MANDESTROBIN (307)

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EXPLANATION

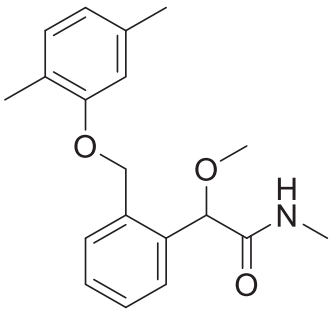
At the Forty-ninth Session of the CCPR (2017), mandestrobin was scheduled for toxicology and residue evaluation as a new compound by the 2018 JMPR. During the 2018 JMPR it appeared that soil degradation studies, field dissipation studies and additional supervised field trials were available that could aid in determination of the residue definition. The residue evaluation was therefore postponed to the 2019 JMPR.

The 2018 JMPR established an ADI of 0–0.2 mg/kg bw and an ARfD of 0.3 mg/kg bw for women of childbearing age. The 2018 Meeting concluded that it was not necessary to establish an ARfD for mandestrobin for the remainder of the population

Mandestrobin is a systemic strobilurin fungicide. It acts on various fungi and also on the control of bacterial nuclear disease. Mandestrobin acts by inhibiting mitochondrial respiration. It binds at the Qo-centre on cytochrome b and blocks electron transfer between cytochrome b and cytochrome c1, disrupting the energy cycle within the fungus by halting the production of ATP.

The Meeting received information from the manufacturer on identity, physical and chemical properties, metabolism in plants and livestock, confined and field rotational crop studies, soil degradation studies, field dissipation studies, residue analysis, storage stability, use patterns, residues resulting from supervised trials on grapes, strawberries, soya beans, rape seed, fate of residues during processing and livestock feeding studies.

IDENTITY

ISO common name:	Mandestrobin
Chemical name	
IUPAC:	(RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
CAS:	2-[(2,5-dimethylphenoxy)methyl]-α-methoxy-N-methylbenzeneacetamide
CAS Registry No:	173662-97-0
CIPAC No:	Not allocated at the time of evaluation
Synonyms and trade names:	S-2200
Structural formula:	
Structure confirmed by UV-VIS, FTIR, 1H-NMR, ^{13}C-NMR and HPLC-MS [Van Meter & Lentz, 2010a, ROP-0009], Mandestrobin (S-2200) is a racemic mixture of S-2167 (R-isomer) and S-2354 (S-isomer). The isomer S-2167 is named as R-isomer of mandestrobin and the isomer S-2354 is named as S-isomer of mandestrobin by JMPR and the same terms are used throughout the evaluation document. The R-S isomer ratio is approximately 50:50.	
Molecular formula:	$C_{19}H_{23}NO_3$
Molecular weight:	313.39 g/mol

PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties of pure mandestrobin (racemic mixture), the pure R-isomer of mandestrobin, the pure S-isomer of mandestrobin and the technical grade mandestrobin (racemic mixture) are listed in Table 1, 2, 3 and 4.

Table 1 Physical and chemical properties for mandestrobin

Parameter	Result	References	Guidelines/method
Appearance:	Purity: 100 % Colour: White (Hue: N 9.5/) Physical state: Powdery solid (20 ºC) No odour (20 ºC)	[Van Meter & Lentz, 2010a, ROP-0009]	OPPTS 830.6302/ visual Munsell colour system, OPPTS 830.6303/ visual and OPPTS 830.6304/ olfactory determination
Vapour pressure:	Purity: 100 % 3.36 × 10–5 mPa at 20 °C (extrapolated) 9.15 × 10–5 mPa at 25 °C (extrapolated) Measured at 40.6, 60.4 and 80.6 °C and extrapolated with Clapeyron-Clausius equation to 20 °C and 25 °C	[Proctor & Lentz, 2011a, ROP-0021]	OPPTS 830.7950 and OECD 104/ gas saturation method
Melting point:	Purity: 100 % 102 °C (n = 3)	[Van Meter & Lentz, 2010a, ROP-0009]	OPPTS 830.7200/ Capillary/liquid bath
Octanol/water partition coefficient:	Purity: 100 % log Pow = 3.51 at 25 ± 1 ºC The measured pH value in the n-octanol-saturated water was 5. The effect of pH on partition coefficient was not determined	[Van Meter & Lentz, 2010d, ROP-0005]	OECD 107 EC Guideline L383A, Method A8 JMAFF 12 Nousan Guideline 8147 OPPTS 830.7550 Shake flask method
Solubility:	Purity: 100 % In water at 20 °C: 15.8 mg/L The measured pH value is 5.	[Lentz & Van Meter, 2009a, ROP-0001]	OECD 105, EC Guideline L383A, Method A6, JMAFF 12Nousan Guideline 8147, OPPTS 830.7840/ Shake flask method
	Purity: 100 % In organic solvents at 20 °C: 275 g/L in acetone 522 g/L in dichloromethane 158 g/L in ethyl acetate 1.46 g/L in hexane 169 g/L in methanol 31.8 g/L in n-octanol 114 g/L in toluene	[Lentz & Van Meter, 2011a, ROP-0014]	OPPTS 830.7840/ Shake flask method
Specific gravity:	Purity: 100 % 1.24 g/cm³ at 20.6 °C (n = 3) Was determined in hexane and then converted to density in water.	[Van Meter & Lentz, 2010a, ROP-0009]	OPPTS 830.7300, Pycnometer
Hydrolysis:	Covered by hydrolysis of R- and S-isomers (see below)		
Photolysis:	Covered by photolysis of R- and S-isomers (see below)		

Parameter	Result	References	Guidelines/method
Dissociation constant:	No dissociative activity in the pH range pH 2 to pH 10 Purity: 100 %, Temperature: 24.3-24.4 °C	[Van Meter & Lentz, 2010a, ROP-0009]	OPPTS 830.7050 and EU 91/414/EEC Directive, Annex II, 2.5 UV/Visible absorption spectra

Table 2 Pure R-isomer of mandestrobin

Parameter	Result	References	Guidelines/method
Appearance:	Purity: 100 % Colour: white (Hue: N 9.5/), Physical state: powdery solid No odour	[Van Meter & Lentz, 2010b, ROP-0010]	OPPTS 830.6302/ visual Munsell colour system, OPPTS 830.6303/ visual and OPPTS 830.6304/ olfactory determination
Vapour pressure:	Purity: 100 % 1.53×10^{-3} mPa at 20 °C 2.33×10^{-3} mPa at 25 °C Measured at 40.7, 60.2 and 80.8 °C and extrapolated with Clapeyron-Clausius equation to 20 °C and 25 °C	[Proctor & Lentz, 2011b, ROP-0022]	OPPTS 830.7950 and OECD 104/ gas saturation method
Melting point:	Purity: 100 % 107 °C (n = 3)	[Van Meter & Lentz, 2010b, ROP-0010]	OPPTS 830.7200/ Capillary/liquid bath
Octanol/water partition coefficient:	Purity: 100 % log Pow = 3.44 at 25 ± 1 ºC The measured pH value in the n-octanol-saturated water was 5. The effect of pH on partition coefficient was not determined	[Van Meter & Lentz, 2010e, ROP-0004]	OECD 107 EC Guideline L383A, Method A8 JMAFF 12 Nousan Guideline 8147 OPPTS 830.7550 Shake flask method
Solubility:	Purity: 100 % 25.8 mg/L in water at 20 ± 0.5 °C The measured pH value is 5. Chiral HPLC showed only one peak, indicating that no isomerisation occurred in the 24, 48 and 72 hour shake flask samples.	[Lentz & Van Meter, 2009b, ROP-0002]	OECD 105, EC Guideline L383A, Method A6, JMAFF 12Nousan Guideline 8147, OPPTS 830.7840/ Shake flask method
	Purity: 100 % In organic solvents at 20 ± 1.0 °C: 424 g/L in acetone 519 g/L in dichloromethane 264 g/L in ethyl acetate 2.57 g/L in hexane 352 g/L in methanol 97.7 g/L in n-octanol 227 g/L in toluene	[Lentz & Van Meter, 2011b, ROP-0015]	OPPTS 830.7840/ Shake flask method
Specific gravity:	Purity: 100 % 1.23 g/cm³ at 20.6 °C (n = 3) Was determined in hexane, which is a solvent in which the sample is insoluble and/or which	[Van Meter & Lentz, 2010b, ROP-0010]	OPPTS 830.7300, Pycnometer

Parameter	Result	References	Guidelines/method
	wets the sample.		
Hydrolysis in water:	[Benzyl-^{14}C] R-isomer of mandestrobin Radiochemical purity: 99.2 % Stable at pH 4, 7 and 9 over 5 days at 50 ± 0.5 ºC in the dark under sterile conditions at 1 mg ai/L. No degradation products were detected and no conversion of *R*-isomer of mandestrobin to *S*-isomer of mandestrobin was observed. Expected DT_{50} at pH 4, 7 and 9 at 25 ºC: >1 year, as <10 % of substance degrades over 5 days at 50 ± 0.5 ºC. No hydrolysis is expected under normal environmental conditions.	[Lewis & Alderman, 2010a, ROM-0005]	OECD 111, OPPTS 835.2110
Photolysis in water:	[Benzyl-^{14}C]-*R*-isomer of mandestrobin with radiochemical purity: 99.2 % [Phenoxy-^{14}C] *R*-isomer of mandestrobin with radiochemical purity: 99.1 % Photodegradation of *R*-isomer of mandestrobin was studied under simulated sunlight in sterile pH 7 buffer at 25 ± 1.0 º for × days with × mg ai/L. The irradiation intensity was adjusted to ca 25 Watts/m2 (300–400 nm) Mandestrobin degrades to less than 3% TRR within 30 days Degradation for Mandestrobin equivalent to UK/US summer sunlight: Bz-^{14}C Ph-^{14}C DT_{50}(days) 5.3 3.6 DT_{75} (days) 10.5 7.2 DT_{90} (days) 17.5 12.0 . %TRR Bz-^{14}C %TRR Ph-^{14}C Mandestrobin-OR 24 22 Mandestrobin-ORC 16 18 Mandestrobin-PR 9.3 9.6 De Xy-mandestrobin 4.6 NA DX-CA-mandestrobin 0.7 NA [a] 5-COOH mandestrobin 0.3 1.5 [a] MCBX 0.3 0.5 [a] Peak A 7.3 5.3 [b] A large number (26–33) of minor unknowns were present at < 10 % of applied radioactivity, whereas largest unknown accounted for 6.6–6.8% TRR	[Lewis & Aldermann, 2010c, ROM-0013]	OECD 316, UV/VIS spectrum

Parameter	Result	References	Guidelines/method
	Mandestrobin was stable in the dark (> 93% recovered). There was no isomerisation of R to the S-isomer Degradation products: DT_{50} (days) Mandestrobin-OR 5.1 Mandestrobin-PR 2.5		
Dissociation constant:	No dissociative activity in the pH range pH 2 to pH 10 Purity: 100 % Temperature: 24.3–24.4 °C	[Van Meter & Lentz, 2010b, ROP-0010]	OPPTS 830.7050 and EU 91/414/EEC Directive, Annex II, 2.5 UV/Visible absorption spectra

[a] Identified with HPLC, but not confirmed by 2D-TLC

[b] Co-chromatographed with 2-COOH-mandestrobin using HPLC, but not confirmed by 2D-TLC. Isolation of the peak showed that the peak contained several degradation products, maximum 2.0% of applied radioactivity.

Table 3 Pure S-isomer of mandestrobin

Parameter	Result	References	Guidelines/method
Appearance:	Purity: 99.7 % Colour: White (Hue: N 9.5/) Physical state: Powdery solid Mild sulfuryl/acidic odour	[Van Meter & Lentz, 2010c, ROP-0011]	OPPTS 830.6302/ visual Munsell colour system, OPPTS 830.6303/ visual and OPPTS 830.6304/ olfactory determination
Vapour pressure:	No data submitted		
Melting point:	Purity: 99.7 % 106 °C (n = 3)	[Van Meter & Lentz, 2010c, ROP-0011]	OPPTS 830.7200/ Capillary/liquid bath
Octanol/water partition coefficient:	No data submitted		
Solubility:	29.1 mg/L in water at 20 ± 0.5 °C Purity: 99.7 % The measured pH was 5. Chiral HPLC showed only one peak, indicating that no isomerisation occurred in the 24, 48 and 72 hour shake flask samples.	[Lentz & Van Meter, 2009c, ROP-0003]	OECD 105, EC Guideline L383A, Method A6, JMAFF 12Nousan Guideline 8147, OPPTS 830.7840/ Shake flask method
	In organic solvents at 20 ± 1.0 °C: Purity: 99.7 % 431g/L in acetone 577 g/L in dichloromethane 266 g/L in ethyl acetate 2.79 g/L in hexane 387 g/L in methanol 83.9 g/L in n-octanol 216 g/L in toluene	[Lentz & Van Meter, 2011c, ROP-0016]	OPPTS 830.7840/ Shake flask method
Specific gravity:	Purity: 99.7 % 1.22 g/cm³ at 20.6 °C (n = 3) Was determined in hexane, which is a solvent in which the sample is insoluble	[Van Meter & Lentz, 2010c, ROP-0011]	OPPTS 830.7300, Pycnometer

Parameter	Result	References	Guidelines/method
	and/or which wets the sample		
Hydrolysis:	[Benzyl-^{14}C] S-isomer of mandestrobin Radiochemical purity: 99.4 % Substance was stable to hydrolysis at pH 4, 7 and 9 over 5 days at 50 ± 0.5 ºC in the dark under sterile conditions at 1 mg ai/L. No degradation products were detected and no conversion of *S*-isomer to *R*-isomer was observed. Expected DT_{50} at pH 4, 7 and 9 at 25 ºC: >1 year, as <10 % of the substance degrades over 5 days at 50 ± 0.5 ºC. No hydrolysis is expected under normal environmental conditions.	[Lewis & Alderman, 2010b ROM-0006]	OECD 111, OPPTS 835.2110
Photolysis:	[Benzyl-^{14}C]-S-isomer of mandestrobin Radiochemical purity > 98% Photodegradation was studied under simulated sunlight in sterile pH 7 buffer at 25 ± 1.0 ºC for 30 days with 1 mg ai/L. The irradiation intensity was adjusted to ca 25 Watts/m^2 (300–400 nm) Mandestrobin degrades to less than 3% TRR within 30 days Degradation for Mandestrobin equivalent to UK/US summer sunlight DT_{50}(days) 4.6 DT_{75} (days) 9.2 DT_{90} (days) 15.3 Degradation products: Max level within 30 day period as %TRR DT_{50} Mandestrobin-OR 19 % 4.0 Mandestrobin-ORC 10 % - Mandestrobin-PR 7.5 % 2.2 De Xy-mandestrobin 7.2% - DX-CA-mandestrobin 3.0% - [a] 5-COOH-mandestrobin 0.7% - [a] MCBX 0.3% - [a] peak A 6.3% - [b] Volatile radioactivity 2.4% up to 33 unknowns with largest unknown 8.1 % Mandestrobin was stable in the dark (> 93% recovered). There was no isomerisation of S to the R-isomer Degradation products: DT_{50} (days) Mandestrobin-OR 4.0 Mandestrobin-PR 2.2	[Lewis & Alderman, 2010d, ROM-0011]	OECD 316, UV/VIS spectrum
Dissociation constant:	No dissociative activity in the pH range pH 2 to pH 10 Purity: 99.7 % Temperature: 24.3–24.4 °C.	[Van Meter & Lentz, 2010c, ROP-0011]	OPPTS 830.7050 and EU 91/414/EEC Directive, Annex II, 2.5 UV/Visible absorption spectra

[a] Identified with HPLC, but not confirmed by 2D-TLC

[b] Co-chromatographed with 2-COOH-mandestrobin using HPLC, but not confirmed by 2D-TLC. Isolation of the peak showed that the peak contained several degradation products, maximum 2.0% of applied radioactivity.

Table 4 Technical grade mandestrobin

Parameter	Result	References	Guidelines/methods
Minimum Purity	Minimum of 94% (n = 5) on dry weight basis (based on constant mass) Minimum of 88 % (n = 5) on wet weight basis (technical concentrate)	[Pilot plant] [Minamisaki, 2012a, ROP-0029]	OPPTS 830.1700/ HPLC (UV spectra)
	Minimum of 95 % (n = 6) on dry weight basis (based on constant mass) Minimum of 86 % (n = 6) on wet weight basis (technical concentrate)	[Onishi, 2015, ROP-0048]	OPPTS 830.1700/ HPLC (UV spectra)
Appearance:	Purity: 98.8% (dry weight basis) Colour: White (Hue: N 9.5/) Physical state: Crystalline powdery solid Odour: No odour	[Crane, 2012b, ROP-0039]	OPPTS 830.6302/ visual Munsell colour system, OPPTS 830.6303/ visual and OPPTS 830.6304/ olfactory determination
	Purity: 93.4% Colour: Very Pale Yellow (Hue:5Y 9/2) Physical state: Powdery solid Odour: Mild, alcoholic	[Van Meter and Lentz, 2011d, ROP-0013]	OPPTS 830.6302 / visual Munsell colour system, OPPTS 830.6303/visual and OPPTS 830.6304/olfactory determination
Density:	Purity: 98.8% (dry weight basis) 1.2015 g/cm^3 at 20 °C (n = 5)	[Crane, 2012b, ROP-0039]	OECD 109, OPPTS 830.7300/ Pycnometer
	Purity: 93.4% 1.23 g/cm^3 at 20.6 °C (n = 3)	[Van Meter and Lentz, 2011d, ROP-0013]	OPPTS 830.7300/ Pycnometer
Solubility	Purity: 98.8 % (dry weight basis) (90.9% when corrected for water and organic volatiles content) In organic solvents at 20 °C 332.30 g/L in acetone 394.56 g/L in dichloromethane 274.11 g/L in ethyl acetate 1.996 g/L in hexane 182.46 g/L in methanol 63.62 g/L in n-octanol 146.93 g/L in toluene:	[Foster and Crane, 2013, ROP-0041]	OPPTS 830.7840/ Shake flask method
Melting range and thermal stability	Purity (reported): 93.4% Endothermic events observed at 100 °C and at 300 °C correspond to melting and boiling point, respectively. Thermally stable within the temperature range 20 to 500 °C.	[Lentz and Meter,2011e, ROP-0017]	OECD 113 OPPTS 830.6316 Differential scanning calorimetry (DSC)
Thermal Stability:	Purity: 98.8% (dry weight basis) Stable for at least 14 days at normal (17.4 to 21.9 °C) and elevated (54 °C)	[Onishi,2012c, ROP-0030]	OPPTS 830.6313/ CIPAC MT 46

Parameter	Result	References	Guidelines/methods
	temperatures in the presence or absence of metals and metal ions (iron powder, nickel powder, iron (II) acetate or nickel (II) acetate).		

Formulations

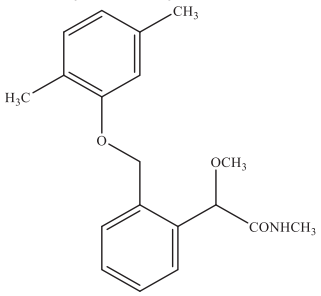
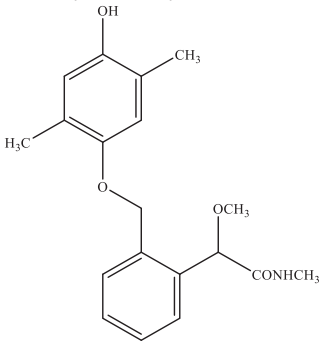
Mandestrobin has not been evaluated by JMPS and therefore no FAO specifications for technical and formulated mandestrobin have been published.

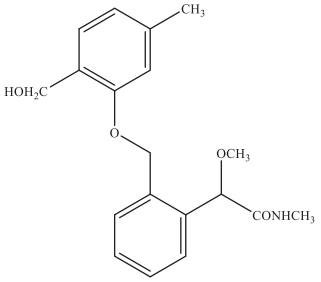
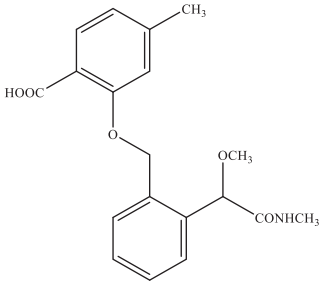
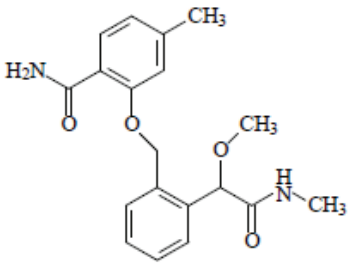
A suspension concentrate (SC) formulation containing 43.4% (w/w) mandestrobin is commercially available in Canada and the USA.

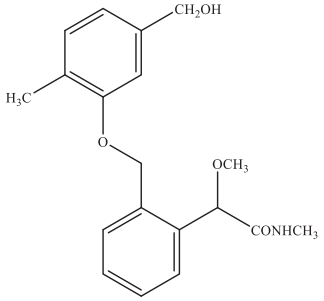
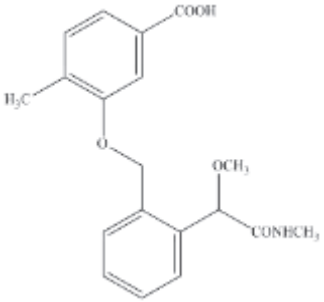
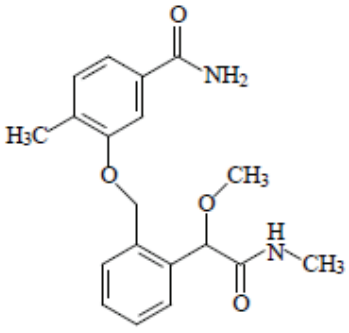
List of reference compounds used in various study reports

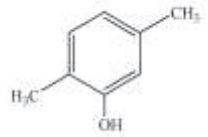
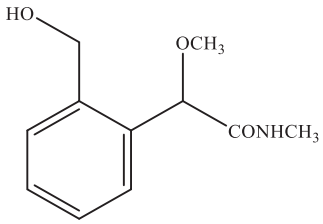
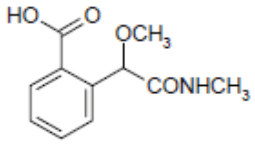
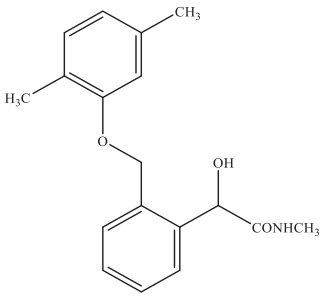
The reference compounds used in the various study reports are listed in Table 5.

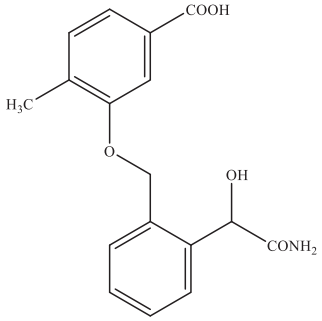
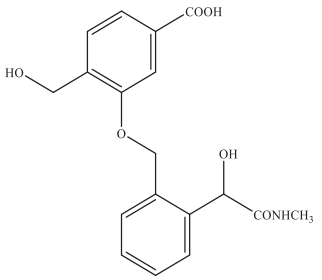
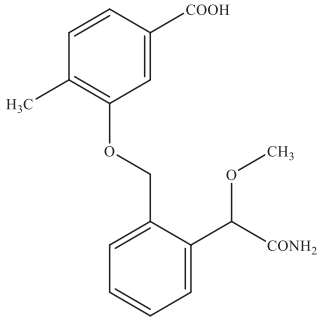
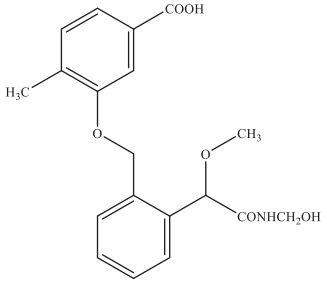
Table 5 List of detected compounds in the various study reports

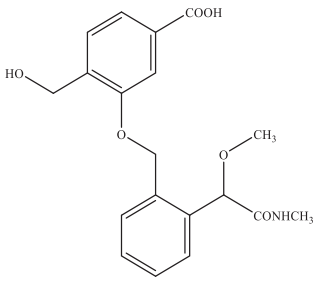
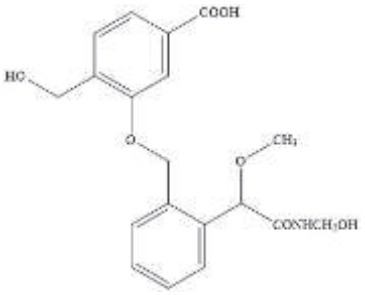
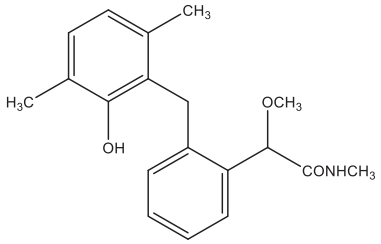
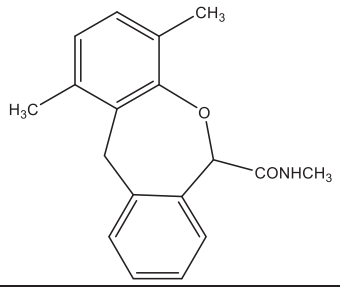
Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
Mandestrobin	IUPAC: (2RS)-2-((2,5-dimethylphenoxy)methyl)phenyl)-2-methoxy-N-methylacetamide  MW = 313.39	Hydrolysis in water Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Tomato fruits; Tomato leaves Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60); Rape seed [application at bloom] Rotated wheat grain; Rotated wheat straw Rotated wheat forage; Rotated wheat hay Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Egg Poultry liver Poultry muscle Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
4-OH-mandestrobin	IUPAC: (2***RS***)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-***N***-methylacetamide  MW = 329.39	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rape seed [application before or at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat grain; Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot leaves Egg Poultry liver Poultry muscle Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney

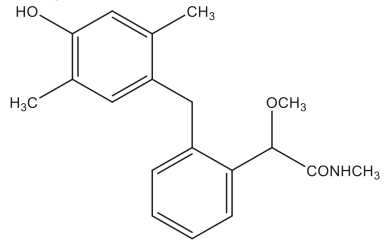
Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
2-CH_2OH-mandestrobin	IUPAC: (2***RS***)-2-[2-(2-hydroxymethyl-5-methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide  MW = 329.39	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Wheat grain Green rape seed fodder (BBCH > 60) Rape seed [application before or at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Poultry liver Poultry muscle Poultry skin Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
2-COOH-mandestrobin	IUPAC: 2-({2-[(1*RS*)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid  MW = 343.38	Photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Egg Poultry liver Poultry skin Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
2-$CONH_2$-mandestrobin	2-amide-mandestrobin IUPAC: 2-({2-[1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzamide 	Laboratory aerobic soil degradation Analysed in US field dissipation and not detected
5-CH_2OH-mandestrobin	IUPAC: (2*RS*)-2-[2-(5-hydroxymethyl-2-methylphenoxymethyl)phenyl]-2-methoxy-*N*-methylacetamide	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rape seed [application at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot leaves Milk fat Ruminant liver; Ruminant kidney

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
	 MW = 329.39	
5-COOH-mandestrobin	IUPAC: 3-({2-[(1*RS*)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid; (RS)-2-{2-[1-methoxy-1-(N-methylcarbamoyl)methyl] benzyloxy}-4-methylbenzoic acid  MW = 343.38	Photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Wheat straw Rape seed [before and at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Poultry skin Ruminant liver; Ruminant kidney Ruminant fat
5-$CONH_2$-mandestrobin	5-amide-mandestrobin IUPAC: 3-({2-[1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzamide 	Laboratory aerobic soil degradation Analysed in US field dissipation and not detected
2,5-dimethylphenol	DMP IUPAC: 2,5-dimethylphenol; 2-hydroxy-p-xylene; p-xylenol; [only visible with phenoxy-label]	Analysed in soil surface photodegradation but not detected Laboratory aerobic soil degradation Analysed in crops (tomato, lettuce, wheat, rape seed) and rotational crops and not detected

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
		
De-Xy-mandestrobin	IUPAC: (2***RS***)-2-(2-hydroxymethylphenyl)-2-methoxy-***N***-methylacetamide [only visible with benzyl label]  MW = 210.253	Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Wheat grain Egg Poultry liver Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
DX-CA-Mandestrobin	(RS)-2-(N-methylcarbamoyl-methoxymethyl)benzoic acid; 2-(1-methoxy-2-(methylamino)-2-oxoethyl)benzoic acid [only visible with benzyl label] 	photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation
MCBX	IUPAC: 2-(2-((2,5-dimethylphenoxy)methyl)phenyl)-2-hydroxy-N-methylacetamide; (*RS*)-2-hydroxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*tolyl] acetamide  MW = 299.37	Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rotated wheat forage ; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Egg Poultry fat Skimmed milk Ruminant liver; Ruminant kidney
5-CA-MCBX-NDM	3-((2-(2-amino-1-hydroxy-2-oxoethyl)benzyl)oxy)-4-methylbenzoic acid	Poultry liver Poultry skin Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant fat Poultry liver Poultry skin

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
	 MW = 315.33	Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant fat
5-CA-2-HM-MCBX	3-((2-(1-hydroxy-2-(methylamino)-2-oxoethyl)benzyl)oxy)-4-(hydroxymethyl)benzoic acid  MW = 345.35	Ruminant liver Poultry liver
5-CA-mandestrobin-NDM	3-((2-(2-amino-1-methoxy-2-oxoethyl)benzyl)oxy)-4-methylbenzoic acid  MW = 329.35	Poultry liver Milk fat Ruminant liver Ruminant fat
5-CA-mandestrobin-NHM	3-((2-(2-((hydroxymethyl)amino)-1-methoxy-2-oxoethyl)benzyl)oxy)-4-methylbenzoic acid  MW = 359.38	Egg Poultry skin; Poultry fat Skimmed milk Ruminant liver

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
5-CA-2-HM-mandestrobin	4-(hydroxymethyl)-3-((2-(1-methoxy-2-(methylamino)-2-oxoethyl)benzyl)oxy)benzoic acid  MW = 359.38	Poultry liver Poultry skin Ruminant liver; Ruminant kidney
5-CA-2-HM-mandestrobin-NHM	4-(hydroxymethyl)-3-((2-(2-((hydroxymethyl)amino)-1-methoxy-2-oxoethyl)benzyl)oxy)benzoic acid  MW = 375.38	Poultry skin Ruminant liver; Ruminant kidney
Mandestrobin-OR	(*RS*)-2-(2-(2-hydroxy-3,6-dimethylbenzyl)phenyl)-2-methoxy-N-methylacetamide  	Photolysis in water Soil surface photodegradation The benzyl radical recombined at the o- position of the phenoxy radical.
Mandestrobin-ORC	(*RS*)-N,1,4-trimethyl-6,11-dihydrodibenzo[b,e]oxepine-6-carboxamide  	Photolysis in water Analysed in soil surface photodegradation but not detected Formed from mandestrobin-OR by cyclisation.
Mandestrobin-PR	(*RS*)-2-(2-(4-hydroxy-2,5-	Photolysis in water

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
	dimethylbenzyl)phenyl)-2-methoxy-N-methylacetamide 	Analysed in soil surface photodegradation but not detected The benzyl radical recombined at the p- position of the phenoxy radical.

METABOLISM AND ENVIRONMENTAL FATE

PLANT METABOLISM

The meeting received plant metabolism studies for mandestrobin after topical and soil application to tomato fruits and foliar spray application on lettuce (leafy crops), wheat (cereals/grass crops) and rape seed (oilseeds and legume crops). Mandestrobin was applied using [-^{14}C] -phenoxy labelled and [^{14}C] -benzyl labelled mandestrobin, indicated as [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin, respectively. The structural formula for both radiolabels is given in Figure 1.

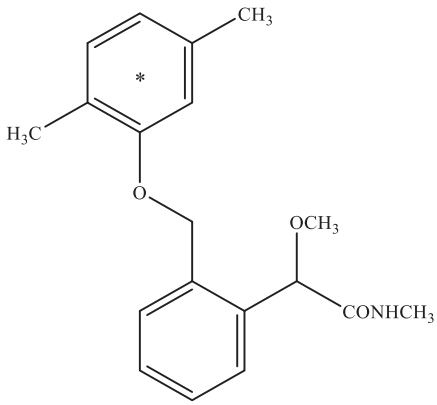
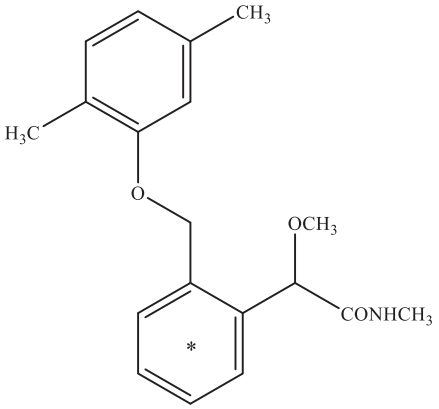
	
[Phenoxy-^{14}C]-mandestrobin, [Ph-^{14}C]-mandestrobin	[Benzyl-^{14}C]-mandestrobin, [Bz-^{14}C]-mandestrobin

Figure 1 Structural formula of [Phenoxy-^{14}C]-mandestrobin and [Benzyl-^{14}C]-mandestrobin

Topical applications of fruits and fruiting vegetables

The metabolic fate of mandestrobin following topical leaf or fruit application was studied in tomato plants of the variety Patio [Ichise *et al*., 2007, ROM-0001]. The tomato plants were grown in pots, filled with a sandy loam soil, in a greenhouse at 25/20 °C (day/night) in February-June 2007 in Takarazuka, Japan. Mandestrobin was separately labelled with ^{14}C at the phenoxy or benzyl rings.

For topical fruit and leaf applications, the plants received 3 topical applications at a rate equivalent to 0.3 kg ai/ha each, with a 10 day interval. The rate equivalence was reported by applicant, without supporting calculation. The ^{14}C labelled SC formulated compounds were applied to two fruits per pot and 5 leaves per pot, using a microsyringe. The dosing amount was determined in proportion to the surface area of fruit and leaves. The first application was conducted at fruit

development stage (fruits 4 cm diameter) and the fruits and leaves were harvested 3 days after the last application (PHI 3 days). The fruits and leaves from the topically treated plants were individually cut from the stem.

Samples were immediately stored at -20 °C. Fruits and leaves were surface rinsed with acetonitrile and extracted three times with acetone/water (80:20; v/v). The radioactivity in the surface rinse and the organic extracts was determined by liquid scintillation counting (LSC). The radioactivity in the untreated fruits and leaves and the post-extraction solids (PES) were quantified by LSC after combustion. Extracted fractions were profiled separately by radio-HPLC and 1D-TLC using co-chromatography with reference standards: mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, De-Xy-mandestrobin, MCBX, 2,5 dimethylphenol. Selected peaks (E06, E07) were treated for 1–5 days with cellulose and beta-glucosidase at pH 5.0 at 39 °C to release the aglycones.

The proportions of radioactivity in surface washes and extracts of samples for topically treated plants and the characterisation of the residues are summarized in Table 6. For the topical, fruit and leaf applications, total radioactive residues in fruit and leaves were 7.4-14 mg eq/kg and 87-199 mg eq/kg, respectively. No residues were detected in the untreated leaves and fruits indicating no translocation from the treated fruits or leaves to the other parts of the plants. The majority of the radioactivity (95-99% TRR) remained on the fruit or leaf surface, suggesting no systemic character in plant. The major compound was identified as parent: 99-100% TRR in fruits and 98-99% TRR in leaves. Chiral HPLC analysis demonstrated no epimerization because the ratios of the R- and S-isomer of the parent did not change on the plant. Metabolites accounted for less than 1% TRR, each. Two minor metabolites (0.85% TRR as E06 and 0.65% TRR as E07) were identified as (malonyl)glucosides of hydroxylated mandestrobin using HPLC-MS/MS in combination with enzymatic treatment. The molecular weight (M+H) for E06 and E07 in positive ion mode decreased from 842 to 578 after cellulase and beta-glucosidase treatment and the enzymatic degradation rate was very slow, indicative of a modified glucose conjugate like malonylglucose. The daughter ions of 192, 160 and 132 are typical fragmentation peaks of the parent compound and indicate no structural changes at the benzyl ring moiety. The definitive position of the OH group could not be determined and the most probable candidates are 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin.

Table 6 Distribution of [^{14}C] mandestrobin residues in extracts of topically treated tomato plants

Treatment	Fraction	Total Radioactive Residue [a]			
		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Topically treated fruits 3 × 0.30 kg ai/ha, PHI 3 days	Acetonitrile surface wash	7.2	98.0%	13	97.1%
	3 × acetone/water extraction	0.13	1.8%	0.36	2.6%
	TRR as sum of extracts	7.4	99.8%	14	99.7%
	..-- parent	-7.4	-100%	-14	-99%
	..-- unknown extracted	-ND	-ND	-0.032	-0.23%
	PES	0.012	0.2%	0.047	0.3%
	TRR as sum of fractions	7.4	100.0%	14	100.0%
Topically treated leaves 3 × 0.30 kg ai/ha, PHI 3 days	Acetonitrile surface wash	86	98.8%	190	95.5%
	3 × acetone/water extraction	0.85	0.98%	8.6	4.3%
	TRR as sum of extracts	87	99.7%	199	99.8%
	..-- parent	-86	-99%	-195	-98%
	..-- E06	-ND	-ND	-1.7	-0.85%
	..-- E07	-ND	-ND	-1.3	-0.65%
	..-- polar region	-0.077	-0.09%	-ND	-ND
	..-- unknown extracted	-0.15	-0.18%	-0.83	-0.42%
	PES	0.22	0.25%	0.37	0.2%
	TRR as sum of fractions	87	100.0%	199	100.0%

%TRR of residues may not add up to 100% due to rounding

[a] %TRR recalculated by the reviewer assuming 100% TRR for the sum of fractions.

Soil treatments of fruits and fruiting vegetables

The metabolic fate of mandestrobin following soil application was studied in tomato plants of the variety Patio [Ichise *et al*., 2007, ROM-0001]. The tomato plants were grown in pots, filled with a sandy loam soil, in a greenhouse at 25/20 °C (day/night) in February-June 2007 in Takarazuka, Japan. Mandestrobin was separately labelled with ^{14}C at the phenoxy or benzyl rings.

The ^{14}C labelled compounds were applied as acetonitrile solution at a rate of 0.9 kg ai/ha when whole fruit was developing. The soil and the soil treated tomato plants were harvested 24 days after the application (PHI 24 days). The soil treated tomato plants were separated into leaves and fruits. The soil was divided in three portions (0–2 cm top layer, 2–10 cm middle layer and 10–18- cm bottom layer) and dried.

Samples were immediately stored at -20 °C. Soil fractions were extracted three times with acetone/water (90:10, v/v), followed by three extractions with acetone/0.1 M HCl (5:1, v/v). The radioactivity in the organic extracts was determined by liquid scintillation counting (LSC). The radioactivity in the fruits, leaves and soil post-extraction solids (PES) were quantified by LSC after combustion. Extracted fractions were profiled separately by radio-HPLC and 1D-TLC using co-chromatography with reference standards: mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, De-Xy-mandestrobin, MCBX, 2,5 dimethylphenol.

The distribution of the applied radioactivity for soil treated plants is summarized in Table 7 and the characterisation of soil residues is summarized in Table 8. Insignificant translocation from soil to plant was observed: 0.06–0.08% TAR in leaves, none in fruits. The majority of the radioactivity (53-72% TAR) remained in the top soil layer (0–2 cm). The major compound identified in all three soil layers was the parent compound. Metabolites 2-COOH-mandestrobin and 5-COOH-mandestrobin were detected at trace levels in the top soil layer only.

Table 7 Distribution of [^{14}C] mandestrobin residues in soil and soil treated tomato plants

Treatment	Fraction	Total Radioactive Residue			
		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TAR	mg eq/kg	%TAR
Fruits from soil treatment 1 × 0.90 kg ai/ha, PHI 24 days	-	ND	ND	ND	ND
Leaves from soil treatment 1 × 0.90 kg ai/ha, PHI 24 days	-	0.23	0.08%	0.21	0.06%
Soil – top layer 1 × 0.90 kg ai/ha, PHI 24 days	acetonitrile/water extract	3.4	43%	2.5	33%
	acetone/0.1 M HCl extract	1.7	21%	1.4	19%
	Unextracted residues	0.54	6.9%	0.090	1.2%
	Total	5.6	72%	4.0	53%
Soil – middle layer 1 × 0.90 kg ai/ha, PHI 24 days	acetonitrile/water extract	0.15	16%	0.17	14%
	acetone/0.1 M HCl extract	0.070	7.3%	0.17	15%
	Unextracted residues	0.016	1.7%	0.013	1.1%
	Total	0.24	25%	0.36	30%
Soil – bottom layer 1 × 0.90 kg ai/ha, PHI 24 days	acetonitrile/water extract	-	-	0.12	12%
	acetone/0.1 M HCl extract	-	-	0.051	5.1%
	Unextracted residues	-	-	0.018	1.8%
	Total	0.064	4.8%	0.19	19%
Total Radioactive Residues	-	6.1	101%	4.7	102%

Table 8 Identification of residues in soil after application of [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin

Treatment	Fraction	Total Radioactive Residue			
		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TRR [a]	mg eq/kg	%TRR [a]
Soil – top layer 1 × 0.90 kg ai/ha, PHI 24 days	parent	5.0	63%	3.9	51%
	2-COOH-mandestrobin	0.012	0.16%	ND	ND

Treatment	Fraction	Total Radioactive Residue			
		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TRR [a]	mg eq/kg	%TRR [a]
	5-COOH-mandestrobin	0.040	0.51%	0.001	0.01%
	unknown extracted	ND	ND	ND	ND
	Unextracted residues	0.54	6.9%	0.090	1.2%
	Total	5.6	71%	4.0	52%
Soil – middle layer 1 × 0.90 kg ai/ha, PHI 24 days	parent	0.22	23%	0.34	29%
	2-COOH-mandestrobin	ND	ND	ND	ND
	5-COOH-mandestrobin	ND	ND	ND	ND
	unknown extracted	ND	ND	ND	ND
	Unextracted residues	0.016	1.7%	0.013	1.1%
	Total	0.24	24%	0.36	30%
Soil – bottom layer 1 × 0.90 kg ai/ha, PHI 24 days	parent	-	-	0.17	16%
	2-COOH-mandestrobin	-	-	ND	ND
	5-COOH-mandestrobin	-	-	ND	ND
	unknown extracted	-	-	ND	ND
	Unextracted residues	-	-	0.018	1.7%
	Total	0.064	4.7%	0.19	18%

[a] %TRR expressed for the total soil (i.e. sum of all three layers)

Foliar applications to leafy vegetables (lettuce)

The metabolic fate of mandestrobin following foliar application was studied in lettuce plants [Panthani and Lentz, 2010b, ROM-0008]. The study was conducted in May-June 2009 in Wareham Massachusetts, USA. In this greenhouse study (28 °C day/21 °C night, relative humidity 19-65 %) lettuce plants (*Lactuca sativa*), of the variety Butter crunch, grown in pots, filled with sandy loam, were grouped in 3 sets, of which groups II and III were foliar sprayed with radiolabel [Ph-^{14}C]-mandestrobin and [Bz-^{14}C]-m

andestrobin, respectively. Group I served as untreated control. The spray was formulated as 25 % (w/v) SC at an actual application rate equivalent to 2 ×0.82 kg ai/ha with an interval of 10 days. Lettuce plants were planted on 15 April 2009 and received the first spray application on 26 May 2009 at BBCH 43. Immature lettuce was harvested 5 days later at BBCH 45 (weight 0.074–0.11 kg). The remainder of the lettuce received a second spray application on 5 June 2009 at BBCH 48. Mature lettuce was harvested 5 days later at BBCH 49 (weight 0.42–0.50 kg).

Immature and mature lettuce samples were collected (cutting 2–4 cm above soil) at the respective harvest intervals, adhering soil was removed and the leaf surface was washed with acetonitrile. Washed lettuce samples were homogenised, extracted twice with acetone/water (80:20; v/v) and once with acetone/water/concentrated hydrochloric acid (80:20:1; v/v/v). Samples were stored frozen (temperature not indicated) between 1–9 months before analysis. The radioactivity in the surface washes and the organic extracts was determined by liquid scintillation counting (LSC) and analysed by radio-HPLC. The radioactivity in the post-extraction solids (PES) was quantified by LSC after combustion.

The proportions of radioactivity in surface washes and extracts of samples are summarized in Table 9 for both radiolabels, [Ph-^{14}C]- and [Bz-^{14C}]-mandestrobin. The distribution of TRR in lettuce leaves was very similar in both radiolabels, from each sampling event, in immature and mature plants. However, a greater proportion of the radioactivity had been absorbed into the foliage at the later harvest timing, with an increase in the relative amount of unextracted residues. The greater part of the radioactive residue (78-88% TRR) can be washed off with the acetonitrile surface wash, for both radiolabels in immature and mature lettuce samples. A total of 98-100% TRR could be extracted with acetone/water (including the acetonitrile surface wash).

Table 9 Proportions of TRR in surface washes and extracts of [^{14}C] mandestrobin residues in lettuce foliage

Plant, Harvest interval and application	Fraction	Total Radioactive Residue			
		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Immature lettuce 5 days after 1st application (1 × 0.82 kg ai/ha)	Acetonitrile surface wash	31	88	25	88
	1st Extraction acetone/water	3.4	9.8	2.8	10
	2nd Extraction acetone /water	0.49	1.4	0.44	1.6
	TRR sum of neutral extractions [a]	**35**	**100**	**28**	**100**
	Extraction acetone/water/HCl	0.095	0.27	0.075	0.27
	PES	0.073	0.21	0.061	0.22
	TRR as sum of fractions	35	100	28	100
Mature lettuce 5 days after 2nd application (2 × 0.82 kg ai/ha)	Acetonitrile surface wash	35	82	33	78
	1st Extraction acetone/water	6.4	15	7.2	17
	2nd Extraction acetone /water	0.73	1.7	1.0	2.4
	TRR sum of neutral extractions [a]	**42**	**98**	**41**	**98**
	Extraction acetone /water / HCl	0.24	0.54	0.34	0.83
	PES	0.49	1.1	0.44	1.1
	TRR as sum of fractions	43	100	42	99

%TRR of residues may not add up to 100% due to rounding

[a] Analytical methods make use of neutral acetone/water extractions

Extracted fractions were profiled separately by radio-HPLC and TLC using co-chromatography with reference standards: mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 4-OH-mandestrobin, De-Xy-mandestrobin, MCBX, (R) MCBX, (S) MCBX, mandestrobin R-isomer, mandestrobin S-isomer and 2,5 dimethylphenol. Chiral HPLC was employed to determine the ratio of R- and S-isomers of mandestrobin, only. The 16-min and 19-min HPLC regions from the mature lettuce extracts were treated with cellulase and beta-glucosidase (at pH 5 for 7 days at 37 °C) and the aglycones were identified by HPLC co-chromatography with authentic reference standards. The remaining solids of the mature harvest samples were further characterised by sequential treatment with Driselase (enzyme mixture of fungal carbohydrolases; twice overnight at pH 4.5 at 37 °C), mild acid hydrolysis (0.1 M HCl, 40 °C, overnight) and mild base hydrolysis (0.1 M NaOH, 40 °C, overnight). The total radioactive residues (TRR) in each fraction were quantified by combustion analysis, but residues released from these solids were not verified against known reference standards.

