processing

High temperature hydrolysis

A high-temperature aqueous hydrolysis study was conducted to determine the nature of degradates of isopyrazam in processed commodities or by-products under conditions typical of common processing practices.

After heating at 90, 100 or 120 °C in acetate buffers of pH 4 and 6 for 20 minutes or in a buffer of pH 5 for 60 minutes, about 95% of recovered radioactivity (> 95% of the initial radioactivity) was isopyrazam. This indicated that isopyrazam was stable against hydrolysis under the above mentioned conditions.

Processing

The Meeting received information on processing of barley to beer and pot barley, and wheat to flour, bread, germ and related by-products.

Processing factors were calculated for the processed commodities of barley and wheat and are shown in the table below. STMR-Ps were calculated for processed commodities of barley and wheat for which maximum residue levels were estimated.

Processed Orange Product	Median Processing factor		STMR-P
	Isopyrazam	Isopyrazam and CSCD459488	
Barley			(0.0375)
Malt	0.55	0.59	0.022
Beer	< 0.13	< 0.12	0.0045
Pot barley	0.37	0.33	0.012
Wheat			(0.015)
Bran (unprocessed)	4.07	4.39	0.066
White flour	0.20	0.23	0.0035
Wholemeal flour	0.73	0.81	0.012
Wholemeal bread	0.50	0.55	0.0083
Wheat germ	0.19	0.25	0.0038

As the residue concentration is higher in bran than in wheat grain, the Meeting estimated a maximum residue level of 0.15 mg/kg by multiplying the maximum residue level for wheat (0.03 mg/kg) by 4.07. A median residue was calculated to be 0.041 mg/kg for the purpose of estimating animal dietary burdens.

Residues in animal commodities

Farm animal dietary burden

Grain, straw and forage of barley, wheat, rye and triticale, and wheat bran may be fed to dairy cattle, beef cattle, broilers and layers. The maximum and mean dietary burdens were calculated using the highest residues or median residues of isopyrazam 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	0.20	0.14	3.65	2.52	12.0[a]	8.40[b]	0.04	0.04
Dairy cattle	2.39	1.71	3.65	2.52	12.0[c]	7.84[d]	0.12	0.09
Broilers	0.04	0.04	0.03	0.03	0.02	0.02	0.00	0.00
Layers	0.04	0.04	1.21[e]	0.87[f]	0.02	0.02	0.01	0.01

[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 levels for milk.

[d] Suitable for estimating STMRs for milk.

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

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

Residues in milk and cattle tissues

Lactating dairy cows were dosed daily for 28 consecutive days via gelatin capsules containing isopyrazam (15–137 ppm in diet corresponding to 1×, 3× and 10×). The syn/anti ratio was approximately 70:30.

In whole milk samples from the 1× and 3× groups, isopyrazam residues were not found above LOQ. However, isopyrazam was found at a slightly higher level than LOQ in milk samples from the 10× group. Isopyrazam was found in cream samples at < 0.01–0.010, 0.018–0.040 and 0.048–0.141 mg/kg from 1×, 3× and 10× group respectively.

The isopyrazam residues in liver were < 0.01–0.010, 0.019–0.036 and 0.092–0.174 mg/kg from the 1×, 3× and 10× groups, respectively, and the isopyrazam residues in kidney were < 0.01,0.01–0.012 and 0.018–0.042 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of isopyrazam in muscle occurred in diaphragm muscle, where residues were < 0.01, 0.010 and 0.024 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of isopyrazam in fat were detected in renal fat, where residues were < 0.01, 0.034 and 0.120 mg/kg from the 1×, 3× and 10× groups, respectively.

Residues of CSAA798670, resulting from the hydrolysis of isopyrazam and structurally-related metabolites were also analysed.

CSAA798670 was present in all milk and tissue samples from treated cows and were generally dose dependent. No residues of this common moiety above the limit of quantification of the method (0.005 mg/kg) were seen in any samples of milk or tissues from the control animal.