The identification and characterisation of radioactive residues in lettuce leaves are shown in Table 10. Both radiolabels gave similar metabolite profiles in immature and mature lettuce leaves, whereas the following residues were identified: De-Xy-mandestrobin, 2-CH_2OH-mandestrobin (conjugated), 4-OH-mandestrobin (conjugated), a mixture of 5-CH_2OH mandestrobin (conjugated) and 5-COOH-mandestrobin (conjugated), MCBX.

The major part of the residue could be assigned to parent (89–94% TRR). The R/S ratio of mandestrobin in the surface wash and neutral extracts remained approximately 1:1, indicating no R/S isomerization. No analysis of R/S isomerization for metabolites was performed. Low levels of metabolites were found in the surface wash plus extractable fraction, each below 3% TRR.

Storage stability: Two representative final harvest samples of mature lettuce, one treated with [Bz-^{14}C]- and the other one with [Ph-^{14}C]-mandestrobin, were stored frozen (temperature not indicated) for approximately 1 month and 6 months, respectively. Neutral and acid extraction was performed for both samples after respective storage times. The extracts were analysed by HPLC. Comparison of HPLC profiles of the 1 and 6 months storage and neutral/acid extracted samples showed a similarity of the metabolite profiles of corresponding analyses, indicating the stability of metabolites in the lettuce samples during storage.

Note by the reviewer: In an addendum to the wheat metabolism study, the 16 and 19 min HPLC peaks were identified as malonylglucoside conjugates.

Table 10 Distribution and identification of residues in immature and mature lettuce leaves after application of [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin

Residues	1 × 0.82 kg ai/ha, at BBCH 43 PHI = 5 days Immature lettuce				2 × 0.82 kg ai/ha; at BBCH 43 and 48 PHI = 5 days Mature lettuce			
	[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent [a]	33	94	26	93	39	91	37	89
De-Xy-mandestrobin [a]	NA	NA	0.20	0.71	NA	NA	0.27	0.65
4-OH-mandestrobin conjugate (16 min) [b]	0.77	2.2	0.44	1.6	1.2	2.7	1.2	2.8
2-CH_2OH-mandestrobin conjugate (16min) [b]	0.15	0.42	0.10	0.36	0.23	0.52	0.27	0.65
Mixture of 5-CH_2OH-mandestrobin conjugate and 5-COOH-mandestrobin conjugate (19 min) [c]	0.33	0.93	0.20	0.71	0.54	1.3	0.63	1.5
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND
MCBX [a]	0.24	0.68	0.16	0.57	0.26	0.59	0.40	0.97
2,4-dimethylphenol	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**34**	**98**	**27**	**97**	**41**	**96**	**40**	**96**
%identified/neutral extract		**98/100 =98**		**97/100 =97**		**96/98 =98**		**96/98 =98**
Unknown 1 (2 min)	0.056	0.16	0.025	0.090	0.11	0.25	0.24	0.59
Unknown 2 (9 min)	ND	ND	0.25	0.90	ND	ND	0.26	0.64
Unknown 3 (11 min)	ND	ND	ND	ND	0.059	0.14	ND	ND
Unknown 4 (14 min) [d]	0.36	1.0	0.11	0.40	0.74	1.7	0.31	0.75
Unknown 5 (22 min)	ND	ND	ND	ND	ND	ND	ND	ND
Unknown 7 (32 min)	0.037	0.10	0.031	0.11	ND	ND	ND	ND
Unknown 8 (37.8 min)	0.029	0.080	ND	ND	ND	ND	ND	ND
Others [e]	0.10	0.29	0.42	1.5	0.26	0.60	0.61	1.5
PES	0.073	0.21	0.061	0.22	0.49	1.1	0.44	1.1
--Enzyme hydrolysis	NA	NA	NA	NA	-–0.058	-–0.13	-–0.060	-–0.15
--Acid hydrolysis	NA	NA	NA	NA	-–0.020	-–0.05	-–0.024	-–0.06
--Weak base hydrolysis	NA	NA	NA	NA	-–0.16	-–0.38	-–0.22	-–0.52
Unextracted Residues	**0.073**	**0.21**	**0.061**	**0.22**	**0.26**	**0.61**	**0.12**	**0.28**
Total unknowns (extracted or hydrolysed)	**0.58**	**1.6**	**0.84**	**3.0**	**1.4**	**3.3**	**1.7**	**4.2**
TRR Total	**35**	**100**	**28**	**100**	**43**	**100**	**42**	**100**

%TRR of residues may not add up to 100% due to rounding

-- = excluded from the total calculation; total calculation is based on the extracted fractions plus PES

ND = not detected; NA = not applicable

[a] Identity confirmed by HPLC and TLC co-chromatography with authentic reference standards.

[b] The 16-min region from the final harvest was subjected to enzyme hydrolysis and the aglycones were identified by HPLC co-chromatography with authentic reference standards. Ratio of products = 2-CH_2OH-mandestrobin: 4-OH-mandestrobin for [Ph-^{14}C] 1 × and 2 × 0.82 kg/ai/ha = 16: 84 and for [Bz-^{14}C] 1 × and 2 × 0.82 kg/ai/ha =19: 81. The 16-min region from the intermediate harvest lettuce was not hydrolysed. The ratio of products is derived from characterization of the 16-min from the final harvest sample

[c] The 19-min region from the final harvest was subjected to enzyme hydrolysis and the aglycone was characterized as a mixture of 5-CH_2OH-mandestrobin and 5-COOH-mandestrobin.

[d] The 14-minute region is comprised of multiple components.

[e] A number of minor peaks.

Foliar applications to cereals

The metabolic fate of mandestrobin following foliar application was studied in wheat plants [Panthani and Lentz, 2010a, ROM-0009]. The study was conducted from April to September 2009 in Wareham, Massachusetts (USA). In this greenhouse study (28 °C day/21 °C night, relative humidity 19-85 %) wheat seeds (Triticum L.), of the variety Promontory, were sown in 27 individual pots (approximately 30 seeds per pot), containing sandy loam soil and grown until maturity. Equally grouped in 3 sets, nine pots were used for the control group and nine for each radiolabel, [Ph-^{14}C]-mandestrobin and [Bz-^{14}C]-mandestrobin, respectively. Wheat plants were sprayed with [^{14}C]-mandestrobin, formulated as a 25 % (w/v) SC, and one single application was made at an actual rate of 0.31 kg ai/ha and 0.30 kg ai/ha, for the phenoxy and for the benzyl label, respectively, at BBCH 32. Wheat forage and wheat hay (each BBCH 37) were harvested 7 days and 14 days after the single spray application, respectively. Wheat grain and straw (BBCH 92) were harvested at maturity 104 days after the single spray application (DAT). At each harvest interval, plant material was collected from three pots per treatment group by cutting approximately 2 to 4 cm above soil level, and any adhering soil removed by gentle shaking or brushing. Sample sizes were 0.19–0.24 kg for wheat forage and hay, 0.10–0.11 kg for wheat straw and 8.9-18 g wheat grain. Storage conditions between harvest and extraction were not indicated.

Wheat forage, hay and straw samples were surface rinsed with acetonitrile. Wheat grain was not surface washed, as this crop part does not get into direct contact with the spray due to the timing of the application. Homogenised samples of wheat forage, hay, grain and straw were extracted twice with acetone/water (80:20; v/v) and once with acetone/water/concentrated hydrochloric acid (80:20:1; v/v/v, approximately 0.1 M HCl). The radioactivity in the surface washes and the organic extracts was determined by LSC. The radioactivity in the PES was quantified by LSC after combustion.

The TRR values are shown in Table 11. The %TRR values as the sum of extraction fractions including the surface wash were presented as mandestrobin equivalents (eq). The distribution of radioactivity in wheat forage, hay, grain and straw was similar between radiolabels. TRR levels for both radiolabels showed a significant decrease with increase of time (DAT). A simple acetonitrile surface wash removed 34-41%, 19-23% and 2.8-3.7% of radioactivity from the surface of wheat forage, hay and straw, respectively. Major amounts of radioactivity could be extracted with simple solvents (acetone/water including acetonitrile wash) resulting in levels in the range of 91–92%, 84-90%, 53-54% and 45-49%, for wheat forage, hay, grain and straw. An increasing proportion of the TRR remained unextracted with increasing PHI, with 5.4-5.8% of the radioactivity in wheat forage, 8.1–11% in hay, 27-33% in wheat grain and 32–38% in wheat straw remaining in the PES.

Table 11 Distribution of [^{14}C]-mandestrobin residues in wheat forage, hay, straw and grain

Commodity	Fractions	Total Radioactive residue			
		[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Immature wheat forage DAT 7 days	Acetonitrile surface wash	4.6	41	3.5	34
	1st Extraction with acetone/water	4.5	40	5.1	49
	2nd Extraction with acetone /water	1.1	9.7	1.0	9.6
	TRR as sum of neutral extractions	10	91	9.7	92
	Extraction with acetone/water/HCl	0.36	3.2	0.22	2.1
	PES	0.64	5.8	0.57	5.4
	TRR as sum of fractions	11	100	10	100
Immature wheat hay DAT 14 days	Acetonitrile surface wash	1.4	23	1.7	19
	1st Extraction with acetone/water	3.0	48	5.4	60
	2nd Extraction with acetone /water	0.79	13	0.93	10
	TRR as sum of neutral extractions [a]	**5.2**	**84**	**8.1**	**90**
	Extraction with acetone/water/HCl	0.29	4.7	0.20	2.2
	PES	0.68	11	0.73	8.1
	TRR as sum of fractions	6.2	100	9.0	100
Mature wheat straw	Acetonitrile surface wash	0.069	3.7	0.070	2.8
	1st Extraction with acetone/water	0.34	19	0.48	19
	2nd Extraction with acetone /water	0.41	22	0.69	28

Commodity	Fractions	Total Radioactive residue			
		[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
DAT 104 days	**TRR as sum of neutral extractions [a]**	**0.84**	**45**	**1.2**	**49**
	Extraction with acetone/water/HCl	0.33	18	0.45	18
	PES	0.70	38	0.81	32
	TRR as sum of fractions	1.9	100	2.5	100
Mature wheat grain DAT 104 days	Acetonitrile surface wash	Not performed			
	1st Extraction with acetone/water	0.004	36	0.024	27
	2nd Extraction with acetone /water	0.002	18	0.024	27
	TRR as sum of neutral extractions [a]	**0.006**	**54**	**0.048**	**53**
	Extraction with acetone/water/HCl	0.002	13	0.017	19
	PES	0.004	33	0.024	27
	TRR as sum of fractions	0.012	100	0.089	100

%TRR of residues may not add up to 100% due to rounding

DAT – days after treatment

[a] Analytical methods make use of neutral acetone/water extractions

The extracted fractions were profiled separately by radio-HPLC and after isolation of regions of interest, metabolites were identified with HPLC and 1D-TLC using co-chromatography with reference standards: mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 4-OH-mandestrobin, De-Xy-mandestrobin, MCBX, I MCBX, (S) MCBX, R-mandestrobin, S-mandestrobin and 2,5-dimethylphenol. Chiral HPLC was employed to determine the ratio of R- and S-isomers of mandestrobin, only. The 16-min and 19-min HPLC regions from the wheat forage and hay extracts were treated with cellulase and beta-glucosidase (pH 5 for 7 days at 39 °C) and the hydrolysis products were analysed by radio HPLC with co-chromatography with reference standards.

The PES of the wheat hay samples were further characterised by sequential treatment with Driselase (enzyme mixture of fungal carbohydrolases, pH 4.5, 38 °C, overnight), mild acid hydrolysis (0.1M HCl, 40 °C overnight) and mild base hydrolysis (0.1 M NaOH, 40 °C overnight). The PES of the wheat straw samples were further characterised by sequential mild acid hydrolysis (0.1M HCl, 40 °C overnight), strong acid hydrolysis (6 M HCl, 80 °C, 4 hours), mild base hydrolysis (0.1M NaOH, 40 °C overnight) and strong base hydrolysis (6 M NaOH, 80 °C overnight). The TRR in each sample was quantified by LSC of extracts and combustion of solids, but residues released from the PES were not verified against known reference standards.

The distribution and identification of residues in wheat forage, wheat hay, wheat straw and wheat grain are given in Table 12 and Table 13, respectively. Following one single application of mandestrobin to wheat, the major residues identified in the wheat forage, hay, straw and grain samples included the parent, De-Xy-mandestrobin, 2-CH_2OH-mandestrobin (conjugated) and 4-OH-mandestrobin (conjugated). MCBX, 2-CH_2OH-mandestrobin (free), 4-OH- mandestrobin (free), 5-CH_2OH-mandestrobin (free and conjugated), 5-COOH-mandestrobin (free) were also found at lower levels.

In wheat grains, parent mandestrobin and MCBX were not detected in either treatment group. De-Xy-mandestrobin accounted for 61 % of the TRR (0.054 mg eq/kg) in the grain sample for the [Bz-^{14}C]-mandestrobin treatment group. The residue of the [Ph-^{14}C]-mandestrobin treated grain comprised of multiple compounds, which were not further characterised as the concentration of each of the fractions was very low (0.003 mg eq/kg.).

In feed commodities, parent accounted for 51 to 60 % of the TRR (5.7-6.2 mg eq/kg) in wheat forage, 23 to 26 % of the TRR (1.6-2.1 mg eq/kg) in the hay and 1.4-2.0 % of TRR (0.026–0.050 mg eq/kg) in wheat straw for both treatment groups. De-Xy-mandestrobin was less than/equal to 12% TRR in the wheat forage, hay and straw samples. MCBX accounted for 0.30–2.8 % of the TRR (0.01–0.30 mg eq/kg) in the wheat forage, hay, and straw samples for both treatment groups. Free 2-CH_2OH-mandestrobin and glycosides of 2-CH_2OH-mandestrobin were identified in wheat

forage and hay, accounting for the free compound up to 1 % of the TRR (< 0.01- 0.08 mg eq/kg) and for the conjugated between 11 and 13% TRR (0.57 -1.2 mg eq/kg), for both radio labels. The glycosides of 4-OH-mandestrobin and 5-CH_2OH-mandestrobin were observed at 5.4 – 6.2 % and 6.8-13 % of TRR (0.56–0.69 mg eq/kg and 0.62–0.81 mg eq/kg) at their maximum, respectively, in wheat forage and hay samples. In wheat straw free 2-CH_2OH-mandestrobin, free 4-OH-mandestrobin, free 5-CH_2OH-mandestrobin and free 5-COOH-mandestrobin were identified accounting each for less than 10% TRR, with 2-CH_2OH-mandestrobin accounting for 6.4-9.4 % of the TRR (0.16 – 0.18 mg eq/kg) for both radiolabels.

Up to eight unidentified metabolite fractions were found in wheat forage and hay, with no individual component exceeding 7.3% TRR. The unknown metabolite 4 (up to 10–12% TRR in hay) was further characterised in both wheat forage and hay, and revealed to contain several unknown metabolite peaks, each below 2% TRR. In wheat straw up to seven unidentified metabolite fractions were found. The unknown metabolite fraction 4 (up to 12 – 21% TRR in straw) comprised multiple compounds, with the largest component of 7.3% TRR (0.14 mg eq/kg). Metabolite fractions (up to 23–33% TRR) of the phenoxy-labelled wheat grain were not further characterised, as each fraction accounted for less than 0.01 mg eq/kg.

Mandestrobin has an *R:S* isomer ratio of approximately 50:50 in the [^{14}C]-mandestrobin test substances. The *R:S* isomer ratio of the parent mandestrobin in wheat forage and hay acetonitrile surface rinses and neutral solvent extracts was investigated using chiral HPLC. No change in the R:S isomer ratio was seen in the wheat forage and hay samples, indicating no R/S isomerization.

Storage stability: A representative [Ph-^{14}C]-mandestrobin sample (wheat hay) was extracted and analysed after approximately 9 months of freezer storage. A representative [Bz-^{14}C]-mandestrobin sample (wheat hay) was extracted and analysed after approximately 2 months of freezer storage. The HPLC profiles were compared to the initial profiles to verify the stability of metabolites in the matrices during storage. The metabolite profiles from the two corresponding analyses were similar, indicating that [^{14}C]-mandestrobin metabolites were stable in the wheat samples.

Notes by reviewer:

A total of 91–92%, 84–90%, 45–49% and 53–54% TRR could be extracted from wheat forage, hay, straw and grains with acetone/water (including the acetonitrile surface wash). Considering the extraction efficiency with organic solvents, the identification levels in wheat hay (51–59% TRR) and straw (18–30% TRR) are low (i.e. less than 80% of the solvent extracted residue). The identification level of ^{14}C-benzyl labelled wheat grain is acceptable. None of the ^{14}C-phenoxy labelled wheat grain residues were identified due to low residue levels (0.012 mg eq/kg).

The 16 and 19 min HPLC regions of the wheat forage and hay extracts were singled out for treatment with enzymes and contained significant amounts (up to 13% TRR) of conjugates of known metabolites. The remainder of these extracts was not subjected to acid, base and enzyme hydrolysis. A radiovalidation study in green rape fodder (see analytical method section), where the whole extract was treated with alkaline and enzymatic hydrolysis, confirmed that the remainder of the extract from the green rape fodder metabolism study did not contain additional aglycones of known metabolites. This suggests that the 16 and 19 min HPLC regions of wheat commodities are the only regions containing aglycones of known metabolites. In an addendum to the wheat metabolism study, the 16 and 19 min HPLC peaks were identified as malonylglucoside conjugates.

The existence of compounds additionally identified in the soil degradation studies was not verified in the metabolism study on lettuce, wheat and rape seed. As some of these metabolites would fit in the plant metabolism scheme, their presence as free or conjugated compounds can therefore not be excluded. Photolysis products are less likely in this study, as the study was conducted in a greenhouse.

Table 12 Distribution and identification of residues in wheat forage and wheat hay after single application of [Ph-^{14}C] (1 × 0.31 kg ai /ha- and [Bz-^{14}C]-mandestrobin (1 × 0.30 kg ai /ha)

Residues	Wheat forage (7 DAT)				Wheat hay (14 DAT)			
	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin		[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	5.7	51	6.3	60	1.6	26	2.1	23
De-Xy-mandestrobin	NA	NA	0.33	3.2	NA	NA	0.14	1.5
4-OH-mandestrobin (conjugate, 16 min) [a]	0.38	3.4	0.56	5.4	0.81	13	0.50	5.5
2-CH_2OH- mandestrobin free conjugate, 16 min [a] --Total (free + conjugate)	 ND 1.2 --1.2	 ND 11 --11	 0.022 0.57 –0.59	 0.22 5.5 --5.7	 0.081 0.70 -–0.78	 1.3 11 --12	 0.083 1.1 --1.2	 0.91 13 --14
Mixture of 5-CH_2OH-mandestrobin conjugate and 5-COOH-mandestrobin conjugate (19 min) [b]	0.69	6.2	0.44	4.3	0.39	6.3	0.62	6.8
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	0.033	0.30	0.30	2.8	0.046	0.74	0.075	0.83
2,5-dimethylphenol	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**8.0**	**71**	**8.5**	**81**	**3.7**	**59**	**4.6**	**51**
%identified/neutral extracts		**71/91 =78**		**81/92 =88**		**59/84 =70**		**51/90 =57**
Unknown 1 (2 min)	0.18	1.6	0.075	0.71	0.075	1.2	0.002	0.030
Unknown 2 (9 min)	0.13	1.2	0.35	3.3	0.037	0.59	0.40	4.4
Unknown 3 (11 min)	0.11	1.0	ND	ND	0.068	1.1	ND	ND
Unknown 4 (14 min) [c]	0.62	5.5	0.25	2.4	0.75	12	0.93	10
Unknown 5 (22 min)	ND	ND	ND	ND	ND	ND	0.028	0.31
Unknown 6 (31 min)	ND	ND	ND	ND	0.041	0.66	0.084	0.92
Unknown 7 (32 min)	0.34	3.0	ND	ND	0.12	2.0	0.24	2.7
Unknown 8 (37.8 min)	0.078	0.70	0.049	0.47	0.045	0.72	0.048	0.53
Others [d]	1.1	9.8	0.66	6.4	0.74	12	2.0	22
PES	0.64	5.8	0.57	5.4	0.68	11	0.73	8.1
--Enzyme Hydrolysis	NA	NA	NA	NA	-–0.091	--1.5	-–0.32	--3.5
--Weak Acid Hydrolysis	NA	NA	NA	NA	-–0.090	--1.4	-–0.13	--1.5
--Weak Base Hydrolysis	NA	NA	NA	NA	-–0.36	--5.8	-–0.21	--2.3
Unextracted Residue	**0.64**	**5.8**	**0.57**	**5.4**	**0.081**	**1.3**	**0.037**	**0.41**
Total unknowns (extracted or hydrolysed)	**2.5**	**23**	**1.4**	**13**	**2.4**	**39**	**4.4**	**48**
Total Radioactive Residues	**11**	**100**	**10**	**100**	**6.2**	**100**	**9.0**	**100**

ND = not detected; NA = not applicable

Total calculation is based on extracted fractions plus PES (hydrolysed fractions do not add up to PES total)

[a] Conjugates of 2-CH_2OH-mandestrobin and 4-OH-mandestrobin confirmed by enzymatic treatment.at a ratio of 76:24 ([Ph-^{14}C]) and at a ratio of 50:50 ([Bz-^{14}C]) in wheat forage and at a ratio of 46:54 ([Ph-^{14}C]) and 70:30 ([Bz-^{14}C]) in wheat hay

[b] Conjugate confirmed by enzymatic treatment in both, wheat forage and hay

[c] Comprises multiple compounds, for [Bz-^{14}C]-mandestrobin largest component in wheat forage 0.48% TRR, 0.050 mg/kg and in wheat hay 1.3% TRR, 0.120 mg/kg; for [Ph-^{14}C]-mandestrobin in wheat forage 0.57% TRR, 0.064 mg eq/kg and 1.6% TRR, 0.099 mg eq/kg in wheat hay

[d] Comprises numerous minor peaks, each peak accounted for or less than 1% TRR (in wheat hay only, in both radiolabels)

Table 13 Distribution and identification of residues in wheat grain and wheat straw after single application of [Ph-^{14}C] (0.306 kg ai /ha- and [Bz-^{14}C]-mandestrobin (0.303 kg ai /ha) at an application rate of 0.3 kg ai/ha

Residues	Grain (104 DAT)				Straw (104 DAT)			
	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin		[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	ND	ND	ND	ND	0.026	1.4	0.050	2.0
De-Xy-mandestrobin	NA	NA	0.054	61	NA	NA	0.29	12
4-OH-mandestrobin (free)	ND	ND	ND	ND	0.023	1.2	0.038	1.5
2-CH_2OH-mandestrobin (free)	ND	ND	0.003	3.1	0.18	9.5	0.16	6.4
5-CH_2OH-mandestrobin (free)	ND	ND	ND	ND	0.039	2.1	0.073	2.9
5-COOH-mandestrobin (free)	ND	ND	ND	ND	0.053	2.9	0.11	4.6
2-COOH-mandestronb	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	ND	ND	0.012	0.66	0.010	0.42
2,5-dimethylphenol	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0**	**0**	**0.057**	**64**	**0.33**	**18**	**0.74**	**30**
%identified/neutral extract		**0/54 =0**		**64/54 =120 [e]**		**18/45 =40**		**30/49 =61**
Unknown 1 (2 min)	0.002	18	0.004	4.2	0.014	0.73	0.002	0.070
Unknown 2 (9 min)	ND	ND	ND	ND	0.018	0.98	0.098	4.0
Unknown 3 (11 min)	0.003	26 [a]	ND	ND	0.025	1.4	ND	ND
Unknown 4 (14 min)	0.003	23 [a]	ND	ND	0.39	21 [b]	0.30	12[(b)]
Unknown 5 (16 min)	ND	ND	0.001	1.3	0.075	4.0	0.070	2.8
Unknown 6 (19 min)	ND	ND	ND	0.23	0.022	1.2	0.001	0.030
Unknown 7 (22 min)	ND	ND	0.002	2.1	0.032	1.7	0.028	1.1
Others [c]	ND	ND	0.001	1.1	0.25	14	0.44	18
PES	0.004 [d]	33 [d]	0.024 [d]	27 [d]	0.70	38	0.81	33
Weak Acid Hydrolysis	NA	NA	NA	NA	–-0.11	--5.9	–-0.12	--4.9
Strong Acid Hydrolysis	NA	NA	NA	NA	–-0.094	--5.1	–-0.078	--3.1
Weak Base Hydrolysis	NA	NA	NA	NA	–-0.21	--11	–-0.26	--10
Strong Base Hydrolysis	NA	NA	NA	NA	–-0.30	--16	–-0.28	--11
Unextracted Residue	0.004	33	0.024	27	0.020	1.1	0.014	0.57
Total unknowns (extracted or hydrolysed)	0.012	100	0.032	36	1.6	85	1.7	68
Total	**0.012**	**100**	**0.089**	**100**	**1.8**	**100**	**2.5**	**100**

ND = not detected; NA = not applicable

%TRR Total of residues may not add up to 100 % due to rounding

[a] Unknown metabolite fraction (11 and 14 min) comprised multiple compounds, which were not further characterised as concentration was very low (0.003 mg eq/kg.)

[b] Comprises multiple compounds in wheat straw; largest component 7.3% TRR, 0.14 mg eq/kg for [Ph-^{14}C]-mandestrobin and 2.6% TRR, 0.065 mg eq/kg for [Bz-^{14}C]-mandestrobin

[c] Comprise numerous minor peaks in wheat straw; each peak accounted for less than 1% TRR.

[d] no further characterisation via hydrolysis

[e] significant amount of additional residues were released with acidified organic solvents

In an addendum to the wheat metabolism study, the identity of the major conjugates of mandestrobin metabolites was further investigated [Ando *et al*., 2010, ROM-0071, original and revised].

The identity of the three major conjugates, whose aglycones were determined as 2-CH_2OH-mandestrobin, 4-OH-mandestrobin and 5-CH_2OH-mandestrobin, was investigated using wheat hay (^{14}C-phenyl-mandestrobin label) from the wheat metabolism study. The wheat hay was harvested on 9 June 2009, stored frozen and taken out of the freezer 9 months later. The wheat hay was extracted three times with acetone/water (80:20, v/v) and the extract was kept refrigerated below 5 °C.

Approximately 200,000 dpm of the wheat hay extract (equivalent to 0.25 g hay) or 50,000 dpm of each isolated conjugate was evaporated to dryness and subjected to hydrolysis. The three conjugates were slowly transformed to their corresponding aglycones after 7 days incubation with 185 U of beta-glucosidase (37 °C; pH 5 acetate buffer) alone. The endogenous moiety of these conjugates was considered a modified form of glucose, such as malonylglucose, because the enzymatic reaction to completely liberate the aglycones required more than 7 days.

To confirm this assumption, a two-step hydrolysis was conducted, i.e. an alkaline hydrolysis followed by a beta-glucosidase treatment which enables stepwise liberation of malonic acid and glucose, respectively. After 1 hour incubation with 0.03 M NaOH (pH 11), quantitative transformation was observed for all three conjugates, which produced the corresponding peaks of simple glucose conjugates. The alkaline hydrolysate of the extract was successively subjected to enzymatic hydrolysis with 185 U of beta-glucosidase (37 °C; pH 5 acetate buffer) and 2-CH_2OH-mandestrobin, 4-OH-mandestrobin and 5-CH_2OH-mandestrobin, respectively, were released from the corresponding peaks within 3 hours. Furthermore, neither significant loss of radioactivity nor remarkable appearance of by-products was observed during the reaction.

In addition, the structure of the three conjugates and their alkaline hydrolysates was confirmed by HPLC-MS. The pseudo-molecular ions for the three conjugates were mainly observed at m/z 600 $[M+Na]^+$ and 578 $[M+H]^+$ for positive ion mode, and 690 $[M+CF_3COO]$- and 576 $[M-H]^-$ for negative ion mode which all suggested its chemical structure as a malonylglucoside conjugate of a mono-hydroxylated mandestrobin. The daughter ion at m/z 312 detected in positive mode for conjugate A and C is characteristic for 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. The daughter ion at m/z 330 for conjugate B in positive ion mode is characteristic for 4-OH-mandestrobin. The daughter ion m/z 532 detected in negative mode is the decarboxylated form $[M-COOH]^-$ typically produced for a malonylglucose conjugate. The pseudo-molecular ions for the alkaline hydrolysate were detected at m/z 514 $[M+Na]^+$ and 492 $[M+H]^+$ for positive mode, and 604 $[M+CF_3COO]^-$, 536 $[M+HCOO]^-$ and 526 $[M+Cl]^-$ for negative ion mode, which indicates its chemical structure as a glucose conjugate of a mono-hydroxylated mandestrobin. The difference in molecular weight between the conjugate and its alkaline hydrolysate is calculated as 86 which clearly shows the release of malonic acid.

The three major conjugates in wheat hay were therefore identified as malonylglucosides of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin.

Foliar applications to pulses and oilseeds (rape seed)

The metabolic fate of mandestrobin following foliar application was studied in rape seed [Panthani and Connor, 2011, ROM-0026]. The study was conducted from March 2009 to July 2009 at a field site, located in Madera, California (USA). Rape seed (Brassica napus L.), of the variety Phoenix Liberty Link were grown under field conditions in sandy loam soil in wooden containers und subdivided in 5 groups. Groups II and IV were sprayed twice with [Ph-^{14}C]- or [Bz-^{14}C]-mandestrobin, respectively, formulated as a 25 % (w/v) SC at actual application rates of 0.39 and 0.38 kg ai/ha, at the 1st and 2nd application, respectively, with an interval of 14 days. The first application was at BBCH 55-61 and the second application at BBCH 66-67. Groups III and V were treated with a single application of [Ph-^{14}C]- or [Bz-^{14}C]-mandestrobin, respectively at a rate of 0.39 kg ai/ha at BBCH 55-61. Group I was the untreated control. Rape seed fodder (sample weight: 1.2–1.5 kg) was harvested from group II and IV only, 14 days after the 2nd application, at BBCH 74. Seeds were harvested at maturity (BBCH 89), 54 days after the single application (group III and V, sample weight: 0.028–0.043 kg) or 40 days after second application (group II and IV, sample weight: 0.061–0.071 kg). Segments (15 cm) of rape seed fodder samples were cut above soil level. For mature harvest samples, seed heads were cut from plants and separated in seeds and pods and only the seeds were analysed. The harvested samples were processed for analysis immediately after the harvest.

Rape seed fodder samples were surface washed with acetonitrile. Homogenised samples of rape seed were extracted four times with hexane, twice with acetone/water (80:20; v/v) and once with

acetone/water/hydrochloric acid (80:20:1; v/v/v). Surface washed homogenised fodder samples were treated in the same way omitting the extraction procedure with hexane. The radioactivity in the surface washes and the organic extracts was determined by LSC. The radioactivity in the PES was quantified by LSC after combustion. The total radioactive residue (TRR) in each sample was determined by liquid scintillation counting (LSC) of extracts (including the surface wash for fodder) and combusted residues.

The distribution of radioactive residues TTR in the surface wash, extracts and PES are shown in Table 14. The distribution of radioactivity in rape seed fodder and seeds was very similar between both radiolabels. In fodder, the acetonitrile surface wash plus acetone/water extractions surface wash removed 87-89% TRR of mandestrobin. The combination of hexane and acetone/water extraction recovered 78-92% TRR of the seeds after two applications (PHI 40 days) and 72–79% TRR of the seeds after one application (PHI 54 days), for Ph-^{14}C and benzyl-labelled plants, respectively.

Table 14 The total radioactive residue (TRR) in rape seed fodder and seeds after one (1st 0.39 kg ai/ha) and two (2nd 0.38 kg ai/ha) application of [Ph-^{14}C]- and [Bz-^{14}C]- mandestrobin

	Fraction	Total Radioactive Residue			
		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Rape seed fodder after two applic at BBCH 55-61 and BBCH 66-67 (PHI 14 days) 14DAT	Acetonitrile surface wash	1.5	37	1.2	34
	1st Extraction with acetone/water	1.6	39	1.6	46
	2nd Extraction with acetone/water	0.46	11	0.32	9.2
	TRR as sum of neutral extractions [a]	**3.5**	**87**	**3.1**	**89**
	Extraction with acetone/water / HCl	0.18	4.5	0.12	3.6
	PES	0.34	8.4	0.26	7.5
	TRR as sum of fractions	4.0	100	3.4	100
rape seed after two applic at BBCH 55-61 and BBCH 66-67 (PHI 40 days)	1st Extraction with hexane	0.097	21	0.10	16
	2nd Extraction with hexane	0.023	4.9	0.031	4.8
	3rd Extraction with hexane	0.007	1.5	0.010	1.6
	1st Extraction with acetone/water	0.18	39	0.24	38
	2nd Extraction with acetone/water	0.055	12	0.16	25
	3rd Extraction with acetone/water	not performed		0.050	7.8
	TRR as sum of neutral extractions [a]	**0.37**	**78**	**0.59**	**92**
	Extraction with acetone /water / HCl	0.033	7.0	0.048	7.5
	PES	0.069	15	0.001	0.11
	TRR as sum of fractions	0.47	100	0.64	100
rape seed after one applic at BBCH 55-61 (PHI 54 days)	1st-3rd Extraction with hexane	<LOQ	<LOQ	<LOQ	<LOQ
	1st Extraction with acetone/water	0.028	56	0.065	59
	2nd Extraction with acetone /water	0.008	16	0.022	20
	TRR as sum of neutral extractions [a]	**0.036**	**72**	**0.087**	**79**
	Extraction with acetone /water / HCl	0.005	10	0.013	12
	PES	0.009	19	0.010	9.3
	TRR as sum of fractions	0.050	101	0.11	100

%TRR Total of residues may not add up to 100 % due to rounding; %TRR has been re-calculated by the reviewer based on radioactive residues (as mg eq/kg) in surface wash, extracts and PES.

LOQ = 2 × Background

[a] Analytical methods make use of neutral acetone/water extractions

The extracted fractions were profiled separately by radio-HPLC and after isolation of regions of interest, metabolites were identified with HPLC and 1D-TLC using co-chromatography with reference standards: mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 4-OH-mandestrobin, De-Xy-mandestrobin, MCBX, (R)-MCBX, (S)-MCBX, mandestrobin *R*-isomer, mandestrobin *S*-isomer and 2,5 dimethylphenol. Chiral HPLC was employed to determine the ratio of R- and S-isomers of mandestrobin, only. The 16-min and 19-min HPLC regions from the rape fodder and seed extracts were treated with 8 U cellulase and 18 U beta-glucosidase (pH 5, 2 days at 39 °C) and the hydrolysis products were analysed by radio HPLC with co-chromatography with reference standards.

The PES of the rape seed samples were further characterised by sequential treatment with amylase (10000 U, pH 7, 22 hrs at 30 °C), protease (130 U, pH 7.2, 22 hrs at 30 °C), weak acid hydrolysis (1 M HCl, 40 °C, overnight), strong acid hydrolysis (6 M HCl, 80 °C, 4 hours), weak base hydrolysis (0.1 M NaOH, 40 °C, overnight) and strong base hydrolysis (6 M NaOH, 80 °C, overnight). Fodder PES was treated in the same manner omitting the enzyme hydrolysis steps. The supernatants of each treatment were analysed by LSC, but not profiled by HPLC against reference standards.

The identification and characterisation of residues in the rape seed after one application (PHI 54 days) or two applications (PHI 40 days) and of [^{14}C]-mandestrobin are shown in Table 15. Both radiolabels, [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin gave similar metabolite profiles for twice sprayed rape seed samples (PHI 54 days). The residue distribution in the two radiolabels profiles of once sprayed rape seed samples (PHI 40 days) deviated from each other.

The majority of residues in rape seed after one application that were detected in the [Ph-^{14}C]-mandestrobin sprayed fractions did not appear in the [Bz-^{14}C]-mandestrobin sprayed samples. The parent was not detected in rape seed after one application in both radio labels. Most metabolites were found below 10% TRR, with exception of unknown 1 and 4 accounting for 0.89–37% TRR (0.003–0.04 mg eq/kg) and 16–40 % TRR (0.021–0.11 mg eq/kg). In both radiolabels the parent, the conjugate of 5-CH_2OH-mandestrobin and MCBX were not detected.

In rape seed after two applications, the parent was found 25-31% TRR (0.14–0.16 mg eq/kg). The glycosides of 2-CH_2OH-mandestrobin, 4-OH-mandestrobin and 5-CH_2OH-mandestrobin were found at 5.1–6.5, 11–14 and 3.1–3.6% TRR (0.031–0.033 mg eq/kg, 0.068–0.071 mg eq/kg and 0.014–0.023 mg eq/kg), respectively. The aglycones of the glycosides were identified after enzymatic hydrolysis. 5-COOH-mandestrobin accounted for only 1.3-3.4% TRR (< 0.008–0.016 mg eq/kg) and MCBX was not detected.

The identification and characterisation of residues in rape fodder after two applications of [^{14}C]-mandestrobin are shown in Table 16. Both radiolabels, [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin gave similar metabolite profiles. The major part of the radioactive residue could be assigned to a 4-OH-mandestrobin conjugate (36 and 27% TRR). The parent and 2-CH_2OH-mandestrobin conjugate made up for 20 and 22% TRR and 12 and 12% TRR, respectively. Free 2-CH_2OH-mandestrobin accounted for less than 0.1 mg eq/kg (≤ 0.16% TRR). MCBX and 5-COOH-mandestrobin glycoside conjugate were detected as minor metabolites, each below 10% TRR.

The R/S ratio of mandestrobin in the surface wash and neutral extracts remained approximately 1:1, indicating no R/S isomerization. No analysis of R/S isomerization for metabolites was performed.

Storage stability: A representative [Ph-^{14}C]-mandestrobin sprayed rape seed fodder sample was stored frozen (temperature not indicated) approximately 7 months, therein after extracted and analysed by HPLC. Oil rape seed samples treated with [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin were stored frozen for approximately 7 months (phenoxy label, 2 applications), 9 months (benzyl label, 1 application) and 11 months (phenoxy label, 1 application), extracted and analysed by HPLC. HPLC profiles of sample extracts were compared to the initial HPLC profile of radiolabelled mandestrobin to verify the stability of metabolites in the matrices during storage. Metabolite profiles from the initial and final analyses for the [Ph-^{14}C]-mandestrobin treated rape seed fodder were very similar, indicating that metabolites were stable in the rape seed fodder samples under frozen storage conditions. Minor differences were observed in the profiles from seeds sampled after one application of [Bz-^{14}C]-mandestrobin after 9 months of frozen storage. The metabolite profiles from these two analyses (initial and final analyses) demonstrate varying concentrations of several of the polar metabolites (retention time from 2 to 11 minutes).

Notes by the reviewer

A total of 87–89%, 78–92% and 72–79% TRR could be extracted from rape seed fodder, double and single treated seeds with hexane-acetone/water (including the acetonitrile surface wash). Considering the good extraction efficiency, the identification levels in rape seed are low as 46–58% TRR and zero-20% TRR could be identified in double and single treated seeds (i.e. less than 80% of the solvent extracted residue).

The 16 and 19 min HPLC regions of the green rape fodder and seed extracts were singled out for treatment with enzymes and contained significant amounts (up to 36% and 14% TRR) of conjugates of known metabolites. The remainder of the extracts was not subjected to acid, base or enzyme hydrolysis. A radiovalidation study in green rape fodder (see analytical method section), where the whole extract was treated with alkaline and enzymatic hydrolysis, confirmed that the remainder of the extract from the green rape fodder metabolism study did not contain additional aglycones of known metabolites. This suggests, that the 16 and 19 min HPLC regions are the only regions containing aglycones of known metabolites. In an addendum to the wheat metabolism study, the 16 and 19 min HPLC peaks were identified as malonylglucoside conjugates.

The existence of compounds additionally identified in the photolysis studies and soil degradation studies was not verified in the metabolism study on lettuce, wheat and rape seed. As some of these metabolites would fit in the plant metabolism scheme, their presence as free or conjugated compounds can therefore not be excluded.

Table 15 Distribution and identification of residues in rape seed after one (1st 0.39 kg ai/ha) or two (1st 0.39 kg ai/ha and 2nd 0.38 kg ai/ha) applications of [Ph-^{14}C] and [Bz-^{14}C]-mandestrobin

	2 × application DALT = 40 days Last application at BBCH 66-67 (flowers)				1 × application DALT = 54 days Application at BBCH 55-61 (no flowers)			
Residues	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin		[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	0.14	31	0.16	25	ND	ND	ND	ND
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin (conjugated, 16 min) [a]	0.068	14	0.071	11	0.004	8.0	ND	ND
2-CH_2OH-mandestrobin (conjugated, 16 min) [a]	0.031	6.5	0.033	5.1	0.002	3.6	ND	ND
5-CH_2OH-mandestrobin (conjugated, 19 min) [b]	0.014	3.1	0.023	3.6	ND	ND	ND	ND
5-COOH-mandestrobin (free)	0.016	3.4	0.008	1.3	0.004	8.7	ND	ND
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	ND	ND	ND	ND	ND	ND
2,5-dimethylphenol	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0.27**	**58**	**0.30**	**46**	**0.010**	**20**	**0**	**0**
%identified/neutral extracts		**58/78 =74**		**46/92 =50**		**20/72 =28**		**0/79 =0**
Unknown 1 (2 min)	0.004	0.89	0.012	1.8	0.003	6.2	0.04	37 [e]
Unknown 2 (9 min)	ND	ND	0.042	6.5	ND	ND	0.006	5.4
Unknown 3 (11 min)	0.005	1.1	0.044	6.8	ND	ND	0.011	9.7
Unknown 4 (14 min) [c]	0.076	16	0.11	18	0.021	40	ND	ND
Unknown 5 (22 min)	ND	ND	ND	ND	0.002	3.8	ND	ND
Others [d]	0.043	9.2	0.13	21	0.005	11	0.043	39
PES	0.069	15	0.001	0.11	0.009	19	0.010	9.3
--Amylase hydrolysis	–-0.014	--3.0	NA	NA	–-0.003	--6.2	NA	NA
--Protease hydrolysis	–-0.008	--1.7	NA	NA	–-0.001	--2.8	NA	NA
--Weak acid hydrolysis	ND	ND	NA	NA	ND	ND	NA	NA
--Strong acid hydrolysis	–-0.006	--1.2	NA	NA	ND	ND	NA	NA
--Weak base hydrolysis	–-0.015	--3.2	NA	NA	–-0.003	--5.5	NA	NA
--Strong base hydrolysis	–-0.023	--4.8	NA	NA	–-0.001	--2.3	NA	NA
Unextracted residue	**0.006**	**1.2**	**0.001**	**0.11**	**0.001**	**1.8**	**0.010**	**9.3**

Residues	2 × application DALT = 40 days Last application at BBCH 66-67 (flowers)				1 × application DALT = 54 days Application at BBCH 55-61 (no flowers)			
	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin		[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Total unknowns (extracted or hydrolysed)	**0.19**	**41**	**0.35**	**54**	**0.039**	**78**	**0.10**	**91**
Total Radioactive Residues	**0.47**	**100**	**0.64**	**100**	**0.050**	**100**	**0.11**	**100**

ND = not detected; NA not applicable

Total radioactive residues was calculated based on extracted and PES (hydrolysed fractions do not add up to 100% PES)

[a] The neutral extract 16 minute region was analysed by HPLC method 4 to separate the conjugates of 2-CH_2OH-mandestrobin and 4-OH-mandestrobin at a ratio of 31:69 (Ph-^{14}C)

[b] Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-mandestrobin

[c] Comprising of multiple compounds, largest component in rape seed twice sprayed 3.6% TRR, 0.017 mg eq/kg (Ph-^{14}C) and 2.2% TRR, 0.014 mg eq/kg (Bz-^{14}C) and once sprayed 2.9% TRR, 0.001 mg eq/kg (Ph-^{14}C)

[d] Comprising numerous minor peaks, each peak accounted for or less than 1% TRR (for Bz-^{14}C-labels in 2 × and 1 × treated rape seed)

[e] This region is characterised as extremely polar from the chromatographic profile. This region was not further analysed, but may contain conjugates of parent or other subsequent metabolite(s), or highly fragmented small molecular degradates, [Response letter RL-01, May 2018]

Table 16 Distribution and identification of residues in rape seed fodder after two applications of [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin at 2 × 0.38–0.39 kg ai/ha) at BCCH 56-61 and BBCH 66-7 with DALT = 14 (harvested at BBCH 74)

Residues	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	0.79	20	0.77	22
De-Xy-mandestrobin	NA	NA	ND	ND
4-OH-mandestrobin (conjugated, 16 min) [a]	1.4	36	0.93	27
2-CH_2OH-mandestrobin Free conjugated, 16 min [a] --Total (free + conjugate)	 0.006 0.48 -–0.49	 0.16 12 --12	 0.004 0.43 -–0.43	 0.11 12 --13
5-CH_2OH-mandestrobin conjugate (19 min) [b]	0.20	5.1	0.096	2.8
5-COOH-mandestrobin	ND	ND	ND	ND
2-COOH-mandestrobin	ND	ND	ND	ND
MCBX	ND	ND	0.006	0.16
2,5-dimethylphenol	ND	ND	NA	NA
Total identified	**2.9**	**73**	**2.2**	**65**
%identified/neutral extracts		**73/87 =84**		**65/89 =73**
Unknown 2 (9 min)	ND	ND	0.16	4.6
Unknown 3 (11 min)	0.012	0.31	0.078	2.3
Unknown 4 (14 min) [c]	0.52	13	0.40	12
Unknown 5 (22 min)	0.028	0.70	0.023	0.66
Others	0.19	4.8	0.28	8.3
PES	0.34	8.4	0.26	7.5
--Weak acid hydrolysis	-–0.049	--1.2	-–0.033	-–0.97
--Strong acid hydrolysis	-–0.048	--1.2	-–0.068	--2.0
--Weak base hydrolysis	-–0.12	--3.0	-–0.068	--2.0
--Strong base hydrolysis	-–0.11	--2.8	-–0.038	--1.1
--Others	ND	ND	-–0.017	-- 0.51
Unextracted residue	**0.11**	**2.8**	**0.034**	**0.98**
Total unknowns (extracted or hydrolysed)	**1.1**	**27**	**1.2**	**34**
Total Radioactive Residue	**4.0**	**100**	**3.4**	**100**

Total Radioactive Residue calculation based on extracted + PES (hydrolysed fractions do not add up to 100%)

[a] Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-mandestrobin and 4-OH-mandestrobin

[b] Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-mandestrobin

[c] Comprising of multiple compounds, largest component 3.6% TRR, 0.15 mg eq/kg (Ph-^{14}C) and 4.6% TRR, 0.16 mg eq/kg (Bz-^{14}C). The largest peaks were further separated into multiple components by HPLC and TLC

Seed treatments of cereal grains, pulses and oilseeds

The metabolic fate of mandestrobin following seed treatment was studied in maize grains and soya bean seeds [Jalal, 2010, ROM-0055]. Batches of 24 g soya bean (number of seeds not reported) seeds (variety Pioneer 93M42) and 12 g (35 seeds) maize seeds (variety TR3026RR, TR2040RR and TR1914) were separately treated with formulated [^{14}C-phenoxy-]-mandestrobin or [^{14}C-benzyl-]mandestrobin at actual rates of 9.3-12 g ai/100 kg of seeds for each radiolabel. The crop seeds from each treatment were planted 6 days after treatment (DAT) in two outdoor field plots in the 2009 season in Porterville, CA, USA. One plot had loamy sand soil and the other plot had sandy clay loam soil. The crops were grown following normal agricultural practices.

Samples of each raw agricultural commodity (RAC) were harvested from individual plots at the appropriate growth stage. Soya bean forage was harvested at DAT=41 and ten plants (129-153 g) were cut from each plot. Soya bean forage for hay was harvested at DAT 67 and air-dried for one day (58-138 g). Succulent green soya bean pods with seeds (135-182 g) were handpicked randomly from each plot at DAT 78. At full maturity, the soya bean stalks with mature seeds were cut an inch above the ground at DAT 103, air-dried for about a week and the mature seeds (177-280 g) were handpicked from the dry plants. Sweetcorn was collected at the milk/succulent stage at DAT 71 as sample of kernels plus cob with husks removed (218-270 g). Maize forage (808-1204 g) was sampled at late dough/early dent stage at DAT 85 by cutting the aerial portions of selected plants above the soil. The remaining maize plants were harvested at maturity at DAT 103, when the grain (466-770 g) was separated from the cobs. The grain-free mature cobs were added to the stalks, and the composite RAC sample was processed as maize fodder (1987-3059 g). Samples were stored frozen for 16-72 days until analysis.

Homogenised samples were combusted followed by liquid scintillation counting. TRR in control soya and control maize RAC samples were equivalent to background while, recovery of radioactivity from soya and maize control RAC samples spiked with ^{14}C-labelled mandestrobin at 0.005 mg eq/kg was 79-100%. TRR levels for each of the RAC samples are shown in Table 17 (treated soya bean seeds) and Table 18 (treated maize seeds).

The results obtained in this study showed that the soya bean food RACs and the maize food and feed RACs grown from seeds treated with either [phenoxy-^{14}C]mandestrobin or [benzyl-^{14}C]-mandestrobin did not take up mandestrobin related residues to any significant extent. The TRR levels were below the LOQ of 0.005 mg eq/kg in all food RAC samples: succulent soya bean pods with seeds, mature soya bean seeds, sweetcorn (kernels plus cob with husks removed) and maize grains. TRR levels were below the LOQ of 0.005 mg eq/kg in all maize feed samples, except the benzyl labelled maize fodder (0.008 mg eq/kg), which was barely above the LOQ.

The soya bean feed RACs, on the other hand, showed low level uptake of mandestrobin related residues from the treated seeds. TRR levels in soya bean forage and soya bean hay samples were 0.027–0.061 mg eq/kg and 0.027–0.050 mg eq/kg, respectively.

Table 17 The total radioactive residue (TRR) in soya grown from seed treated with [Ph-^{14}C]- or [Bz-^{14}C]- mandestrobin

	[Ph-^{14}C] mandestrobin seed treatment at 9.3 g ai/100 kg	[Bz-^{14}C] mandestrobin seed treatment at 11 g ai/100 kg
Soya bean forage	0.027; 0.038 mean 0.032	0.040; 0.061 mean 0.051
Soya bean hay	0.030; 0.027 mean 0.028	0.045; 0.050 mean 0.048

Soya bean succulent pods with seeds	< 0.005; < 0.005 mean < 0.005	< 0.005; < 0.005 mean < 0.005
Soya bean mature seeds	< 0.005; < 0.005 mean < 0.005	< 0.005; < 0.005 mean < 0.005

Table 17 The total radioactive residue (TRR) in maize grown from seed treated with [Ph-^{14}C]- or [Bz-^{14}C]- mandestrobin

	[Ph-^{14}C] mandestrobin seed treatment at 11 g ai/100 kg	[Bz-^{14}C] mandestrobin seed treatment at 12 g ai/100 kg
Sweetcorn (kernels plus cob with husk removed)	< 0.005; < 0.005 mean < 0.005	< 0.005; < 0.005 mean < 0.005
Maize forage	< 0.005; < 0.005 mean < 0.005	< 0.005; < 0.005 mean < 0.005
Maize fodder (corn stover)	< 0.005; < 0.005 mean < 0.005	0.011; 0.005 mean 0.008
Maize grain	< 0.005; < 0.005 mean < 0.005	< 0.005; < 0.005 mean < 0.005

In a follow-up study, the radio-active residues in soya bean forage, soya bean hay and maize fodder were further characterized [Jalal, 2013, ROM-0056]. Prior to extraction, the total radioactive residue (TRR) in the crop samples were freshly determined using combustion analyses. The TRR levels were comparable to the levels determined in the original study.

Samples were extracted twice with acetonitrile and once with 50% aqueous acetonitrile. Table 19 shows that a large part of the radioactivity could be extracted: 87–89% TRR from soya bean forage; 48–53% TRR from soya bean hay and 71% TRR from maize fodder.

Table 19 Distribution of radioactivity in extracts and solids of crops grown from seeds treated with mandestrobin

		[Ph-^{14}C] mandestrobin		[Bz-^{14}C] mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Soya bean forage from seed treated with 9.3-11 g ai/100 kg	Acetonitrile extract	0.024	62.4	0.041	53.6
	Acetonitrile/water extract	0.009	24.8	0.026	35.0
	TRR as sum of neutral extractions	**0.033**	**87.2**	**0.067**	**88.6**
	PES	0.005	12.8	0.009	11.4
	TRR as sum of fractions	0.038	100	0.076	100
	TRR from combustion	0.040	-	0.072	-
	TRR from combustion (original study)	0.038	-	0.061	-
Soya bean hay from seed treated with 9.3-11 g ai/100 kg	Acetonitrile extract	0.004	13.0	0.005	9.2
	Acetonitrile/water extract	0.010	35.1	0.025	43.7
	TRR as sum of neutral extractions	**0.014**	**48.1**	**0.030**	**52.8**
	PES	0.015	51.9	0.026	47.2
	TRR as sum of fractions	0.030	100	0.056	100
	TRR from combustion	0.034	-	0.057	-
	TRR from combustion (original study)	0.030	-	0.050	-
Maize fodder from seed treated with 11–12 g ai/100 kg	Acetonitrile extract	not performed		0.005	51.2
	Acetonitrile/water extract			0.002	19.8
	TRR as sum of neutral extractions			**0.007**	**71.0**
	PES			0.003	29.0
	TRR as sum of fractions			0.010	100
	TRR from combustion			0.010	-
	TRR from combustion (original study)			0.011	-

The extracts that contained 0.005 mg eq/kg or more, were further analysed by HPLC and 2D-TLC by co-chromatography with reference standards for parent (RT 21.2), De-Xy-mandestrobin (RT 7.3), DX-CA-mandestrobin (RT 7.6), 2-COOH-mandestrobin (RT 14.9), 2,5-dimethylphenol (RT 15.3), 5-COOH-mandestrobin (RT 16.1) and MCBX (RT 18.0). Results are shown in Table 20.

Mandestrobin was found at 1.2–7.9% TRR, but never exceeded 0.003 mg eq/kg. Metabolites identified were De-Xy-mandestrobin (0.6-12% TRR), 2-COOH-mandestrobin (1.6–5.1% TRR) and 5-COOH-mandestrobin (3.3% TRR), but none of them exceeded 0.005 mg eq/kg. HPLC profiles of both acetone and aqueous extracts showed two major peaks with approximate retention times of 12 and 13 min, which accounted for 14–29% TRR and 9.6–22% TRR. The remainder of the radioactivity was associated with multi-component bands. All of these major and minor compounds remained at or near the origin of the 2D TLC indicative of polar conjugated compounds.

Notes by the reviewer:

A total of 87–89%, 48–53% and 71% TRR could be extracted from soya bean forage, soya bean hay and maize fodder with acetonitrile/water. Considering the good extraction efficiency with organic solvents, the identification level in soya bean forage (29% TRR), soya hay (3.7% TRR) and maize fodder (8.2% TRR) is low (i.e. less than 80% of the solvent extracted residue).

The existence of compounds additionally identified in the photolysis studies, plant metabolism studies with foliar treatments and soil degradation studies was not verified in the metabolism study on seed treatment. As some of these metabolites would fit in the plant metabolism scheme, their presence as free or conjugated compounds can therefore not be excluded.

Table 20 Identification and characterisation of radioactivity in crops grown from seeds treated with mandestrobin

	soya bean forage [Ph]		soya bean forage [Bz]		soya bean hay [Ph]		soya bean hay [Bz]		maize fodder [Bz]	
	mg eq/kg	%TRR	mg eq/kg	TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	0.003	7.9%	0.002	2.3%	< 0.001	1.5%	0.001	1.2%	< 0.001	2.8%
2-COOH-mandestrobin	0.002	5.1%	ND	ND	0.001	2.2%	0.001	1.6%	ND	ND
5-COOH-mandestrobin	0.001	3.3%	ND	ND	ND	ND	ND	ND	ND	ND
De-Xy-mandestrobin	0.005	12.3%	0.004	5.3%	NA	NA	< 0.001	0.6%	0.001	5.4%
Unk RT 1.8	0.001	3.2%	ND	ND	ND	ND	ND	ND	ND	ND
Unk RT 2.5	0.001	2.7%	0.003	3.8%	ND	ND	< 0.001	0.3%	ND	ND
Unk RT 4.8	ND	ND	0.003	3.7%	ND	ND	0.001	2.7%	ND	ND
Unk RT 5.8	ND	ND	0.002	3.1%	ND	ND	0.002	3.4%	ND	ND
Unk RT 6.3	ND	ND	0.008	10.1%	ND	ND	0.003	4.7%	ND	ND
Unk RT 11.0	0.003	8.9%	0.005	6.4%	0.001	4.4%	0.001	2.5%	< 0.001	4.5%
Unk RT 12.3	0.009	22.8%	0.022	28.6%	0.004	14.5%	0.010	18.0%	0.001	14.8%
Unk RT 13.3	0.007	18.0%	0.016	21.7%	0.003	9.6%	0.007	12.0%	0.002	17.9%
Unk RT 15.8	0.001	2.9%	0.003	3.4%	0.001	2.8%	0.002	2.8%	0.001	5.8%
Unanalysed	ND	ND	ND	ND	0.004	13.0%	0.002	3.0%	0.002	19.8%
PES	0.005	12.8%	0.009	11.4%	0.015	51.9%	0.026	47.2%	0.003	29.0%
Total identified	**0.011**	**29%**	**0.006**	**7.6%**	**0.002**	**3.7%**	**0.003**	**3.4%**	**0.002**	**8.2%**
%identified/ neutral extracts		**29/87 =33**		**7.6/89 =8.5**		**3.7/48 =7.7**		**3.4/53 =6.4**		**8.2/71 =12**
Total	0.038	100%	0.076	100%	0.030	100%	0.056	100%	0.010	100%

Overview of the metablic pathway of mandestrobin in plants

Plant metabolism studies have been presented covering foliar treatments to the crop categories of leafy crops (lettuce), cereal/grass crops (wheat) and pulses and oilseeds (rape seed) as well as seed treatments to cereal/grass crops (maize) and pulses and oilseeds (soya beans). In addition studies with topical and soil treatments of fruits and fruiting vegetables (tomatoes) have been presented.

No R/S epimerization of mandestrobin occurred as the R/S ratio of mandestrobin remained approximately 1:1.

The main route of metabolism in crops (depicted in Figure 2) is via a series of monohydroxylation of the dimethylphenoxy ring and oxidations of the methyl group attached to the phenoxy ring, and subsequent glycoside conjugation, to yield the metabolites 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and 5-COOH-mandestrobin and their malonylglucoside conjugates. De-Xy-mandestrobin is formed via cleavage of the phenoxy-ring from mandestrobin or its free metabolites.

Although the route of metabolism of mandestrobin has been shown to be qualitatively similar in the leafy parts of the four crop groups, the quantitative formation of metabolites differs as a result of the differing sampling times.

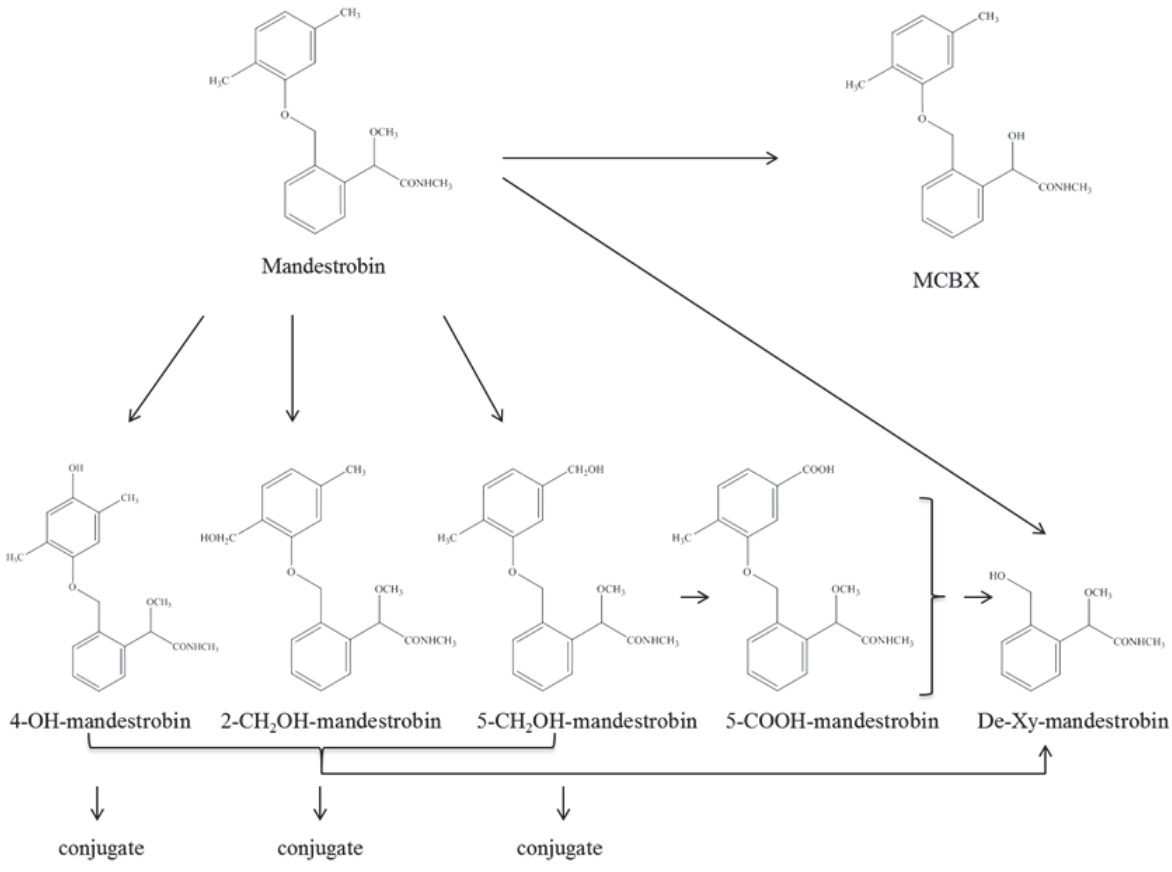


Figure 2 Metabolic pathway of mandestrobin in primary crops lettuce, rape seed (seed, green fodder (BBCH > 60)) and wheat (forage, hay, grains and straw) as well as rotational crops lettuce (foliage), carrots (root and foliage), broccoli, and barley (forage, hay, grains, straw)

Rotational crops

The Meeting received information on confined and field rotational crops.

Confined rotational crop studies

A confined rotational crop study was designed to provide information on the uptake and metabolism of mandestrobin in rotational crops [Panthani *et al*, 2011, ROM-0032]. The study was carried out in outdoor pots containing sandy loam soil (pH 7.5, 1.4% organic matter (om), CEC 10.1 meq/100g) at Fresno, CA, USA, in the period from May 2009 till December 2010. The pots were divided in three treatment groups, whereas group I was used as an untreated control. Bare soil was sprayed with a single application of a 25% SC formulation of [Ph-^{14}C]-mandestrobin (group II) or [Bz-^{14}C]-mandestrobin (group III) each at an actual rate of 1.6 kg ai/ha. After ageing the soil (plant back interval, PBI) for 30, 120 and 365 days, lettuce (variety Salad Bowl), carrots (variety: Danvers Half Long 126) and wheat (variety: Blanca Royale) were sown in the soils. No soil samples were taken for %TRR identification of parent and metabolites. Crops were sampled and analysed at early harvest of 50% of maturity (immature lettuce (sample weight for 30, 120 and 365 PBI (s.w.) 0.15–0.25 kg), wheat forage (for all PBI s.w. 0.17–0.20 kg) and hay (s.w. 0.21–0.26 kg); (BBCH growth stage not indicated) and final harvest (mature lettuce, wheat straw and wheat grain, carrot root and carrot foliage). Samples of carrot, lettuce and wheat were extracted and analysed within 3, 6 and 9 months of freezer storage, respectively. The storage temperature was not stated.

Homogenised plant samples were extracted 2–3 times with acetone/water (80:20; v/v) and up to three times with acetone/water/concentrated hydrochloric acid (80:20:1; v/v/v). Following the centrifugation the TRR was determined in plant extracts by LSC. Unextracted residues were analysed by combustion analysis.

The TRRs (sum of neutral and acidic extracts and PES) of the rotated crops on treated soils are shown in Table 21. Negligible levels of radioactivity were present in the control crops. Residues > 0.01 mg eq/kg are found in all rotational crops at all plant back intervals (PBI), except in carrot roots and wheat grain at 365 PBI. The TRR levels were consistently higher in immature lettuce compared to mature lettuce at all PBI. Residue levels at the 120 and 365 BPI were generally higher in crops from the benzyl-label treated plots compared to the phenoxy-label treated plots. Twice an (neutral) extraction of crop samples with acetone:water (80:20, v:v), extracted already 71–81%, 45-66%, 34-64% of TRR from wheat forage, hay and straw (range over all PBI), shown in Table 21. In immature and mature lettuce samples of PBI 30 and 365, 75-92% of TRR was determined in the neutral extracts and 59-81% of TRR in PBI 120. Also in carrot root and foliage samples, 77-90% and 64-83% of TRR were determined in the neutral extracts. In wheat grain only 5.2–37% TRR and 5.2–36% of TRR were determined in the neutral for [Ph-^{14}C]- and [Bz^{14}C]-mandestrobin treated wheat grain, respectively. The major part of %TRR was detected in the PES fraction, with 89–90% and 30–32% of TRR in the [Ph-^{14}C]- and [Bz^{14}C]-mandestrobin treated wheat grain, respectively.

Table 21 Total radioactive residues (based on extraction) in confined rotational crops grown in aged soil treated with [phenoxy-^{14}C]- or [benzyl-^{14}C]-mandestrobin

PBI	Rotational Crop/ Matrix	DAT	DAS	mg eq/kg (%TRR) in extraction fractions							
				[Ph-^{14}C]-mandestrobin				[Bz-^{14}C]-mandestrobin			
				Neutral total [a]	Acid Total [b]	PES	TRR as sum of fractions	Neutral total [a]	Acid total [b]	PES	TRR as sum of fractions
30	Wheat										
	Forage (immature)	55	25	**2.0 (73)**	0.23 (8.4)	0.52 (19)	2.7 (100)	1.9 (73)	0.23 (9.1)	0.45 (18)	2.5 (100)
	Hay (immature)	137	107	**0.82 (52)**	0.24 (15)	0.50 (32)	1.6 (100)	2.7 (59)	0.77 (17)	1.1 (24)	4.5 (100)
	Grain (mature)	299	269	**0.002 (5.8)**	0.003 (5.4)	0.032 (89)	0.037 (100)	0.038 (31)	0.043 (36)	0.039 (32)	0.12 (100)
	Straw (mature)			**0.46 (35)**	0.16 (12)	0.70 (53)	1.3 (100)	0.35 (43)	0.12 (15)	0.34 (42)	0.82 (100)
	Lettuce										
	Foliage (immature)	60	30	**0.31 (92)**	0.008 (2.3)	0.019 (5.6)	0.33 (100)	0.29 (92)	0.007 (2.2)	0.019 (6.0)	0.32 (100)
	Foliage	74	44	**0.074**	0.002	0.008	0.084	0.20	0.003	0.015	0.22

PBI	Rotational Crop/ Matrix	DAT	DAS	mg eq/kg (%TRR) in extraction fractions							
				[Ph-^{14}C]-mandestrobin				[Bz-^{14}C]-mandestrobin			
				Neutral total [a]	Acid Total [b]	PES	TRR as sum of fractions	Neutral total [a]	Acid total [b]	PES	TRR as sum of fractions
	(mature)			**(88)**	(2.1)	(10)	(100)	(92)	(1.6)	(6.9)	(100)
	Carrot										
	Foliage (mature)	194	164	**0.093 (83)**	0.012 (10)	0.008 (6.8)	0.11 (100)	0.057 (78)	0.008 (12)	0.008 (11)	0.073 (100)
	Root (mature)			**0.044 (87)**	0.002 (3.9)	0.005 (9.3)	0.051 (100)	0.035 (89)	0.001 (3.8)	0.003 (6.9)	0.039 (100)
	Wheat										
120	Forage (immature)	160	40	**0.10 (71)**	0.006 (4.0)	0.035 (25)	0.14 (100)	0.25 (81)	0.012 (3.8)	0.048 (16)	0.31 (100)
	Hay (immature)	194	74	**0.18 (62)**	0.034 (12)	0.074 (26)	0.28 (100)	0.48 (66)	0.099 (14)	0.15 (20)	0.72 (100)
	Grain (mature)	252	132	**0.002 (5.2)**	0.003 (5.2)	0.032 (90)	0.037 (100)	0.075 (37)	0.067 (33)	0.059 (30)	0.20 (100)
	Straw (mature)			**0.22 (64)**	0.030 (8.7)	0.093 (27)	0.34 (100)	0.38 (64)	0.070 (12)	0.14 (24)	0.59 (100)
	Lettuce										
	Foliage (immature)	169	49	**0.022 (67)**	0.002 (4.9)	0.010 (29)	0.034 (100)	0.062 (81)	0.004 (4.9)	0.011 (14)	0.077 (100)
	Foliage (mature)	198	78	**0.013 (59)**	0.002 (8.1)	0.007 (33)	0.022 (100)	0.036 (75)	0.004 (7.1)	0.009 (18)	0.049 (100)
	Carrot										
	Foliage (mature)	247	127	**0.040 (77)**	0.007 (12)	0.005 (11)	0.052 (100)	0.059 (76)	0.010 (14)	0.008 (10)	0.077 (100)
	Root (mature)			**0.024 (84)**	0.001 (3.6)	0.003 (12)	0.028 (100)	0.036 (90)	0.001 (2.9)	0.003 (6.8)	0.040 (100)
	Wheat										
365	Forage (immature)	390	25	**0.069 (72)**	0.003 (3.5)	0.024 (25)	0.10 (100)	0.21 (80)	0.010 (3.7)	0.041 (16)	0.26 (100)
	Hay (immature)	431	66	**0.16 (45)**	0.049 (14)	0.15 (41)	0.35 (100)	0.37 (50)	0.13 (18)	0.23 (32)	0.74 (100)
	Grain (mature)	489	124	**Not extracted**			0.003 (100)	Not extracted			0.005 (100)
	Straw (mature)			**0.10 (34)**	0.049 (16)	0.15 (50)	0.31 (100)	0.15 (40)	0.075 (20)	0.15 (40)	0.38 (100)
	Lettuce										
	Foliage (immature)	390	25	**0.051 (77)**	0.002 (3.2)	0.013 (19)	0.066 (100)	0.059 (80)	0.002 (2.4)	0.013 (18)	0.074 (100)
	Foliage (mature)	406	41	**0.015 (79)**	0 (0)	0.004 (21)	0.019 (100)	0.016 (75)	0 (0)	0.006 (24)	0.022 (100)
	Carrot										
	Foliage (mature)	476	111	**0.021 (71)**	0.004 (10)	0.006 (18)	0.031 (100)	0.017 (64)	0.004 (16)	0.005 (19)	0.026 (100)
	Root (mature)			**0.006 (77)**	ND	0.002 (23)	0.008 (100)	0.007 (84)	ND	0.001 (16)	0.008 (100)

DAS- Days after sowing, DAT-days after treatment, PBI-plant back interval

[a] Sum of extractions with acetone:water (80:20, v:v): 3 neutral extractions for wheat hay and wheat straw; 2 neutral extractions for all other commodities. Analytical methods use acetone/water as extraction solvent.

[b] Sum of extractions with acetone:water:concentrated HCl (80:20:1, v:v:v); 3 acid extractions for wheat grain (30 and 120 PBI), 2 acid extractions for wheat hay, wheat straw, mature lettuce (120 PBI) and carrot foliage; single extraction for all other commodities.

Extracts were combined, concentrated and profiled by radio-HPLC (HPLC-1). Specific regions of interest were then isolated and analysed using HPLC with a different chromatic column to better resolve individual components (HPLC-4). Metabolites were identified by HPLC and confirmed by 1D-TLC using co-chromatography with reference standards: mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin,

De-Xy-mandestrobin, MCBX, I-MCBX, (S)-MCBX and R-isomer of mandestrobin, S-isomer of mandestrobin and 2,5-dimethylphenol. The R:S isomer ratio of mandestrobin was determined by chiral HPLC.

The 15, 16 and 19 min HPLC 1 regions from 30 PBI wheat hay, wheat forage or mature lettuce were treated with cellulase and beta-glucosidase (5-7 days, pH 5, 39 °C). The 2–14 min HPLC 1 regions from wheat forage and hay were subjected separately to 0.1 M HCl (20 hrs, 40 °C) and enzyme hydrolysis. The 11 min HPLC 1 region from wheat hay was subjected separately to 6 M NaOH (2 hrs, 80 °C), 6 M HCl (2 hrs, 80 °C) and 6 M HCl (overnight, reflux). The hydrolysed products were characterised by HPLC co-chromatography and 1D-TLC against authentic reference standards. The 15, 16 and 19 min HPLC 1 regions were shown to contain glycosides of known metabolites. The chromatographic profile of the 2–14 min HPLC 1 (i.e. 2–29 min HPLC 4) regions did not change during enzymatic and acidic hydrolysis and did not co-chromatograph with any known metabolites before or after hydrolysis.

Unextracted residues of 30 PBI wheat forage, hay and straw samples were further characterised by sequential enzyme hydrolysis with Driselase (except for straw) followed by weak acid hydrolysis (0.1 M HCl, 40 °C, overnight), strong acid hydrolysis (6 M HCl, 80 °C, 4 hours), weak base hydrolysis (0.1 M NaOH, 40 °C, overnight) and strong base hydrolysis (6 M NaOH, 80 °C, 4 hours). The 30 DAT wheat grain PES samples were further characterised by sequential enzyme hydrolysis with amylase followed by protease, weak acid hydrolysis (1 M HCl, 40 °C, overnight) and weak base hydrolysis (1 M NaOH, 40 °C, overnight). The radioactivity in the hydrolysates was determined by LSC, but not profiled by HPLC against reference standards.

The identification and characterisation of the of radioactive residues is given for wheat forage, hay and straw PBI 30 days in Table 22, for wheat forage, hay and straw PBI 120 days in Table 23 for wheat forage, hay and straw PBI 365 days in Table 24. The residue characterisation for wheat grain, immature lettuce and mature lettuce at 30, 120 and 365 days PBI is presented in Table 25, Table 26 and Table 27, respectively. Radioactive residues found in carrot root and carrot foliage at 30, 120, 365 days PBI were summarized in Table 28.

Parent mandestrobin was generally found at low levels in all rotational crops at all PBI. Parent was identified in 30 and 120 PBI wheat forage, 365 PBI wheat hay, 120 and 365 PBI wheat straw, at max 0.045 mg eq/kg (1.8% TRR) in 30PBI wheat forage (Table 24). Parent was only detected in 30 PBI wheat grain samples (<LOD) with <1% of TRR (Table 18). In immature (0.003–1.3 mg eq/kg, 1.3–20% TRR, Table 26) and mature lettuces (0.002–0.014 mg eq/kg, 0.49-13% TRR, Table 27), as well as in carrot roots (0.015–0.04 mg/kg eq, 53-78% TRR,) and carrot foliage (≤ 0.001–0.01 mg eq/kg, 1.2–12% of TRR) the parent was identified at all PBI (except 365 days PBI for carrot roots, because not profiled), shown in Table 28. The R:S isomer ratios for the parent mandestrobin were found to be 41:59 in 30 PBI benzyl-labelled mature lettuce and 42:58 in 30 PBI phenoxy-labelled wheat forage. The R:S isomer ratio in both radiolabelled test substances was 50.3:49.7.

The metabolites 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and 4-OH- mandestrobin were found in free and conjugated form with a total accounting for 0.001–0.39 mg eq/kg (1.2–14% TRR), 0.002- 0.69 mg eq/kg (2.2–28% TRR) and at 0.008–0.6 mg eq/kg (1.7-34% TRR), respectively in all crops (Table 22-Table 28), except wheat grain. In wheat, only 4-OH-mandestrobin glycoside was identified as metabolite, among 10 not identified peaks, but below 0.01 mg eq/kg (3.8% TRR). The glycoside conjugate 2-CH_2OH-mandestrobin was the major compound in residue of wheat forage, hay and straw samples only for PBI 120. In immature lettuce at all PBI mainly the malonylglycoside was the major compound in the residue.

At all PBI the conjugated forms of 5-CH_2OH-mandestrobin, and the glycoside with up to 3 glycosides was the major compound in the residue, in carrot foliage (not detected in carrot root), immature and mature lettuce, wheat forage, wheat hay and wheat straw. For all crops, except wheat grain 4-OH- mandestrobin-glycoside and –malonylglycoside accounted for most of the 4-OH-mandestrobin.

The metabolites 5-COOH-mandestrobin and MCBX were found only in free form at < 0.001–0.14 mg eq/kg (0.43-9.0% TRR) and ≤ 0.001–0.028 mg eq/kg (1.3-4.8% TRR), respectively in all crops (Table 22-Table 28), except wheat grain. De-Xy-mandestrobin was only found in the benzyl-labelled 30 PBI immature and mature lettuce sample, in small amounts in a multi-component peak (11 min) which accounted for 0.010–0.012 mg eq/kg (3.9-4.8% TRR).

Representative [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin treated lettuce and wheat samples were stored up to approximately 6 and 9 months in the freezer (exact temperature not indicated), extracted and analysed, respectively. The two metabolite profiles of the initial and final analyses were very similar indicating that [^{14}C]-labelled metabolites were stable in the lettuce and wheat matrices under freezer storage conditions. Carrot samples were all analysed within 3 months of sample collection.

Notes by the reviewer:

The 11, 15, 16 and 19 min HPLC regions of the plant commodity extracts were singled out for treatment with enzymes and contained conjugates of known metabolites. The remainder of these extracts was not subjected to acid, base and enzyme hydrolysis. A radiovalidation study in green rape fodder (see analytical method section), where the whole extract was treated with alkaline and enzymatic hydrolysis, confirmed that the remainder of the extract from the green rape fodder metabolism study did not contain additional aglycones of known metabolites. This suggests, that the selected HPLC regions are the only regions containing aglycones of known metabolites.

Residues from wheat grains could not be extracted to a significant extent with organic solvents from the phenoxy label (5.2–5.8% TRR). In wheat grains with the benzyl label the extraction efficiency was better, with 31–37% TRR extracted with organic solvents. Considering the extraction efficiency with organic solvents, the identification level in wheat grains is low, since only zero to 3.8% TRR was identified in the benzyl labelled wheat grains.

A total of 77–87% TRR (carrot roots), 59–92% (forage/leaves), 45-62% TRR (wheat hay), 34–64% TRR (wheat straw) could be extracted from the indicated commodities. Considering the extraction efficiency with organic solvents, the identification level in these commodities is low since only 47–65% TRR (carrot roots, benzyl label), 6.9–53% TRR (wheat forage), 16–71% TRR (lettuce), 5.4–26% TRR (carrot foliage), 11–45% TRR (wheat hay), 14-33% TRR (wheat straw) could be identified in the respective commodities (i.e. less than 80% of the solvent extracted residue). Only the 30 day PBI samples from the phenoxy labelled wheat hay, mature lettuce and carrot roots and 120 day PBI samples from the phenoxy labelled carrot roots had a high identification level (> 80% of the extracted residue).

The existence of compounds additionally identified in the soil degradation studies was not verified in the confined rotational crop study. As some of these metabolites would fit in the plant metabolism scheme, their presence as free or conjugated compounds can therefore not be excluded.

Table 22 Identification and distribution in wheat forage, hay and straw 30 days PBI

Metabolite/ Residue fraction	Wheat forage PBI 30 days				Wheat hay PBI 30 days				Wheat straw PBI 30 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR
Parent	ND	ND	0.045	1.8	ND	ND	ND	ND	ND	ND	ND	ND
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin												
- free	0.012	0.43	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
- glc	0.027	0.99	ND	ND	0.22	14	0.46	10	0.036	2.7	0.067	8.1
- malonyl-glc	0.39	14	0.60	24	ND	ND	0.072	1.6	ND	ND	ND	ND
- total	–-0.43	--15	–-0.6	--24	–-0.22	--14	–-0.53	--12	-- 0.036	--2.7	–-0.067	--8.1
2-CH_2OH-mandestrobin												

Metabolite/ Residue fraction	Wheat forage PBI 30 days				Wheat hay PBI 30 days				Wheat straw PBI 30 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR
- free	ND	ND	ND	ND	0.16	10	0.20	4.5	0.035	2.6	0.006	0.69
- glc	0.39	14	ND	ND	ND	ND	0.038	0.85	ND	ND	ND	ND
- total	–-0.39	--14	--ND	--ND	–-0.16	--10	–-0.24	--5.3	–-0.035	--2.6	–-0.006	–-0.69
5-CH_2OH-mandestrobin												
- free	ND	ND	ND	ND	0.018	1.2	0.056	1.2	0.029	2.2	0.011	1.4
- glc 1	0.24	9.0	0.019	0.76	0.16	10	0.17	3.8	0.046	3.4	0.016	1.9
- glc 2	0.043	1.6	0.52	21	ND	ND	0.072	1.6	ND	ND	ND	ND
- glc 3	0.14	5.1	0.15	5.8	ND	ND	0.068	1.5	ND	ND	ND	ND
- total	–-0.43	--16	–-0.69	--27	–-0.18	--11	–-0.37	--8.2	–-0.075	--5.7	–-0.027	--3.3
5-COOH-mandestrobin	0.012	0.43	ND	ND	0.14	9.0	0.060	1.3	0.067	5.1	0.014	1.7
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**1.3**	**46**	**1.3**	**53**	**0.70**	**45**	**1.2**	**26**	**0.21**	**16**	**0.11**	**14**
%identified/neutral extracts		**46/73 =63**		**53/73 =73**		**45/ 52 =87**		**26/ 59 =44**		**16/ 35 =46**		**14/ 43 =33**
2 min	ND	ND	0.23 [f]	9.2 [f]	0.016	1.0	ND	ND	0.018	1.4	ND	ND
8 min	0.002	0.06	ND	ND	0.005	0.29	0.037	0.83	0.037	2.8	ND	ND
9 min	0.008	0.30	ND	ND	ND	ND	0.10	2.3	0.007	0.54	0.009	1.1
9.5 min	ND	0.02	0.039	1.5	ND	ND	0.24 [c]	5.4 [c]	0.006	0.47	0.034 [c]	4.1 [c]
10 min	0.050	1.8	0.026	1.0	ND	ND	0.48 [c]	11 [c]	ND	ND	0.047 [c]	5.7 [c]
10.5 min	ND	ND	0.12	4.9	NA	NA	ND	ND	ND	ND	ND	ND
11 min	0.010	0.37	0.077	3.0	0.082	5.3	0.41 [c]	9.1 [c]	0.016	1.2	0.072 [c]	8.8 [c]
14 min	0.25 [a]	9.3 [a]	0.22 [d]	8.7 [d]	0.11 [b]	6.8 [b]	0.65 [g]	14 [g]	0.020	1.4	0.014	1.8
20 min	0.023	0.83	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22 min	0.009	0.34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
40 min	0.008	0.28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Others	0.47 [c]	17 [c]	0.026	1.0	0.030	1.9	0.30	6.6	0.30 [c]	23 [c]	0.19 [c]	23 [c]
PES	0.52	19	0.45	18	0.50	32	1.1	24	0.70	53	0.34	42
Driselase hydrolysis	–-0.23	--8.4	–-0.19	--7.5	–-0.067	--4.3	–-0.26	--5.6	--	--	--	--
0.1M acid hydrolysis	–-0.063	--2.3	–-0.068	--2.7	–-0.038	--2.4	–-0.12	--2.6	--	--	--	--
1M acid hydrolysis	--	--	--	--	--	--	--	--	–-0.053	--4.0	–-0.037	--4.5
6M acid hydrolysis	–-0.011	–-0.39	–-0.059	--2.3	–-0.013	–-0.83	–-0.16	--3.6	–-0.030	--2.3	–-0.023	--2.8
0.1M base hydrolysis	–-0.10	--3.9	–-0.07	--2.8	–-0.043	--2.8	–-0.14	--3.1	--	--	--	--
1M base hydrolysis	--	--	--	--	--	--	--	--	–-0.29	--22	–-0.12	--15
6M base hydrolysis	–-0.096	--3.5	–-0.059	--2.3	–-0.32	--20	–-0.30	--6.6	–-0.21	--16	–-0.11	--14
Unextracted ted residue	**–-0.016**	**–-0.59**	**–-0.006**	**–-0.25**	**–-0.023**	**--1.5**	**–-0.12**	**--2.6**	**–-0.12**	**--9.1**	**–-0.048**	**--5.8**
Total unknowns (extracted or hydrolysed)	**1.3**	**49**	**1.2**	**47**	**0.72**	**49**	**3.2**	**71**	**0.99**	**75**	**0.66**	**80**
TRR Total	**2.6**	**96**	**2.5**	**100**	**1.4**	**95**	**4.5**	**100**	**1.3**	**100**	**0.82**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

ND = not detected, NA = not applicable, -- = not included in calculation of TRR total

%TRR Total of residues may not add up to 100% due to rounding; Total calculated based on extracted and PES.

[a] 14 minute region comprised 8 components, the largest of which accounted for 0.049 mg eq/kg, 1.8% TRR.

[b] 14 minute region comprised 7 components, the largest of which accounted for 0.034 mg eq/kg, 2.2% TRR.

[c] Consists of multiple components. No further characterisation of the components and their level of TTR were given.

[d] 14 minute region comprised 4 components, the largest of which accounted for 0.12 mg eq/kg, 4.6% TRR.

[f] 2 minute region comprised multiple components.

[g] 14 minute region comprised 6 components, the largest of which accounted for 0.24 mg eq/kg, 5.2% TRR.

Table 23 Identification and distribution in wheat forage, Hay and Straw 120 DAT

Metabolite / Residue fraction	Wheat forage PBI 120 days				Wheat hay PBI 120 days				Wheat straw PBI 120 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
parent	0.001	0.89	ND	ND	ND	ND	ND	ND	0.004	1.2	0.009	1.5
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin - free - glc - malonyl glc - total	ND 0.016 0.008 –- 0.024	ND 12 5.9 --18	ND 0.009 0.011 –-0.02	ND 2.8 3.6 --6.4	0.005 0.027 0.015 –- 0.047	1.8 9.4 5.2 --16	0.002 0.048 ND –- 0.050	0.28 6.7 ND --7.0	0.009 0.017 ND –- 0.026	2.7 4.9 ND --7.7	0.016 0.030 ND –- 0.046	2.7 5.0 ND --7.7
2-CH_2OH-mandestrobin - free - glc - total	0.008 0.014 –- 0.022	5.9 9.9 --16	0.010 0.010 –- 0.020	3.2 3.3 --6.5	0.014 ND –- 0.014	4.9 ND --4.9	0.003 0.002 –- 0.005	0.42 0.31 –- 0.73	0.007 0.016 –- 0.023	2.0 4.8 --6.8	0.011 0.033 –- 0.044	1.9 5.5 --7.4
5-CH_2OH-mandestrobin -free - glc 1 - glc 2 - glc 3 - total	ND 0.011 0.001 0.003 –- 0.015	ND 8.1 1.0 2.5 --12	0.004 0.009 ND 0.008 –- 0.021	1.3 3.1 ND 2.5 --6.9	0.005 0.023 0.006 0.010 –- 0.044	1.9 8.0 2.2 3.4 --15	ND 0.020 0.001 ND –- 0.021	ND 2.8 0.08 0 ND --2.9	0.016 0.008 ND ND –- 0.024	4.7 2.3 ND ND --7.0	0.030 0.013 ND ND –- 0.043	5.1 2.2 ND ND --7.4
5-COOH-mandestrobin	ND	ND	0.002	0.56	0.003	1.1	0.004	0.59	0.013	3.8	0.024	4.0
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	ND	ND	ND	ND	ND	ND	0.015	4.5	0.028	4.8
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0.062**	**12**	**0.021**	**6.9**	**0.11**	**38**	**0.080**	**11**	**0.10**	**31**	**0.19**	**33**
%identified/ neutral extracts		**12/ 71 =17**		**6.9/ 81 =8.5**		**38/ 62 =61**		**11/ 66 =17**		**31/ 64 =48**		**33/ 64 =52**
2 min	ND	ND	ND	ND	0.010	3.6	ND	ND	ND	ND	ND	ND
8 min	ND	ND	ND	ND	ND	ND	0.018	2.4	0.013	3.9	0.02	3.4
9 min	ND	ND	0.008	2.7	0.003	0.98	0.058	8.0	0.013	3.7	0.028	4.7
9.5 min	ND	ND	0.025	8.2	ND	ND	0.018	2.4	ND	ND	ND	ND
10 min	0.001	0.53	0.009	2.9	ND	ND	0.052	7.2	ND	ND	ND	ND
11 min	0.001	0.69	0.019	6.4	ND	ND	0.063	8.7	0.008	2.3	0.018	3.0
14 min	0.021 [a]	14 [a]	0.083 [a]	27 [a]	0.088 [b]	31 [b]	0.13 [b]	18 [b]	0.059 [c]	18 [c]	0.12 [c]	20 [c]
19 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Metabolite / Residue fraction	Wheat forage PBI 120 days				Wheat hay PBI 120 days				Wheat straw PBI 120 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
22 min	ND	ND	0.002	0.56	ND	ND	ND	ND	ND	ND	ND	ND
Others	0.020	14	0.05	16	0.002	0.81	0.16	22	0.049	14	0.074	12
PES	**0.035**	**25**	**0.048**	**16**	**0.074**	**26**	**0.15**	**20**	**0.093**	**27**	**0.14**	**24**
Total unknowns (extracted)	**0.043**	**30**	**0.20**	**64**	**0.10**	**36**	**0.50**	**69**	**0.14**	**42**	**0.26**	**43**
TRR Total	**0.14**	**100**	**0.31**	**100**	**0.28**	**100**	**0.72**	**100**	**0.34**	**100**	**0.59**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

PES was not further characterised, ND= not detected, NA = not applicable, glc = glycoside, -- = not included in calculation of TRR total

%TRR Total of residues may not add up to 100% due to rounding

[a] 14-minute region in wheat forage comprised 7 components, the largest of which accounted for 0.006 mg/kg, 4.3% TRR ([Ph-^{14}C]-label) and comprised 7 components, the largest of which accounted for 0.031 mg eq/kg, 10% TRR ([Bz-^{14}C]-label)

[b] 14-minute region in hay comprised 3 components, the largest of which accounted for 0.053 mg eq/kg, 19% TRR ([Ph-^{14}C]-label) and comprised 7 components, the largest of which accounted for 0.10 mg eq/kg, 14% TRR ([Bz-^{14}C]-label)

[c] 14- minute region in straw comprised 3 components, the largest of which accounted for 0.026 mg eq/kg, 7.7% TRR ([Ph-^{14}C]-label) and comprised 3 components, the largest of which accounted for 0.056 mg eq/kg, 9.4% TRR ([Bz-^{14}C]-label)

Table 24 Identification and distribution in wheat forage, hay and straw 365 PBI

Metabolite / Residue fraction	Wheat forage PBI 365 days				Wheat hay PBI 365 days				Wheat straw PBI 365 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
parent	ND	ND	ND	ND	0.002	0.85	0.002	0.37	ND	0.24	0.005	2.2
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin	0.002	2.5	ND	ND	ND	ND	0.005	1.1	ND	ND	ND	ND
- free	0.005	5.5	0.009	3.6	0.007	3.4	0.003	0.62	0.006	4.4	0.020	8.8
- glc	0.003	3.4	0.006	2.3	0.003	1.2	ND	ND	0.002	1.7	0.004	1.6
- malonyl glc	--	--11	--	--5.9	--0.01	--4.6	--	--1.7	--	--6.1	--	--10
- total	0.010		0.015				0.008		0.008		0.024	
2-CH_2OH-mandestrobin	0.006	6.2	0.003	1.1	0.012	6.6	0.013	2.5	0.006	4.7	0.003	1.4
- free	ND	ND	0.008	3.1	0.002	1.1	ND	ND	ND	ND	0.003	1.4
- glc	--	--6.2	--	--4.2	--	--7.7	--	--2.5	--	--4.7	--	--2.8
- total	0.006		0.011		0.014		0.013		0.006		0.006	
5-CH_2OH-mandestrobin	ND	ND	ND	ND	0.004	1.8	0.007	1.4	ND	ND	0.006	2.5
- free	0.005	5.0	0.008	3.0	0.009	4.4	0.007	1.4	0.006	4.3	0.006	2.6
- glc 1	0.004	4.3	ND	ND	ND	ND	0.007	1.4	0.003	2.6	0.004	1.7
- glc 2	0.002	2.0	0.004	1.7	0.005	2.4	ND	ND	ND	0.27	0.002	0.90
- glc 3	--	--11	--	--4.7	--	--8.6	--	--4.2	--	--7.2	--	--7.7
- total	0.011		0.012		0.018		0.021		0.009		0.018	
5-COOH-mandestro	0.002	1.8	ND	ND	0.007	3.2	0.003	0.55	0.004	3.4	0.005	2.3

Metabolite / Residue fraction	Wheat forage PBI 365 days				Wheat hay PBI 365 days				Wheat straw PBI 365 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
bin												
2-COOH mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	0.001	1.3	ND	ND	0.004	1.7	ND	ND	0.003	2.6	0.003	1.5
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0.030**	**32**	**0.038**	**15**	**0.055**	**27**	**0.047**	**9.3**	**0.030**	**24**	**0.061**	**27**
%identified/ neutral extracts		**32/ 72 =44**		**15/ 80 =19**		**27/ 45 =60**		**9.3 50 =19**		**24/ 34 =71**		**27/ 40 =68**
2 min	0.003	2.8	ND	ND	0.004	2.1	ND	ND	0.002	1.2	0.002	1.1
8 min	ND	ND	0.003	1.1	ND	ND	0.001	0.29	ND	ND	ND	ND
9 min	0.003	2.8	0.003	1.1	0.001	0.45	0.002	0.43	0.001	0.77	0.002	0.75
9.5 min	0.001	0.95	0.027 [a]	10 [a]	0.001	0.65	0.047	9.4	0.003	2.4	0.005	2.0
10 min	ND	ND	0.025	9.8	0.003	1.6	0.006	1.1	0.001	0.89	ND	ND
11 min	0.004	4.0	0.003	1.2	0.008	3.7	0.018	3.7	0.002	1.5	0.025	11
14 min	0.018 [a]	19 [a]	0.095 [a]	37 [a]	0.032 [b]	16 [b]	0.065 [b]	13 [b]	0.019 [c]	15 [c]	0.034 [c]	15 [c]
20 min	0.002	2.0	0.005	1.9	ND	ND	ND	ND	ND	ND	ND	ND
37.8 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.005	2.3
42 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.001	0.39
Others	0.012	12	0.018	7.0	0.10	7.7	0.32	31	0.071	4.7	0.093	0.63
PES	**0.024**	**25**	**0.041**	**16**	**0.15**	**41**	**0.23**	**32**	**0.15**	**50**	**0.15**	**40**
Total unknowns (extracted)	**0.043**	**43**	**0.18**	**69**	**0.15**	**32**	**0.46**	**59**	**0.099**	**26**	**0.17**	**33**
TRR Total	**0.097**	**100**	**0.26**	**100**	**0.35**	**100**	**0.74**	**100**	**0.28**	**100**	**0.38**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

ND= Not detected, NA = not applicable, glc = glycoside, -- = not included in calculation of TRR total

[a] 14-minute region in wheat forage comprised 8 components, the largest of which accounted for 0.023 mg eq/kg, 9.0% TRR ([Bz-^{14}C]-label)

[b] 14=-minute region in hay PBI 365 comprised 4 components, the largest of which accounted for 0.015 mg eq/kg, 7.1% TRR ([Ph-^{14}C]-label) and comprised 7 components, the largest of which accounted for 0.019 mg eq/kg, 3.8% TRR ([Bz-^{14}C]-label)

[c] 14-minute region in straw PBI 365 comprised 4 components, the largest of which accounted for 0.006 mg eq/kg, 4.5% TRR. ([Ph-^{14}C]-label) and comprised 2 components, the largest of which accounted for 0.025 mg eq/kg, 11% TRR ([Bz-^{14}C]-label)

Table 25 Identification and distribution of residues in wheat grain at 30, 120 and 365 PBI with [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin

Metabolite / Residue fraction	Grain PBI 30 days				Grain PBI 120 days				Grain PBI 365 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	ND	0.43	ND	ND	0.004	10	ND	ND			0.005	
De-Xy-mande strobin	NA	NA	ND	ND			ND	ND	0.003	100		100
4-OH-	ND	ND	ND	ND	NP	NP	0.008	3.8	NP	NP	NP	NP

Metabolite / Residue fraction	Grain PBI 30 days				Grain PBI 120 days				Grain PBI 365 days			
	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/ kg	%TRR	mg eq/ kg	%TRR	mg eq/ kg	%TRR	mg eq/ kg	%TRR	mg eq/ kg	%TRR	mg eq/ kg	%TRR
mandestrobin-glc												
2-CH_2OH-mandestrobin	ND	ND	ND	ND			ND	ND				
5-CH_2OH-mandestrobin	ND	ND	ND	ND			ND	ND				
5-COOH-mandestrobin	ND	ND	ND	ND			ND	ND				
2-COOH-mandestrobin	ND	ND	ND	ND			ND	ND				
MCBCX	ND	ND	ND	ND			ND	ND				
2,5-dimethyl phenol	ND	ND	NA	NA			NA	NA				
Total identified	**0**	**0.43**	**0**	**0**			**0.008**	**3.8**				
%identified/ neutral extracts		**0.43/5.8 =7.4**		**0/ 31 =0**				**3.8/ 37 =10**				
2 min	0.002	5.1	ND	ND			ND	ND				
8 min	ND	0.47	ND	ND			ND	ND				
9 min	ND	ND	ND	ND			0.003	1.5				
9.5 min	ND	ND	0.013 [b]	11 [b]			0.003	1.6				
10 min	ND	ND	0.010 [b]	8.2 [b]			0.007	3.4				
11 min	0.001	1.5	0.027 [b]	22 [b]			0.003	1.7				
14 min region	0.001	1.8	0.032 [b]	26 [b]			0.11 [a]	55 [a]				
16 min	ND	0.75	ND	ND			ND	ND				
19 min	ND	0.13	ND	ND			ND	ND				
Others	ND	1.1	ND	0.01			0.007	3.1				
PES	0.032	89	0.039	32	0.032	90	0.059	30				
Amylase hydrolysis	-- 0.002	--6.7	-- 0.009	--7.2	Not extract.		Not extract.					
Protease hydrolysis	-- 0.002	--5.1	-- 0.005	--3.8								
1M acid hydrolysis	-- 0.001	--3.0	-- 0.002	--1.4								
1M base hydrolysis	-- 0.012	--33	-- 0.012	--10								
Unextracted residue	**0.015**	**41**	**0.012**	**9.7**								
Total unknowns (extracted or hydrolysed)	**0.021**	**58**	**0.11**	**90**	**0.004**	**10**	**0.13**	**67**	**0.003**	**100**	**0.005**	**100**
TRR Total	**0.036**	**100**	**0.12**	**100**	**0.036**	**100**	**0.20**	**100**	**0.003**	**100**	**0.005**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

NP = Not profiled, glc = glycoside, ND= not detected, NA = not applicable, -- = not included in calculation of TRR total

[a] 14 minute region comprised 6 components, of which one component accounted for 0.044 mg eq/kg, 22% TRR, another component accounted for 0.034 mg eq/kg (17% TRR) and the remaining 4 components were maximum 4.2% TRR (0.008 mg eq/kg).