Residues of CSAA798670 in whole milk, expressed as isopyrazam equivalents, reached a maximum after 3 days of dosing in all three dose groups with mean CSAA798670 residues of 0.039, 0.120 and 0.340 mg/kg from the 1×, 3× and 10× groups, respectively. The mean residues decreased by day 5 to 0.026, 0.067 and 0.184 mg/kg from the 1×, 3× and 10× groups, respectively, and remained approximately at that level during the remainder of the dosing period.

Residue levels in cream were higher than in skimmed milk. The mean residues of CSAA798670 in skimmed milk, expressed as isopyrazam equivalents, were 0.022, 0.069 and 0.189 mg/kg, from the 1×, 3× and 10× groups, respectively. The mean residues in cream were 0.024, 0.081 and 0.262 mg/kg from the 1×, 3× and 10× groups, respectively.

The mean CSAA798670 residues in liver, expressed as isopyrazam equivalents, were 0.219, 0.597 and 1.907 mg/kg from the 1×, 3× and 10× groups, respectively, and the mean residues in kidney were 0.060, 0.162 and 0.658 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of CSAA798670 in muscle expressed as isopyrazam equivalents were detected in diaphragm muscle, where residues were 0.022, 0.052 and 0.174 mg/kg from the 1×, 3× and 10× groups, respectively.

The highest mean residues of CSAA798670 in fat expressed as isopyrazam equivalents were detected in renal fat, where residues were 0.028, 0.089 and 0.346 mg/kg from the 1×, 3× and 10× groups, respectively.

Using the dietary burdens for beef and dairy cattle and the results in the lactating cattle feeding study, the maximum residue levels and STMRs were estimated. The calculated residues in cattle tissues and milk are summarized below.

	Feed level (ppm) for milk residues	Residues in milk (mg/kg)	Residues in cream (mg/kg)	Feed level (ppm) for tissue residues	Residues (mg/kg) in			
					Muscle	Liver	Kidney	Fat
Maximum residue level, beef or dairy cattle								
Feeding study [a]	15	< 0.01	0.01	15	< 0.01	0.01	< 0.01	< 0.01
Dietary burden and residue estimate	12	< 0.008	< 0.008	12	< 0.008	0.008	< 0.008	< 0.008
STMR beef or dairy cattle								
Feeding study [b]	15	< 0.01	< 0.01	15	< 0.01	< 0.01	< 0.01	< 0.01
Dietary burden and residue estimate	7.8	0.0042	0.0042	8.4	0.0056	0.0056	0.0056	0.0056

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

[b] Mean residues for tissues and milk

The Meeting estimated a maximum residue level for isopyrazam in milks, mammalian meat and mammalian fats (except milk fats) at 0.01* mg/kg, and for milk fats at 0.02 mg/kg. The Meeting also estimated a maximum residue level of 0.02 mg/kg for edible offal (mammalian) on a basis of residues in liver.

STMRs were estimated to be 0.0056 mg/kg for mammalian meat, liver, kidney and mammalian fats (except milk fats) and 0.0042 mg/kg for milks and milk fats. HRs were estimated to be 0.008 mg/kg for mammalian meat, liver, kidney and mammalian fat (except milk fats).

Residues in eggs and poultry tissues

No feeding study on laying hens was conducted as the expected dietary burden for hens was low.

In the hen metabolism study conducted at an actual dose of 11 ppm dry matter in the feed, the highest residue (as total radioactive residue, TRR) found was 0.164 mg/kg in parent equivalent in liver.

In the extracts of egg white, egg yolk, liver, skin and attached fat, and peritoneal fat, the highest concentration of isopyrazam observed was 0.004 mg/kg. Muscle was not subject to characterization or identification of radioactive residues as the TRR in muscle was 0.004–0.006 mg/kg in isopyrazam equivalents.

As the calculated maximum and mean dietary burden for estimating a maximum residue level and STMR/HRs for poultry were 1.21 and 0.87 ppm, significantly lower than 11 ppm, the Meeting estimated a maximum residue level of 0.01* mg/kg for isopyrazam in eggs, poultry meat, edible offal of poultry and fat.