[b] The unknown peaks and/or regions eluting at 9.5, 10, 11 and 14 minutes (HPLC method 1) did not correspond to any known metabolite standards of mandestrobin, and ranged from 0.01 and 0.03 mg eq/kg (8.2–26% TRR)

Table 26 Identification and distribution of residues in immature lettuce 30, 120, 365 days PBI with [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin (M)

Immature lettuce	PBI 30 days				PBI 120 days				PBI 365 days			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	0.003	1.0	0.021	6.5	ND	1.3	ND	ND	0.013	20	0.009	12
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin												
- free	ND	ND	ND	ND	ND	ND	ND	ND	0.002	2.8	0.001	0.70
- glc	0.007	2.1	0.004	1.3	ND	0.31	0.006	8.2	ND	ND	0.002	2.4
- malonyl glc	0.11	32	0.080	25	0.002	7.0	0.008	10	0.008	13	0.013	17
- total	–-0.12	--34	–-0.084	--27	–-0.002	--7.3	–-0.014	--19	–-0.010	--15	–-0.016	--20
2-CH_2OH-mandestrobin – glc	0.018	5.3	0.008	2.5	ND	0.22	ND	ND	ND	ND	ND	ND
5-CH_2OH-mandestrobin												
- free	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
- glc 1	0.003	0.81	0.001	0.41	0.001	4.3	0.002	2.6	ND	ND	0.001	1.4
- glc 2	0.041	12	0.024	7.5	ND	0.22	ND	ND	0.012	18	0.002	2.6
- glc 3	0.043	13	0.026	8.4	0.001	3.0	0.002	2.5	0.001	2.0	0.002	3.1
- total	–-0.087	--26	–-0.051	--16	–-0.002	--7.6	–-0.004	--5.1	–-0.013	--20	–-0.005	--7.1
5-COOH-mandestrobin	ND	ND	0.002	0.50	ND	ND	ND	ND	ND	ND	ND	ND
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	0.004	1.2	ND	ND	ND	ND	0.002	3.4	ND	ND
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0.22**	**67**	**0.17**	**54**	**0.004**	**16**	**0.018**	**24**	**0.038**	**59**	**0.030**	**39**
%identified/ neutral extracts		**67/ 92 =73**		**54/ 92 =59**		**16 67 =24**		**24/ 81 =30**		**59/ 77 =77**		**39/ 80 =49**
2 min	0.004	1.3	0.003	0.99	0.003	10	0.008	11	0.001	1.6	0.004	5.2
8 min	ND	ND	0.004	1.1	ND	ND	ND	ND	ND	ND	0.003	3.9
9 min	ND	ND	0.006	2.1	<ND	0.34	ND	ND	ND	ND	0.001	1.3
9.5 min	ND	ND	0.014	4.5	ND	0.37	ND	ND	ND	0.11	0.005	7.4
10 min	ND	ND	0.005	1.6	ND	ND	ND	ND	ND	ND	0.001	1.2
11 min	ND	ND	0.012 [b]	3.9 [b]	ND	ND	0.004	5.7	ND	ND	ND	0.64
14 min	0.017 [a]	5.1 [a]	0.027 [c]	8.9 [c]	0.003 [a]	12 [a]	0.030 [c]	38 [c]	0.006 [a]	8.3 [a]	0.009 [d]	13 [d]
16 min	ND	ND	ND	ND	ND	0.22	ND	ND	ND	ND	ND	ND
20 min	0.024	7.1	0.008	2.5	0.001	1.6	ND	ND	0.004	5.8	0.006	8.4
22 min	0.006	1.8	0.004	1.2	0.001	4.4	ND	ND	-	-	-	-
Others	0.041	12	0.043	14	0.009	25	0.005	7.6	0.004	6.0	0.002	2.1
PES	0.019	5.6	0.019	6.0	0.010	29	0.011	14	0.013	19	0.013	18
Total unknowns (extracted or	**0.092**	**28**	**0.13**	**40**	**0.017**	**55**	**0.047**	**62**	**0.015**	**22**	**0.031**	**43**

Immature lettuce	PBI 30 days				PBI 120 days				PBI 365 days			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
hydrolysed)												
TRR Total	**0.33**	**100**	**0.32**	**100**	**0.031**	**100**	**0.076**	**100**	**0.066**	**100**	**0.074**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

ND: Not detected, NA = not applicable, glc = glycoside, -- = not included in calculation of TRR total

[a] 14-minute region in PBI 30 comprised 4 components, the largest of which accounted for 0.006 mg/kg, 1.9% TRR, in PBI 120 comprised 7 components, the largest of which accounted for 0.001 mg/kg, 4.1% TRR and in PBI 365 comprised 4 components, the largest of which accounted for 0.002 mg/kg, 3.0% TRR.

[b] The 11-min peak (in lettuce 30PBI) contains multiple components including a small amount of De-Xy-mandestrobin.

[c] The 14-minute region in PBI 30 comprised 8 components, the largest of which accounted for 0.008 mg/kg, 2.6% TRR, in PBI 120 comprised 4 components, the largest of which accounted for 0.015 mg/kg, 20% TRR.

[d] 14 minute region comprised 5 components, the largest of which accounted for 0.005 mg/kg, 6.9% TRR.

Table 27 Identification and distribution of residues in mature lettuce 30, 120, 365 days PBI with [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin

Mature lettuce	30 days PBI				120 days PBI				365 days PBI			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR
Parent	0.013	13	0.014	6.5	0.002	8.4	0.004	9.4	ND	0.49	ND	ND
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin												
- free	ND	ND	ND	ND	ND	ND	0.001	2.5	ND	ND	ND	ND
- glc	ND	0.32	0.006	2.8	0.001	2.7	0.005	9.9	0.001	3.3	ND	0.57
- malonyl glc.	0.019	23	0.054	25	ND	ND	ND	ND	0.002	8.2	0.001	6.8
- total	–-0.019	--23	–-0.060	--28	–-0.001	--2.7	–-0.006	--12	–-0.003	--12	–-0.001	--7.4
2-CH_2OH-mandestrobin												
- free	ND	ND	ND	ND	0.001	3.4	0.001	1.7	ND	ND	ND	ND
- glc	ND	0.32	0.004	1.7	ND	ND	ND	0.73	ND	ND	ND	1.2
- total	--ND	–-0.32	–-0.004	--1.7	–-0.001	--3.4	–-0.001	--2.4	--ND	--ND	--ND	--1.2
5-CH_2OH-mandestrobin												
-free	ND	ND	ND	ND	ND	ND	ND	0.98	ND	ND	ND	ND
- glc 1	0.002	1.9	ND	ND	ND	1.7	0.002	5.0	ND	2.0	ND	0.89
- glc 2	0.006	7.7	0.014	6.2	ND	ND	ND	0.17	0.003	15	ND	2.0
- glc 3	0.018	22	0.032	15	ND	ND	ND	ND	0.001	6.9	0.001	4.2
- total	–-0.026	--32	–-0.046	--21	--ND	--1.7	–-0.002	--6.2	–-0.004	--24	–-0.001	--7.2
5-COOH-mandestrobin	ND	ND	ND	ND	ND	1.5	ND	0.97	ND	ND	ND	0.95
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	0.002	2.6	0.005	2.2	ND	1.6	0.001	2.9	ND	2.4	ND	ND
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0.060**	**71**	**0.13**	**59**	**0.004**	**19**	**0.014**	**34**	**0.007**	**38**	**0.002**	**17**
%identified/ neutral extracts		**71/88 =81**		**59/92 =64**		**19/59 =32**		**34/75 =45**		**38/79 =48**		**17/75 =23**
2 min	0.003	3.3	0.001	0.68	0.004	18	0.001	2.9	0.002	9.6	0.004	19
8 min	ND	ND	0.001	0.68	ND	ND	0.001	1.0	ND	ND	ND	1.0

Mature lettuce	30 days PBI				120 days PBI				365 days PBI			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR	mg eq/kg.	%TRR
9 min	ND	ND	0.003	1.4	ND	2.2	0.003	6.0	ND	0.49	0.001	6.4
9.5 min	ND	ND	0.010	4.7	ND	ND	0.001	2.0	ND	ND	ND	ND
10 min	ND	ND	0.003	1.5	ND	ND	0.001	1.8	ND	ND	ND	ND
11 min	ND	ND	0.01 [b]	4.8 [b]	ND	ND	ND	ND	ND	1.0	0.001	4.0
14 min	0.002 [a]	2.5 [a]	0.006 [c]	3.0 [c]	0.002 [d]	11 [d]	0.005 [e]	12 [e]	0.004 [f]	15 [f]	0.004 [g]	19 [g]
16 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20 min	0.003	3.8	0.011	4.9	ND	ND	ND	ND	ND	ND	ND	ND
22 min	ND	ND	ND	ND	ND	ND	0.001	2.8	0.002	11	0.001	5.9
Others	0.006	7.2	0.028	13	0.004	17	0.009	20	0.001	3.8	0.001	2.6
PES	0.008	10	0.015	6.9	0.007	33	0.009	18	0.004	21	0.006	25
Total unknowns (extracted)	**0.014**	**17**	**0.073**	**34**	**0.010**	**48**	**0.022**	**48**	**0.009**	**41**	**0.012**	**58**
TRR Total	**0.082**	**97.8**	**0.22**	**100**	**0.022**	**99.8**	**0.045**	**100**	**0.020**	**100**	**0.020**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

ND= Not detected, NA = not applicable, glc.=glycoside, -- = not included in calculation of TRR total

[a] 14-minute region comprised 1 component, 0.002 mg/kg, 2.5% TRR.

[b] A portion was identified as De-Xy-mandestrobin using HPLC co-chromatography and TLC analysis,

[c] 14-minute region comprised 3 components, the largest of which accounted for 0.002 mg/kg, 1.1% TRR.

[d] 14-minute region comprised 1 component, 0.002 mg/kg, 11% TRR.

[e] 14-minute region comprised 7 components, the largest of which accounted for 0.003 mg/kg, 6.8% TRR.

[f] 14-minute region comprised 4 components, the largest of which accounted for 0.001 mg/kg, 4.1% TRR.

[g] 14-minute region comprised 5 components, the largest of which accounted for 0.002 mg/kg, 7.1% TRR.

Table 28 Identification and distribution of residues in carrot root at 30, 120, 365 days PBI with [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin

Carrot root	PBI 30 days				PBI 120 days				PBI 365 days			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	0.040	78	0.018	47	0.015	53	0.024	61	0.006	77	0.008	84
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NP	NP	NP	NP
4-OH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND				
2-CH_2OH-mandestrobin (free)	ND	ND	ND	ND	0.004	14	0.001	3.0				
5-CH_2OH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND				
5-COOH-mandestrobin	ND	ND	ND	ND	0.003	9.4	0.001	1.6				
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND				
MCBX	ND	ND	ND	ND	ND	1.7	ND	ND				
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA				
Total identified	**0.040**	**78**	**0.018**	**47**	**0.022**	**79**	**0.026**	**65**				
%identified/ neutral extracts		**78/87 =90**		**47/89 =53**		**79/84 =94**		**65/90 =72**				
2 min	0.006	12	0.018 [a]	46 [a]	ND	ND	ND	ND				
11 min	ND	ND	ND	ND	ND	ND	0.004	11				

Carrot root	PBI 30 days				PBI 120 days				PBI 365 days			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
14 min	ND	ND	ND	ND	0.001	1.9	0.005 [b]	11 [b]				
Others	ND	ND	ND	ND	0.002	5.6	0.002	5.2				
PES	**0.005**	**9.3**	**0.003**	**6.9**	**0.003**	**12**	**0.003**	**6.8**	**0.002**	**23**	**0.001**	**16**
Total unknowns (extracted)	**0.006**	**12**	**0.018**	**46**	**0.003**	**7.5**	**0.011**	**28**	**0.006**	**77**	**0.009**	**100**
TRR Total	**0.051**	**100**	**0.039**	**100**	**0.028**	**100**	**0.040**	**100**	**0.008**	**100**	**0.009**	**100**

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

NP = Not profiled, ND= not detected, -- = not included in calculation of TRR total

[a] The 2-minutes peak consisted of numerous components, and maximum peak amounted to 0.011 ppm or 29% TRR.

[b] The 14-minute region comprised 3 components, the largest of which accounted for 0.003 mg/kg, 6.4% TRR.

Table 1 Identification and distribution of residues in carrot foliage at 30, 120, 365 days PBI with [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin

Carrot foliage	PBI 30 days				PBI 120 days				PBI 365 days			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Parent	0.001	1.2	0.002	3.2	0.003	4.9	0.010	12	ND	ND	ND	1.7
De-Xy-mandestrobin	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND
4-OH-mandestrobin												
- free	ND	ND	ND	ND	0.001	1.4	ND	ND	ND	ND	ND	ND
- glc	0.002	1.4	ND	ND	ND	ND	ND	ND	0.001	4.7	0.001	4.2
- malonyl glyc	0.003	2.4	ND	ND	0.001	2.2	0.002	2.6	0.001	3.8	0.001	4.7
- total	–-0.005	--3.8	--ND	--ND	–-0.002	--3.6	–-0.002	--2.6	–-0.002	--8.5	–-0.002	--8.9
2-CH_2OH-mandestrobin												
- free	ND	ND	ND	ND	0.002	3.1	ND	ND	0.001	2.1	ND	0.70
- glc	ND	ND	ND	ND	ND	ND	0.001	1.5	0.001	2.2	0.001	2.2
- total	--ND	--ND	--ND	--ND	–-0.002	--3.1	–-0.001	--1.5	–-0.002	--4.2	–-0.001	--2.9
5-CH_2OH-mandestrobin												
- free	ND	ND	ND	ND	ND	ND	0.001	1.5	ND	ND	ND	ND
- glc 1	0.008	7.2	0.002	2.2	ND	ND	ND	ND	0.001	2.2	0.001	2.7
- glc 2	0.001	0.93	ND	ND	0.003	6.2	0.002	2.0	0.002	5.8	0.002	6.6
- glc 3	ND	ND	ND	ND	0.001	1.2	ND	ND	0.001	1.8	ND	1.8
- total	–-0.009	--8.1	–-0.002	--2.2	–-0.004	--7.4	–-0.003	--3.6	–-0.004	--9.9	–-0.003	--11
5-COOH-mandestrobin	ND	ND	ND	ND	0.001	1.3	ND	ND	ND	1.1	ND	1.3
2-COOH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.4	ND	ND
2,5-dimethyl phenol	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA
Total identified	**0.015**	**13**	**0.004**	**5.4**	**0.012**	**20**	**0.016**	**20**	**0.008**	**25**	**0.006**	**26**
%identified/ neutral extracts		**13/83 =16**		**5.4/78 =6.9**		**20/77 =26**		**20/76 =26**		**25/71 =35**		**26/64 =41**
2 min	0.014	13	0.010	13	ND	ND	0.001	1.7	0.002	5.1	0.001	3.9
8 min	0.003	2.3	0.007	9.8	ND	0.47	0.001	0.96	ND	0.43	ND	1.4
9 min	0.004	3.4	0.003	4.3	ND	ND	0.002	2.9	ND	ND	ND	1.7
9.5 min	ND	ND	0.004	6.1	0.001	1.9	0.004	4.8	ND	ND	ND	ND

Carrot foliage	PBI 30 days				PBI 120 days				PBI 365 days			
Metabolite/ Residue fraction	[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M		[Ph-^{14}C]-M		[Bz-^{14}C]-M	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
10 min	0.004	3.2	ND	ND	0.001	1.4	0.007	9.3	ND	ND	ND	ND
11 min	0.006	5.4	0.006	7.6	0.001	1.8	0.003	3.8	0.001	3.6	0.001	5.1
14 min	0.058 [a]	51 [a]	0.031 [b]	43 [b]	0.021 [c]	40 [c]	0.024 [d]	31 [d]	0.011 [e]	36 [e]	0.009 [f]	34 [f]
42 min	ND	ND	ND	ND	0.001	2.8	0.001	0.80	ND	ND	ND	ND
20 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.46	ND	0.65
22 min	ND	ND	ND	ND	0.002	3.5	0.001	0.96	ND	0.47	ND	0.63
Others	0.001	1.9	ND	ND	0.009	17	0.011	14	0.003	10	0.002	7.5
PES	0.008	6.8	0.008	11	0.005	11	0.008	10	0.006	18	0.005	19
Total unknowns (extracted)	**0.090**	**80**	**0.061**	**84**	**0.036**	**69**	**0.055**	**70**	**0.017**	**57**	**0.013**	**55**
TRR Total	0.11	100	0.074	100	0.051	100	0.077	100	0.031	100	0.027	100

[Ph-^{14}C]-M = [Ph-^{14}C]-Mandestrobin, [Bz-^{14}C]-M= [Bz-^{14}C]-Mandestrobin

ND = Not detected, NA = not applicable, -- = not included in calculation of TRR total

[a] 14-minute region comprised 5 components, the largest of which accounted for 0.027 mg/kg, 24% TRR and 0.013 mg eq/kg (11% TRR). The remaining 3 components were 0.005–0.007 mg eq/kg (4.2–6.6% TRR)

[b] 14 minute region comprised 6 components, the largest two accounted for 0.008 mg eq/kg, 11% TRR; the remaining 4 components were < 0.001–0.007 mg eq/kg (0.0–9.5% TRR)

[c] 14 minute region comprised 4 components, the largest two of which accounted for 0.007 mg eq/kg, 13% TRR and 0.006 mg eq/kg (11% TRR). The other 2 components were 0.004 mg eq/kg (7.1–8.4% TRR)

[d] 14 minute region comprised 7 components, the largest of which accounted for 0.007 mg/kg, 8.9% TRR.

[e] 14 minute region comprised 2 components which accounted for 0.007 mg eq/kg, 24% TRR and 0.004 mg eq/kg (12% TRR).

[f] 14 minute region comprised 3 components, the largest of which accounted for 0.004 mg eq/kg, 16% TRR; the remaining 2 components were below 10% TRR and < 0.01 mg eq/kg

Field rotational crop studies

Study 1: Field rotational crop studies were conducted in 2010–2011 in Northern France (trial FR01) and Spain (trial SP01) to provide information on the uptake of mandestrobin in rotational crops [Roussel, 2012, ROR-0202]. Each trial consisted of 2 plots planted with winter rape seed. One plot served as untreated control and the other was treated with a single foliar application of an SC formulation of mandestrobin at a rate of 0.20–0.21 kg ai/ha with a volume of 300 L/ha. Rape seed was treated at growth stage BBCH 65 (flowering). No adjuvant was added to the spray mixture. The application was performed using hand-carried boom sprayers closely simulating commercial-type treatments. All foliage was sprayed thoroughly and uniformly and without excessive run-off. The preceding crop, winter rape seed (whole plant), was sampled 3-4 hours after the application to confirm the appropriate application. A representative soil sample of 1 kg minimum weight obtained from at least 10 cores – 10–20 cm depth – was collected at each trial site from the untreated plot. The characteristics of the soil are shown in Table 30. The rape seed crop was mechanically destroyed (crushed) 14 days after application (= PBI 14) and incorporated into the soil and succeeding crops (carrot, lettuce, broccoli and spring or winter barley) were sown or transplanted into the plots at PBI of 14, 120 and 365 days. Samples of succeeding crops were harvested at their respective harvest time (Table 31). Commodities were collected from at least 12 plants (carrots roots and leaves, lettuce heads, broccoli inflorescences) or from 12 different areas of the plot (barley grain and straw). The minimum weight was at least 1 kg per sample, except 0.5 kg straw, 2 kg lettuce heads and 2 kg carrot roots. Soil was not collected.

Table 30 Soil characteristics

	02700 Mennessis, Picardie, Northern France	01213 Lantaron, Alava, Spain

	FLN-10–6268-FR01	FLN-10–6268-SP01
Soil type (USDA)	loam	sandy loam
pH (water)	7.5	7.6
pH (KCl)	7.0	7.5
pH (0.01 M CaCl2)	7.0	7.3
CEC (meq/100 g)	10.5	6.8
Water holding capacity pF0 (maximum water holding capacity) pF2 (0.1 bar) pF2.5 (0.33 bar)	 51.2 32.5 16.1	 47.2 22.4 10.3
Organic Carbon (%)	1.0	1.3
Organic matter (%)	1.7	2.2

Table 31 Summary of PBI, DAT and DAS in field rotational crops (lettuce, carrots, broccoli and barley) grown in aged soil (14, 120, 365 days PBI) treated with primary crop rape seed treated with mandestrobin at two field sites in Northern France (FR01) and Spain (SP01)

Trial conditions		02700 Mennessis, Picardie, Northern France FLN-10–6268-FR01 1 × 0.20–0.21 kg ai/ha loam soil		01213 Lantaron, Alava, Spain FLN-10–6268-SP01 1 × 0.21 kg ai/ha sandy loam soil	
Winter rape seed (whole plant) (primary crop; DAT=0)		1.4 mg/kg R-isomer 1.2 mg/kg S-isomer = 2.6 mg/kg mandestrobin		0.97 mg/kg R-isomer 0.94 mg/kg S-isomer = 1.9 mg/kg mandestrobin	
Rotational crop sample	Plant back interval PBI	Harvest at Days after application DAT	Harvest at Days after sowing DAS	Harvest at Days after application DAT	Harvest at Days after sowing DAS
Lettuce	14	74	60	58	44
Carrot roots		144	130	106	92
Carrot foliage		144	130	106	92
Broccoli		144	130	103	89
Barley grain		151	137	106	92
Barley straw		151	137	106	92
Lettuce	120	194	74	173	53
Carrot roots		382	262	326	206
Carrot foliage		382	262	326	206
Broccoli		167	47	240	120
Barley grain		416	296	296	176
Barley straw		416	296	296	176
Lettuce	365	441	76	414	49
Carrot roots		508	143	454	89
Carrot foliage		508	143	454	89
Broccoli		441	76	452	87
Barley grain		480	115	456	91
Barley straw		480	115	456	91

Weather conditions did not generally alter the growth, development and maturity of the winter rape seed (preceding crop) and of rotational crops at the trial sites. Some exceptions were observed in France on the PBI 14 day barley that was sown too late for a normal development even under perfect weather conditions and PBI 102 day lettuce was less developed due to frost. In Spain PBI 120 day barley and carrots had suffered from cold during winter. Some samples were taken smaller than expected: 0.06 kg for PBI 14 day and 0.66 kg for PBI 265 day barley in France, 1.1–1.4 kg for PBI 102 day lettuce in France.

Samples were kept frozen (-18 °C) until extraction. Extraction for the analysis of mandestrobin and its metabolites occurred within a maximum of 332 days after the corresponding

harvest and extracts were analysed within a maximum storage of 10 days. This storage period is covered by the storage stability studies for all crop commodities.

Samples of broccoli, lettuce, carrot leaves, green rape fodder (whole plant, BBCH 65), carrot roots, barley grains and barley straw were analysed for R- and S-isomers of mandestrobin using chiral HPLC-MS/MS method DFG-S19 with an LOQ of 0.005 mg/kg for each isomer. The same samples, except green rape fodder, were analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates using HPLC-MS/MS method S10–02011 with an LOQ of 0.01 mg/kg for each analyte. Residues of the metabolites were expressed as the free aglycones.

Average concurrent fresh recoveries for each matrix ranged between: 74-99% for the R- and S-isomer of mandestrobin at 0.005–0.05 mg/kg; 70–106% for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin at 0.01–0.10 mg/kg. Control samples had residues below 0.3LOQ.

The level of the parent compound found in the preceding crop rape seed (whole plant) was 1.9-2.6 mg/kg on the day of application. Mandestrobin and its metabolites 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin including their (malonyl)glucoside conjugates, expressed as the free aglycones, were not found (< 0.01 mg/kg each) in any of the succeeding crop samples (lettuce, carrot root, carrot foliage, broccoli, barley grain and barley straw) planted at a 14, 120 or 365 PBI of mandestrobin to preceding crop winter rape seed.

Notes by the reviewer:

The application rate in the rotational field trials (1 × 0.21 kg ai/ha) did **not** match the maximum seasonal total rate for uses for which rotation is foreseen (1 × 0.42 kg ai/ha for rape seed). Furthermore, since the application was not conducted on bare soil, and the soil has not been analysed, the actual soil treatment levels remain unknown.

HPLC-MS/MS method DFG-S19 is valid for the determination of mandestrobin in the range 0.01–0.10 mg/kg in rape seed. The high levels up to 2.6 mg/kg found in the primary crop rape seed were not validated. However, since these levels are analysed for indication only, this is considered acceptable.

Samples were not analysed for De-Xy-mandestrobin, or for the metabolites found in photolysis studies or soil degradation studies. Photolysis products might be relevant, since mandestrobin was not applied to bare soil but to primary crop rape seed. Soil degradation products might be relevant, since rape seed plants were mixed with the soil.

Study 2: Field rotational crop studies were conducted in 2011–2012 in Fresno, CA, USA to provide information on the uptake of mandestrobin in rotational crops [Green, 2013, ROR-0240]. Each trial consisted of 2 plots planted with leaf lettuce. One plot served as untreated control and the other was treated with four foliar applications of an SC formulation of mandestrobin at a rate of 0.42 kg ai/ha (0.17 kg ai/A) each, with an interval of 7 days and a volume of 235 L/ha. The total seasonal rate was 1.7 kg ai/ha (0.69 kg ai/A). The leaf lettuce was planted on 12 Sept 2011 and treated on 18 and 25 October 2011 and 1 and 8 November 2011 at BBCH 15, 17, 19 and 49, respectively. Non-ionic surfactant (0.125% v/v) was included in the spray mixture. The application was performed using a bicycle boom or CO_2 powered boom. The characteristics of the soil are shown in Table 31. The leaf lettuce was grown to maturity, harvested, and destroyed one day after the last application. Succeeding crops (spinach, red beets and sorghum or wheat) were sown or transplanted into the plots at a PBI of 101, 253, and 356 days.

Samples of succeeding crops were harvested at their respective harvest time (Table 33). Commodities were collected by hand from at least 12 plants (red beet, spinach and mature sorghum commodities) or from 12 different areas of the plot (wheat commodities and sorghum forage). Wheat commodities were cut using scissors or knives. Wheat hay was left to dry in the field. Wheat grain was separated from the wheat straw by placing the cut plants between two tarps or into a bag, and

then shaking and lightly beating the sample. Sorghum forage samples were collected using pruning shears to cut the bottom of the plants. Sorghum grain was collected by cutting 25 grain heads. After drying at approximately 70°F for two weeks, the grain was removed from the heads and the samples sieved to remove fine chaff. The sorghum fodder was cut at the same time as the grain heads. The stalks and leaves were sectioned into approximately 18 inch pieces and allowed to dry at approximately 70°F for two weeks. Spinach leaves were collected using scissors using a zig-zag pattern across the plot for sampling. The beets were collected also using a zig-zag collection pattern. The beets were pulled by hand, the tops were cut off, and roots and the leaves were collected. Sample weights were 1.2–1.5 kg wheat forage, 0.65–0.79 kg wheat hay, 1.2–1.4 kg wheat grains, 0.57–0.74 kg wheat straw, 0.45-1.4 kg spinach leaves, 2.0–2.2 kg red beet roots, 1.2–2.2 kg red beet leaves, 1.6-1.8 kg sorghum forage, 1.1–1.4 kg sorghum grain, 1.1–1.5 kg sorghum fodder. Soil was not collected.

Table 32 Soil characteristics

	Fresno, CA, USA Trial V-38087-A soil 0–30 cm (untreated plot)
Soil type (USDA)	sandy loam
pH (water)	7.1
CEC (meq/100 g)	10.6
Water holding capacity %moisture at 1/3 bar	14.4
Organic matter (%)	0.53

Table 33 Summary of PBI, DAT and DAS in field rotational crops (spinach, red beets, wheat, sorghum) grown in aged soil (101, 253, 356 days PBI) with primary crop leaf lettuce treated with mandestrobin a field site in the USA

Trial conditions		Fresno, CA, USA Trial V-38087-A 4 × 0.42 kg ai/ha to leaf lettuce	
Rotational crop sample	Plant back interval PBI	Harvest at Days after application DAT	Harvest at Days after sowing DAS
Spinach	101	167	66
Red beet roots		195	94
Red beet leaves		195	94
Wheat forage		167	66
Wheat hay		196	95
Wheat straw		224	123
Wheat grain		224	123
Spinach	253	308	55
Red beet roots		316	63
Red beet leaves		316	63
Sorghum forage		343	90
Sorghum fodder		385	132
Sorghum grain		385	132
Spinach	356	407	51
Red beet roots		519	163
Red beet leaves		519	163
Wheat forage		489	133
Wheat hay		500	144
Wheat straw		580	204
Wheat grain		580	204

Weather conditions did not generally alter the growth, development and maturity of the rotational crops at the trial sites. Some exceptions were observed in spinach, where only 0.45 kg of spinach leaves could be collected from the PBI 235 plot due to weather damage.

Samples were kept frozen (-23 °C or lower) until extraction. Extraction for the analysis of mandestrobin and its metabolites occurred within a maximum of 505 days after the corresponding harvest and extracts were analysed within a maximum storage of 27 days.

Samples of wheat forage, wheat hay, wheat straw, wheat grains, sorghum forage, sorghum fodder, sorghum grain, red beets roots, red beets leaves, and spinach leaves were analysed for mandestrobin and De-Xy-mandestrobin using HPLC-MS/MS method RM-48C-2 with an LOQ of 0.02 mg/kg for each analyte. Only the 253 PBI samples (all matrices) and the 356 PBI samples (wheat only) were analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates using HPLC-MS/MS method RM-48A with an LOQ of 0.02 mg/kg for each analyte. Residues of the metabolites were expressed as the free aglycones.

Average concurrent fresh recoveries for each matrix ranged between: 70–120% with and RSD below 20% for mandestrobin, De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin at 0.02–0.20 mg/kg. An exception is sorghum forage, where the average recovery was 69% (range 67-74%, n = 3) for De-Xy-mandestrobin and the precision was 23% (recovery range 66–104%) for 4-OH-mandestrobin. Another exception is sorghum fodder, where the average recovery was 69% (range 64–78%) for 4-OH-mandestrobin. Control samples had residues below 0.5LOQ.

Mandestrobin and its metabolites De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin including their (malonyl)glucoside conjugates, expressed as the free aglycones, were not found (< 0.01 mg/kg each) in any of the succeeding crop samples (wheat forage, wheat hay, wheat straw, wheat grains, sorghum forage, sorghum fodder, sorghum grain, red beets roots, red beets leaves, and spinach leaves) planted at a 101, 253 and 359 PBI of mandestrobin to preceding crop leaf lettuce.

Notes by the reviewer:

- The application rate in the rotational field trials (4 × 0.42 kg ai/ha = 1.7 kg ai/ha) is higher than the maximum seasonal total rate for uses for which rotation is foreseen (1 × 0.42 kg ai/ha for rape seed). Since the short PBI of 30 days was not covered in this study, the USA and Canada use patterns indicate a plant back restriction of 4 months covering the PBI of 101 days.
- The application was not conducted on bare soil and the treated primary lettuce crop was removed from the plot. The leaf lettuce primary crop was treated at BBCH 15, 17, 19 and 49. Given the crop growth stage of the primary crop, the soil was not fully covered by the lettuce at the first three treatments and part of the application reached the soil.
- The storage period of the samples of 505 days is NOT covered by the storage stability studies as storage stability is only investigated for a maximum of 12 months. Storage stability studies with incurred plant residues suggest that this period can be extended to 2 years.
- Analytical methods used are fit for purpose.
- Samples were not analysed for photolysis products or for soil degradation products. As part of the application reached the soil, soil degradation products might be taken up by the plants. Photolysis products are not expected in this study, since the treated leaf lettuce was removed from the plots.

Overview of the metabolic pathway of mandestrobin in rotational crops

The proposed rotational crop metabolism (depicted in Figure 2) studies have been presented covering the crop categories cereals/grass crops (barley), roots and tubers (carrots) and leafy crops (broccoli, lettuce).

No R/S epimerization of mandestrobin as the R/S ratio of mandestrobin remained approximately 1:1.

The metabolism in primary and confined rotational crops was essentially the same. For the non-edible plant parts of wheat (forage, straw and hay), carrot (foliage) and the edible plant parts of lettuce and carrot (roots) the metabolic pathway of mandestrobin included monohydroxylation of the dimethylphenoxy ring to form 4-OH-mandestrobin followed by formation of the glycoside and (malonyl)glucoside conjugates. Also oxidation of the methyl groups attached to the 2- and 5-positions on the dimethylphenoxy ring to form 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and 5-COOH-mandestrobin was form followed by formation of the corresponding glycoside conjugates. Minor metabolic pathways included the O-demethylation of the methoxy-group of the side chain to form MCBX and cleavage of the ether linkage to form De-Xy-mandestrobin. Further metabolism occurred to form other minor metabolites and minor polar products.

Metabolism in livestock

The Meeting received information on the metabolic fate of mandestrobin in ruminants (lactating goats) and poultry (laying hens).

Lactating goats

The metabolism of mandestrobin was studied in lactating goats [Hardwick, 2012b, ROM-0039]. Two lactating goats were orally dosed with either [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin approximately the same time each day for 7 consecutive days with a gelatin capsule.

Administered doses were recalculated in amendment 2 of the original report. The average feed consumption/day during the dosing period was 1.3 and 2.4 kg dry diet/day for phenoxy and benzyl label, respectively). The actual mean daily dose administered was 16 mg ai/animal/day for the phenoxy or 35 mg ai/animal day for the benzyl label. Based on feed consumption during the dosing period, the actual average dose level of [Ph-^{14}C]-mandestrobin was 13 ppm in the diet, and the actual average dose level of [Bz-^{14}C]-mandestrobin was 14 ppm in the diet. The age of the goats was 2–4 year. Body weights were 51 and 59 kg at the start of dosing and 52 and 62 kg at sacrifice for the goats given the phenoxy and benzyl labelled test substance, respectively.

Urine, faeces, cage debris and cage washes were collected once daily, and milk was collected twice daily. The two milk samples collected each day (am and pm) were kept separate. At the end of the in-life phase, a methanol cage wash was collected. Approximately 6 hours after the final dose, the animals were terminated by anaesthetic overdose, and the following tissues collected for analysis: liver, kidneys, muscle (flank and loin muscle), fat (omental, renal and subcutaneous fat), blood and plasma. Milk samples were separated into fat and aqueous fractions by centrifugation (480g, 10 min) on the day of collection. Tissue, milk, excreta and cage wash samples were stored frozen prior to analysis.

Blood and homogenised tissue samples were solubilised prior to analysis by LSC. Faeces samples were homogenised and aliquots analysed by combustion. Aliquots of urine, milk fractions and cage wash were analysed directly for radioactivity by LSC.

A total of 79% of the total administered dose was recovered from both the phenyl and benzyl label. The radioactivity was recovered in essentially equal proportions in the urine (35%-40% TAR) and faeces (38-42% TAR). Radioactivity recovered in the cage washings accounted for 0.68-1.2% TAR. Recovery of radioactivity from milk was low with 0.002–0.005% and 0.024–0.073% TAR, in milk fat and aqueous fraction of milk, respectively. The radioactivity remaining associated with the excised tissues was 0.27–0.33% TAR with highest recovered radioactivity in liver (0.22–0.29% TAR).

Following administration of [Ph-^{14}C]-mandestrobin, concentrations were 0.32 mg eq/kg in liver, 0.17 mg eq/kg in kidneys, 0.012 mg eq/kg in flank muscle, 0.0079 mg eq/kg in loin muscle, 0.012 mg eq/kg in omental fat, 0.013 mg eq/kg in renal fat and 0.0097 mg eq/kg in subcutaneous fat.

Following administration of [Bz-^{14}C]-mandestrobin, concentrations were 0.61 mg eq/kg in liver, 0.41 mg eq/kg in kidneys, 0.016 mg eq/kg in flank muscle, 0.014 mg eq/kg in loin muscle, 0.028 mg eq/kg in omental fat, 0.034 mg eq/kg in renal fat and 0.033 mg eq/kg in subcutaneous fat.

The residue levels for [^{14}C-Ph]- and [^{14}C-Bz]-mandestrobin determined in milk, both fat and aqueous fraction, versus sampling times (AM and PM) are given in Table 34 below. Within one day of administration of the first dose, a plateau was reached in both aqueous and fat fractions in milk. Concentrations of the residues were highest in milk collected in the afternoon, indicating a rapid elimination. The ratio between the total radioactive residues in milk fat and milk aqueous had a mean of 2.5 (range 1.7-3.7) and 1.6 (0.86-2.3) for the phenoxy and benzyl label, respectively.

Table 34 Residue levels of for milk, both fat and aqueous fraction

Milk Sample Time Point	Milk Fat Fraction				Milk – Aqueous Fraction			
	[^{14}C-Ph]-mandestrobin mg eq/kg		[^{14}C-Bz]-mandestrobin mg eq/kg		[^{14}C-Ph]-mandestrobin mg eq/kg		[^{14}C-Bz]-mandestrobin mg eq/kg	
	am	pm	am	pm	am	pm	am	pm
0-24 hours	NA	0.019	NA	0.025	NA	0.007	NA	0.016
24-48 hours	0.008	0.028	0.006	0.028	0.004	0.009	0.007	0.015
48-72hours	0.008	0.026	0.007	0.031	0.004	0.009	0.006	0.018
72 – 96 hours	0.008	0.025	0.009	0.030	0.004	0.009	0.007	0.014
96 – 119 hours	0.011	0.025	0.010	0.035	0.004	0.009	0.007	0.015
119 – 143 hours	0.009	0.023	0.009	0.031	0.004	0.010	0.007	0.018
143 – 167 hours	0.010	0.033	0.008	0.035	0.006	0.009	0.006	0.016

Tissue samples containing TRRs > 0.01 mg eq/kg were subjected to solvent extraction, protease digestion, acid and base hydrolysis, and the extracts profiled by HPLC.

The fat (pool of omental, perirenal and subcutaneous fat), muscle and milk fat samples from day 7 were sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile. The hexane extract was partitioned against acetonitrile. The acetonitrile partition and the ethyl acetate, acetonitrile and acid acetonitrile extracts were combined, concentrated and analysed by HPLC as “pooled organic fraction”. The benzyl-labelled organic extracts of fat and milk fat were also analysed by LC/MS.

The benzyl-labelled aqueous milk fraction from day 6 (pm) was partitioned against hexane. The aqueous residue was freeze-dried and reconstituted in organic solvent/water, concentrated and centrifuged prior to analysis by HPLC. The phenoxy-labelled aqueous milk fraction was not extracted due to the low residue level in the sample (< 0.01 mg eq/kg).

Liver and kidney samples were sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, ethyl acetate, water, 1 M HCl and 1 M ammonia solution. The hexane extract was partitioned against acetonitrile. The acetonitrile partition and the ethyl acetate, acetonitrile and acid acetonitrile extracts were combined and concentrated for analysis by HPLC as “pooled organic fraction”. The water, 1 M HCl and 1 M ammonia solution extracts were combined, freeze-dried, reconstituted in organic solvent/water, reduced in volume under nitrogen and centrifuged prior to analysis by HPLC as “aqueous extracts”. The benzyl-labelled organic liver extract was also analysed by LC/MS. The benzyl-labelled organic kidney extract was also analysed by LC/MS.

The remaining solids from liver were digested with protease (pH 7.2, 18 hours at 37 °C), then partitioned with ethyl acetate. The aqueous fraction (freeze-dried and centrifuged) was analysed by HPLC. Remaining digestion residues were extracted with acetonitrile and sequentially hydrolysed under strong acid conditions (10 M HCl, reflux) followed by strong base conditions (10 M NaOH reflux). The acetonitrile extract and the acid hydrolysate were analysed by HPLC.

Extractability of the radioactive residues with neutral solvents ranged from 60% TRR in liver to 95% in kidney. Sequential treatment of remaining solids with protease, acid and base released an additional 23/26, 4.7/7.2, 0.5/1.2% TRR, respectively with the ^{14}C-Ph and ^{14}C-Bz label in liver.

Identified radio-components present in extracted residues (combined solvent extracts and radioactivity released from PES by harsh treatment) are summarized Table 35.

Table 35 Distribution of [Ph-^{14}C]-mandestrobin and [Bz-^{14}C]-mandestrobin residues in goat commodities (residues expressed as mg/kg mandestrobin equivalents and %TRR)

Sample	Fraction	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Liver	Pooled organic fraction [c]	0.19	59	0.29	47
	Hexane partition	< 0.001	0.2	< 0.001	0.2
	Aqueous fraction [d]	0.036	11	0.075	12
	Total extracted	**0.22**	**70**	**0.36**	**60**
	PES	0.095	30	0.25	40
	Protease digest-Aqueous phase	–-0.062	--20	–-0.13	--22
	Protease digest-Organic phase	–-0.002	–-0.5	–-0.003	–-0.5
	Organic extract of protease debris	–-0.009	--2.7	–-0.025	--4.1
	Acid hydrolysis (10M HCl)	–-0.015	--4.7	–-0.044	--7.2
	Base hydrolysis (10M NaOH)	–-0.002	–-0.5	–-0.008	--1.2
	Remaining solids	–-0.005	--2.0	–-0.036	--5.8
	Total	0.32	100	0.61	100
Kidney	Pooled organic fraction [c]	0.14	82	0.34	82
	Hexane partition	< 0.001	< 0.1	< 0.001	< 0.1
	Aqueous fraction [d]	0.021	12	0.055	13
	Total extracted	**0.16**	**94**	**0.39**	**95**
	PES	0.096	5.7	0.020	4.8
	Total	0.17	100	0.41	100
Muscle [a]	Pooled organic fraction [c]	0.007	72	0.013	87
	Hexane partition	< 0.001	0.3	< 0.001	0.1
	Total extracted	**0.007**	**72**	**0.013**	**87**
	PES	0.0028	28	0.0019	13
	Total	0.010	100	0.015	100
Fat [b]	Pooled organic fraction [c]	0.010	84	0.028	90
	Hexane partition	< 0.001	3.4	< 0.001	2.1
	Total extracted	**0.01**	**87**	**0.028**	**92**
	PES	0.002	13	0.002	8.3
	Total	0.012	100	0.031	100
Milk -fat (day 7, pm)	Pooled organic fraction [c]	0.026	80	0.035	91 [e]
	Hexane partition	0.004	11	0.003	8.7 [e]
	Total extracted	**0.03**	**92**	**0.038** [e]	**100** [e]
	PES	0.003	8.3	ND	ND
	Total	0.033	100	0.038 [e]	100
Milk – aqueous (Day 6, pm)	Aqueous fraction	Sample not extracted		0.017	96
	Hexane partition			< 0.001	3.6
	PES			ND	ND
	Total extracted =Total	**0.010**	**100**	**0.018**	**100**

ND = not detected, -- = not included in the calculation of TRR total

[a] Pool of flank and loin muscle

[b] Pool of omental, perirenal and subcutaneous fat

[c] Pooled after sequential extraction with ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, ethyl acetate and also the acetonitrile fraction that was left after partitioning with hexane.

[d] Pooled after sequential extraction with water, 1 M HCl and 1 M ammonia [for liver and kidney)

[e] Was recalculated by the reviewer, based on the sum of residues being 100% TRR.

Analysis and identification of residues in the extracts was conducted by radio-HPLC using co-chromatography with the reference standards: De-Xy-mandestrobin, Dx-CA- mandestrobin, 5-CA-2-HM-MCBX, 5-CA-2-HM-mandestrobin-NHM, 5-CA-2-HM-mandestrobin, 5-CA-MCBX-NDM, 2-COOH-mandestrobin, 2-CH_2OH-mandestrobin, 4-OH- mandestrobin, 5-CH_2OH-mandestrobin, 5-CA-mandestrobin-NHM, 5-CA-mandestrobin-NDM, 5-COOH-mandestrobin, MCBX, R/S-mandestrobin. Selected extracts were also analysed by LC-MS in positive ion mode for metabolite identification.

The identification and characterisation of the of radioactive residues in the tissues liver, kidney, muscle and fat is given in Table 36 for both radiolabels, [Ph-^{14}C]- and [Bz-^{14}C]- mandestrobin respectively. Analysis with LC/MS confirmed the presence of the parent in milk fat, liver and fat, 2-CH_2OH-mandestrobin in liver, 5-COOH-mandestrobin in liver and kidney and 4-OH-mandestrobin-glucuronide in kidney.

Total radioactive residue levels in milk fat and the aqueous fraction of milk were low (0.01–0.038 mg/kg) and the major residues identified in milk fat was parent accounting for 0.011–0.012 mg eq/kg (32–33% TRR). In the aqueous fraction of milk no metabolite residue stands out, each accounting for < 0.01 mg eq/kg (<4.5% TRR), except the free form of metabolite 5-CA-mandestrobin-NHM identified with 15% TRR (0.003 mg eq/kg).

Total radioactive residue levels in muscle and fat were low (0.010–0.034 mg eq/kg) and the major residues identified in muscle and fat were parent (18–50% TRR) and 2-CH_2OH-mandestrobin (2.6–10% TRR). Total radioactive residues in liver and kidney (Table 37) were much higher 0.17–0.61 mg eq/kg and parent compound was metabolized to 1.6–7.7% TRR. Besides parent, major residues in liver and kidney were: 5-COOH-mandestrobin (11–25% TRR), 2-CH_2OH-mandestrobin (3.5–7.8% TRR), De-Xy-mandestrobin (4.5–8.2% TRR) and free and conjugated forms of 4-OH-mandestrobin (0.8–17% TRR).

Storage stability: All samples were analysed within 6 months after collection, and therefore storage stability investigations were not performed.

Note by the reviewer:

A total of 60–70% TRR (liver), 94-95% (kidney), 72–87% TRR (muscle), 87-92% TRR (fat) could be extracted from the indicated commodities with organic solvents. Considering the extraction efficiency with organic solvents, the identification level in milk and tissues is low as only 32–53% TRR (milk), 31–36% TRR (muscle), 37-65% TRR (fat tissue), 41–48% TRR (liver), 53-59% TRR (kidney) could be identified in the respective commodities (i.e. less than 80% of the solvent extracted residue).

R/S isomerisation of parent compound was not investigated.

Table 36 Characterization and identification of residues of metabolites in the milk fat and the milk aqueous fraction; and in goat muscle and fat from [Ph-^{14}C]- and [Bz-^{14}C]- mandestrobin-dosed goats.

Compounds	milk fat %TRR (mg eq/kg)		skim milk %TRR (mg eq/kg)		Muscle %TRR (mg eq/kg)		Fat %TRR (mg eq/kg)	
	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]
Parent (free)	33 (0.011)	32 (0.012)		4.5 (0.0008)	23 (0.0023)	18 (0.0027)	50 (0.0057)	23 (0.0072)
De-Xy-mandestrobin (free)	NA	4.5 (0.0017)		2.8 (0.0005)	NA	4.7 (0.0007)	NA	1.9 (0.0006)
4-OH-mandestrobin (free)	6.1 (0.0020)	ND		2.3 (0.0004)	ND	ND	ND	ND
2-CH_2OH-mandestrobin (free)	5.8 (0.0019)	2.9 (0.0011)		2.8 (0.0005)	6.0 (0.0006)	10 (0.0015)	2.6 (0.0003)	ND
5-CH_2OH-mandestrobin (free)	ND	2.4 (0.0009)		ND	ND	ND	ND	ND
5-CA-2-HM-mandestrobin-NHM (free)	ND	ND		ND	ND	ND	ND	ND
5-CA-2-HM-mandestrobin (free)	ND	ND		ND	ND	ND	ND	ND
5-CA-mandestrobin-NHM (free)	ND	ND		15 (0.0026)	ND	ND	ND	ND
5-CA-mandestrobin-NDM (free)	4.0 (0.0013)	2.4 (0.0009)		ND	ND	ND	2.6 (0.0003)	1.9 (0.0006)
2-COOH-mandestrobin (free)	ND	ND		ND	2.0 (0.0002)	3.4 (0.0005)	7.0 (0.0008)	2.2 (0.0007)

Compounds	milk fat %TRR (mg eq/kg)		skim milk %TRR (mg eq/kg)		Muscle %TRR (mg eq/kg)		Fat %TRR (mg eq/kg)	
	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]
5-COOH-mandestrobin (free)	ND	ND		ND	ND	ND	3.5 (0.0004)	3.8 (0.0012)
MCBX (free)	ND	ND		2.8 (0.0005)	ND	ND	ND	ND
5-CA-MCBX-NDM (free)	4.3 (0.0014)	2.6 (0.0010)		2.3 (0.0004)	ND	ND	ND	4.8 (0.0015)
5-CA-MCBX (free)	ND	ND		ND	ND	ND	ND	ND
Total identified	**53 (0.017)**	**47 (0.018)**		**32 (0.0057)**	**31 (0.0031)**	**36 (0.0054)**	**65 (0.0075)**	**37 (0.012)**
%identified/ neutral extracts	**53/92 =58**	**47/100 =47**		**32/100 =32**	**31/72 =43**	**36/87 =41**	**65/87 =75**	**37/92 =40**
Unknowns								
-Hexane partition	11 (0.0037)	8.7 (0.0033)		3.4 (0.0006)	0.3 (<LOQ)	0.1 (<LOQ)	3.5 (<LOQ)	2.2 (<LOQ)
-Organic extracts [a]	28 (0.0090)	44 (0.017)		ND	41 (0.0041)	51 (0.0075)	18 (0.0021)	52 (0.016)
-Aqueous extracts	ND	ND		64 (0.011) [b]	ND	ND	ND	ND
PES	**8.3 (0.0027)**	**ND**		**ND**	**28 (0.0028)**	**13 (0.0019)**	**13 (0.0015)**	**7.9 (0.0025)**
Total unknowns (extracted)	**39 (0.013)**	**53 (0.020)**	**100 (0.010)**	**68 (0.012)**	**41 (0.004)**	**51 (0.0075)**	**22 (0.0025)**	**55 (0.017)**
Total TRR	**100 (0.033)**	**100 (0.038)**	**100 (0.010)**	**100 (0.018)**	**100 (0.010)**	**100 (0.015)**	**100 (0.012)**	**100 (0.032)**

NA = not applicable, ND = not detected; LOQ = 0.0001 mg eq/kg

[a] Organic extracts contained 3-6 unknown peaks or regions of interest, none exceeded 0.003 mg eq/kg, except for one region. The fat (Bz label) contains one unknown region of 10.7 ppb = 38% TRR. Exhaustive efforts were made by LC/MS to identify the region accounting for 10.7 ppb but no usable identification data could be produced.

[b] Aqueous extract of milk contained 8 unknown peaks or regions of interest, none exceeded 0.002 mg eq/kg

Table 37 Characterization and identification of residues of metabolites in the goat tissues liver and kidney from [Ph-^{14}C]- and [Bz-^{14}C]- mandestrobin-dosed goats

Compounds	Liver %TRR (mg eq/kg)	Liver %TRR (mg eq/kg)	Kidney %TRR (mg eq/kg)	Kidney %TRR (mg eq/kg)
	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]
Parent	--3.1 (0.010)	--7.7 (0.047)	--2.1 (0.0036)	--1.6 (0.0065)
- free	0.94 (0.0030)	4.3 (0.026)	2.1 (0.0036)	1.6 (0.0065)
- released by 1 M HCl and 1 M NH_3	0.69 (0.0022)	0.41 (0.0025)	ND	ND
- released by protease (and acetonitrile)	1.2 (0.0037)	2.6 (0.016)	ND	ND
- released by 10 M HCl	0.34 (0.0011)	0.38 (0.0023)	ND	ND
De-Xy-mandestrobin	NA	--8.1 (0.050)	NA	--4.5 (0.018)
- free		2.6 (0.016)		4.5 (0.018)
- released by 1 M HCl and 1 M NH_3		1.1 (0.0070)		ND
- released by protease		3.9 (0.024)		ND
- released by 10 M HCl		0.46 (0.0028)		ND
4-OH-mandestrobin	-–0.82 (0.0026)	-–0.82 (0.0050)	--17 (0.028)	--13 (0.055)
- free	0.82 (0.0026)	0.82 (0.0050)	1.7 (0.0029)	ND
- glucuronide	ND	ND	15 (0.025) [e]	13 (0.055) [e]
2-CH_2OH-mandestrobin	--7.8 (0.025)	--6.3 (0.038)	--3.5 (0.0059)	--3.6 (0.015)
- free	6.3 (0.020)	3.6 (0.022)	3.5 (0.0059)	3.6 (0.015)
- released by 1 M HCl and 1 M NH_3	1.2 (0.0037)	ND	ND	ND

Compounds	Liver %TRR (mg eq/kg) [^{14}C-Ph]	Liver %TRR (mg eq/kg) [^{14}C-Bz]	Kidney %TRR (mg eq/kg) [^{14}C-Ph]	Kidney %TRR (mg eq/kg) [^{14}C-Bz]
- released by protease - released by 10 M HCl	0.34 (0.0011) ND	2.4 (0.015) 0.31 (0.0019)	ND ND	ND ND
5-CH_2OH-mandestrobin (free)	2.0 (0.0064)	1.5 (0.0091)	0.59 (0.0010)	ND
5-CA-2-HM-mandestrobin-NHM (free)	0.88 (0.0028)	0.51 (0.0031)	3.6 (0.0061)	5.2 (0.021)
5-CA-2-HM- mandestrobin - free - released by 1 M HCl and 1 M NH_3 - released by protease	--3.4 (0.011) 2.3 (0.0074) ND 1.1 (0.0036)	--1.3 (0.0081) 0.83 (0.0051) 0.49 (0.0030) ND	ND	-–0.92 (0.0038) 0.92 (0.0038) ND ND
5-CA-mandestrobin- NHM - free - released by 10 M HCl	-–0.094 (0.0003) ND 0.094 (0.0003)	ND	ND	ND
5-CA-mandestrobin-NDM - free - released by 10 M HCl	--1.5 (0.0049) 1.4 (0.0044) 0.16 (0.0005)	--1.3 (0.0078) 1.3 (0.0078) ND	ND	ND
2-COOH-mandestrobin - free - released by 1 M HCl and 1 M NH3 - released by protease - released by 10 M HCl	--4.2 (0.014) 1.6 (0.0052) ND 1.9 (0.0062) 0.66 (0.0021)	--1.7 (0.011) ND 1.2 (0.0074) 0.26 (0.0016) 0.26 (0.0016)	--2.2 (0.0037) 2.2 (0.0037) ND ND ND	--2.8 (0.012) 2.8 (0.012) ND ND ND
5-COOH-mandestrobin - free - released by protease	--20 (0.064) 19 (0.062) 0.75 (0.0024)	--11 (0.065) 11 (0.065) ND	--25 (0.042) 25 (0.042) ND	--20 (0.083) 20 (0.083) ND
MCBX (free)	0.50 (0.0016)	0.67 (0.0041)	0.94 (0.0016)	0.58 (0.0024)
5-CA-MCBX-NDM (free)	2.8 (0.0088)	ND	4.3 (0.007)	ND
5-CA-2-HM-MCBX - released by 10 M HCl - released by 1 M HCl and 1 M NH_3	-–0.94 (0.0030 0.66 (0.0021) 0.28 (0.0009)	ND	ND	ND
Total identified	**48 (0.15)**	**41 (0.25)**	**59 (0.10)**	**53 (0.22)**
% identified/neutral extracts	**48/70 =69**	**41/60 = 68**	**59/94 =63**	**53/95 =56**
Unknowns -Hexane partition -Organic extracts -Aqueous extracts -Protease aqueous -Protease organic -Acid hydrolysate -Basic hydrolysate	 0.25 (0.0008) 20 (0.063) [a] 8.8 (0.028) [d] 15 (0.048) [c] 2.4 (0.0078) [d] 3.2 (0.010) [d] 0.53 (0.0017)	 0.15 (0.0009) 20 (0.13) [a] 9.0 (0.055) [d] 13 (0.082) 3.6 (0.022) [d] 5.8 (0.035) [d] 1.2 (0.0075) ND ND	 0.06 (<LOQ) 26 (0.044) [b] 9.8 (0.017) [d] ND ND ND ND	 0.05 (<LOQ) 34 (0.14) [b] 8.1 (0.033) [d] ND ND ND ND
PES	**1.9 (0.0060)**	**5.9 (0.036)**	**5.6 (0.0096)**	**4.8 (0.020)**
Total unknowns (extracted and hydrolysed)	**50 (0.16)**	**54 (0.33)**	**36 (0.061)**	**42 (0.17)**
Total TRR	**100 (0.32)**	**100 (0.61)**	**100 (0.17)**	**100 (0.41)**

LOQ = 0.0001 mg eq/kg; ND = not detected, NA = not applicable, -- = not included in the calculation of Total TRR

"-"=not treated with protease or 10 M HCl.

[a] Organic extracts in liver contained 13-14 known peaks or regions of interest, none exceeded 0.011 mg eq/kg ([^{14}C-Ph]) or 0.018 mg eq/kg ([^{14}C-Bz])

[b] Organic extracts in kidney contained 9-10 known peaks or regions of interest; none exceeded 0.010 mg eq/kg ([^{14}C-Ph]) or 0.027 mg eq/kg ([^{14}C-Bz]

[c] Aqueous protease digest in liver contained 6-9 known peaks or regions of interest; none exceeded 0.009 mg eq/kg ([^{14}C-Ph]) or 0.018 mg eq/kg ([^{14}C-Bz])

[d] Extract, digests or hydrolysates contained multiple components, each not exceeding 0.001–0.007 mg eq/kg.

[e] In comparison with the data (same retention time and same HPLC method) from the kidney from the animals dosed with [Bz-^{14}C]-mandestrobin was tentatively identified as glucuronide in [^{14}C-Ph]-label, 4-OH-mandestrobin-glucuronide was tentatively identified by LC/MS, based on comparison with results from a rat metabolism study using the same HPLC methodology ([^{14}C-Bz]-label)

Laying hens

The metabolism of mandestrobin was studied in laying hens [Hardwick, 2012a, ROM-0040]. Two groups of ten laying hens (Lohman Brown, weighing 1.5–2.1 kg) were dosed orally with either [Ph-^{14}C]- or [Bz-^{14}C]-mandestrobin. The hens were dosed once daily during 14 days via capsules containing 1.8 mg [^{14}C-Ph]- or 1.8 mg [Bz-^{14}C]-mandestrobin as actual dose, respectively,. The total and mean dose administered was 25 mg/animal and 13 ppm in the diet, respectively based on an actual feed consumption of 143–146 g dry feed/animal/day. Excreta and eggs were collected twice daily (before and 5–8 hours after dosing) during the test period until 6 hours after the last dose on day 14. Six to seven hours after the last treatment, the hens were sacrificed and tissues (peritoneal fat, breast and thigh muscle, skin including subcutaneous fat, and liver) were collected. All samples were stored at <-10 °C.

Excreta samples were mixed with water, and tissue samples homogenized and then solubilized prior to analysis by LSC. Aliquots of egg and cage wash were analysed directly for radioactivity by LSC.

Following 14 consecutive daily doses of [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin, from hens an averaged total of 85% (81–89 %) and an averaged total of 100% (95-103%) of the administered dose was recovered for the radiolabels , respectively. The majority of the administered dose was excreted, with 83% (78–88%) of the total dose of [Ph-^{14}C]-mandestrobin and 98 % (94-101%) of the total dose of [Bz-^{14}C]-mandestrobin being recovered in the excreta. In [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin dosed hens, 1.3% (0.43–3.3%) and 0.99% (0.46-1.8%) was recovered cage wash, 0.21% (0.14–0.26%) and 0.18% (0.097–0.25%) was recovered in eggs, 0.070% (0.044–0.11%) and 0.090% (0.062–0.15%) was recovered tissues, respectively.

The total radioactive residues in eggs and tissues are presented in Table 38. The highest residue concentration was found in the liver, amounting to 0.29–0.30 mg eq/kg for [Ph-^{14}C]- and [Bz-^{14}C]-radiolabel, respectively. Lower residue concentrations were found in muscle (0.013–0.025 mg eq/kg), fat (0.032 mg eq/kg) and skin (0.048–0.054 mg eq/kg). Radioactive residues in egg increased from 0.050 mg eq/kg on Day 2 to 0.11 mg eq/kg on Day 11 for [Ph-^{14}C]-mandestrobin and from 0.050 mg eq/kg on Day 2 to 0.081 mg eq/kg on Day 7 for [Bz-^{14}C]-mandestrobin. For both radiolabels residues in eggs reached a steady state within 7 days.

Tissue and egg samples were subjected to various extractions, and the extracts analysed by LSC. The distribution of the radioactivity in the various extracts of eggs and tissues is presented in Table 38.

Egg samples from day 11 (phenoxy label) or day 12 (benzyl label), fat and muscle samples were pooled according to radiolabel, and sequentially extracted with hexane, ethyl acetate, acetonitrile and 1 % formic acid in acetonitrile. The hexane extract was partitioned against acetonitrile. The acetonitrile partition was combined with the ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts. The pooled organic fractions were concentrated and analysed by HPLC-UV. The phenoxy-labelled egg extract was also analysed by LC/MS.

Liver and skin samples were pooled by radiolabel and sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, water, 1 M HCl and 1 M ammonia solution. The hexane extract was partitioned against acetonitrile. The acetonitrile partition and the ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were combined and concentrated

for analysis by HPLC-UV. For liver (both labels) and skin (phenoxy label only), the water, 1 M HCl and 1 M ammonia solution extracts were combined, freeze dried, reconstituted in organic solvent/water, reduced in volume under nitrogen and centrifuged. The top phase of the resulting biphasic extract was discarded, and the bottom phase analysed by HPLC-UV.

The remaining solids from liver were subjected to protease digestion (18 hours, 37 °C) and then partitioned with ethyl acetate. The aqueous fraction was reduced in volume by freeze-drying and centrifuged prior to analysis by HPLC-UV. The solids remaining after protease digestion was extracted with acetonitrile and then subjected to acid hydrolysis with 10 M HCl under reflux. The acid hydrolysate was analysed by HPLC-UV. The solids remaining after acid hydrolysis was subjected to base hydrolysis with 10 M NaOH under reflux, but not profiled by HPLC.

The extracted residue in fat, eggs and skin accounted for 86–96% TRR, in liver and muscle for 63–66 %TRR and 52–59% TRR.

Table 38 Total Radioactive residues and extractability in samples from laying hens treated with [^{14}C-Ph]- and [^{14}C-Bz]- mandestrobin

Sample	Fraction	[Ph-^{14}C]-mandestrobin		[Bz-^{14}C]-mandestrobin	
		mg eq/kg	%TRR	mg eq/kg	%TRR
Liver	Pooled organic fraction [c]	0.15	50	0.15	50
	Hexane partition	0.002	0.6	0.003	0.9
	Aqueous extracts [d]	0.036	12	0.045	15
	Total extracted	**0.18**	**63**	**0.20**	**66**
	PES	0.11	37	0.10	34
	Protease digest – Aqueous phase	-–0.047	--16	-–0.052	--17
	Organic extract of protease debris	-–0.010	--3.4	-–0.013	--4.2
	Acid hydrolysis (10M HCl)	-–0.013	--4.4	-–0.020	--6.7
	Base hydrolysis (10M NaOH)	-–0.004	--1.5	-–0.002	-–0.7
	unextracted	-–0.036	--12	-–0.017	--5.6
	Total TRR	0.29	100	0.30	100
Egg	Pooled organic fraction [c]	0.10	92	0.067	89
	Hexane partition	< 0.001	0.1	< 0.001	< 0.1
	Total extracted	**0.10**	**92**	**0.067**	**89**
	PES	0.009	8.4	0.008	11
	Total TRR	0.11	100	0.075	100
Muscle [a]	Pooled organic fraction [c]	0.007	51	0.014	58
	Hexane partition	< 0.001	0.9	< 0.001	0.5
	Total extracted	**0.007**	**52**	**0.014**	**59**
	PES	0.006	48	0.010	41
	Total TRR	0.014	100	0.024	100
Fat [b]	Pooled organic fraction [c]	0.030	91	0.029	89
	Hexane partition	0.002	5.9	< 0.001	2.8
	Total extracted	**0.032**	**96**	**0.029**	**92**
	PES	0.001	3.4	0.003	8.4
	Total TRR	0.033	100	0.032	100
Skin	Pooled organic fraction [c]	0.033	69	0.040	73
	Hexane partition	< 0.001	1.4	< 0.001	1.0
	Aqueous extracts [d]	0.010	21	0.007	12
	Total extracted	**0.043**	**92**	**0.047**	**86**
	PES	0.004	8.0	0.008	14
	Total TRR	0.048	100	0.054	100

-- = not included in the calculation of Total TRR

[a] Pool of flank and loin muscle

[b] Pool of omental, perirenal and subcutaneous fat

[c] Pooled after sequential extraction with ethyl acetate, acetonitrile and 1 % formic acid in acetonitrile, ethyl acetate and also the acetonitrile fraction that was left after partitioning with hexane.

[d] Pooled after sequential extraction with water, 1 M HCl and 1 M ammonia

Tissue and egg extracts containing TRRs > 0.01 mg eq/kg were profiled by HPLC-UV. Retention times of radiolabelled metabolites were compared with those of reference standards: mandestrobin, De-Xy-mandestrobin, Dx-CA-mandestrobin, 5-CA-2-HM-MCBX, 5-CA-HM-mandestrobin-NHM, 5-CA-2-HM-mandestrobin, 5-CA-MCBX-NDM, 2-COOH-mandestrobin, 2-CH_2OH-mandestrobin, 4-OH-mandestrobin, 5-CH_2OH-mandestrobin, 5-CA-mandestrobin-NHM, 5-CA-mandestrobin-NDM, 5-COOH-mandestrobin, MCBX. Portions of selected extracts were examined by LC/MS to confirm the identity of relevant metabolites.

The identification and characterisation of radioactive residue in liver (Table 39) and in the tissue kidney, muscle, fat and in egg (day 11 and 12) in Table 40 is given for [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin, respectively.

For both radiolabels, the main component of the radioactive residue in eggs was the parent mandestrobin, accounting for 0.025–0.058 mg eq/kg (33-51% TRR). The metabolites 2-COOH-mandestrobin, 4-OH-mandestrobin and MCBX were found in egg samples from hens dosed with [Ph-^{14}C]-mandestrobin, and De-Xy-mandestrobin, 4-OH-mandestrobin and 5-CA-mandestrobin –NHM were found in egg samples from hens dosed with [Bz-^{14}C]-mandestrobin. All metabolites identified in egg were present at levels < 0.01 mg/kg.

In liver samples for both radio labels the main component of the extracted residue was free De-Xy-mandestrobin (0.36 mg eq/kg, 12% TRR), 4-OH-mandestrobin (0.008–0.045 mg eq/kg (2.7-15% TRR). Mandestrobin, 2-COOH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CA-mandestrobin-NDM, 5-CA-2-HM-MCBX and 5-CA-MCBX-NDM were present as minor residues (< 0.01 mg eq/kg; < 2.9% TRR). Total radioactive residue levels in muscle were low, for parent and the metabolites 2-CH_2OH-mandestrobin and 4-OH-mandestrobin were found in muscle at very low levels (< 0.001 mg eq/kg, < 3.7% TRR). Skin contained a number of very minor metabolites, the most significant of which were 2-COOH-mandestrobin, present at 0.0044 mg eq/kg (9.2% TRR), and 4-OH-mandestrobin, present at 0.0029 mg eq/kg (6.1% TRR at max).

For both radiolabels, the main component of the radioactive residue in fat was parent mandestrobin, accounting for 0.011–0.016 mg eq/kg (34-50% TRR). All metabolites identified in fat were present at very low levels (< 0.001–0.003 mg eq/kg, < 10% TRR), comprising 4-OH-mandestrobin, 5-CA-mandestrobin-NHM and MCBX, De-Xy-mandestrobin.

Storage stability: All samples were analysed within 4–5months after collection, and therefore storage stability investigations were not performed.

Note by the reviewer:

A total of 63–66% TRR (liver), 52–59% TRR (muscle), 86–96% (eggs, fat, skin) could be extracted from the indicated commodities with organic solvents. Considering the extraction efficiency with organic solvents, the identification level in eggs and tissues is low as only 19–23% TRR (liver), 5.0–5.9% TRR (muscle), 42–59% TRR (eggs, fat) and 10–33% TRR (skin) could be identified in the respective commodities (i.e. less than 80% of the solvent extracted residue).

R/S isomerisation of parent compound was not investigated.

Table 39 Amount and nature of residues in the tissue liver of laying hens given an actual dose of 1.8 mg [^{14}C-Ph]- or [^{14}C-Bz]-mandestrobin as %TRR and in (mg eq/kg)

Compounds	Liver %TRR (mg eq/kg)	
	[^{14}C-Ph]	[^{14}C-Bz]
Parent (total)	--3.0 (0.0087)	--2.1 (0.0064)
- free	0.61 (0.0018)	ND
- released by 1 M HCl and 1 M NH_3	0.81 (0.0024)	0.74 (0.0022)
- released by protease	1.5 (0.0045)	0.9 (0.0027)
- released by 10 M HCl	ND	0.5 (0.0015)
De-Xy-mandestrobin	NA	--12 (0.036)

Compounds	Liver %TRR (mg eq/kg)	
	[^{14}C-Ph]	[^{14}C-Bz]
- free		8.6 (0.026)
- released by 1 M HCl and 1 M NH_3		1.2 (0.0035)
- released by protease		1.9 (0.0057)
- released by 10 M HCl		0.47 (0.0014)
4-OH-mandestrobin	--15 (0.045)	--2.7 (0.0080)
- free	13 (0.039)	ND
- glucuronide	0.37 (0.0011)	0.8 (0.0024)
- released by protease	1.0 (0.0031)	1.3 (0.0039)
- released by 10 M HCl	0.58 (0.0017)	0.6 (0.0017)
2-CH_2OH-mandestrobin	-–0.95 (0.0028)	ND
- free	ND	
- released by 1 M HCl and 1 M NH_3	0.95 (0.0028)	
5-CH_2OH-mandestrobin (free)	ND	ND
5-CA-2-HM-mandestrobin NHM (free)	ND	ND
5-CA-2-HM-mandestrobin (free)	--1.1 (0.0032)	ND
- free	ND	
- released by 1M HCl and 1 M NHe	1.1 (0.0032)	
- released by protease	ND	
5-CA-mandestrobin-NHM (free)	ND	ND
5-CA-mandestrobin-NDM (free)	ND	0.4 (0.0012)
2-COOH-mandestrobin (free)	2.4 (0.007)	ND
Total identified	**23 (0.067)**	**19 (0.057)**
%identified/neutral extracts	**23/63 =37**	**19/66 =29**
Unknowns		
- Hexane partition	0.60 (0.0017)	0.90 (0.0026)
-Organic extracts	34 (0.099) [a]	40 (0.12) [a]
- Aqueous extracts	10 (0.030) [b]	12 (0.036) [b]
- Protease aqueous	12 (0.036) [c]	13 (0.039) [c]
- Protease organic	3.4 (0.010)	4.2 (0.013)
- Acid hydrolysate	3.9 (0.011) [d]	5.1 (0.015)
- Basic hydrolysate	1.5 (0.0043)	0.7 (0.0021)
PES	**12 (0.036)**	**5.6 (0.017)**
total unknowns (extracted and hydrolysed)	**65 (0.19)**	**75 (0.23)**
Total TRR	**100 (0.29)**	**100 (0.30)**

Note: ND=not detected, NA= not applicable, -- = not included in the calculation of Total TRR

[a] Organic extracts contained 12–18 unknown peaks or regions of interest , each not exceeding 0.018 mg eq/kg ([^{14}C-Ph]-label) or 0.026 mg eq/kg ([^{14}C-Bz]-label).

[b] Aqueous extracts contained 6-7 unknown peaks or regions of interest, each not exceeding 0.004 mg eq/kg.

[c] Aqueous residues of the protease digest contained 6- 10 unknown peaks or regions of interest, each not exceeding 0.006 mg eq/kg.

[d] Acid hydrolysate residues contained 7-15 unknown peaks or regions of interest, each not exceeding 0.002 mg eq/kg ([^{14}C-Ph]-label) or 0.003 mg eq/kg ([^{14}C-Bz]-label).

Table 40 Amount and nature of residues (%TRR and in (mg eq/kg)) in muscle, fat, skin and eggs of laying hens administered with an actual dose of 1.8 mg [^{14}C-Ph]- or [^{14}C-Bz]-mandestrobin

Compounds	**Muscle %TRR (mg eq/kg)**		**Fat %TRR (mg eq/kg)**		**Skin %TRR (mg eq/kg)**		**Egg %TRR (mg eq/kg)**	
	[^{14}C-Ph]	**[^{14}C-Bz]**	**[^{14}C-Ph]**	**[^{14}C-Bz]**	**[^{14}C-Ph]**	**[^{14}C-Bz]**	**[^{14}C-Ph] g**	**[^{14}C-Bz] g**
Parent	2.2 (0.0003)	1.3 (0.0003)	50 (0.016)	34 (0.011)	3.1 (0.0015)	1.5 (0.0008)	51 (0.058)	33 (0.025)
-free	2.2	1.3	50	34	1.0	1.5	51	33

Compounds	Muscle %TRR (mg eq/kg)		Fat %TRR (mg eq/kg)		Skin %TRR (mg eq/kg)		Egg %TRR (mg eq/kg)	
	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph]	[^{14}C-Bz]	[^{14}C-Ph] g	[^{14}C-Bz] g
-released by 1 M HCl and 1 M NH3	(0.0003) ND	(0.0003) ND	(0.016) ND	(0.011) ND	(0.0005) 2.1 (0.0010)	(0.0008) ND	(0.058) ND	(0.025) ND
De-Xy-mandestrobin (free)	NA	ND	NA	9.6 (0.0031)	NA	2.6 (0.0014)	NA	0.40 (0.0003)
4-OH-mandestrobin (free)	ND	2.5 (0.0006)	4.3 (0.0014)	6.5 (0.0021)	6.1 (0.0029)	4.8 (0.0026)	1.5 (0.0017)	4.4 (0.0033)
2-CH_2OH-mandestrobin - free - released by 1 M HCl and 1 M NH_3	--3.7 (0.0005) 3.7 (0.0005) ND	--1.3 (0.0003) 1.3 (0.0003) ND	ND	ND	--2.7 (0.0013) ND 2.7 (0.0013)	ND	ND	ND
5-CH_2OH-mandestrobin	ND	ND	ND	ND	ND	ND	ND	ND
5-CA-2-HM-mandestrobin-NHM (free)	ND	ND	ND	ND	2.9 (0.0014)	ND	ND	ND
5-CA-2-HM-mandestrobin (free)	ND	ND	ND	ND	4.6 (0.0022)	0.013 (0.0007)	ND	ND
5-CA-mandestrobin-NHM (free)	ND	ND	2.5 (0.0008)	ND	2.1 (0.0010)	ND	ND	3.7 (0.0028)
5-CA-mandestrobin-NDM (free)	ND	ND	ND	ND	ND	ND	ND	ND
2-COOH-mandestrobin (free)	ND	ND	ND	ND	9.2 (0.0044)	ND	0.71 (0.0008)	NA
5-COOH-mandestrobin (free)	ND	ND	ND	ND	0.6 (0.0003)	ND	ND	ND
MCBX (free)	ND	ND	2.8 (0.0009)	1.6 (0.0005)	1.9 (0.0009)	ND	1.3 (0.0015)	ND-
5-CA-2-MCBX-NDM (free)	ND	ND	ND	ND	ND	ND	ND	ND
5-CA-2-HM-MCBX (total)	ND	ND	ND	ND	ND	ND	ND	ND
Total identified	**5.9 (0.0008)**	**5.0 (0.0012)**	**59 (0.019)**	**52 (0.017)**	**33 (0.016)**	**10 (0.0055)**	**55 (0.062)**	**42 (0.031)**
%identified/ neutral extracts	**5.9/52 =11**	**5.0/59 =8.4**	**59/96 =61**	**52/92 =57**	**33/92 =36**	**10/86 =12**	**55/92 =60**	**42/89 =47**
Unknowns								
-Hexane partition	0.9 (<LOQ)	0.5 (<LOQ)	5.9 (<LOQ)	2.8 (<LOQ)	1.4 (<LOQ)	1.0 (<LOQ)	0.1 (<LOQ)	0.1 (<LOQ)
-Organic extracts	45 (0.0061) a	53 (0.013) a	32 (0.010) b	37 (0.012) b	41 (0.020) c	63 (0.034) c	37 (0.042) f	47 (0.035) f
-Aqueous extracts	ND	ND	ND	ND	16 (0.0079) d	12 (0.0067) e	ND	ND
PES	**48 (0.0065)**	**41 (0.0099)**	**3.4 (0.0011)**	**8.4 (0.0027)**	**7.9 (0.0038)**	**14 (0.0076)**	**8.4 (0.0095)**	**11 (0.0085)**
Total unknowns	**46 (0.0062)**	**54 (0.013)**	**38 (0.012)**	**40 (0.013)**	**59 (0.028)**	**76 (0.041)**	**37 (0.042)**	**47 (0.036)**
Total TRR	**100 (0.014)**	**100 (0.024)**	**100 (0.032)**	**100 (0.032)**	**100 (0.048)**	**100 (0.054)**	**100 (0.11)**	**100 (0.075)**

ND = not detected, LOQ = 0.0001 mg eq/kg; NA= not applicable, -- = not included in the calculation of Total TRR

[a] Organic extracts contained 9 unknown peaks or regions of interest, each not exceeding 0.002 mg eq/kg.

[b] Organic extracts contained 8-12 unknown peaks or regions of interest, each not exceeding 0.002 mg eq/kg.

[c] Organic extracts contained 9-10 unknown peaks or regions of interest, each not exceeding 0.005 mg eq/kg ([^{14}C-Ph]-label) or 0.006 mg eq/kg ([^{14}C-Bz]-label).

[d] Aqueous extracts contained 3 unknown peaks or regions of interest, each not exceeding 0.002 mg eq/kg ([^{14}C-Ph]-label).

[e] Aqueous extracts were not further characterised.

[f] Organic extracts contained 11–14 unknown peaks or regions of interest, each not exceeding 0.007 mg eq/kg ([^{14}C-Ph]-label) or 0.004 mg eq/kg ([^{14}C-Bz]-label).

[g] Eggs were sampled on day 11 and 12 from laying hens dosed with [^{14}C-Ph]- and [^{14}C-Bz]-mandestrobin, respectively.

Overview of the metabolic pathway of mandestrobin in livestock

The proposed metabolic pathway (depicted in Figure 3) of mandestrobin in lactating goats and laying hens proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation, ether hydrolysis and glucuronide conjugation.

Hydroxylation of the phenoxy ring gives 4-OH-mandestrobin, and hydroxylation of the methyl groups on the phenoxy ring gives 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. Further oxidation of the hydroxymethyl groups to the carboxylic acid gives 2-COOH-mandestrobin, 5-COOH- mandestrobin and 5-CA-2-HM- mandestrobin. Hydroxylation also occurs on the N-methyl group, to give 5-CA- mandestrobin -NHM and 5-CA-2-HM- mandestrobin -NHM.

Mandestrobin is also subject to hydrolysis of the phenoxy ether link, yielding De-Xy-mandestrobin.

N-demethylation of 5-COOH- mandestrobin results in the formation of 5-CA- mandestrobin -NDM, while O-demethylation of mandestrobin results in the formation of MCBX.

The primary metabolites are further metabolized by conjugation. Mandestrobin, De-Xy-mandestrobin, 5-CA-2-HM-MCBX, 5-CA-2-HM-mandestrobin, 2-COOH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CA-mandestrobin-NDM and 5-COOH-mandestrobin were present in goat liver in bound/conjugated form. Mandestrobin, De-Xy-mandestrobin, 4-OH-mandestrobin and 5-CA-2-HM-mandestrobin were present in hen liver in bound/conjugated form. The 4-OH-mandestrobin was found as the glucuronide conjugate in goat kidney.

R/S isomerisation of parent compound was not investigated.

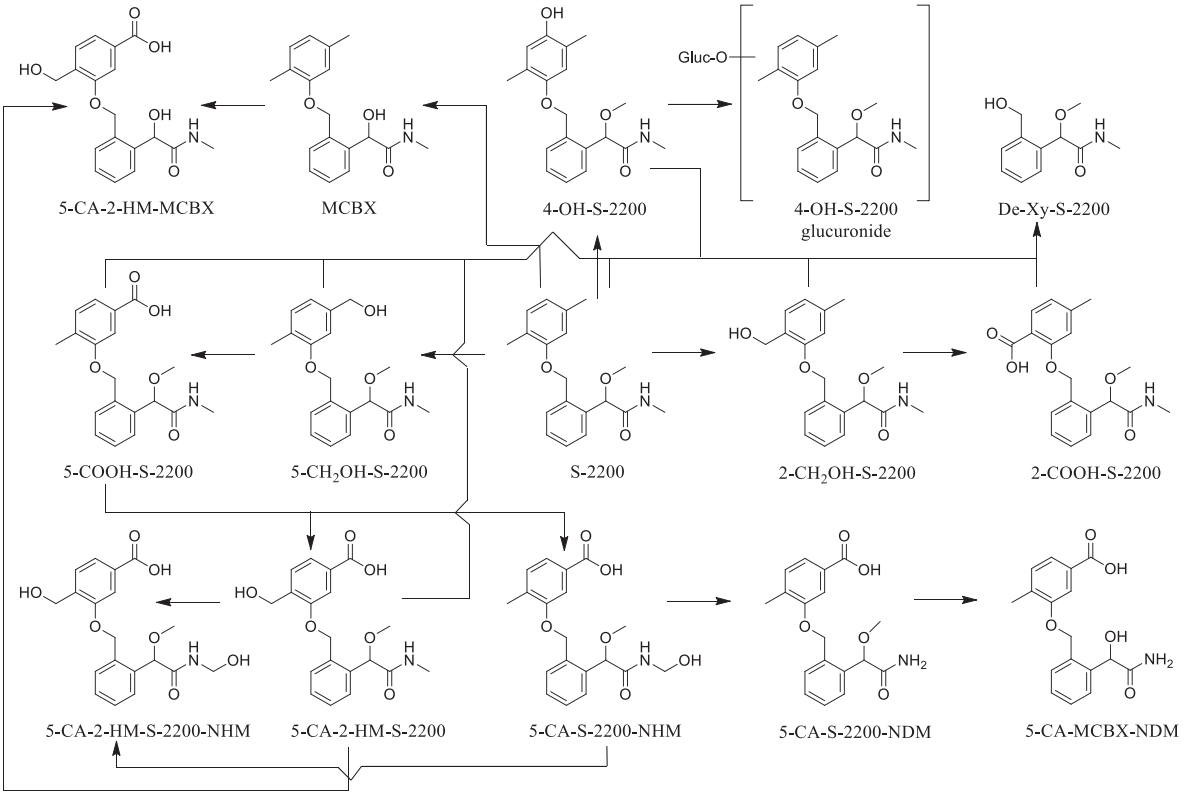


Figure 3 Proposed metabolic pathway of mandestrobin (=S-2200) in lactating goats and laying hens

Environmental fate in soil

The Meeting received information on photodegradation of mandestrobin (R or S isomer) on the soil surface, aerobic degradation of mandestrobin (R or S isomer) and its metabolites in soil under laboratory conditions and dissipation of mandestrobin under field conditions. The fate and behaviour of the R- and S-isomer of mandestrobin in or on soil was investigated using [^{14}C-benzyl]-R- and S-isomer of mandestrobin and [^{14}C-phenoxy]-R- and S-isomer of mandestrobin (see Figure 1).

Photodegradation on the soil surface

Study 1–2. The photodegradation of the R-isomer or S-isomer of mandestrobin was studied in a soil from Europe [Graham & Gilbert, 2011a, ROM-0020; Graham & Gilbert, 2010, ROM-0019]. Soil characteristics are reported in Table 41. The [^{14}C-benzyl]-R- or S-isomer of mandestrobin or the [^{14}C-phenoxy]-R-isomer of mandestrobin were applied to the surface of a 3 mm thin layer of (3 g) soil at an application rate of 8.4 mg ai/kg dry weight soil, each (equivalent to a field application rate of 0.2 kg ai/ha). Duplicate samples were maintained at 20 ± 2 °C and irradiated for 30 days using light from a Xenon arc lamp with a 290 nm cut-off filter. The average intensity over the 300 to 400 nm range was 20.5–28.3 Watts/m^2 in ROM-0020 and 22.7–28.6 Watts/m^2 in ROM-0019, which is approximately equivalent to one US or UK summer day. Soil samples were sieved to 2 mm and the soil moistures were adjusted to 75 % of the moisture holding capacity at 1/3 bar. Soil moistures were checked daily and if necessary adjusted. Moistened air was pulled over the surface of the soil samples and the units were connected to two 2 M sodium hydroxide traps. Control samples were incubated in the dark. Soil and trapping solutions were sampled immediately after treatment and at 3, 5, 8, 13, 17, 22–23 and 30 days of irradiation. Soil samples were analysed immediately after sampling.

Table 41 Soil characteristics for studies ROM-0019 and ROM-0020

Soil name	Chelmorton
Study	ROM-0019

Soil name	Chelmorton
	ROM-0020
Location	Chelmorton, Derbyshire, UK
Soil texture (USDA) [a]	silt loam
-- Sand (%)	26
-- Silt (%)	54
-- Clay (%)	20
Organic Carbon (%) [b]	3.5
Organic Matter (%)	6.0
CEC (meq/100 g)	29.4
pH (H_2O)	7.0
pH (KCl)	5.8
pH ($CaCl_2$)	6.1
Water Holding Capacity at pF 0 (0.001 bar) [%]	76.1
Water Holding Capacity at pF 2.0 (0.1 bar) [%]	35.8
Water Holding Capacity at pF 2.5 (0.33 bar) [%]	34.3
Disturbed bulk density (g/cm^3)	0.9
Microbial biomass initial [mg microbial C/kg] at 26 March 2009	471.0
Microbial biomass final [mg microbial C/kg] at 28 May 2009	415.4

[a] Classification according to United States Department of Agriculture (USDA)

[b] Organic C = organic matter/1.724 based on the certificate values

Soils were extracted twice with acetone/water (9:1, v/v), once with acetone, twice with acetone/0.1 M HCl (5:1, v/v) and once with acetone. Extracts, post-extracted solids and trapping solutions were analysed by LSC. Recovery of applied activity for the irradiated test systems ranged from 91–98% TAR for the [^{14}C-benzyl]-R-isomer of mandestrobin, 94–98% TAR for the [^{14}C-benzyl]-S-isomer of mandestrobin and 95–99% TAR for the [^{14}C-phenoxy]-R-isomer of mandestrobin.

Extracts were analysed by HPLC and selected extracts were analysed by chiral HPLC and 2D-TLC using co-chromatography with reference standards. Selected HPLC fractions were isolated and analysed by TLC to investigate the number and type of compounds within these fractions. Reference standards used were *R*-isomer of mandestrobin, *S*-isomer of mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, mandestrobin-OR, mandestrobin-ORC, mandestrobin-PR, De-Xy-mandestrobin, DX-CA-mandestrobin, MCBX and 2,5-dimethylphenol. Results are shown in Table 42.

No isomerisation of R-isomer to the S-isomer or the S-isomer to the R-isomer occurred.

The route of degradation on the soil surface is similar for both light exposed and dark soil samples. The R-or S-isomer of mandestrobin underwent oxidation of the methyl group at 2- or 5-position of the phenoxy-ring. The resulting 2-COOH-mandestrobin and 5-COOH-mandestrobin compounds steadily increased to 3.5–5.9% TAR and 3.5–8.1% TAR after 23–30 days. Other possible pathways are cleavage of the phenyl-ether-bond leading to the formation of De-Xy-mandestrobin, followed by oxidation to form DX-CA-mandestrobin. DX-CA-mandestrobin steadily increased to 2.8–5.5% TAR after 23–30 days. De-Xy-mandestrobin, MCBX, mandestrobin-OR und unknown degradates were observed at low levels (< 5% TAR) throughout the study. Mandestrobin-ORC, mandestrobin-PR and 2,5-dimethylphenol were not detected in any of the extracts. Unextracted residues and residues in the NaOH trap steadily increased to 4.4–9.6% TAR and 0.7–4.8% TAR after 23–30 days, respectively.