STMRs were estimated to be at LOQ of 0.01 mg/kg for eggs, poultry meat, liver and fat. HRs were also estimated to be 0.01 mg/kg (same level as the maximum residue levels) for these commodities.

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 compliance with the MRL*): Isopyrazam (sum of syn-isomer and anti-isomer).*

Definition of the residue (for estimation of dietary intake) for plant commodities: *Sum of isopyrazam and 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid [9-(1-hydroxyl-1-methylethyl)-(1RS, 4RS, 9RS)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]amide expressed as isopyrazam.*

Definition of the residue (for compliance with the MRL and estimation of dietary intake) for animal commodities: *Isopyrazam (sum of syn-isomer and anti-isomer).*

Residue is fat-soluble.

Commodity		Recommended MRL, mg/kg		STMR/STMR-P	HR/HR-P
CCN	Name	New	Previous	mg/kg	mg/kg
FI 0327	Banana	0.06	-	0.015	0.015
GC 0640	Barley	0.07	-	0.0375 0.022 [ab]	-
	Malt			0.022	-
	Beer			0.0045	-
	Pot barley			0.012	-
	Barley forage	-		2.45 [ab]	3.63 [ab]
AS 0640	Barley straw and fodder, dry	3	-	0.4285 [ab]	1.06 [ab]
MO 0105	Edible offal (Mammalian)	0.02		0.0056	0.008
MF 0100	Mammalian fats (except milk fats)	0.01*		0.0056	0.008
MM 0095	Meat (from mammals other than marine mammals)	0.01*		0.0056 (fat) 0.0056 (muscle)	0.008
ML 0106	Milks	0.01*		0.0042	-

Commodity		Recommended MRL, mg/kg		STMR/STMR-P mg/kg	HR/HR-P mg/kg
CCN	Name	New	Previous		
FM 0183	Milk fats	0.02		0.0042	-
PF 0111	Poultry fats	0.01*		0.01	0.01
PM 0110	Poultry meat	0.01*		0.01 (fat) 0.01 (muscle)	0.01
PO 0111	Poultry, Edible offal of	0.01*		0.01	0.01
PE 0112	Eggs	0.01*		0.01	0.01
GC 0650	Rye	0.03		0.015 0.01 [a]	-
AF 0650	Rye forage (green)			2.10 [a]	2.95 [a]
AS 0650	Rye straw and fodder, dry	3		0.952 [a]	1.51[a]
GC 0653	Triticale	0.03		0.015 0.01 [a]	-
	Triticale forage			2.10 [a]	2.95 [a]
AS 0653	Triticale straw and fodder, dry	3		0.952 [a]	1.51[a]
GC 0654	Wheat	0.03		0.015 0.01 [a]	-
	White flour			0.0035	-
	Wholemeal flour			0.012	-
CP 1212	Wholemeal bread			0.0083	-
CF 1210	Wheat germ			0.0038	-
	Wheat forage			2.10 [a]	2.95 [a]
AS 0654	Wheat straw and fodder, dry	3		0.952 [a]	1.51[a]
CM 0654	Wheat bran, unprocessed	0.15		0.066 0.041[a]	-

[a] for the purpose of calculating animal dietary burdens. Expressed on an "as received" basis.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of isopyrazam were calculated for the 13 GEMS/Food cluster diets using STMRs and STMR-Ps estimated by the current Meeting (see Annex 3 of the 2011 JMPR Report). The ADI is 0–0.06 mg/kg bw and the calculated IEDIs were 0% of the maximum ADI. The Meeting concluded that the long-term intake of residues of isopyrazam resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intakes (IESTI) of isopyrazam were calculated for food commodities and their processed commodities using HRs/HR-Ps or STMRs/STMR-Ps estimated by the current Meeting (see Annex 4 of the 2011 JMPR Report). The ARfD is 0.3 mg/kg bw and the calculated IESTIs were 0% of the ARfD. The Meeting concluded that the short-term intake of residues of isopyrazam, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.

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