Table 42 Average (n = 2) total 14C distribution in irradiated soils and dark controls

Time point [days]	R-isomer of M (%AR)	2-COOH-M (%AR)	5-COOH-M (%AR)	De-Xy-M (%AR)	DX-CA-M (%AR)	MCBX (%AR)	M-OR (%AR)	Unk (%AR)	PES (%AR)	NaOH trap (%AR)	Total (%AR)

Time point [days]	R-isomer of M (%AR)	2-COOH-M (%AR)	5-COOH-M (%AR)	De-Xy-M (%AR)	DX-CA-M (%AR)	MCBX (%AR)	M-OR (%AR)	Unk (%AR)	PES (%AR)	NaOH trap (%AR)	Total (%AR)
benzyl-14C-]-R-isomer of mandestrobin, irradiated soil; ROM-0020											
0	95.8	ND	ND	ND	ND	ND	ND	0.9	0.1	-	97.1
5	86.0	1.6	1.7	1.3	1.4	ND	ND	1.4	1.5	0.2	98.4
8	81.0	2.3	2.5	1.0	1.7	ND	ND	1.2	2.4	0.4	96.4
13	78.4	3.6	4.0	1.2	2.8	ND	ND	2.1	3.3	0.6	96.8
17	72.7	4.3	4.4	1.4	3.8	ND	ND	2.8	3.8	1.0	95.4
23	67.6	5.1	5.6	1.3	4.5	ND	ND	2.4	6.7	1.1	95.6
30	62.6	5.0	5.0	1.8	4.0	ND	ND	2.9	5.7	2.4	90.6
benzyl-14C-]-R-isomer of mandestrobin, dark control; ROM-0020											
0	95.8	ND	ND	ND	ND	ND	ND	0.9	0.1	-	97.1
5	87.9	1.1	1.7	ND	0.6	ND	ND	0.6	1.5	0.1	96.5
8	85.3	1.5	2.5	ND	0.9	ND	ND	0.6	2.0	ND	96.1
13	82.1	2.6	4.4	ND	1.9	0.1	ND	2.1	3.2	0.1	96.6
17	77.4	3.2	5.2	ND	1.9	0.2	ND	3.1	4.0	ND	95.0
23	70.7	4.1	6.2	ND	2.2	ND	ND	3.1	6.5	0.7	93.5
30	69.3	4.6	6.9	0.1	2.8	0.2	ND	4.1	5.7	1.0	94.6
phenoxy-14C-]-R-isomer of mandestrobin, irradiated soil; ROM-0020											
0	94.0	ND	ND	NA	NA	ND	ND	0.8	0.1	-	95.2
3	88.7	0.8	1.2	NA	NA	ND	ND	0.9	2.3	0.5	96.2
8	83.1	2.3	2.7	NA	NA	ND	0.9	1.4	4.5	1.3	99.0
13	75.8	4.1	4.4	NA	NA	ND	1.3	1.6	7.4	1.4	95.9
17	72.3	5.2	5.3	NA	NA	0.2	1.1	1.8	7.9	1.9	95.7
23	68.2	6.7	5.9	NA	NA	ND	0.8	2.1	8.8	2.4	94.7
30	66.7	5.9	6.4	NA	NA	ND	0.8	0.6	9.6	4.8	94.7
phenoxy-14C-]-R-isomer of mandestrobin, dark control; ROM-0020											
0	94.0	ND	ND	NA	NA	ND	ND	0.8	0.1	-	95.2
3	90.3	1.1	1.4	NA	NA	ND	ND	0.7	2.0	0.2	97.4
8	83.9	1.5	2.5	NA	NA	ND	ND	1.5	3.2	0.4	95.9
13	81.2	2.9	4.6	NA	NA	ND	ND	1.6	5.0	0.7	95.9
17	81.3	3.1	5.2	NA	NA	ND	0.2	1.3	5.4	0.6	96.9
23	78.4	3.6	5.8	NA	NA	0.2	0.9	0.6	6.5	0.9	96.9
30	71.6	4.9	8.1	NA	NA	ND	0.5	1.1	8.2	1.5	95.9
[benzyl-14C-]-S-isomer of mandestrobin, irradiated soil; ROM-0019											
Time point [days]	S-isomer of M (%AR)	2-COOH-M (%AR)	5-COOH-M (%AR)	De-Xy-M (%AR)	DX-CA-M (%AR)	MCBX (%AR)	M-OR (%AR)	Unk (%AR)	PES (%AR)	NaOH trap (%AR)	Total (%AR)
0	96.5	ND	ND	ND	ND	ND	ND	1.4	0.1	-	98.3
3	87.2	0.9	1.1	1.4	0.7	0.2	0.8	1.4	1.4	0.2	97.5
8	83.0	2.1	1.8	0.9	2.4	ND	0.9	2.9	2.0	0.4	96.5
13	77.7	2.5	2.4	3.0	2.5	ND	1.0	2.7	2.6	0.5	95.0
17	78.1	2.6	2.0	2.0	3.3	0.4	1.0	2.7	2.5	0.5	95.1
22	76.4	2.7	2.8	2.4	3.2	ND	1.2	2.8	3.3	0.8	95.6
30	65.9	4.8	3.5	3.0	5.5	0.6	1.6	4.1	4.4	1.2	94.5
[benzyl-14C-]-S-isomer of mandestrobin, dark control; ROM-0019											
0	96.5	ND	ND	ND	ND	ND	ND	1.4	0.1	-	98.3
3	89.4	0.7	0.6	ND	0.3	ND	ND	1.9	1.3	0.2	96.7
8	85.3	1.5	2.4	0.5	1.7	ND	ND	2.0	2.0	0.1	95.5
13	86.2	2.5	3.7	ND	2.8	ND	ND	1.7	3.5	0.5	100.9
17	80.6	2.6	3.7	ND	2.3	ND	ND	1.7	3.4	ND	94.1
22	74.3	3.5	4.7	0.2	4.0	0.1	ND	2.6	4.4	0.4	93.7
30	75.8	2.9	4.6	ND	3.8	ND	ND	2.1	3.9	0.7	93.1

M = mandestrobin; ND = not detected; NA = not applicable (radiolabel is not on this molecule);-not analysed

Unk = total unknowns, includes largest unknown (max 0.2–2.6% AR), minor unknowns, aqueous fraction and unresolved background

Total = sum of neutral and acidic extracts (before HPLC separation), PES and traps

DT_{50} values were calculated assuming single first order (SFO) kinetics and using KinGUI version 1.1 software. The fit of the SFO model is based on visual assessment of goodness of fit and on Chi-squared error. Results are shown in Table 43. DT_{50} values are applicable to Europe and North America at latitudes of 30°, 40°, and 50°. No correction of irradiation days into equivalent sunlight days for Europe or North America was required because the irradiation intensity was equivalent to natural sunlight at these latitudes.

Table 43 Degradation rate in irradiated soils for the R-isomer of mandestrobin

	Irradiated soils			Dark controls		
	DT_{50} (days)	DT_{90} (days)	χ^2 (%)	DT_{50} (days)	DT_{90} (days)	χ^2 (%)
[Benzyl-^{14}C] R-isomer of mandestrobin; ROM-0020	49	163	1.54	62	205	1.37
[Phenoxy-^{14}C] R-isomer of mandestrobin; ROM-0020	56	185	1.95	85	281	1.54
[Benzyl-^{14}C] S-isomer of mandestrobin; ROM-0019	64	212	2.53	83	275	2.37

Overview of the degradation pathway of mandestrobin after soil surface photolysis

Mandestrobin was degraded by several routes following soil surface photolysis (depicted in Figure 4).

- Oxidation of the methyl groups on the phenoxy ring formed 2-COOH-mandestrobin and 5-COOH-mandestrobin.
- Cleavage of the ether linkage formed De-Xy-mandestrobin, which was subsequently oxidized to DX-CA-mandestrobin.
- Demethylation of the 2-methoxy group to form MCBX (minor route).
- Photolytic reactions to form mandestrobin-OR (minor route)
- Mineralization (formation of bound residues and CO_2).

R/S isomerisation of parent compound did not occur.

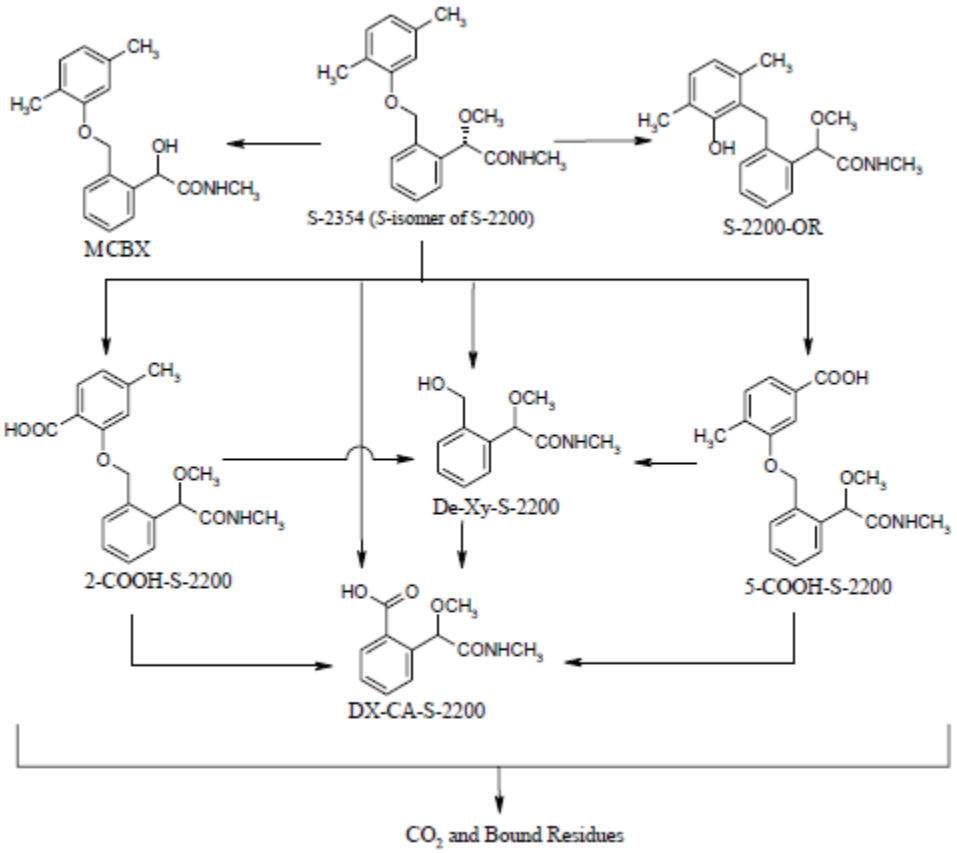


Figure 4 Overview of the degradation pathway of mandestrobin (S-2200) after soil surface photolysis.

Aerobic degradation in soil-laboratory studies with mandestrobin

Study 1–2: The degradation of the R-isomer of mandestrobin under aerobic laboratory conditions was studied in six terrestrial soils from Europe [Graham & Gilbert, 2011b, ROM-0028; Graham & Gilbert, 2011d, ROM-0030]. Soil characteristics are reported in Tables 44 and 45. The soil (50 g) was treated with [^{14}C-benzyl]-R-isomer of mandestrobin or [^{14}C-phenoxy]-R-isomer of mandestrobin, each at an application rate of 0.8 mg ai/kg dry weight soil (equivalent to a field application rate of 0.2 kg ai/ha assuming an incorporation depth of 2.5 cm and a bulk density of 1.0 g/cm^3). The active substance was dispensed on the soil surface and mixed with the soil. Soil samples were incubated under aerobic conditions in the dark at 20 ± 2 °C for 120–126 days. Volatile compounds were passed through a series of trapping solutions (ethanediol trap, 2% liquid paraffin in xylene trap, 2 M sodium hydroxide solutions) to collect any volatile degradation products including CO_2. Soil and trapping solutions were sampled at 0, 7, 14, 29–30, 59–61, and 120–126 days after treatment. Soil moisture was adjusted to the water holding capacity (WHC) at pF2 every 8–11 days.

Table 44 Soil characteristics for studies ROM-0028, ROM-0029, ROM-0030 and ROM-0031

Soil name	Speyer 5M	Speyer 2.2	SK920191	Chelmorton	Aschard	Monteil
Study	ROM-0028 ROM-0029	ROM-0028 ROM-0029	ROM-0028 ROM-0029	ROM-0028 ROM-0029	ROM-0030 ROM-0031	ROM-0030 ROM-0031
Location	Mechters heim, Rheinland-Pfalz Germany	Hanhofen, Rheinland-Pfalz Germany	South Witham, Lincolnshire, UK	Chelmorton, Derbyshire, UK	Aschard, Grezille, France	Monteil, Latour, Bas, Elne, France
Soil texture (USDA) [a]	sandy loam	loamy sand	clay loam	silt loam	loam	silty clay

Soil name	Speyer 5M	Speyer 2.2	SK920191	Chelmorton	Aschard	Monteil
						loam
-- Sand (%)	62	82	43	25	38	15
-- Silt (%)	28	12	27	55	39	53
-- Clay (%)	11	6.0	30	20	23	32
Organic Carbon (%) [b]	1.3	2.1	3.8	3.4	1.3	1.4
Organic Matter (%)	2.2	3.6	6.6	5.9	2.2	2.4
CEC (meq/100 g)	15	10	33.9	25.0	15.4	18.3
pH (H_2O)	8.2	6.0	7.9	6.6	8.1	8.5
pH (KCl)	7.6	5.1	7.7	5.7	7.2	7.7
pH ($CaCl_2$)	7.2	5.5	7.6	5.9	7.4	7.7
Water Holding Capacity at pF 0 (0.001 bar) [%]	36.6	46.5	65.7	71.0	41.1	56.0
Water Holding Capacity at pF 2.0 (0.1 bar) [%]	24.8	20.0	34.2	38.0	30.4	36.1
Water Holding Capacity at pF 2.5 (0.33 bar) [%]	14.5	13.6	26.9	28.6	18.9	27.7
Disturbed bulk density (g/cm^3)	1.3	1.2	1.1	0.9	1.3	1.1

[a] Classification according to United States Department of Agriculture (USDA)

[b] Organic C = organic matter/1.724 based on the certificate values

Table 45 Soil viability

Soil name	Speyer 5M	Speyer 2.2	SK920191	Chelmorton	Aschard	Monteil
Study	ROM-0028	ROM-0028	ROM-0028	ROM-0028	ROM-0030	ROM-0030
Microbial biomass (mg microbial carbon/kg soil) at DAT=0 [c]	171	838	720	941	130	202
Microbial biomass (mg microbial carbon/kg soil DM) at DAT = 120 [c]	170	194	810	353	257	207
Organic carbon (%) at DAT = 0	1.3	4.0	1.9	2.8	1.0	1.4
Organic carbon (%) at DAT = 120	1.3	1.0	2.1	1.0	2.0	1.5

[a] Fumigation/Extraction method

Soils were extracted sequentially with acetone/water (9:1, v/v), acetone and acetone/0.1 M HCl (5:1, v/v). Unextracted residues of the DAT 120–126 samples were treated with 0.5 M NaOH for 16–26 hrs and the precipitate was separated off as the humic acid fraction. The supernatant was acidified to pH 1 with 5 M HCl and the supernatant was separated off as the fulvic acid fraction. The precipitate was reconstituted in 0.5 M NaOH as the humic acid fraction. Soil samples and trapping solutions were analysed by (combustion) LSC. Recovery of applied activity ranged from 94% to 102%.

All extracts containing ≥5% AR were analysed by (chiral) HPLC and TLC techniques. The nature of the radioactivity in the NaOH traps was investigated by barium chloride solution addition. Dissolved $^{14}CO_2$ would be precipitated as insoluble barium ^{14}C-carbonate. Reference standards used were R-isomer of mandestrobin, *S*-isomer of mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, De-Xy-mandestrobin, DX-CA-mandestrobin, 2.5-dimethylphenol, MCBX, or the R- and S-isomer of MCBX. Results are shown in Table 46.

In six European soils, the R-isomer of mandestrobin declined to 15–64% AR at the end of the study. No transformation of the R-isomer to the S-isomer occurred. The ^{14}C-benzyl- or phenoxy-labelled R-isomer of mandestrobin degraded mainly to CO_2 (up to 34% AR), unextracted residues (up to 33%) and three metabolites. Metabolite 5-COOH-mandestrobin was the major metabolite and was

found up to a mean maximum of 20% of applied radioactivity after 60 days. Metabolite 2-COOH-mandestrobin was present at a mean maximum of 8.7% of applied radioactivity after 60 days and metabolite DX-CA-mandestrobin was found at a maximum of 3.3%. Metabolite MCBX did not exceed 1% AR in any soil at any time point. All other metabolites individually accounted for less than 3% AR. Levels of CO_2 after 120 days incubation were similar for both radio labels, ranging from 4.2–34 % of AR. No other volatile compounds were detected. Levels of unextracted residues showed greater variation between soil types than between label positions and were between 6.7 % and 33 % of AR at 120–126 days incubation. The partitioning of the unextracted residues into humic acid, fulvic acid and humin like fractions is presented in Table 47.

DT_{50} and DT_{90} values for the *R*-isomer of mandestrobin were in the range 40 to 227 days and 132 to 754 days, respectively, for SFO kinetics and are summarized in Table 63.

Notes by the reviewer:

Within EU the equivalent field application rates for this study was calculated based on an incorporation depth of 5 cm and a bulk density 1.5 g/cm^3. Using these default values, the equivalent field application rate for a treatment of 0.8 mg ai/kg soil would be 0.6 kg ai/ha.

The presence of the plant metabolites 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin was not verified in soil. Unknown B and C were identified as 2-$CONH_2$-mandestrobin and 5-$CONH_2$-mandestrobin, respectively, in soil degradation study ROM-0051.

Table 46 Distribution of radioactivity (% of applied radioactivity) in 6 European soils after application of ^{14}C-benzyl or ^{14}C-phenoxy labelled R-isomer of mandestrobin (means of duplicate samples)

Speyer 5 M (14C-phenoxy) ROM-0028	Interval in days	0	7	14	30	59	120
	R-isomer of mandestrobin [b]	98.1	86.4	77.4	72.8	58.4	35.3
	5-COOH-mandestrobin	ND	3.9	6.9	9.4	12.7	12.5
	2-COOH-mandestrobin	ND	2	3.7	4.9	5.4	4.9
	MCBX	ND	ND	0.1	ND	0.2	0.3
	2,5-dimethylphenol	ND	ND	ND	ND	ND	0.2
	Extracted unknown A	ND	0.2	0.5	ND	ND	0.4
	Extracted minor unknowns [c]	1.0	1.4	1.1	1.5	0.6	0.6
	Un-extracted	0.2	3.2	4.4	6.8	12.6	21.3
	CO_2 [a]	NA	0.6	1.5	2.9	6.7	19.6
	Mass balance [e]	100	101	98.7	98.3	97.5	95.5
Speyer 5M (14C-benzyl) ROM-0028	Interval in days	0	7	14	30	59	120
	R-isomer of mandestrobin [b]	98.9	89.1	81.7	71	39.4	24.6
	5-COOH-mandestrobin	ND	3.6	6.4	10.9	16.9	11.6
	2-COOH-mandestrobin	ND	2.1	3.3	4.7	5.7	3.6
	MCBX	ND	ND	ND	0.6	0.5	0.2
	DX-CA-mandestrobin [d]	ND	ND	ND	ND	0.6	0.3
	Extracted unknown A	ND	ND	0.3	0.8	0.7	0.3
	Extracted minor unknowns [c]	0.6	1.3	1.4	0.4	1.4	0.5
	Unextracted	0.2	2.6	3.8	7.2	18.5	29.2
	CO_2 [a]	NA	0.9	1.7	3.7	9	25.1
	Mass balance [e]	100	102	102	99.2	95.1	96.2
Speyer 2.2 (14C-benzyl) ROM-0028	Interval in days	0	7	14	30	59	120
	R-isomer of mandestrobin [b]	95.2	83.2	80.3	80	69.6	63.8
	5-COOH-mandestrobin	ND	3.3	5.6	6.1	5.7	4.9
	2-COOH-mandestrobin	ND	0.9	1.5	1.8	2.1	2.8
	MCBX	ND	ND	0.2	ND	0.3	0.6

Speyer 5 M (14C-phenoxy) ROM-0028	Interval in days	0	7	14	30	59	120
	DX-CA-mandestrobin [d]	ND	ND	0.5	0.8	3.5	5.0
	Extracted unknown A	ND	0.2	0.5	0.3	0.3	0.6
	Extracted unknown B	ND	ND	0.5	0.8	2.6	2.8
	Extracted unknown C	ND	0.2	ND	ND	ND	0.1
	Extracted minor unknowns [c]	1.8	1.1	1.0	0.6	2.0	1.6
	Unextracted	0.1	2.1	3.1	3.4	4.5	6.7
	CO_2 [a]	NA	1.3	2.3	2.8	2.6	4.2
	Mass balance [e]	97.9	95.8	95.5	96.6	94.1	94.5
SK920191 (14C-benzyl) ROM-0028	Interval in days	0	7	14	30	59	120
	R-isomer of mandestrobin [b]	96.7	80.7	70.6	56.1	41.2	22.5
	5-COOH-mandestrobin	ND	9.4	12.7	15.9	16.9	11.5
	2-COOH-mandestrobin	ND	4.3	6.2	7.2	8.6	3.3
	MCBX	ND	ND	0.1	0.4	ND	0.2
	De-Xy-mandestrobin/ DX-CA-mandestrobin	ND	ND	ND	0.1	ND	ND
	Extracted unknown A	ND	0.2	0.2	0.2	ND	0.1
	Extracted minor unknowns [c]	1.7	1.0	0.9	0.4	3.4	0.3
	Unextracted	0.3	4.4	6.8	13.3	20.1	33.2
	CO_2 [a]	NA	1.1	1.8	4.9	7.1	23.2
	Mass balance [e]	99.9	101	99.3	98.5	97.2	96.3
Chelmorton (14C-benzyl) ROM-0028	Interval in days	0	7	14	30	59	120
	R-isomer of mandestrobin [b]	92.1	76.1	67.9	59.2	50.3	43.1
	5-COOH-mandestrobin	ND	4.8	5.3	6.3	7.0	7.3
	2-COOH-mandestrobin	ND	3.2	3.1	3.6	4.6	4.8
	MCBX	ND	ND	0.4	ND	0.6	0.4
	DX-CA-mandestrobin [d]	ND	0.2	0.1	0.3	2.2	3.1
	Extracted unknown A	ND	0.4	0.7	ND	0.8	0.7
	Extracted unknown B	ND	ND	0.1	ND	0.2	0.1
	Extracted unknown C	ND	ND	0.2	ND	0.4	0.6
	Extracted minor unknowns [c]	1.3	0.5	0.6	0.9	0.2	0.6
	Unextracted	0.2	6.8	10.4	14.5	16.3	19.0
	CO_2 [a]	NA	3.2	5.7	9.6	11.1	14.4
	Mass balance [e]	95.0	95.2	94.6	94.3	93.8	94.0
Aschard (14C-benzyl) ROM-0030	Interval in days	0	7	14	29	60	126
	R-isomer of mandestrobin [b]	99.2	84	76.8	60.3	40.5	21.7
	5-COOH-mandestrobin	ND	5.8	10.8	15.9	19.7	15.7
	2-COOH-mandestrobin	ND	3.0	5.0	7.1	8.7	7.0
	MCBX	ND	ND	ND	0.2	0.2	0.1
	De-Xy-mandestrobin/ DX-CA-mandestrobin	ND	ND	0.1	0.2	ND	0.1
	Extracted unknown A	ND	ND	0.7	0.6	0.5	ND
	Extracted unknown B	ND	ND	ND	ND	0.1	ND
	Extracted minor unknowns [c]	0.5	0.8	1.2	0.8	1.0	0.8
	Unextracted	0.1	1.8	3.5	7.8	15.1	24.5
	CO_2 [a]	NA	0.6	1.5	4.1	10.7	24.9
	Mass balance [e]	100.4	98.6	99.5	69.9	96.8	96.4
Monteil (14C-benzyl) ROM-0030	Interval in days	0	7	14	29	60	120
	R-isomer of mandestrobin [b]	96.8	83.3	75.2	56.3	32.3	15.1
	5-COOH-mandestrobin	ND	4.9	7.6	11.2	13.1	8.2
	2-COOH-mandestrobin	ND	1.7	2.7	4.4	5.1	3.2
	MCBX	ND	ND	0.4	0.4	0.3	0.2

Speyer 5 M (14C-phenoxy) ROM-0028	Interval in days	0	7	14	30	59	120
	De-Xy-mandestrobin/ DX-CA-mandestrobin [d]	ND	ND	ND	ND	0.1	ND
	Extracted unknown A	ND	0.3	0.7	1.1	0.5	0.2
	Extracted unknown B	ND	ND	ND	ND	0.2	ND
	Extracted minor unknowns [c]	0.2	0.6	0.8	1.0	1.4	0.6
	Unextracted	0.1	6.7	8.4	15.2	25.1	32.9
	CO_2 [a]	NA	1	2.2	7.4	17.9	34.4
	Mass balance [e]	98.8	98.6	98	97.2	96.5	96.1

NA – not analysed; ND – not detected

[a] Ethanediol and paraffin xylene traps did not contain any radioactivity

[b] No isomerisation of the R-isomer to the S-isomer of mandestrobin occurred

[c] Includes unresolved background

[d] Only DX-CA-mandestrobin (up to 3.3% AR in Speyer 2.2) and other unknown metabolites were found by further chromatographic analysis; De-Xy-mandestrobin was not detected

[e] Sum of radioactivity in extracts, post extracted solids and volatiles before HPLC separation

Table 47 Distribution of unextracted residues (as % of applied radioactivity) into fulvic acids, humic acids and humin after 120–126 days incubation

Soil	fulvic acid fraction	humic acid fraction	humin fraction	Study
Speyer 5M (^{14}C-benzyl)	10.7	6.2	13.1	ROM-0028
Speyer 5M (^{14}C-phenoxy)	7.0	5.6	10.0	ROM-0028
Speyer 2.2 (^{14}C-benzyl)	[a]	[a]	[a]	ROM-0028
SK920191 (^{14}C-benzyl)	8.6	5.6	21.9	ROM-0028
Chelmorton (^{14}C-benzyl)	7.6	5.6	6.2	ROM-0028
Aschard (^{14}C-benzyl))	8.4	4.3	13.9	ROM-0030
Monteil (^{14}C-benzyl)	9.2	4.6	19.1	ROM-0030

[a] no data, since <4% AR detected as carbon dioxide.

Study 3-4: The rate of aerobic degradation of S-isomer of mandestrobin was studied in six European soils [Graham & Gilbert, 2011c, ROM-0029; Graham & Gilbert, 2011e, ROM-0031]. Soil characteristics are reported in Table 44 and Table 48. The soil was treated with [^{14}C-benzyl]-S-isomer of mandestrobin as described for aerobic soil degradation studies 1 and 2 (R-isomer of mandestrobin).

Table 48 Soil viability for studies ROM-0029 and ROM-0031

Soil name	Speyer 5M	Speyer 2.2	SK920191	Chelmorton	Aschard	Monteil
Study	ROM-0029	ROM-0029	ROM-0029	ROM-0029	ROM-0031	ROM-0031
Location	Mechters heim, Rheinland-Pfalz Germany	Hanhofen, Rheinland-Pfalz Germany	South Witham, Lincolnshire, UK	Chelmorton, Derbyshire, UK	Aschard, Grezille, France	Monteil, Latour, Bas, Elne, France
Microbial biomass (mg microbial carbon/kg soil) at DAT=0 [a]	173	891	785	967	191	198
Microbial biomass (mg microbial carbon/kg soil) at DAT = 120 [a]	159	185	819	419	307	263
Organic carbon (%) at DAT = 0	1.3	4.2	2.1	2.9	1.5	1.4
Organic carbon (%) at DAT = 120	1.2	0.9	2.2	1.2	2.4	1.9

[a] Fumigation/Extraction method

Soil and trapping solutions were sampled, extracted and analysed as described for study 1–2. Mass balances ranged from 95% to 100%. Results are shown in Table 49.

In six European soils, the S-isomer of mandestrobin declined to 28-72% AR at the end of the study. No transformation of the S-isomer to the R-isomer occurred. The ^{14}C-benzyl-labeled S-isomer of mandestrobin degraded mainly to CO_2 (up to 27% AR), unextracted residues (up to 30% AR) and one metabolite. Metabolite 5-COOH-mandestrobin was the major metabolite and was found up to a mean maximum of 16% AR at DAT 60. All other metabolites, including the known metabolites 2-COOH-mandestrobin (max 4.8% AR), metabolite DX-CA-mandestrobin (max 4.1% AR), De-Xy-mandestrobin, MCBX and unknown metabolites, individually accounted for less than 5% AR. Levels of CO_2 after 120 days incubation ranged from 4.4–27 % of AR. No other volatile compounds were detected. Levels of unextracted residues ranged between 6.4% and 30% of AR at 120–126 days incubation. The partitioning of the bound residue into humic acid, fulvic acid and humin like fractions is presented in Table 50.

DT_{50} and DT_{90} values for the S-isomer of mandestrobin were in the range 60–323 days and 200 to >1000 days, respectively, for SFO kinetics and are summarized in Table 63.

Note by the reviewer:

The presence of the plant metabolites 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin was not verified in soil. Unknown B and C were identified as 2-$CONH_2$-mandestrobin and 5-$CONH_2$-mandestrobin, respectively, in soil degradation study ROM-0051.

Table 49 Distribution of radioactivity (% of applied radioactivity) in six European soils after application of ^{14}C-benzyl labelled S-isomer of mandestrobin (means of duplicate samples

Speyer 5M (^{14}C-benzyl) ROM-0029	**Interval in days**	**0**	**7**	**14**	**29**	**60**	**120**
	S-isomer of mandestrobin [b]	96.5	84.7	80.8	74.5	58.2	34.7
	5-COOH-mandestrobin	ND	2.8	4.4	5.0	10.5	7.0
	2-COOH-mandestrobin	ND	1.2	1.8	2.4	3.4	2.2
	MCBX	ND	ND	ND	ND	0.3	0.3
	DX-CA-mandestrobin [d]	ND	ND	ND	0.4	ND	0.3
	Extracted unknown A	ND	0.5	0.6	0.7	0.7	0.5
	Extracted minor unknowns [c]	1.5	0.3	1.8	1.2	0.6	1.0
	Unextracted	0.2	2.9	3.9	6.0	14.4	25.4
	CO_2 [a]	NA	0.9	1.7	2.4	9.3	19.9
	Mass balance [e]	98.9	96.2	98.5	96.9	97.5	95.6
Speyer 2.2 (^{14}C-benzyl) ROM-0029	**Interval in days**	**0**	**7**	**14**	**30**	**61**	**120**
	S-isomer of mandestrobin [b]	96.4	87.6	83.9	86	77.7	71.7
	5-COOH-mandestrobin	ND	2.0	2.8	3.8	3.3	3.5
	2-COOH-mandestrobin	ND	0.5	0.9	1.3	2.1	2.8
	MCBX	ND	0.1	ND	ND	0.5	0.4
	DX-CA-mandestrobin [d]	ND	ND	ND	0.8	2.9	4.0
	Extracted unknown A	ND	ND	0.3	ND	0.4	0.4
	Extracted unknown B	ND	ND	0.3	0.6	1.5	1.8
	Extracted unknown C	ND	ND	ND	ND	0.2	0.3
	Extracted minor unknowns [c]	1.2	1.3	1.0	0.7	1.1	1.0
	Unextracted	ND	2	2.5	3.6	4.6	6.4
	CO_2 [a]	NA	1.1	1.7	2.7	3.4	4.4
	Mass balance [e]	98.7	97.6	97.3	99.5	98.1	97
SK920191 (^{14}C-benzyl) ROM-0029	Interval in days	0	7	14	29	60	120
	S-isomer of mandestrobin [b]	94	83.3	80.1	70.3	55	39.4
	5-COOH-mandestrobin	ND	3.2	6.5	8.6	10.2	4.7
	2-COOH-mandestrobin	ND	0.8	1.6	1.5	1.5	0.7

Speyer 5M (^{14}C-benzyl) ROM-0029	**Interval in days**	**0**	**7**	**14**	**29**	**60**	**120**
	MCBX	ND	0.3	0.2	0.3	ND	0.1
	De-Xy-mandestrobin/ DX-CA-mandestrobin	ND	ND	ND	ND	ND	ND
	Extracted unknown A	ND	0.5	0.4	0.6	0.4	0.1
	Extracted minor unknowns [c]	2.5	1.0	1.1	0.9	1.0	0.4
	Unextracted	0.3	4.1	6.5	10.6	19.0	27.3
	CO_2 [a]	NA	1.1	1.7	3.8	9	18.1
	Mass balance [e]	98.1	97.7	97.9	96.5	96.1	95.1
Chelmorton (^{14}C-benzyl) ROM-0029	**Interval in days**	**0**	**7**	**14**	**30**	**61**	**120**
	S-isomer of mandestrobin [b]	95.8	78.8	70.6	64.1	54.8	49.0
	5-COOH-mandestrobin	ND	3.3	4.5	5.4	5.9	6.0
	2-COOH-mandestrobin	ND	3.7	2.8	3.0	3.8	4.8
	MCBX	ND	ND	ND	0.3	0.4	0.4
	DX-CA-mandestrobin [d]	ND	ND	0.2	0.5	2.1	4.1
	Extracted unknown A	ND	ND	0.5	0.4	0.5	0.5
	Extracted unknown B	ND	ND	ND	ND	0.1	ND
	Extracted unknown C	ND	ND	0.4	0.2	0.4	0.5
	Extracted minor unknowns [c]	0.4	1.3	0.8	0.3	0.3	0.9
	Unextracted	0.1	7.4	11.5	14.5	17.6	18.4
	CO_2 [a]	NA	2.8	5.8	8.6	11.3	12.2
	Mass balance [e]	97.9	97.2	96.9	97.3	97.3	96.8
Aschard (^{14}C-benzyl) ROM-0031	**Interval in days**	**0**	**7**	**14**	**29**	**60**	**126**
	S isomer of mandestrobin [b]	97.7	89.3	81.8	70.6	54.5	31.1
	5-COOH-mandestrobin	ND	3.2	6.1	12.1	16.1	14.6
	2-COOH-mandestrobin	ND	1.6	2.4	4.0	4.7	3.6
	MCBX	ND	ND	ND	ND	ND	0.2
	De-Xy-mandestrobin/ DX-CA-mandestrobin	ND	ND	ND	ND	0.1	0.5
	Extracted unknown A	ND	ND	0.4	0.5	0.3	0.2
	Extracted unknown B	ND	ND	ND	ND	0.6	0.5
	Extracted minor unknowns [c]	0.5	1.1	0.4	1.5	0.7	1.2
	Unextracted	0.1	1.6	3	6.3	11.8	22.8
	CO_2 [a]	NA	0.4	1.2	2.9	7.4	20.0
	Mass balance [e]	98.9	99.6	98.9	97.9	96.9	95.5
Monteil (^{14}C-benzyl) ROM-0031	**Interval in days**	**0**	**7**	**14**	**29**	**60**	**120**
	S-isomer of mandestrobin [b]	95.8	88	82	68.3	42.7	28.5
	5-COOH-mandestrobin	ND	3.6	5.5	8.4	8.7	5.8
	2-COOH-mandestrobin	ND	1.0	1.8	3.2	3.4	2.6
	MCBX	ND	ND	ND	0.4	0.3	0.3
	De-Xy-mandestrobin/ DX-CA-mandestrobin	ND	ND	ND	ND	ND	0.1
	Extracted unknown A	ND	0.4	0.6	0.9	0.9	0.5
	Extracted unknown B	ND	ND	ND	ND	0.4	0.4
	Extracted minor unknowns [c]	0.7	0.6	1.0	0.6	0.5	0.5
	Unextracted	0.2	4.3	6.2	12.1	23.4	29.7
	CO_2 [a]	NA	0.7	1.1	5.6	15.7	27.1
	Mass balance [e]	98.6	98.5	98.2	99.5	96.8	96.1

[a] Ethanediol and paraffin xylene traps did not contain any radioactivity

[b] No isomerisation of the S-isomer to the R-isomer of mandestrobin occurred

[c] Includes unresolved background

[d] Only DX-CA-mandestrobin was found by further chromatographic analysis; De-Xy-mandestrobin was not detected

[e] Sum of radioactivity in extracts, post extracted solids and volatiles before HPLC separation

[f] Unknown B was identified as

Table 50 Distribution of unextracted residues (as percentage of applied radioactivity) into fulvic acids, humic acids and humin after 120 days incubation

Soil	fulvic acid fraction	humic acid fraction	humin fraction	Study
Speyer 5M (^{14}C-benzyl)	10.1	6.2	11.2	ROM-0029
Speyer 2.2 (^{14}C-benzyl)	[a]	[a]	[a]	ROM-0029
SK920191 (^{14}C-benzyl)	7.3	3.5	17.3	ROM-0029
Chelmorton (^{14}C-benzyl)	6.8	4.8	6.9	ROM-0029
Aschard (^{14}C-benzyl)	7.8	2.9	13.2	ROM-0031
Monteil (^{14}C-benzyl)	8.4	3.8	18.3	ROM-0031

[a] no data, since <4% AR detected as carbon dioxide.

Study 5-6: The degradation of the R- and S-isomer of mandestrobin under aerobic laboratory conditions was studied in four terrestrial soils from the USA [Gohre, 2013, ROM-0050; Maurer & Gohre, 2013, ROM-0051]. Soil characteristics are reported in Tables 51 and 52. The soil (50 g) was treated with the [benzyl-^{14}C]-R-isomer of mandestrobin (RB), the [benzyl-^{14}C]-S-isomer of mandestrobin (SB) or the [phenoxy-^{14}C]-R-isomer of mandestrobin (RP), each at an application rate of 9.0 mg ai/kg dry weight soil (based on a field application rate of 2.24 kg ai/ha, an incorporation depth of 2.5 cm and a bulk density of 1.0 g/cm^3). The active substance was dispensed on the soil surface and mixed with the soil. Soil samples were incubated under aerobic conditions in the dark at 20 ± 2 °C for 362–364 days in chambers equipped with 2 M NaOH traps for collection of $^{14}CO_2$ and tetraglyme for other volatiles. Soil and trapping solutions were sampled at 0, (7), 13–15, (34), 62–63, (96), 120–131, 181, (274), and 362–364 days after treatment (DAT), depending on the study. Soil moisture was adjusted periodically to 50 ± 10 % of maximum water holding capacity (WHC).

Table 51 Soil characteristics for 4 USA soils

Soil name	Penn series	Atwater	Sharkey	KD Manning
Study	ROM-0050	ROM-0051	ROM-0051	ROM-0051
Location	Batistown, NJ, USA	Madera, CA, USA	Leland, MS, USA	Plaza, ND, USA
Soil texture (USDA) [a]	loam	sand	silt loam	sandy loam
-- Sand (%)	26	87	20	73
-- Silt (%)	49	11	54	13
-- Clay (%)	25	2.0	26	14
Organic Matter (%)	2.0	0.67	1.4	3.1
Organic Carbon (%) [b]	1.2	0.39	0.81	1.8
CEC (meq/100 g)	8.7	5.8	14.7	16.3
pH (H_2O)	6.9	7.4	6.4	7.3
pH (KCl);	-	-	-	-
pH ($CaCl_2$);	--	-	-	-
Disturbed bulk density (g/cm^3)	1.08	1.38	0.98	1.19
Water Holding Capacity at pF 0 (0.001 bar) [%]	-	-	-	-
Soil moisture at 1/10 bar [%] = Water Holding Capacity at pF 2.0 (0.1 bar) [%]	27.0	-	-	-
Soil moisture at 1/3 bar [%] = Water Holding Capacity at pF 2.5 (0.33 bar) [%]	21.3	6.1	22.0	14.9

[a] Classification according to United States Department of Agriculture (USDA)

[b] Organic C = organic matter/1.724 based on the certificate values

Table 52 Soil viability for studies ROM-0050 and ROM-0051

Soil name	Penn series	Atwater	Sharkey	KD Manning
Study	ROM-0050	ROM-0051	ROM-0051	ROM-0051
Location	Batistown, NJ,	Madera,	Leland,	Plaza,

Soil name	Penn series	Atwater	Sharkey	KD Manning
	USA	CA, USA	MS, USA	ND, USA
Microbial biomass (mg microbial carbon/kg dw soil) at DAT=1 [a]	321.7; 552.3	61.1	125.8	308.6
Microbial biomass (mg microbial carbon/kg dw soil) at DAT 364-365 [a]	20.5	10.2	114.6	36.8
Organic carbon (%) at DAT = 0	1.2	0.39	0.81	1.8
Organic carbon (%) at DAT = 364-365	-	-	-	-

[a] based on fumigation/extraction method

Soil samples 0–63 DAT from ROM-0050 (Penn series) were extracted twice with acetone/water (9:1, v/v) and twice with acetone: 0.1 M HCl (5:1, v/v). Samples from 96–362 DAT from ROM-0050 (Penn series) were extracted twice with acetonitrile: water (9:1, v/v) and then twice with acetonitrile/0.1 M hydrochloric acid (5:1, v/v). Soil samples from ROM-0051 were extracted twice with methanol:water (9:1, v/v) and twice with methanol:0.5M HCl (5:1, v/v). An additional soxhlet or dismembrator extraction with acetone:0.5M HCl (5:1, v/v) was performed on the post extracted solids (PES) at 362–364 DAT. The remaining unextracted residue was fractionated into humin, fulvic acid and humic acid components as described for aerobic degradation studies 1–2. Soil extracts, PES and volatiles were analysed by (combustion) LSC. Recovery of the applied activity ranged from 99 to 104% of AR.

Extracts were analysed by (chiral) HPLC and 2D-TLC techniques using co-chromatography with reference standards. Reference substance used were mandestrobin (racemic), *R*-isomer of mandestrobin, *S*-isomer of mandestrobin, 5-COOH-mandestrobin, 2-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, 5-$CONH_2$-mandestrobin, MCBX (R/S, R, S), De-Xy-mandestrobin, DX-CA-mandestrobin and 2,5-dimethylphenol (DMP). The nature of the radioactivity in the NaOH traps was investigated by barium chloride solution addition. Dissolved $^{14}CO_2$ would be precipitated as insoluble barium ^{14}C-carbonate. The findings are summarized in Table 53 (ROM-0050) and Table 54 (ROM-0051).

In the four US soils, mandestrobin declined to 24–51% AR at the end of the study. Chiral HPLC analysis confirmed that mandestrobin *R*- or *S*-isomer did not isomerize during the incubation. The principal early degradation routes were oxidation of the methyl groups on the phenoxy ring to form 2-COOH-mandestrobin and 5-COOH-mandestrobin. The phenoxy acids reached their peaks at 34–96 days in the Penn series, but increased until the end of the test for the other 3 USA soils (up to 7.6% AR for 2-COOH-mandestrobin and up to 10% AR for 5-COOH –mandestrobin). The corresponding amides steadily increased until the end of the study (up to 14% AR for 2-$CONH_2$-mandestrobin and up to 14% AR for 5-$CONH_2$-mandestrobin). Cleavage of the ether linkage formed De-Xy-mandestrobin (up to 2.5% AR, benzyl label only) which oxidized to DX-CA-mandestrobin. DX-CA-mandestrobin peaked at 125-267 days (up to 12% AR, benzyl label only). Demethylation of mandestrobin to MCBX was a minor route (up to 1.6% AR). Levels of CO_2 after 362–364 days incubation ranged from 10–21 % of AR and unextracted residues increased slowly up to 8.4–18% AR. The partitioning of the unextracted residues into humic acid, fulvic acid and humin like fractions is presented in Table 55.

DT_{50} and DT_{90} values for the *R*- and S-isomers of mandestrobin were in the range 144–378 days and 479–1255 days, respectively, for SFO kinetics and are summarized in Table 63.

Table 53 Average (n = 2) radioactivity distribution of ^{14}C-mandestrobin under aerobic conditions in a single USA soil

Interval (days)	0	7	13	34	63	96	131	181	274	362
Characterisation	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR

Interval (days)	0	7	13	34	63	96	131	181	274	362
Characterisation	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR
[benzyl-^{14}C] R-isomer of mandestrobin, RB, Penn Series, ROM-0050										
mandestrobin	96.9	92.3	91.0	77.5	62.7	50.4	45.9	41.4	33.0	26.4
5-COOH-M	ND	3.4	4.6	9.1	5.7	2.7	2.0	1.8	1.5	1.1
2-COOH-M	ND	1.5	2.1	5.2	7.6	5.8	3.9	3.2	1.8	1.1
5-CONH$_2$-M	ND	ND	ND	ND	5.2	9.8	11.2	11.6	12.2	13.5
2-CONH$_2$-M	ND	ND	ND	ND	0.4	4.5	7.4	9.3	12.3	13.7
MCBX	ND	ND	0.1	0.3	0.5	1.1	0.9	0.9	1.0	0.7
De-Xy-M	ND	ND	ND	ND	0.5	ND	ND	ND	ND	0.3
DX-CA-M	ND	0.8	1.1	3.4	8.5	10.0	10.9	10.8	7.9	6.4
unknown A	ND	ND	ND	0.4	0.5	1.5	3.0	3.1	5.6	7.5
Others [a]	1.4	0.6	0.5	1.6	2.2	5.7	6.9	5.0	6.6	9.5
PES	0.4	2.0	2.2	4.1	6.3	5.4	6.4	6.8	9.0	10.1
Volatiles (CO_2)	na	0.4	0.7	1.4	2.6	4.0	5.3	7.1	10.0	12.8
Total	98.7	101.3	101.9	103.2	102.2	101.9	103.4	101.3	101.6	103.6
[benzyl-^{14}C] S-isomer of mandestrobin, SB, Penn Series, ROM-0050										
mandestrobin	94.1	84.7	81.8	71.6	62.4	49.0	42.9	36.6	30.9 [b]	25.4
5-COOH-M	0.8	2.7	4.5	7.9	6.6	2.9	2.2	1.9	1.4 [b]	1.2
2-COOH-M	ND	1.3	1.7	3.7	4.6	6.5	5.7	5.2	3.6 [b]	3.0
5-CONH$_2$-M	ND	ND	ND	ND	1.7	8.3	9.3	10.2	10.8 [b]	10.9
2-CONH$_2$-M	ND	ND	ND	ND	ND	2.2	3.9	5.6	8.6 [b]	9.8
MCBX	0.4	ND	0.2	0.2	0.4	0.9	1.1	1.0	1.1 [b]	0.7
De-Xy-M	2.5	ND	ND	ND	ND	0.6	ND	ND	ND [b]	ND
DX-CA-M	0.4	2.1	2.4	3.5	7.7	8.4	11.5	10.8	8.0 [b]	8.2
unknown A	ND	ND	0	0.7	0.9	1.4	1.3	1.5	2.2 [b]	3.6
Others [a]	2.0	1.1	1.6	1.8	4.3	4.9	6.7	7.8	8.8 [b]	10.0
PES	1.6	4.9	4.9	6.1	8.4	6.7	8.1	8.4	9.7	11.2
Volatiles (CO_2)	-	2.4	3.4	4.8	6.6	8.2	9.6	11.1	13.5	15.8
Total	100.3	99.6	101.0	100.2	102.2	101.4	100.9	100.0	100.0	100.3
[phenoxy-^{14}C] R-isomer of mandestrobin, RP, Penn Series, ROM-0050										
mandestrobin	92.2	84.5	78.8	68.5	56.8	47.1	41.5	35.3	27.8	24.3
5-COOH-M	0.4	3.0	5.8	10.2	9.9	4.0	2.8	1.9	1.6	1.4
2-COOH-M	ND	1.9	3.2	5.3	6.2	6.3	4.2	2.6	1.4	1.2
5-CONH$_2$-M	ND	ND	ND	ND	2.0	9.8	11.9	12.8	12.3	13.5
2-CONH$_2$-M	ND	ND	ND	ND	ND	4.3	7.9	10.4	12.9	12.8
MCBX	0.8	ND	ND	0.1	0.7	0.8	1.0	0.9	1.0	0.7
2,5-DMP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Others [a]	1.8	0.3	0.3	0.3	3.4	1.7	2.2	2.3	3.1	5.3
PES	95.2	7.7	7.6	9.9	13.5	12.2	14.0	14.7	16.8	17.5
Volatiles (CO_2)	-	2.4	3.3	5.1	8.0	11.0	13.3	15.6	18.2	20.6
Total	96.6	99.9	99.5	99.8	100.5	99.0	99.1	97.6	99.8	99.4

M= mandestrobin, ND = not detected; PES = post extraction solids

[a] Apart from Unknown A up to 21–24 individual unknowns were observed, with the highest individual unknown being 2.6-3.3% of AR.

[b] Single sample

Table 54 Average (n = 2) radioactivity distribution of ^{14}C-mandestrobin under aerobic conditions in three USA soils

Interval (days)	0	15	34	62	125	181	267	364
Characterisation	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR
[benzyl-^{14}C] R-isomer of mandestrobin, RB, Atwater, ROM-0051								
mandestrobin	96.6	91.8	94.3	88.5	76.1	56.1	49.4	47.0
5-COOH-M	0.2	2.3	2.6	2.5	5.4	8.7	9.8	9.2
2-COOH-M	ND	1.1	1.3	1.4	4.1	6.6	7.6	6.4
5-CONH$_2$-M	ND	ND	ND	ND	0.1	0.1	0.3	1.0
2-CONH$_2$-M	ND	ND	ND	0.1	0.1	0.3	0.4	0.4
MCBX	ND	0.3	0.9	0.6	0.4	0.7	0.5	1.1
De-Xy-M	1.1	ND	ND	ND	0.6	ND	ND	0.5
DX-CA-M	ND	ND	0.9	0.4	1.4	5.5	7.3	5.6

Interval (days)	0	15	34	62	125	181	267	364
Characterisation	%AR	%AR	%AR	%AR	%AR	%AR	%AR	%AR
unknown A	ND	0.1	0.2	0.3	0.8	1.6	1.9	1.8
Other unknowns	2.5	4.7	2.6	3.6	4.3	4.5	3.1	7.6
Soil bound PES	0.5	1.4	1.6	2.1	3.6	7.3	8.3	8.4
Volatiles (CO_2)	-	0.8	1.1	1.4	4.1	6.9	10.7	14.6
Total	100.9	101.0	103.2	100.9	101.0	98.4	99.6	103.2
[benzyl-^{14}C] R-isomer of mandestrobin, RB, Sharkey, ROM-0051								
mandestrobin	98.7	89.0	81.5	73.9	68.1	61.1	56.6	51.4
5-COOH-M	ND	1.6	5.5	5.7	5.0	5.1	5.7	6.1
2-COOH-M	ND	1.3	3.5	4.2	4.4	5.7	6.1	6.9
5-$CONH_2$-M	ND	ND	ND	ND	1.6	2.4	2.9	2.9
2-$CONH_2$-M	ND	ND	ND	ND	0.7	1.2	1.6	1.5
MCBX	ND	1.6	0.4	0.7	0.5	0.4	0.6	0.6
De-Xy-M	1.1	ND	ND	0.2	ND	ND	ND	ND
Dx-CA-M	ND	1.6	3.4	4.6	5.5	4.8	4.5	5.2
unknown A	ND	0.7	0.6	1.1	1.8	2.4	2.3	2.0
Other unknowns	2.6	2.5	2.0	3.0	2.5	3.5	4.0	3.3
Soil bound PES	0.9	2.2	3.9	5.0	6.9	8.4	9.9	12.2
Volatiles (CO_2)	-	1.0	2.0	2.9	4.5	6.2	6.7	10.2
Total	103.3	100.8	102.1	101.6	102.2	101.3	100.6	103.0
[phenoxy-^{14}C] R-isomer of mandestrobin, RP, KD Manning, ROM-00051								
mandestrobin	98.0	94.2	90.8	82.1	70.8	61.4	51.2	51.4
5-COOH-M	ND	3.6	5.2	7.2	7.9	8.2	9.4	10.1
2-COOH-M	ND	1.4	2.1	3.1	4.0	5.2	6.4	6.6
5-$CONH_2$-M	ND	ND	ND	ND	0.8	1.3	1.9	0.7
2-$CONH_2$-M	ND	ND	ND	ND	0.3	0.6	1.0	0.6
MCBX	ND	0.2	0.4	0.4	0.4	0.5	0.6	0.8
De-Xy-M	1.4	0.3	ND	ND	0.1	ND	ND	ND
Dx-CA-M	ND	0.2	1.1	1.6	4.8	6.3	8.3	7.6
unknown A	ND	0.1	0.1	0.4	0.8	1.0	1.7	1.4
Other unknowns	2.5	3.1	1.8	2.3	3.0	5.5	4.9	4.6
Soil bound PES	0.4	1.5	2.1	2.7	4.3	5.7	8.0	9.0
Volatiles (CO_2)	-	0.7	1.2	2.0	3.8	5.2	7.6	11.0
Total	102.4	101.5	102.3	101.4	101.2	101.1	102.4	104.3

- not analysed

ND = not detected

Table 55 Distribution of unextracted residues (as % of applied radioactivity) after 362–364 days incubation

Sample	RB 362D-34 Soxhlet	SB 362D-53 Soxhlet	RP 362–74 Soxhlet	RP 362D-88 dismembrator	D-364D-42 Soxhlet	E-364D-50 Soxhlet	F-364D-68 Soxhlet
Soil name	Penn Series	Penn Series	Penn Series	Penn Series	Atwater	Sharkey	KD Manning
PES	10.4	11.5	17.6	17.4	11.43	12.67	9.36
Soxhlet or dismembrator extract	2.1	2.1	2.1	2	0.97	2.37	1.12
humin (PFD)	3.6	3.7	6	-	3.25	3.73	3.59
humic acid (HA)	1.7	2	4	-	2.91	1.72	2.4
fulvic acid (FA)	2.6	3.3	4.9	-	2.95	2.06	2.27
- FA organosoluble	1.3	1.3	1.6	-	-	-	-
- FA aqueous	1.2	1.8	3.1	-	-	-	-
Total recovery	9.9	11.1	16.9	-	10.08	9.89	9.38
Mass balance	95.3	96	96.2	-	88.1	78.1	100.20%

Sample	RB 362D-34 Soxhlet	SB 362D-53 Soxhlet	RP 362–74 Soxhlet	RP 362D-88 dismembrator	D-364D-42 Soxhlet	E-364D-50 Soxhlet	F-364D-68 Soxhlet
(%)							
Soxhlet or dismembrator extract identification							
mandestrobin	0.1	0.1	0.7	ND	-	-	-
5-COOH-M	ND	0.1	0.2	0.1	-	-	-
2-COOH-M	ND	0.1	ND	ND	-	-	-
5-$CONH_2$-M	0.2	0.2	0.7	0.1	-	-	-
2-$CONH_2$-M	0.1	0.1	ND	ND	-	-	-
MCBX	ND	ND	ND	ND	-	-	-
De-Xy-M	ND	ND	na	na	-	-	-
DX-CA-M	ND	ND	na	na	-	-	-
DMP	na	na	ND	ND	-	-	-
Others [a]	1.5	1.4	0.5	1.8	-	-	-
Total	1.9	2	2	1.9	-	-	-

na = not applicable (label not on this molecule);-not analysed

Aerobic degradation in soil-laboratory studies with 2-COOH-mandestrobin

Study 7: The degradation of 2-COOH-mandestrobin under aerobic laboratory conditions was studied in three terrestrial soils from Europe [Lewis & Gilbert, 2010 a, ROM-0017]. Soil characteristics are reported in Tables 56 and 57. The soil (50 g) was treated with [^{14}C-benzyl]-2-COOH-mandestrobin at an application rate of 0.88 mg ai/kg dry weight soil (equivalent to a field application rate of 0.2 kg ai/ha, assuming an incorporation depth of 2.5 cm, a bulk density of 1.0 g/cm^3 and assuming 100% conversion to the metabolite and considering the different molecular weights). The active substance was dispensed on the soil surface and mixed with the soil. Soil samples were incubated under aerobic conditions in the dark at 20 ± 2 °C for 120 days. Volatile compounds were passed through a series of trapping solutions (ethanediol trap, 2% liquid paraffin in xylene trap, 2 M NaOH solutions) to collect any volatile degradation products including CO_2. Soil and trapping solutions were sampled at 0, 7, 14, 30, 60–62, and 120 days after treatment. Soil moisture was adjusted to the water holding capacity (WHC) at pF2 every 8 days.

Table 56 Soil characteristics for studies ROM-0017

Soil name	Speyer 5M	SK920191	SK104691
Study	ROM-0017 ROM-0018	ROM-0017 ROM-0018	ROM-0017 ROM-0018
Location	Mechters heim, Rheinland-Pfalz Germany	South Witham, Lincolnshire, UK	Chelmorton, Derbyshire, UK
Soil texture (USDA) [a]	sandy loam	clay loam	silt loam
-- Sand (%)	62	38	26
-- Silt (%)	28	27	55
-- Clay (%)	11	35	19
Organic Carbon (%) [b]	1.2	4.1	3.2
Organic Matter (%)	2.1	7.1	5.5
CEC (meq/100 g)	15	30.6	20.8
pH (H_2O)	8.3	7.6	6.5

Soil name	Speyer 5M	SK920191	SK104691
pH (KCl)	7.8	7.3	5.3
pH ($CaCl_2$)	7.2	7.5	5.6
Water Holding Capacity at pF 0 (0.001 bar) [%]	40.9	62.8	63.1
Water Holding Capacity at pF 2.0 (0.1 bar) [%]	19.6	31.5	39.0
Water Holding Capacity at pF 2.5 (0.33 bar) [%]	14.6	26.8	26.9
Disturbed bulk density (g/cm^3)	not analysed	1.2	0.9

[a] Classification according to United States Department of Agriculture (USDA)

[b] Organic C = organic matter/1.724 based on the certificate values

Table 57 Soil viability

Soil name	Speyer 5M	SK920191	SK104691
Study	ROM-0017	ROM-0017	ROM-0017
Microbial biomass (mg microbial carbon/kg soil) at DAT=0 [a]	322	1421	759
Microbial biomass (mg microbial carbon/kg soil DM) at DAT = 120 [a]	155	800	304
% organic carbon at DAT=0	2.7	3.5	2.4
% organic carbon at DAT = 120	1.3	2.0	1.0

[a] Fumigation/Extraction method

Soils were extracted three times with acetone/water (9:1, v/v), twice with acetone/0.1 M HCl (5:1, v/v) and once with acetone. Unextracted residues of the DAT 60–120 samples were treated with 0.5 M NaOH for 24 hrs and the precipitate was separated off as the humic acid fraction. The supernatant was acidified to pH 1 with 5 M HCl and the supernatant was separated off as the fulvic acid fraction. The precipitate was reconstituted in 0.5 M NaOH as the humic acid fraction. Soil samples and trapping solutions were analysed by (combustion) LSC. Recovery of applied activity ranged from 93% to 100%.

Extracts were analysed by HPLC and 2D-TLC techniques using co-chromatography with reference standards. Reference substance used were 2-COOH-mandestrobin, De-Xy-mandestrobin and DX-CA-mandestrobin. The nature of the radioactivity in the NaOH traps was investigated by barium chloride solution addition. Dissolved $^{14}CO_2$ would be precipitated as insoluble barium ^{14}C-carbonate. The findings are summarized in Table 58.

In three European soils, 2-COOH-mandestrobin declined to 3.2–4.4% AR at the end of the study. The ^{14}C-benzyl-labeled 2-COOH-mandestrobin degraded mainly to CO_2 (up to 43–54% AR) and unextracted residues (up to 37–47% AR). Up to four unknown peaks represented up to 13% AR, but the maximum of any single degradation product was < 5% AR. De-Xy-mandestrobin and DX-CA-mandestrobin were detected at low levels (< 0.5% AR). The partitioning of the bound residue into humic acid, fulvic acid and humin like fractions is presented in Table 50.

DT_{50} and DT_{90} values for 2-COOH-mandestrobin were in the range 18–26 days and 60–86 days, respectively, for SFO kinetics and are summarized in Table 64.

Note by the reviewer:

The presence of the plant metabolites 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin and the presence of soil metabolites 2-$CONH_2$-mandestrobin and 5-$CONH_2$-mandestrobin was not verified in these degradation studies.

Table 58 Distribution of radioactivity (% of applied radioactivity) in 3 European soils after application of ^{14}C-benzyl labelled 2-COOH-mandestrobin (means of duplicate samples

Speyer 5M (^{14}C-benzyl) ROM-0017	Interval in days	0	7	14	30	60	120
	2-COOH-mandestrobin	97.6	87.5	72.3	45.0	17.0	4.4
	De-Xy-mandestrobin	ND	0.2	ND	ND	ND	ND
	DX-CA-mandestrobin	ND	ND	0.1	ND	ND	ND
	Extracted unknowns [b]	0.8	3.2	8.1	12.8	8.2	0.4
	Unextracted	0.3	6.4	12.4	24.5	39.3	42.5
	CO_2 [a]	-	1.7	5.4	14.7	32.3	47.8
	Mass balance [c]	98.8	98.9	98.3	97.0	96.7	95.1
SK920191 (^{14}C-benzyl) ROM-0017	Interval in days	0	7	14	30	60	120
	2-COOH-mandestrobin	97.3	75.9	59.2	35.1	12.8	4.4
	De-Xy-mandestrobin	ND	ND	ND	ND	ND	ND
	DX-CA-mandestrobin	ND	ND	ND	ND	0.8	ND
	Extracted unknowns [b]	0.8	4.5	6.7	7.1	2.6	0.6
	Unextracted	1.0	15.1	24.3	36.1	46.5	46.7
	CO_2 [a]	-	2.9	7.5	17.7	32.9	43.2
	Mass balance [c]	99.1	98.4	97.7	96.1	95.6	94.8
SK104691 (^{14}C-benzyl) ROM-0017	Interval in days	0	7	14	30	60	120
	2-COOH-mandestrobin	96.7	71.3	54.2	32.9	8.2	3.2
	De-XY-mandestrobin	ND	ND	ND	ND	ND	ND
	DX-CA-mandestrobin	ND	1.3	0.9	0.6	ND	ND
	Extracted r unknowns [b]	1.3	2.1	3.4	3.2	3.3	0.8
	Unextracted	1.5	19.3	27.7	37.2	42.9	36.7
	CO_2 [a]	-	4.0	10.6	19.9	42.4	53.7
	Mass balance [c]	99.5	97.9	96.8	92.8	96.7	94.4

- not analysed; ND = not detected

[a] Ethanediol and paraffin xylene traps did not contain any radioactivity

[b] Includes unresolved background; largest unknown is 0.2–4.8% AR

[c] Sum of radioactivity in extracts, post extracted solids and volatiles before HPLC separation

Table 59 Distribution of unextracted residues (as % of applied radioactivity) into fulvic acids, humic acids and humin after 120 days incubation

Soil	fulvic acid fraction	humic acid fraction	humin fraction	Study
Speyer 5M (^{14}C-benzyl) – 120 DAT	11.3	12.6	15.6	ROM-0017
SK920191 (^{14}C-benzyl) – 120 DAT	8.7	13.2	24.9	ROM-0017
SK104691 (^{14}C-benzyl) – 60 DAT	10.8	21.2	9.4	ROM-0017

Notes by the reviewer:

Within EU the equivalent field application rates for this study was calculated based on an incorporation depth of 5 cm and a bulk density 1.5 g/cm^3. Using these default values and assuming 100% conversion to the metabolite and considering the different molecular weights, the equivalent field application rate for a treatment of 0.88 mg ai/kg soil would be 0.6 kg ai/ha.

Aerobic degradation in soil-laboratory studies with 5-COOH-mandestrobin

Study 8: The rate of aerobic degradation of 5-COOH-mandestrobin was studied in three European soils [Lewis & Gilbert, 2010b, ROM-0018]. Soil characteristics are reported in Table 56 and Table 60.

The soil was treated with [^{14}C-benzyl]-5-COOH-mandestrobin as described for aerobic soil degradation study 7.

Table 60 Soil viability for study ROM-0018

Soil name	Speyer 5M	SK920191	SK104691
Study	ROM-0018	ROM-0018	ROM-0018
Location	Mechters heim, Rheinland-Pfalz Germany	South Witham, Lincolnshire, UK	Chelmorton, Derbyshire, UK
Microbial biomass (mg microbial carbon/kg soil) at DAT=0 [a]	340	1655	851
Microbial biomass (mg microbial carbon/kg soil) at DAT = 120 [a]	189	1040	428
Organic carbon (%) at DAT = 0	2.8	4.0	2.7
Organic carbon (%) at DAT = 120	1.6	2.5	1.3

[a] Fumigation/Extraction method

Soil and trapping solutions were sampled, extracted and analysed as described for study 6. Mass balances ranged from 95% to 104%. Results are shown in Rable 61

In three European soils, 5-COOH-mandestrobin declined to 8.2–16% AR at the end of the study. The ^{14}C-benzyl-labeled 5-COOH-mandestrobin degraded mainly to CO_2 (up to 38–52% AR) and unextracted residues (up to 37–48% AR). Up to three unidentified degradation products were detected; one of them reached up to 5.9% AR at one sampling time (DAT 14 in Speyer soil); all other degradation products did not exceed 5% AR. De-Xy-mandestrobin was not detected; DX-CA-mandestrobin was detected at low levels (< 0.5% AR). The partitioning of the bound residue into humic acid, fulvic acid and humin like fractions is presented in Table 62.

DT_{50} and DT_{90} values for 5-COOH-mandestrobin were in the range 22–41 days and 73–136 days, respectively, for SFO kinetics and are summarized in Table 64.

Note by the reviewer:

The presence of the plant metabolites 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin and the presence of soil metabolites 2-$CONH_2$-mandestrobin and 5-$CONH_2$-mandestrobin was not verified in these degradation studies.

Table 61 Distribution of radioactivity (% of applied radioactivity) in 3 European soils after application of ^{14}C-benzyl labelled 5-COOH-mandestrobin (means of duplicate samples)

Speyer 5M (^{14}C-benzyl) ROM-0018	Interval in days	0	7	14	30	62	120
	5-COOH-mandestrobin	102.7	91.9	77.4	60.4	36.2	16.4
	De-Xy-mandestrobin	ND	ND	ND	ND	ND	ND
	DX-CA-mandestrobin	ND	ND	ND	ND	ND	ND
	Extracted unknowns [b]	1.3	0.8	5.7	4.7	5.8	1.9
	Unextracted	0.4	6.6	12.4	22.3	31.4	36.6
	CO_2 [a]	-	1.5	4.7	12.2	24.6	40.6
	Mass balance [c]	104.4	100.8	100.2	99.5	98.0	95.4
SK920191 (^{14}C-benzyl) ROM-0018	Interval in days	0	7	14	30	62	120
	5-COOH-mandestrobin	98.5	79.6	65.1	39.5	30.9	11.9
	De-Xy-mandestrobin	ND	ND	ND	ND	ND	ND

Speyer 5M (^{14}C-benzyl) ROM-0018	Interval in days	0	7	14	30	62	120
	DX-CA-mandestrobin	ND	ND	ND	ND	ND	ND
	Extracted unknowns [b]	2.4	0.7	2.9	1.7	2.1	0.3
	Unextracted	2.1	16.7	24.3	39.5	41.8	48.4
	CO_2 [a]	-	2.1	7.1	18.1	22.9	38.0
	Mass balance [c]	103.1	99.1	99.3	98.7	97.6	98.6
SK104691 (^{14}C-benzyl) ROM-0018	Interval in days	0	7	14	30	62	120
	5-COOH-mandestrobin	99.0	75.8	62.9	36.1	15.6	8.2
	De-XY-mandestrobin	ND	ND	ND	ND	ND	ND
	DX-CA-mandestrobin	ND	0.3	ND	ND	ND	ND
	Extracted unknowns [b]	1.6	1.2	0.7	0.3	0.6	ND
	Unextracted	3.1	20.5	27.7	38.7	41.0	37.6
	CO_2 [a]	-	3.4	9.0	20.5	38.8	52.2
	Mass balance [c]	103.7	101.1	100.3	95.6	95.9	98.0

ND = not detected

[a] Ethanediol and paraffin xylene traps did not contain any radioactivity

[b] Includes unresolved background; largest unknown represents 0.1–5.9% AR

[c] Sum of radioactivity in extracts, post extracted solids and volatiles prior to HPLC separation

Table 62 Distribution of unextracted residues (as % of applied radioactivity) into fulvic acids, humic acids and humin after 120 days incubation

Soil	fulvic acid fraction	humic acid fraction	humin fraction	Study
Speyer 5M (^{14}C-benzyl) – 120 DAT	10.5	11.1	17.2	ROM-0018
SK920191 (^{14}C-benzyl) – 120 DAT	8.2	13.1	25.7	ROM-0018
SK104691 (^{14}C-benzyl) – 62 DAT	9.3	21.8	8.1	ROM-0018

Note by the reviewer:

Within EU the equivalent field application rates for this study was calculated based on an incorporation depth of 5 cm and a bulk density 1.5 g/cm^3. Using these default values and assuming 100% conversion to the metabolite and considering the different molecular weights, the equivalent field application rate for a treatment of 0.88 mg ai/kg soil would be 0.6 kg ai/ha.

Kinetic endpoints for aerobic degradation in soil – laboratory studies

The rate of degradation of the R- and S-isomers of mandestrobin was calculated using kinetic modelling of the residue data for six soils in Europe [Graham & Gilbert, 2011a-d, ROM-0028, ROM-0029, ROM-0030 and ROM-0031] and four soils in the USA [Gohre, 2013, ROM-0050; Maurer & Gohre, 2013, ROM-0051]. Curves were established through the data points assuming single first order using KinGUI software (version 1.1 for EU soils, version 2.2012.216.1027 for USA soils). The goodness of fit was assessed by visual inspection and an error criterion based on a chi-squared test at a probability of 0.05. The findings are summarized in Table 63.

In a second study [Jarvis *et al*., 2012, ROM-0035] data from the six EU soil studies on the R- and S-isomer of mandestrobin were combined and fitted to SFO (single first order), FOMC (first order multicompartment) and DFOP (double first order in parallel) kinetics according to the FOCUS (2006) guidance to determine DT_{50} values. Curves were established through the data points using KinGUII software (version 2). The findings are also summarized Table 63.

In a third study [Jarvis and Montesano, 2019, UK0–4844] data from the 4 USA soil studies were fitted to SFO (single first order), FOMC (first order multicompartment) and DFOP (double first order in parallel) kinetics according to the FOCUS (2014) guidance to determine DT_{50} values. The

data from the three labels used in the Penn series were combined (R-isomer benzyl label, S-isomer benzyl label, R-isomer phenoxy label). The other three USA soils were based on the R-isomer benzyl label. Curves were established through the data points using the CAKE 3.3 model. The FOMC model was invalid as the DT90 value was not reached during the study lifetime. The slow phase for the DFOP fit prioritises the experimental data at the later time points to provide a highly conservative DT_{50} value for simulation modelling. In the case of these studies, incubations continued well beyond the 120 days recommended in the OECD 307 study guideline for aerobic soil studies (studies were 365 days duration) and the slower degradation at later times can be explained by the loss of microbial activity. Overall therefore, the SFO kinetics are considered appropriate. The findings are summarized Table 63.

Best fit DT_{50} values for the R- and S-isomer of mandestrobin ranged from 40–378 days and 60–323 days respectively. Corresponding DT_{90} values of the *R*- and S-isomer of mandestrobin ranged from 132–1255 days and 200->1000 days, respectively.

When the data for the R- and S-isomer of mandestrobin were combined, the geometric mean DT_{50} for mandestrobin is 102 days for the EU soils, 269 days for the US soils and 151 days for all soils together.

Table 63 Rate of degradation of the *R*- and S-isomer of mandestrobin

Soil	ref	pH ($CaCl_2$)	C_{org} (%)	Clay (%)	label position	Model	DT_{50} (days)	DT_{90} (days)	χ^2 (%)	R^2
R-isomer of mandestrobin										
EU-Speyer 5 M	ROM-0028	7.2	1.3	11	^{14}C-benzyl	SFO	53.4	177.3	4.18	0.9807
	ROM-0028				^{14}C-phenoxy	SFO	84.1	279.4	3.53	0.9659
EU-Speyer 2.2	ROM-0028	5.5	2.1	6.0	^{14}C-benzyl	SFO	227.1	754.4	4.04	0.8278
EU-SK920191	ROM-0028	7.6	3.8	30	^{14}C-benzyl	SFO	50.6	167.9	5.26	0.9697
EU-Chelmorton (SK104691)	ROM-0028	5.9	3.4	20	^{14}C-benzyl	SFO	102.1	339.2	8.18	0.8335
EU- Aschard	ROM-0030	7.4	1.3	23	^{14}C-benzyl	SFO	50.2	166.9	4.05	0.9849
EU- Monteil	ROM-0030	7.7	1.4	32	^{14}C-benzyl	SFO	39.8	132.1	2.28	0.9960
USA-Penn Series	ROM-0050	nr	2.0	25	^{14}C-benzyl	SFO	151.3	502.7	7.33	0.9481
	ROM-0050				^{14}C-phenoxy	SFO	144.3	479.2	7.57	0.95
USA-Atwater	ROM-0051	nr	0.39	2.0	^{14}C-benzyl	SFO	295.0	980.1	4.574	0.906
USA-Sharkey	ROM-0051	nr	0.81	26	^{14}C-benzyl	SFO	377.8	1254.9	5.25	0.8991
USA-KD Manning	ROM-0051	nr	1.8	14	^{14}C-benzyl	SFO	324.9	1079.2	3.702	0.9561
S-isomer of mandestrobin										
EU-Speyer 5 M	ROM-0029	7.2	1.3	11	^{14}C-benzyl	SFO	85.4	283.7	2.45	0.9702
EU-Speyer 2.2	ROM-0029	5.5	2.1	6.0	^{14}C-benzyl	SFO	322.6	>1000	3.00	0.8256
EU-SK920191	ROM-0029	7.6	3.8	30	^{14}C-benzyl	SFO	92.0	305.5	2.91	0.9773
EU-Chelmorton (SK104691)	ROM-0029	5.9	3.4	20	^{14}C-benzyl	SFO	120.0	398.6	8.17	0.7927
EU-Aschard	ROM-0031	7.4	1.3	23	^{14}C-benzyl	SFO	74.4	247.1	1.87	0.9940
EU-Monteil	ROM-	7.7	1.4	32	^{14}C-benzyl	SFO	60.1	199.7	3.33	0.9806

Soil	ref	pH ($CaCl_2$)	C_{org} (%)	Clay (%)	label position	Model	DT_{50} (days)	DT_{90} (days)	χ^2 (%)	R^2
	0031									
USA-Penn Series	ROM-0050	nr	1.2	25	^{14}C-benzyl	SFO	147.3	489.2	7.06	0.9555
R- and S-isomer of mandestrobin (combined data)										
EU-Speyer 5 M	ROM-0035	7.2	1.3	11	^{14}C-benzyl ^{14}C-phenoxy	SFO	75.7	251.5	2.4	-
EU-Speyer 2.2	ROM-0035	5.5	2.1	6.0	^{14}C-benzyl	SFO	268.5	892.0	3.6	-
EU-SK920191	ROM-0035	7.6	3.8	30	^{14}C-benzyl	DFOP	76.5 [b]	241.9	2.0	-
EU-Chelmorton (SK104691)	ROM-0035	5.9	3.4	20	^{14}C-benzyl	DFOP	245.8 [b]	681.1	1.1	-
EU-Aschard	ROM-0035	7.4	1.3	23	^{14}C-benzyl	SFO	61.6	204.7	3.1	-
EU-Monteil	ROM-0035	7.7	1.4	32	^{14}C-benzyl	SFO	49.0	162.9	2.9	-
USA – Penn	UK0–4844	nr	2.0	25	^{14}C-benzyl ^{14}C-phenoxy	SFO	145	482	7.1	-
USA-Atwater	UK0–4844	nr	0.39	2.0	^{14}C-benzyl	SFO	295	980	4.6	-
USA-Sharkey	UK0–4844	nr	0.81	26	^{14}C-benzyl	SFO	378	1250	5.3	-
USA-KD Manning	UK0–4844	nr	1.8	14	^{14}C-benzyl	SFO	325	1080	3.7	-
Geometric mean EU soils [a]							102	-		
Geometric mean USA soils [a]							269			
Geometric mean all soils [a]							151			

[a] calculated by the reviewer.

[b] DT_{50} values of 76.5 and 245.8 days are the DT_{50} values resulting from the k2 (slow phase) rate constants in the soils where DFOP kinetics are selected (soils SK920191 and Chelmorton) – this is the standard FOCUS kinetics approach for deriving DT_{50} values that will be used as modelling inputs for environmental exposure assessment. DT_{50} values of 64.4 and 110.1 days are the overall DT_{50} values (i.e. mixture of K1 and K2 rate constants for the DFOP fit for soils SK920191 and Chelmorton). This direct DT_{50} calculation from DFOP kinetics would not be used in the environmental exposure assessment.

The rate of degradation of 2-COOH-mandestrobin and 5-COOH-mandestrobin was calculated in a similar way using kinetic modelling of the residue data for three soils in Europe [Lewis & Gilbert, 2010a/b, ROM-0017; ROM-0018]. The findings are summarized in Table 64.

In a second study [Jarvis *et al*., 2012, ROM-0035] data from the EU soil studies on R- and S-mandestrobin were combined with the 3 EU soil studies on 2-COOH-mandestrobin and 5-COOH-mandestrobin and fitted to SFO, FOMC and DFOP kinetics according to the FOCUS (2006) guidance to determine the DT_{50} values for the metabolites. Curves were established through the data points using KinGUII software (version 2). The findings are also summarized Table 64.

DT_{50} values for 2-COOH and 5-COOH-mandestrobin ranged from 18-30 days and 11–41 days respectively. Corresponding DT_{90} values 2-COOH and 5-COOH-mandestrobin ranged from 60–148 days and 36-136 days, respectively.

When the all data were combined, the geometric mean DT_{50} is 27 days for 2-COOH-mandestrobin in EU soils and 26 days for 5-COOH-mandestrobin in EU soils. US soils were not investigated.

Table 64 Rate of degradation of 2-COOH- and 5-COOH-mandetrobin based on single first order (SFO) kinetics

Soil	ref	pH ($CaCl_2$)	C_{org} (%)	Clay (%)	label position	DT_{50} (days)	DT_{90} (days)	χ^2 (%)	R^2
2-COOH-mandestrobin									
EU-Speyer 5 M [a]	ROM-0017	7.2	1.2	11	^{14}C-benzyl	25.9	86.1	4.01	0.993
EU-SK920191 [a]	ROM-0017	7.5	4.1	35	^{14}C-benzyl	20.4	67.8	2.03	0.999
EU-SK104691 [a]	ROM-0017	5.6	3.2	19	^{14}C-benzyl	18.1	60.0	3.36	0.995
EU-Speyer 2.2 [b]	ROM-0035	5.5	2.1	6.0	^{14}C-benzyl	44.7	148.5	12.1	-
EU – Aschard [b]	ROM-0035	7.4	1.3	23	^{14}C-benzyl	29.5	97.9	4.3	-
EU – Monteil [b]	ROM-0035	7.7	1.4	32	^{14}C-benzyl	29.0	96.5	3.0	-
Geometric mean						23.0 [c] 26.7 [e]			
5-COOH-mandestrobin									
EU-Speyer 5 M	ROM-0018	7.2	1.2	11	^{14}C-benzyl	41.0	136.1	2.37	0.993
EU-SK920191	ROM-0018	7.5	4.1	35	^{14}C-benzyl	30.3	100.5	7.95	0.968
EU-SK104691	ROM-0018	5.6	3.2	19	^{14}C-benzyl	21.9	72.7	4.50	0.994
EU-Speyer 2.2 [b]	ROM-0035	5.5	2.1	6.0	^{14}C-benzyl	10.8	35.9	8.6	-
EU – Aschard [b]	ROM-0035	7.4	1.3	23	^{14}C-benzyl	38.3	127.1	1.4	-
EU – Monteil [b]	ROM-0035	7.7	1.4	32	^{14}C-benzyl	24.9	82.7	2.9	-
Geometric mean						30.4 [d] 25.6 [e]			

[a] DT_{50} values calculated based on results from 2-COOH-mandestrobin or 5-COOH-mandestrobin applied to soils

[b] DT_{50} values estimated based on results from R- and S-mandestrobin applied to soils

[c] Geometric mean as reported in ROM-0035 including degradation data from the EU soil studies on R- and S-mandestrobin from Aschard and excluding Monteil and Speyer 2.2 as these soils had metabolite percentages below 10% AR.

[d] Geometric mean as reported in ROM-0035 including degradation data from the EU soil studies on R- and S-mandestrobin from Aschard and Monteil and excluding Speyer 2.2 as this soil had metabolite percentages below 10% AR.

[e] Geometric mean calculated by the reviewer, based on all available data.

Overview of the metabolism pathway of mandestrobin in aerobic soil

Mandestrobin was metabolized by several routes in aerobic soil (depicted in Figure 5).

- Oxidation of the methyl groups on the phenoxy ring formed 2-COOH-mandestrobin and 5-COOH-mandestrobin, which were further metabolized to the corresponding amides (2-CONH2-mandestrobin and 5-CONH2-mandestrobin). The acid degradates (2-COOH-mandestrobin and 5-COOH-mandestrobin) were formed primarily during the early part of the aerobic exposure.

- Cleavage of the ether linkage formed De-Xy-mandestrobin, which was subsequently oxidized to DX-CA-mandestrobin.
- Demethylation of the 2-methoxy group to form MCBX was a minor route of degradation under aerobic conditions.
- Mineralization (formation of bound residues and CO_2) were significant, reaching maximums of 23–39% of the AR.

R/S isomerisation of parent compound did not occur.

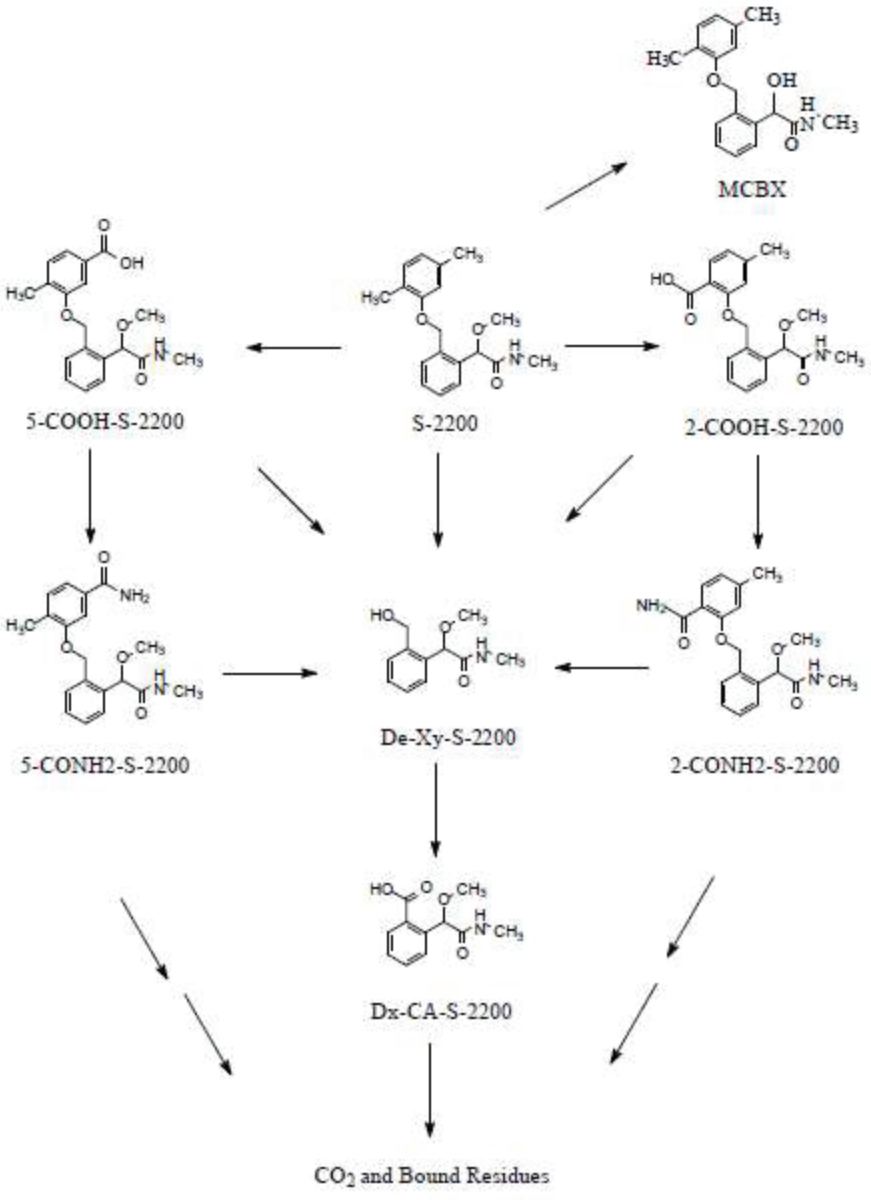


Figure 5 Overview of the degradation pathway of mandestrobin (S-2200) in aerobic soil

Field dissipation studies

Study 1: Four field dissipation trials were carried out in Southern France, Spain, Austria and Germany in May 2009 to June 2010 to study the behaviour of mandestrobin in soil [Lewis, 2012, ROR-0010]. Mandestrobin was sprayed as an SC formulation to bare soil at an actual rate of 0.20–0.21 kg ai/ha. Soil characteristics for the four soils are summarized in Table 65. The biomass determinations showed that all soils had a viable microbial biomass throughout the one year duration of the field phase. Soil cores were taken immediately prior to spraying, within 3 hours post-application and at 2–4, 7, 14-15, 29-33, 61–62, 84-98, 182–190, 272–280, and 365–372 days after application. All soil cores were frozen at -10 °C or lower within 8 hrs of collection. Soil samples were analysed within 320 days of field collection, which was within the 12 month period of storage stability demonstrated in study 8202025.

Table 65 Soil characteristics for European soils in field dissipation studies

Soil name	8202031/1	8202031/2	820203/3	8202031/4
Location	Nimes, Languedoc Roussillon, France	Carlet, Valencia, Spain	Gemeinlebarn, Lower Austria	Kutenholz, Niedersachsen, Germany
Soil texture (USDA) [a]	clay	loam	silt loam	loamy sand
-- Sand (%)	23	43	35	84
-- Silt (%)	35	31	51	13
-- Clay (%)	42	26	14	3
Organic Carbon (%) [b]	1.4	1.3	2.5	1.7
Organic Matter (%)	2.4	2.2	4.3	2.9
CEC (meq/100 g)	9.5	10	11	5.6
pH (H_2O)	8.6	8.7	8.3	6.4
pH (KCl)	7.8	7.8	7.9	5.1
pH ($CaCl_2$)	7.6	7.8	7.7	5.4
Water Holding Capacity at pF 0 (FC at 0.001 bar) [%]	58.1	46.8	65.4	39.3
Water Holding Capacity at pF 2.0 (FC at 0.1 bar) [%]	27.5	23.4	38.2	12.4
Water Holding Capacity at pF 2.5 (FC at 0.33 bar) [%]	25.7	18.8	28.0	7.2
Microbial biomass (µg microbial carbon/g soil) prior to application [c]	235.4	182.7	410.2	143.1
Microbial biomass (µg microbial carbon/g soil DM) at 12 months post-application [c]	305.1	261.5	469.3	150.3

[a] Classification according to United States Department of Agriculture (USDA)

[b] Organic C = organic matter/1.724

[c] Fumigation/Extraction method

For residue analysis, the soil cores were cut into soil horizons (0–10 cm, 10–20 cm and 20–30 cm) and homogenised by 4 mm and 2 mm mesh size sieves. Soil samples were analysed for the R- and S-isomer of mandestrobin, De-Xy-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin using LC-MS/MS method CLE 8231772–01V with a reported LOQ of 0.005 mg/kg for the metabolites and 0.0025 mg/kg for the R- and S-isomers of mandestrobin. All residue results are corrected for moisture content and are presented as mg/kg on a dry weight basis.

The results for the 0–10 cm and 0–30 cm soil layers for the four soils are shown in Table 66. The residues for the 0–30 cm soil layers are obtained by summing the residues found in the 0–10, 10–20 and 20–30 cm soil layers.

Residues in control samples (collected prior to application) were generally below 0.3LOQ, except for 2 out of 12 soil samples from the Spanish trials (0.0014–0.0024 mg/kg and 0.0015–0.0024 mg/kg for the R- and S-isomers, respectively). The majority of the procedural recovery values

for each analytical batch were within the acceptance criteria of 70–120%, except for 2–4 out of 10–12 batches in each of the soils for the R- and S-isomer of mandestrobin (126–224%) and/or 5-COOH-mandestrobin (152–321%).

A flood occurred at the Spanish trial site which was caused by a leaking irrigation pipe. The study author indicated that the results of the samples taken before and after the flood occurred (84 and 98 days), demonstrated that the flooding had no adverse effect upon the outcome of the study.

Parent isomers were mainly found in the 0–10 cm soil layers. Maximum levels of parent isomers were found in the 0–10 cm soil layers on day 0–2 and ranged from 0.0431 mg/kg (German trial, day 2) to 0.0889 mg/kg (Austrian trial, day 0). Soil samples (0–10 cm) collected after 187 days in the Spanish trial or 365 days in the French trial did not contain parent isomers above the LOQ of 0.0025 mg/kg. In soil samples (0–10 cm) collected after 369-372 days, parent isomers were slightly above the LOQ (0.0027–0.0042 mg/kg) in the Austrian and German trials. In the 10–20 cm layers, levels of both isomers were below LOQ of 0.0025 mg/kg for the duration of the study in all trials, except for the samples collected on day 4 and 7 (0.0029–0.0034 mg/kg) in the Spanish trial and day 0 and 2 in the German trial (0.0037–0.0050 mg/kg). In the 20–30 cm layers, levels of both isomers were below the LOQ of 0.0025 mg/kg in all trials at all time points.

Metabolite 5-COOH-mandestrobin appeared at 61–190 days after application and was mainly found in the 0–10 cm soil layers. Metabolite 5-COOH-mandestrobin was found in the French trial between 61–183 days (up to 0.0072 mg/kg), the Spanish trial between 62–98 days (up to 0.0079 mg/kg) and the German trial at 190–369 days (up to 0.0074 mg/kg). In the Austrian trial metabolite 5-COOH-mandestrobin was not found (< 0.005 mg/kg). At all other time points no residues above LOQ of 0.005 mg/kg were observed in any of the soil layers.

Metabolite 2-COOH-mandestrobin appeared at 61–280 days after application and was mainly found in the 0–10 cm soil layers. Metabolite 2-COOH-mandestrobin was found in the French trial between 61–183 days (up to 0.012 mg/kg), the Spanish trial at 98 days (0.0051 mg/kg), the Austrian trial at 62–278 days (up to 0.0074 mg/kg), and the German trial at 280–369 days (up to 0.0094 mg/kg). At all other time points no residues above LOQ of 0.005 mg/kg were observed in any of the soil layers.

Metabolite De-Xy-mandestrobin remained below the LOQ of 0.005 mg/kg in all trials at all time points at all sampling depths, except in the Spanish trial, where metabolite De-Xy-mandestrobin was mainly found in the 0–10 cm layer between 4-62 days at levels up to 0.012 mg/kg.

Metabolite DX-CA-mandestrobin and the amide metabolites 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin were not analysed for.

Notes by the reviewer:

LC-MS/MS method CLE 8213772–01V for the determination of mandestrobin, De-XY-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin in soil is considered:

- Valid for the determination of De-Xy-mandestrobin and 2-COOH-mandestrobin in the range 0.005–0.5 mg/kg.
- Valid for the determination of the R- and S-isomer of mandestrobin in the range 0.1–0.25 mg/kg. The method is not valid at the reported LOQ of 0.0025 mg/kg (see analytical method section). As the residue levels in some of the control samples had levels up to 0.0024 mg/kg, the LOQ for mandestrobin is taken as 0.005 mg/kg.
- Not valid for the determination of 5-COOH-mandestrobin (see analytical method section).

The validity of the results for soil batches where the procedural recoveries for the R- and S-isomer of mandestrobin and 5-COOH-mandestrobin were above 120% was not affected: a) because the residue values in the soil samples were below the LOQ although the concurrent recoveries were

high; b) the duplicate sample was within the acceptance criteria in another batch and there was no significant difference between the duplicate samples.

Table 66 Soil residues (mg/kg dry weight soil) in field dissipation studies

DAT (days)	Depth (cm)	R-isomer of mandestrobin mg/kg dw	S-isomer of mandestrobin mg/kg dw	mandestrobin mg/kg dw	5-COOH-mandestrobin mg/kg dw	2-COOH-mandestrobin mg/kg dw	De-Xy-mandestrobin mg/kg dw
Trial 8202031/1, Nîmes, Southern France, single application, 0.20 kg ai/ha, 294-301 L/ha, at 11 June 2009							
0	0–10	0.0658	0.0661	0.132	< 0.005	< 0.005	< 0.005
	0–30	0.0669	0.0665	0.134	< 0.005	< 0.005	< 0.005
4	0–10	0.0284	0.0284	0.057	< 0.005	< 0.005	< 0.005
	0–30	0.0284	0.0284	0.057	< 0.005	< 0.005	< 0.005
7	0–10	0.0292	0.0277	0.057	< 0.005	< 0.005	< 0.005
	0–30	0.0292	0.0277	0.057	< 0.005	< 0.005	< 0.005
14	0–10	0.0205	0.0187	0.039	< 0.005	< 0.005	< 0.005
	0–30	0.0205	0.0187	0.039	< 0.005	< 0.005	< 0.005
29	0–10	0.0123	0.0121	0.024	< 0.005	< 0.005	< 0.005
	0–30	0.0123	0.0121	0.024	< 0.005	< 0.005	< 0.005
61	0–10	< 0.0025	0.0032	< 0.005	0.0072	0.0077	< 0.005
	0–30	< 0.0025	0.0038	0.006	0.0072	0.0077	< 0.005
92	0–10	0.0049	0.0069	0.012	0.0065	0.0053	< 0.005
	0–30	0.0058	0.0069	0.013	0.0065	0.0053	< 0.005
183	0–10	< 0.0025	< 0.0025	< 0.005	< 0.005	0.0097	< 0.005
	0–30	< 0.0025	< 0.0025	< 0.005	0.0068	0.012	< 0.005
278	0–10	0.0029	0.0033	0.006	< 0.005	< 0.005	< 0.005
	0–30	0.0029	0.0033	0.006	< 0.005	< 0.005	< 0.005
365	0–10	< 0.0025	< 0.0025	< 0.005	< 0.005	< 0.005	< 0.005
	0–30	< 0.0025	< 0.0025	< 0.005	< 0.005	< 0.005	< 0.005
Trial 8202031/2, Carlet, Spain, single application, 0.21 kg ai/ha, 320 L/ha, at 28 May 2009							
0	0–10	0.0804	0.0802	0.161	< 0.005	< 0.005	< 0.005
	0–30	0.0820	0.0825	0.164	< 0.005	< 0.005	< 0.005
4	0–10	0.0258	0.0265	0.052	< 0.005	< 0.005	0.010
	0–30	0.0305	0.0314	0.062	< 0.005	< 0.005	0.012
7	0–10	0.0178	0.0207	0.039	< 0.005	< 0.005	0.0090
	0–30	0.0207	0.0245	0.046	< 0.005	< 0.005	0.0090
14	0–10	0.0080	0.0088	0.017	< 0.005	< 0.005	0.0069
	0–30	0.0098	0.0113	0.021	< 0.005	< 0.005	0.0069
29	0–10	0.0128	0.0130	0.026	< 0.005	< 0.005	0.011
	0–30	0.0128	0.0130	0.026	< 0.005	< 0.005	0.011
62	0–10	0.0042	0.0085	0.013	0.0069	< 0.005	0.011
	0–30	0.0042	0.0090	0.013	0.0069	< 0.005	0.011
84	0–10	0.0039	0.0079	0.012	0.0075	< 0.005	< 0.005
	0–30	0.0047	0.0086	0.013	0.0075	< 0.005	< 0.005
98	0–10	0.0068	0.0045	0.011	0.0079	0.0051	< 0.005
	0–30	0.0084	0.0059	0.014	0.0079	0.0051	< 0.005
178	0–10	< 0.0025	< 0.0025	< 0.005	< 0.005	< 0.005	< 0.005
	0–30	< 0.0025	< 0.0025	< 0.005	< 0.005	< 0.005	< 0.005
272	0–10	< 0.0025	< 0.0025	< 0.005	< 0.005	< 0.005	< 0.005
	0–30	< 0.0025	< 0.0025	< 0.005	< 0.005	< 0.005	< 0.005
365	0–10	< 0.0025	< 0.0025	< 0.005	0.0052	< 0.005	< 0.005
	0–30	< 0.0025	< 0.0025	< 0.005	0.0052	< 0.005	< 0.005
Trial 8202031/3, Gemeinlebarn, Austria, single application, 0.20 kg ai/ha, 300 L/ha, at 4 June 2009							
0	0–10	0.0889	0.0885	0.177	< 0.005	< 0.005	< 0.005
	0–30	0.0914	0.0909	0.182	< 0.005	< 0.005	< 0.005
4	0–10	0.0644	0.0643	0.129	< 0.005	< 0.005	< 0.005
	0–30	0.0664	0.0663	0.133	< 0.005	< 0.005	< 0.005
7	0–10	0.0477	0.0472	0.095	< 0.005	< 0.005	< 0.005
	0–30	0.0483	0.0472	0.095	< 0.005	< 0.005	< 0.005
15	0–10	0.0325	0.0311	0.064	< 0.005	< 0.005	< 0.005
	0–30	0.0325	0.0311	0.064	< 0.005	< 0.005	< 0.005
33	0–10	0.0163	0.0171	0.033	< 0.005	< 0.005	< 0.005
	0–30	0.0163	0.0171	0.033	< 0.005	< 0.005	< 0.005

DAT (days)	Depth (cm)	R-isomer of mandestrobin mg/kg dw	S-isomer of mandestrobin mg/kg dw	mandestrobin mg/kg dw	5-COOH-mandestrobin mg/kg dw	2-COOH-mandestrobin mg/kg dw	De-Xy-mandestrobin mg/kg dw
62	0–10	0.0062	0.0161	0.022	< 0.005	0.0074	< 0.005
	0–30	0.0062	0.0180	0.024	< 0.005	0.0074	< 0.005
90	0–10	0.0044	0.0079	0.012	< 0.005	0.0057	< 0.005
	0–30	0.0050	0.0090	0.014	< 0.005	0.0057	< 0.005
182	0–10	< 0.0025	0.0049	0.005	< 0.005	< 0.005	< 0.005
	0–30	< 0.0025	0.0049	0.005	< 0.005	< 0.005	< 0.005
278	0–10	0.0037	0.0034	0.007	< 0.005	0.0060	< 0.005
	0–30	0.0037	0.0034	0.007	< 0.005	0.0060	< 0.005
372	0–10	0.0027	0.0034	0.006	< 0.005	< 0.005	< 0.005
	0–30	0.0027	0.0034	0.006	< 0.005	< 0.005	< 0.005
Trial 8202031/4, Kutenholz, Germany, single application, T1 0.20 kg ai/ha, 296 L/ha, at 27 May 2009							
0	0–10	0.0411	0.0397	0.081	< 0.005	< 0.005	< 0.005
	0–30	0.0469	0.0454	0.092	< 0.005	< 0.005	< 0.005
2	0–10	0.0431	0.0427	0.086	< 0.005	< 0.005	< 0.005
	0–30	0.0468	0.0464	0.093	< 0.005	< 0.005	< 0.005
7	0–10	0.0080	0.0097	0.018	< 0.005	< 0.005	< 0.005
	0–30	0.0085	0.0097	0.018	< 0.005	< 0.005	< 0.005
14	0–10	0.0107	0.0130	0.024	< 0.005	< 0.005	< 0.005
	0–30	0.0107	0.0130	0.024	< 0.005	< 0.005	< 0.005
30	0–10	0.0089	0.0083	0.017	< 0.005	< 0.005	< 0.005
	0–30	0.0096	0.0089	0.018	< 0.005	< 0.005	< 0.005
61	0–10	0.0097	0.0088	0.019	< 0.005	< 0.005	< 0.005
	0–30	0.0097	0.0088	0.019	< 0.005	< 0.005	< 0.005
86	0–10	0.0081	0.0088	0.017	< 0.005	< 0.005	< 0.005
	0–30	0.0081	0.0088	0.017	< 0.005	< 0.005	< 0.005
190	0–10	< 0.0025	< 0.0025	< 0.005	0.0074	< 0.005	< 0.005
	0–30	< 0.0025	< 0.0025	< 0.005	0.0074	< 0.005	< 0.005
280	0–10	0.0035	0.0064	0.010	0.0055	0.0094	< 0.005
	0–30	0.0045	0.0088	0.013	0.0055	0.0094	< 0.005
369	0–10	0.0042	0.0037	0.008	0.0068	0.0062	< 0.005
	0–30	0.0042	0.0037	0.008	0.0068	0.0062	< 0.005

The data obtained for R- and S-isomers of mandestrobin from analysis of the 0–10 cm, 10–20 cm, and 20–30 cm soil study samples were summed to get the mandestrobin concentrations. Simple first order kinetics (SFO), first order multi-compartment kinetics (FOMC) and bi-exponential double first order in parallel kinetics (DFOP) were used in order to determine the best-fit values for 50% (DT_{50}) and 90% (DT_{90}) dissipation for each trial site. All calculations were performed using KINGUI v1.1 software. Following FOCUS (2006), the suitability of the model was established based on the minimum error to pass the Chi-squared test at a probability of 0.05. The calculated DT_{50} and DT_{90} values for each trial and model are summarized Table 67.

The kinetics with visual acceptance and with the lower Chi-squared error % values were selected in each case. For all trial sites the DFOP model gave the best fit. No significant difference in the degradation rates of the R- and S-isomers of mandestrobin was observed. The DT_{50} and DT_{90} values calculated for mandestrobin (racemic mixture) for the DFOP model ranged from 2.3 to 8.3 days and 48 to 226 days, respectively.

Table 67 Summary of kinetics data for DT_{50} values of the *R*- and S-isomer of mandestrobin and mandestrobin (racemic mixture)

Model	χ^2 error (%)	visual fit	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)	visual fit	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)	visual fit	DT_{50} (days)	DT_{90} (days)
	R-isomer of mandestrobin				S-isomer of mandestrobin				mandestrobin (racemic mixture)			
8202031/1, Nimes, France												
SFO	27.95	2	6.58	21.86	28.01	2	5.90	19.61	27.75	2	6.26	20.81
FOMC	18.31	3	2.81	128.5	19.35	4	1.50	130.4	12.71	3	3.25	67.62

Model	χ^2 error (%)	visual fit	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)	visual fit	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)	visual fit	DT_{50} (days)	DT_{90} (days)
	R-isomer of mandestrobin				S-isomer of mandestrobin				mandestrobin (racemic mixture)			
DFOP	11.27	5	2.61	46.46	11.69	5	1.84	50.57	11.14	5	2.29	47.92
2802031/2, Carlet, Spain												
SFO	27.62	2	3.31	11.00	29.77	2	3.63	12.04	28.27	2	3.54	11.77
FOMC	13.52	4	1.63	38.61	13.11	3	1.63	56.84	11.59	4	1.84	40.36
DFOP	11.20	5	2.69	49.39	8.57	5	2.92	66.05	8.73	5	2.82	55.09
8202031/3, Gemeinlebarn, Austria												
SFO	12.13	4	10.21	33.93	19.97	2	10.52	34.95	15.61	3	10.46	34.75
FOMC	10.26	4	6.89	72.16	12.18	4	6.28	136.6	9.29	4	7.16	97.89
DFOP	8.90	5	9.35	45.46	7.66	5	8.60	107.5	5.72	5	8.28	81.63
8202031/4, Kutenholz, Germany												
SFO	37.44	2	4.63	15.38	37.49	2	5.42	17.99	37.40	2	5.12	17.02
FOMC	36.44	2	4.38	149.8	33.11	2	5.21	109.8	33.81	2	5.28	65.66
DFOP	30.20	3	4.01	175.0	28.76	3	4.74	281.0	29.11	4	4.53	225.9

Studies 2–6: Five field dissipation studies were carried out in Canada and the USA from June 2011 to June 2013 to study the behaviour of mandestrobin in soil [Bitter, 2013 d-h, ROR-0245, ROR-0246, ROR-0247, ROR-0248, ROR-0249; Bitter, 2015d, ROR-0267]. Mandestrobin was sprayed as an SC formulation to bare soil with four applications of 0.54–0.58 kg ai/ha (total 2.2–2.3 kg ai/ha) with an interval of 13-15 days. The spray volume per application was 756-807 L/ha in the Canadian trials and 426-511 L/ha in the USA trials. A Non Ionic Surfactant at 0.125% v/v was added as adjuvant in each of the trials. Soil characteristics for the five soils are summarized in Table 65. Soil cores were taken immediately prior to the first spray, within 3 hours and at 1, 6-7 and/or 12–14 days after the first, second and third spray, within 3 hours and at 1, 3, 7, 14, 28-30, 59-61, 79–93, 120–121, 150, 181–183, 211, 240–245, 272, 297-305, 358-362, 419-427 , 479-482, 535-539 and 664-666 days after the last spray. All soil cores were stored frozen at -10 °C or lower for a maximum of 616-818 days. The storage period of 616-818 days is covered by the storage stability studies.

Table 68 Application rates and soil characteristics for the top 30 cm of soil

Soil name	Saskatchewan	Ontario	Georgia	North Dakota	California
Location	Saskatoon Saskatchewan, CAN, 2011	Branchton, Ontario, CAN, 2011	Tift county Georgia, USA, 2011	Northwood North Dakota, USA, 2011	Madera California, USA, 2011
Reference	ROR-0245	ROR-0246	ROR-0247	ROR-0248	ROR-0249
Application rate	0.58, 0.57, 0.57 and 0.57 kg ai/ha	0.55, 0.54, 0.57 and 0.55 [a] kg ai/ha	0.56, 0.56, 0.56, and 0.56 kg/ai/ha	0.56, 0.55, 0.55, 0.56 kg ai/ha	0.57, 0.57, 0.57, and 0.56 kg ai/ha
Interval	14-14-14 days	13-14-14 days	14-14-14 days	14-14-14 days	15-13-14 days
Total seasonal rate	2.29 kg ai/ha	2.20 kg ai/ha	2.24 kg ai/ha	2.22 kg ai/ha	2.27 kg ai/ha
Soil texture (USDA) [a]	loam	sandy loam	sand	loam	sandy loam
-- Sand (%)	33	69	89	27	72
-- Silt (%)	40	26	6	46	15
-- Clay (%)	27	5	5	27	13
pH (H_2O)	6.4	6.8	6.7	6.8	7.7
Organic Matter (%)	2.4	1.4	0.70	5.2	0.22
CEC (meq/100 g)	16.7	7.1	3.8	22.9	8.8
Bulk density (disturbed, g/cm^3)	1.05	1.27	1.47	0.99	1.27
Moisture at 1/3 bar (%)	22.7	11.8	4.8	43.7	10.8

[a] last application of 0.55 kg ai/ha applied in two separate applications of 0.38 kg ai/ha plus 0.17 kg ai/ha, but on the same day.

For residue analysis, the soil cores were cut into soil horizons 0–15 cm; 15–30 cm; 30–45 cm; 45–60 cm; 60–75 cm and 75–90 cm. Soil samples were analysed for mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin using LC-MS/MS method RM-48S-3 with an LOQ of 0.02 mg/kg for each analyte. All residue results are corrected for moisture content and are presented as mg/kg on a dry weight basis.

Residues were almost exclusively found in the top 15-cm surface soil layer. The average results from the 0–15 cm soil layers are summarized in Tables 69, 70 and 71.

LC-MS/MS method RM-48S-3 is considered valid in the range 0.02–1.0 mg/kg for each analyte. All soil residues fell within this range. Residues in control samples (collected prior to application) were below the LOQ. Concurrent average recoveries at 0.02, 0.1 0.2, 0.5 and/or 1.0 mg/kg fell within the range of 70–120%.

Maximum levels of parent compound were found at day 0–1 after the last application and ranged from 0.41 mg/kg (California, DALA 0) to 0.81 mg/kg (Saskatchewan, DALA 1) in the 0–15 cm soil layers. Levels of parent compound decreased slowly, but remained above the LOQ of 0.02 mg/kg at all locations at all time points (up to 419-666 days after the last application). In the Saskatchewan trial, low levels of parent compound (0.02–0.06 mg/kg) appeared in the 15-30 cm layers at 0–3 days after the last application. In all other trial locations, parent compound only appeared in the 0–15 cm soil layers.

Metabolite 5-COOH-mandestrobin appeared at 13-42 days after the **first** application and was only found in the 0–15 cm soil layers. Metabolite 5-COOH-mandestrobin was found at low levels of 0.02–0.07 mg/kg in the 0–15 cm soil layer throughout the study until 183-427 days after the last application.

Metabolite 2-COOH-mandestrobin appeared at 13-35 days after the **first** application and was only found in the 0–15 cm soil layers. Metabolite 2-COOH-mandestrobin was found at low levels of 0.02–0.04 mg/kg throughout the study until 14-420 days after the last application.

Metabolite DX-CA-mandestrobin appeared at 21 days after the **first** application in the Saskatchewan trial and was only found in the 0–15 cm soil layers. Metabolite DX-CA-mandestrobin was found at low levels of 0.02–0.03 mg/kg up to 79 days after the last application. At all other locations, only a single occasion occurred, where DX-CA-mandestrobin was found at the LOQ of 0.02 mg/kg.

Metabolite De-Xy-mandestrobin was not analysed for. The amide metabolites 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin were not detected throughout the duration of the study.

Table 69 Average concentration of residues in 0–15 cm soil layers from Saskatchewan and Ontario

DALA [a]	parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]	DALA [a]	parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]
ROR-0245, Saskatoon, Saskatchewan, CAN					ROR-0246, Branchton, Ontario, CAN				
0 [c]	- [d]	- [d]	- [d]	- [d]	0 [c]	0.18	< 0.02	< 0.02	< 0.02
7	0.16	< 0.02	< 0.02	< 0.02	7	0.16	< 0.02	< 0.02	< 0.02
13 [b]	0.14	< 0.02	< 0.02	< 0.02					
0 [c]	0.31	< 0.02	< 0.02	< 0.02	0 [c]	0.10	< 0.02	< 0.02	< 0.02
					1	0.48	< 0.02	< 0.02	< 0.02
7	0.21	0.02	< 0.02	< 0.02	7	0.18	< 0.02	< 0.02	< 0.02
13 [b]	0.07	< 0.02	< 0.02	< 0.02	13 [b]	0.21	< 0.02	< 0.02	< 0.02
0 [c]	0.20	< 0.02	< 0.02	< 0.02	0 [c]	0.51	< 0.02	0.02	0.02
6	0.44	< 0.02	0.03	< 0.02	7	0.32	< 0.02	< 0.02	0.02
13 [b]	0.19	< 0.02	< 0.02	< 0.02	13 [b]	0.38	< 0.02	0.02	0.03
0 [c]	0.66	0.02	0.02	0.02	0 [c]	0.55	< 0.02	0.02	0.03
1 [e]	0.81	0.02	0.03	0.02	1 [e]	0.60	< 0.02	0.02	0.03
3	0.39	0.02	0.03	0.02	3	0.47	< 0.02	0.03	0.03
7	0.44	0.02	0.03	0.02	7	0.44	0.02	0.04	0.04
14	0.35	0.02	0.02	0.02	14	0.39	< 0.02	0.03	0.03

DALA [a]	parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]	DALA [a]	parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]
29	0.39	0.03	0.03	0.02	28	0.40	< 0.02	0.03	0.04
59	0.19	0.03	0.03	< 0.02	59	0.30	< 0.02	0.04	0.07
79	0.25	0.02	0.03	0.02	93	0.29	< 0.02	0.03	0.06
120	- [f]	- [f]	- [f]	- [f]	120	0.19	< 0.02	0.02	0.05
150	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]
180	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]
240	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]	- [f]
297	0.11	< 0.02	0.02	< 0.02	302	0.15	< 0.02	< 0.02	0.03
360	0.13	< 0.02	0.02	< 0.02	358	0.10	< 0.02	< 0.02	0.02
420	0.10	< 0.02	0.02	0.02	427	0.11	< 0.02	< 0.02	0.02
480	- [f]	- [f]	- [f]	- [f]	479	0.04	< 0.02	< 0.02	< 0.02
540	- [f]	- [f]	- [f]	- [f]					
					666	0.05	< 0.02	< 0.02	< 0.02

[a] DALA = Days After Last Application; M=-mandestrobin

[b] One day prior to the next application

[c] Core sampled at the application when the spray has dried – typically collection started between 1 and 3 hours after application

[d] Rainstorm after first application prevented sampling.

[e] One day after the last application

[f] Frozen ground conditions prevented sampling.

Table 70 Mean concentration of residues in 0–15 cm soil layers from Georgia and North Dakota

DALA [a]	parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]	DALA [a]	parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]
ROR-0247, Tift County, Georgia, USA					ROR-0248, ROR-0267, Northwood, North Dakota, USA				
0 [c]	0.22	< 0.02	< 0.02	< 0.02	0 [c]	0.06	< 0.02	< 0.02	< 0.02
					7	0.14	< 0.02	< 0.02	< 0.02
13 [b]	0.13	< 0.02	0.02	0.03	13 [b]	0.30	< 0.02	< 0.02	< 0.02
0 [c]	0.27	< 0.02	< 0.02	0.02	0	0.47	< 0.02	< 0.02	< 0.02
					7	0.52	< 0.02	< 0.02	< 0.02
13 [b]	0.11	< 0.02	0.02	0.02	13 [b]	0.22	< 0.02	< 0.02	< 0.02
0 [c]	0.25	< 0.02	0.02	0.02	0 [c]	0.46	< 0.02	< 0.02	< 0.02
					7	0.31	< 0.02	0.02	< 0.02
13 [b]	0.17	< 0.02	0.02	0.03	13 [b]	0.37	< 0.02	0.02	< 0.02
0 [c]	0.47	< 0.02	< 0.02	0.03	0 [c]	0.66	< 0.02	0.02	0.02
1 [d]	0.40	< 0.02	0.02	0.04	1 [d]	0.38	< 0.02	< 0.02	< 0.02
3	0.35	< 0.02	0.02	0.04	3	0.61	0.02	0.03	0.02
7	0.32	< 0.02	0.02	0.04	7	0.19	< 0.02	< 0.02	< 0.02
14	0.18	< 0.02	0.02	0.03	14	0.14	< 0.02	0.03	0.02
28	0.23	< 0.02	< 0.02	0.04	30	0.32	< 0.02	0.03	0.02
59	0.26	< 0.02	< 0.02	0.04	61	0.06	< 0.02	< 0.02	< 0.02
					80	0.04	< 0.02	< 0.02	< 0.02
120	0.22	< 0.02	< 0.02	0.04	120	- [e]	- [e]	- [e]	- [e]
					150	- [e]	- [e]	- [e]	- [e]
181	0.13	< 0.02	< 0.02	0.04	180	- [e]	- [e]	- [e]	- [e]
211	0.14	< 0.02	< 0.02	0.02					
240	0.12	< 0.02	< 0.02	0.02	245	0.10	< 0.02	0.02	< 0.02
272	0.12	< 0.02	< 0.02	0.02					
305	0.12	< 0.02	< 0.02	< 0.02	303	0.29	< 0.02	0.03	0.03
359	0.08	< 0.02	< 0.02	< 0.02	362	0.10	< 0.02	0.02	< 0.02
420	0.08	< 0.02	< 0.02	< 0.02	419	0.12	< 0.02	0.02	< 0.02
479	0.04	< 0.02	< 0.02	< 0.02	480	- [e]	- [e]	- [e]	- [e]
539	0.06	< 0.02	< 0.02	< 0.02					
					664	0.09	< 0.02	0.02	0.02

na = not analysed

[a] DALA = Days After Last Application; M=-mandestrobin;

[b] One day prior to the next application

[c] Core sampled at the application when the spray has dried – typically collection started between 1 and 3 hours after application

[d] One day after the last application

[e] Frozen ground conditions prevented sampling.

Table 71 Mean concentration of residues in 0–15 cm soil layers from California

DALA [a]	Parent [a]	DX-CA-M [a]	2-COOH-M [a]	5-COOH-M [a]
ROR-0249, Madera, California, USA				
0 [c]	0.18	< 0.02	< 0.02	< 0.02
7	0.08	< 0.02	< 0.02	< 0.02
14 [b]	0.11	< 0.02	< 0.02	< 0.02
0 [c]	0.24	< 0.02	< 0.02	< 0.02
7	0.15	< 0.02	0.02	0.02
12 [b]	0.22	< 0.02	0.02	0.03
0 [c]	0.29	< 0.02	0.02	0.03
7	0.26	< 0.02	0.03	0.04
13 [b]	0.26	< 0.02	0.02	0.03
0 [c]	0.38	< 0.02	0.02	0.03
1 [d]	0.41	< 0.02	0.02	0.03
3	0.33	< 0.02	0.03	0.04
7	0.30	< 0.02	0.03	0.04
14	0.26	< 0.02	0.02	0.04
28	0.19	< 0.02	0.02	0.04
59	0.13	< 0.02	< 0.02	0.02
90	0.08	< 0.02	< 0.02	0.02
121	0.08	< 0.02	< 0.02	< 0.02
150	0.07	< 0.02	< 0.02	< 0.02
183	0.10	< 0.02	< 0.02	0.02
240	0.06	< 0.02	< 0.02	< 0.02
300	0.04	< 0.02	< 0.02	< 0.02
360	0.03	< 0.02	< 0.02	< 0.02
421	0.03	< 0.02	< 0.02	< 0.02
482	< 0.02	< 0.02	< 0.02	< 0.02
535	0.02	< 0.02	< 0.02	< 0.02

na = not analysed

[a] DALA = Days After Last Application; M=mandestrobin

[b] One day prior to the next application

[c] Core sampled at the application when the spray has dried – typically collection started between 1 and 3 hours after application

[d] One day after the last application

At first, a single first-order (SFO) model was used in order to determine the best-fit values for 50% (DT_{50}) and 90% (DT_{90}) dissipation for each trial site based on the measured mandestrobin concentrations. The goodness of fit was assessed by the coefficient of determination (r^2) and an error criterion based on a chi-squared significance test. All calculations were performed using KinGUII software (version 2.2012.320.1629).

In a second study [Jarvis and Montesano, 2019, UK0–4844] data from the 5 USA and Canadian soil studies were fitted to SFO (single first order), FOMC (first order multicompartment) and DFOP (double first order in parallel) kinetics according to the FOCUS (2014) guidance to determine DT_{50} values. Curves were established through the data points using the CAKE 3.3 model. In all cases the SFO fits show similar DT_{50} and DT_{90} values to those in the original reports. Since environmental conditions (primarily soil temperature and moisture content) are variable under field conditions there is no expectation of constant degradation rates. In all cases except California, the FOMC kinetics approach would be considered invalid since the key criterion is not met; namely that the DT_{90} (overall) value is reached during the study lifetime. The statistical evaluation of the DFOP

kinetics was considered unacceptable when the probability value from the t- test exceeded 0.05 for either of the two fitted rate constants. In these conditions SFO kinetics remain the most appropriate, with the possible exception of California where FOMC could be more appropriate. The calculated DT_{50} and DT_{90} values for each trial are summarized in Table 72.

The DT_{50} and DT_{90} values calculated for mandestrobin (racemic mixture) for the best fit models ranged from 14 to 165 days and 46 to 548 days, respectively.

Table 72 Summary of kinetics data for DT_{50} values of mandestrobin using SFO or FOMC

Model	Study reference	χ^2 error (%)	DT_{50} (days)	DT_{90} (days)
SFO	ROR-0245 (Saskatchewan) UK0–4844 (modelling)	24.2	45.1	150
SFO	ROR-0246 (Ontario) UK0–4844 (modelling)	14.0	130	432
SFO	ROR-0247 (Georgia) UK0–4844 (modelling)	22.9	165	548
SFO	ROR-0248 (North Dakota) UK0–4844 (modelling)	49.4	14	46.5
FOMC	ROR-0249 (California) UK0–4844 (modelling)	100	25.8	332

nr = not reported.

Environmental fate in water/sediment systems

The Meeting did not receive information on the environmental fate in water/sediment systems, except for hydrolysis and photolysis in water. These studies were summarized in the physico-chemical properties section. Studies on the environmental fate in water/sediments are not required for the envisaged uses.

METHODS OF RESIDUE ANALYSIS

The Meeting received information on enforcement/monitoring methods for the determination of mandestrobin and its metabolites in plant and animal commodities. In addition the Meeting received information on analytical methods for the determination of mandestrobin and its metabolites as used in the various study reports (supervised residue trials, storage stability studies, processing studies, feeding studies). The analytical residue methods have been evaluated according to the guidance provided by OECD (Series on Pesticides number 39) as indicated on page 27 of the FAO manual 2016.

Validation results are required for every commodity submitted for MRL-setting: at least one full validation for a commodity within the five defined crop groups (high acid content, high water content, high oil content, high protein content, high starch content) and a reduced validation for every other commodity within a certain crop group. Where validation results do not meet the criteria given below, this is indicated.

When the analytical method is validated according to a full validation scheme, it means that:

- at least FIVE recovery experiments per level were conducted on at least 2 levels (LOQ and 10× LOQ) and average recovery per level was shown to be between 70–120% and the relative standard deviation (RSD_r or CV) per level was shown to be < 20%,
- at least two control samples were analysed and were shown to be below 0.3× LOQ and
- the calibration was conducted with at least 5 single points or at least 3 duplicate points and was shown to be linear (either standards in solvent or matrix matched standards).

When the analytical method is validated according to a reduced validation scheme, it means that:

- a full validation is available for a crop in the same crop group (high acid content, high water content, high oil content, high protein content, high starch content);
- at least 3 recovery experiments per level were conducted on at least two levels (LOQ and 10× LOQ) and the average recovery per level was shown to be between 70–120% and the relative standard deviation (RSD_r or CV) per level was < 20%;
- at least two control samples were analysed and shown to be below 0.3× LOQ
- the calibration was conducted with at least five single points or at least three duplicate points and was shown to be linear (only relevant for matrix matched standards; standards in solvent are already covered by full validation).

Radiovalidation for green rape fodder and rape seed

Extraction efficiency and hydrolysis efficiency of mandestrobin and its metabolites were determined using radiolabelled samples from green rape fodder and rape seed [Gohre and Aston, 2013, ROA-0039].

Radio-labelled green rape fodder and rape seed samples were obtained from a metabolism study [Panthani and Connor, 2011, ROM-0026]. In this metabolism study, rape seed plants were treated with 2 foliar spray applications of SC formulated [benzyl-^{14}C]-mandestrobin. Actual application rates were 0.39 and 0.38 kg ai/ha at the 1st and 2nd application, respectively, with an interval of 14 days. The green rape fodder and rape seeds were harvested 14 and 40 days after the last application, respectively. Green rape fodder and rape seeds were stored frozen (temperature not stated) and taken 3.8 years after the initial analyses (which were immediately after harvest).

In the original metabolism study, these levels were 3.4 mg eq/kg in green rape fodder and 0.64 mg eq/kg in rape seed (based on the sum of extracted fractions and post extracted solids). Green rape fodder and rape seed were taken from the freezer, 3.8 years after the initial analyses and homogenised. The total radioactive residue (TRR) was determined by combustion LSC as 5.1 mg eq/kg in green rape fodder and 0.30 mg eq/kg in rape seed.

In the original metabolism study, green rape fodder samples were surface washed with acetonitrile, homogenised and then extracted twice with acetone/water (80:20; v/v). Homogenised rape seed samples were extracted three times with hexane and then three times with acetone/water (80:20; v/v). The extraction efficiency for these neutral extraction methods was 89% TRR for green rape fodder and 92% TRR for rape seed.

Almost 4 years after the initial analyses, radiolabelled green rape fodder samples were subjected to extractions with acetone/water (70:30 or 80:20, v/v) with a variety of extraction conditions (see Table 73). Rape seed was extracted using smaller sample sizes and smaller extraction volumes (see Table 73). Extracts and post-extracted solids (PES) were analysed by LSC and combustion LSC.

Analysis in rape seed were more difficult than that in green rape fodder due to lower amounts of radioactivity and the oily matrix. Greater analytical variability was observed in the rape seed extracts, compared to the green rape fodder extracts and the HPLC analyses had to be performed by fraction collection LSC.

In general, the extraction efficiencies for the Tissumizer and the reciprocal shaker with both solvent systems were similar, while the smaller sample sizes and extraction volumes resulted in slightly higher extractabilities. The various methods extracted 82–90% of the green rape fodder TRR and 88-90% of the rape seed TRR. As the extraction efficiency is similar to the extraction efficiency in the original metabolism study, each of these extraction conditions is capable of extracting sufficient radioactivity from green rape fodder and rape seed.

Table 73 Extraction efficiencies for green rape fodder and rape seed under various conditions

Extraction Code	Matrix; Equipment	Amount	Acetone/water	Volume	Duration	%TRR extracted [a]	%TRR PES [a]	%TRR total [a]
OSRA	Green rape fodder; Tissumizer	50 g	80:20, v/v	3 ×100 mL	3 × 5 min	90 (84.8)	10 (9.7)	100.0 (94.5)
OSRA70		20 g	70:30, v/v	3 ×40 mL	3 × 5 min	88 (88.0)	12 (11.9)	100.0 (99.9)
OSRA80		20 g	80:20, v/v	3 ×40 mL	3 × 5 min	89 (90.9)	11 (11.3)	100.0 (102.2)
OSRC	Green rape fodder; Reciprocal shaker	50 g	80:20, v/v	2 ×100 mL	overnight, 1hr	83 (85.6)	17 (17.6)	100.0 (103.2)
OSRC70		20 g	70:30, v/v	2 ×40 mL	overnight, 1hr	84 (87.4)	16 (16.5)	100.0 (103.9)
OSRC80		20 g	80:20, v/v	2 ×40 mL	overnight, 1hr	84 (88.6)	16 (16.3)	100.0 (104.9)
OSRB		50 g	80:20, v/v	2 ×100 mL	2 × 1 hr	82 (80.3)	18 (17.1)	100.0 (97.4)
OSRS1	Rape seed; Tissumizer	2.5 g	70:30, v/v	3 ×15 mL	3 × 5 min	90 (112.1)	10 (13.0)	100.0 (125.1)
OSRS2		1.9 g	70:30, v/v	3 ×15 mL	3 × 5 min	88 (104.1)	12 (13.7)	100.0 (117.8)

[a] Values between brackets represent the values presented in the study report. The reviewer recalculated these values to 100% recovery.

The radiolabelled green rape fodder samples were extracted with a Tissumize using acetone/water (80:20 or70:30, v:v) with an extraction efficiency of 88-90% TRR (see OSRA and OSRA70 in Table 73).

Aliquots of the green rape fodder extracts (equivalent to 0.4 g green rape fodder) were concentrated by rotary evaporation to near dryness. The hydrolysis procedure was conducted in two steps; starting with an alkaline hydrolysis to cleave any malonyl groups off and followed by an enzymatic hydrolysis (beta-glucosidase) to cleave any glucose groups off. The hydrolysed extracts were then analysed by HPLC equipped with a radio flow detector to quantitate aglycone release. An unhydrolysed green rape fodder extract was analysed as well as a green rape fodder extract subjected only to alkaline hydrolysis in order to evaluate the efficiency of both hydrolyses at releasing the aglycones. Reference standards used were mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, De-Xy-mandestrobin, DX-CA-mandestrobin and MCBX.

In the OSRA extract, nine HPLC peaks appeared of which the 42 min peak could be assigned to parent compound (27% TRR). The rest of the radioactivity could not be assigned to any of the reference standards used. After the extract was subjected to alkaline hydrolysis, the majority of the radioactivity eluting in the 19 min peak (containing malonylglucosides) eluted at 16 min (containing glucosides). The number of HPLC peaks did not change after alkaline hydrolysis, but some of the unidentified peaks shifted to higher retention times indicating that other conjugates have been cleaved off as well. After the extract was subsequently subjected to enzyme hydrolysis, 20 HPLC peaks appeared and the majority of the radioactivity eluting in the 16 min peak had disappeared. The 42 min HPLC peak could be assigned to parent, the 28 min HPLC peak to 4-OH-mandestrobin, the 27 min HPLC peak to 2-CH_2OH-mandestrobin and the 11 min peak to De-Xy-mandestrobin. Results are shown in Table 74.

Table 74 shows that increasing the molarity of the NaOH from 0.03 M to 0.06 M and increasing the alkaline hydrolysis time from 1 hr to 2 hrs, increasing the beta-glucosidase hydrolysis time from 3 hrs to 15-16 hrs (overnight) and increasing the beta-glucosidase concentration from 1000 to 2000 units resulted in the highest yields of aglycones. Under these conditions parent seemed to degrade as well and small amounts of De-Xy-mandestrobin appeared.

In the original metabolism study, green rape fodder extracts were separated by radio-HPLC and only the 16-min and 19-min HPLC regions were subjected to enzyme hydrolysis (8 U cellulase and 18 U beta-glucosidase, pH 5, 2 days at 39 °C). Extracts contained 22% TRR as parent, 27% TRR as 4-OH-mandestrobin (from conjugates), 12% TRR as 2-CH_2OH-mandestrobin (from conjugates), 2.8% TRR as 5-CH_2OH-mandestrobin (from conjugates) and 0.2% TRR as MCBX, which led to 64% TRR being identified in the green rape fodder. Residues of 2-COOH-mandestrobin, 5-COOH-mandestrobin, De-Xy-mandestrobin and 2,5-dimethylphenol were not detected in the metabolism study; the presence of DX-CA-mandestrobin was not investigated.

The present study recovered 18-28% TRR as parent, 25-30% TRR as 4-OH-mandestrobin, 4.0–7.0% TRR as 2-CH_2OH-mandestrobin and 2.0–3.6% TRR as De-Xy-mandestrobin, whereby the full extract was treated with alkaline and enzyme hydrolysis. In the present study, residues of 5-CH_2OH-mandestrobin, MCBX, 2-COOH-mandestrobin, 5-COOH-mandestrobin and DX-CA-mandestrobin were not detected.

Conclusion: Hydrolysis efficiency was shown to be sufficient for the malonylglucoside and glucoside conjugates of hydroxylated mandestrobin present in green rape fodder, since it was shown that malonylglucoside metabolites appeared in the 19 min peak and that the majority of the radioactivity in this peak and the corresponding 16 min peak for the glucose conjugates disappeared.

The quantitative recovery of the metabolites in the present study is similar to or lower than the recovery for these metabolites in the green rape fodder metabolism study. Since only selected HPLC peaks in the green rape fodder metabolism study were treated with enzymes and the extract as a whole was not treated with enzymes, acids and/or base, this radiovalidation study shows that the remainder of the extract from the metabolism study does not contain any additional aglycones of known metabolites.

Table 74 Recovery after alkaline and enzyme hydrolysis of the green rape fodder extracts

	OSRA %TRR [b]	OSRA 1 hr 0.03 M NaOH %TRR [b]	OSRA 1 hr 0.03 M NaOH 3 hrs 1000 U beta-glucosidase %TRR [b]	OSRA70 2 hrs 0.06 M NaOH; 3 hrs 2000 U beta-glucosidase %TRR	OSRA70 2 hrs 0.06 M NaOH 15-16 hrs 2000 U beta-glucosidase %TRR	metabolism study
Parent	27.06 (25.58)	24.29 (22.96)	28.06 (26.53)	19.10 [a]	18.44 [a]	22
De-Xy-mandestrobin	ND	ND	1.95 (1.84)	3.63	3.58	ND
4-OH-mandestrobin	ND	ND	25.19 (23.81)	29.02 [a]	29.55 [a]	27
2-CH_2OH-mandestrobin	ND	ND	4.81 (4.55)	3.98 [a]	7.01 [a]	12
5-CH_2OH-mandestrobin	ND	ND	ND	ND	ND	2.8
MCBX	ND	ND	ND	ND	ND	0.16
16 min peak	6.28 (5.94)	41.11 (38.86)	1.86 (1.76)	9.47 [a]	5.98 [a]	
19 min peak	37.83 (35.76)	1.18 (1.12)	7.85 (7.42)	4.20 [a]	5.03 [a]	
other unknowns	18.43 (17.42) [c]	20.89 (19.75) [c]	19.23 (18.18) [c]	22.23	21.99	
Total extracted	89.70 (84.80)	89.70 (84.80)	89.70 (84.80)	88.00	88.00	93 [d]
Total identified	27.06 (25.58)	24.29 (22.96)	58.07 (54.90)	52.10	55.00	64

OSRA and OSRA 70 are extraction conditions, explained in Table 73

[a] average of three replicates

[b] Values between brackets represent the values presented in the study report. The values without brackets represent the values as recalculated by the reviewer; the reviewer recalculated these values to match with Table 73

[c] Consists of 6 HPLC peaks after extraction or alkaline hydrolysis and 15 HPLC peaks after glucosidase treatment, with the highest peak 5.31 (5.02) %TRR in any of the extracts.

[d] In the metabolism study the green rape fodder was extracted with acetonitrile, acetone/water (twice) and acetone/water/HCl. The last extraction released an additional 3.6% TRR.

The radiolabelled rape seed samples were extracted with a Tissumize using acetone/water (70:30 v:v) with an extraction efficiency of 88-90% TRR (see OSRS1 and OSRS2 in Table 73).

A hexane partition was added to remove the oils. Aliquots of the rape seed extracts (equivalent to 1 g rape seed each) were concentrated by rotary evaporation at 20–30 °C to remove the acetone. One aliquot (OSRS1-A) was partitioned with hexane. Three other aliquots (OSRS1-B and ORS2-A/B) were first mixed with acetonitrile and then partitioned with hexane. Without acetonitrile addition, parent mandestrobin partitioned into the hexane fraction and consequently the hexane fraction was analysed for this sample (OSRS1-A). When acetonitrile was added to the hexane/water partition, the radioactivity identified as mandestrobin partitioned into the acetonitrile/water fraction. A minor amount of activity (≤ 1.0% of TRR) remained in the hexane fraction and these fractions were not analysed.

The hexane fractions, the water fraction and the acetonitrile/water fraction from the hexane partition were concentrated by rotary evaporation to near dryness. The residue was subjected to alkaline and enzymatic hydrolysis under conditions similar to the green rape fodder method. Because of the lower radioactivity in rape seed compared to green rape fodder (and consequently larger amount of matrix), it was necessary to increase the amount of the base (5 mL 0.06 M NaOH) and enzyme (5,000–10,000 units of beta-glucosidase). Results are shown in Table 75.

In the original metabolism study, rape seed extracts were separated by radio-HPLC and only the 16-min and 19-min HPLC regions were subjected to mild enzyme hydrolysis enzyme hydrolysis (8 U cellulase and 18 U beta-glucosidase, pH 5, 2 days at 39 °C). Extracts contained 25% TRR as parent, 11% TRR as 4-OH-mandestrobin (from conjugates), 5.1% TRR as 2-CH_2OH-mandestrobin (from conjugates), 3.6% TRR as 5-CH_2OH-mandestrobin (from conjugates) and 1.3% TRR as 5-COOH-mandestrobin, which led to 46% TRR being identified in the rape seed. Residues of 2-COOH-mandestrobin, De-Xy-mandestrobin, MCBX and 2,5-dimethylphenol were not detected in the metabolism study; the presence of Dx-CA-mandestrobin was not investigated.

The present study recovered 0.83-4.4% TRR as parent, 1.3-1.9% TRR as 4-OH-mandestrobin, 0.40–0.72% TRR as 2-CH_2OH-mandestrobin, 1.9-3.0% TRR as 5-COOH-mandestrobin and 0.72–0.79% TRR as 2-COOH-mandestrobin, whereby the full extract was treated with alkaline and enzyme hydrolysis. In the present study, residues of 5-CH_2OH-mandestrobin, MCBX, De-Xy-mandestrobin and DX-CA-mandestrobin were not detected.

Although the extraction efficiency has not changed during the storage, the current extracts contained much lower amounts of parent compared to the extracts derived in the metabolism study. It was noticed that the HPLC analyses showed a high degree of variability. Since validation of analytical methods did not show this variability, this suggests that the samples were not properly homogenised or the composition of the radioactive residues in rape seed has changed upon four-year storage. Therefore, no conclusions can be drawn on the hydrolysis efficiency for rape seed.

Table 75 Recovery after alkaline and enzyme hydrolysis of the rape seed extracts

	OSRS1 %TRR [b]	OSRS1 2 hrs 0.06 M NaOH; 3 hrs 5000–10000 U beta-glucosidase %TRR	OSRS2 2 hrs 0.06 M NaOH 15-16 hrs 10000 U beta-glucosidase %TRR	metabolism study
Parent	2.52 (3.03)	4.38 (5.27) [a]	0.83 (1.03) [a]	25
4-OH-mandestrobin	ND	1.93 (2.32) [a]	1.27 (1.57) [a]	11

	OSRS1 %TRR [b]	OSRS1 2 hrs 0.06 M NaOH; 3 hrs 5000–10000 U beta-glucosidase %TRR	OSRS2 2 hrs 0.06 M NaOH 15-16 hrs 10000 U beta-glucosidase %TRR	metabolism study
2-CH_2OH-mandestrobin	0.72 (0.86)	0.40 (0.49) [a]	ND	5.1
5-CH_2OH-mandestrobin	ND	ND	ND	3.6
2-COOH-mandestrobin	0.72 (0.86)	ND	0.79 (0.97) [a]	ND
5-COOH-mandestrobin	1.89 (2.27)	3.02 (3.63) [a]	2.64 (3.25) [a]	1.3
16 min peak	ND	ND	ND	
19 min peak	5.76 (6.92)	0.99 (1.19) [a]	2.24 (2.76) [a]	
other unknowns	78.39 (94.16) [c]	79.27 (95.22)	80.22 (98.54)	
Total extracted	90.00 (108.10)	90.00 (108.10)	88.00 (108.10)	99.9
Total identified	5.84 (7.02)	9.74 (11.70)	5.54 (6.81)	46

OSRS1 and OSRS2 are extraction conditions, explained in Table 73

[a] average of two replicates

[b] Values between brackets represent the values presented in the study report (based on the average extraction efficiency of 108.1%). Values without brackets represent the values as recalculated by the reviewer; the reviewer recalculated these values to match with Table 73

[c] In the metabolism study the rape seed were extracted with three times with hexane and three times with acetone/water followed by an extraction with acetone/water/HCl. This last extraction released an additional 7.5% TRR.

The analytical methods used in the supervised trials use the following extraction methods:

- Methods RM-48C-1, RM-48C-2 and RM-48C-2B use a mixture of acetone/water (70:30, v/v) and extract 5 g samples twice on a reciprocal shaker for 1 hour.
- Methods RM-48G uses a mixture of acetone/water (70:30, v/v) and extracts 2.5 g samples twice on a reciprocal shaker for 1 hour.
- Methods SUM-1023V, SUM-1021V, SUM-1022V, SUM-1027V, S10–02011 use a mixture of acetone/water (80:20, v/v) and extracts 20 g samples (5 g barley straw) twice on a reciprocal shaker for 10 min.
- Methods RM-48A uses a mixture of acetone/water (80:20, v/v) and extracts 2.5 g samples twice on a reciprocal shaker for 1 hour.

The extraction efficiency for these methods is shown to be sufficient.

The analytical methods proposed for enforcement and monitoring use different extraction solvents:

- HPLC-MS/MS multi-residue method QuEChERS uses acetonitrile.
- GC-MS multi-residue method DFG-S19 uses cyclohexane/ethyl acetate (1:1, v/v) with accelerated solvent extraction at high temperature under pressure.

The extraction efficiency for these methods has not been validated.

The analytical methods used in the supervised trials for the determination of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin use the following hydrolysis methods:

- Methods SUM-1021V, SUM-1022V, SUM-1027V, S10–02011 evaporate the acetone off. The aqueous phase was hydrolysed for 1 hr with 0.1 M NaOH at pH 11. After adjustment of the pH to 5 (acetate buffer), samples were treated with 250 units of beta-glucosidase for 3 hrs.
- Methods RM-48A evaporates the acetone off and the aqueous phase was mixed with acetonitrile. For oily crops the mixture was partitioned twice with hexane to remove the oils. The acetonitrile/water phase was evaporated to near dryness. The residue was hydrolysed for 2 hrs with 0.06 M NaOH at pH 11. After adjustment of the pH to 5 (acetate buffer), samples were treated with 2500 units of beta-glucosidase for 3 hrs.

The hydrolysis efficiency for these methods has not been shown. The effect of 0.1 M sodium hydroxide solution has not been shown, nor the effect of 250 or 2500 units of beta-glucosidase. Oily crops showed a high variability in the analytical results, possibly due to homogenisation effects and further proof of sufficient hydrolysis efficiency is desirable.

Radiovalidation for animal commodities

In a reply to a request from PMRA Canada to submit radio-validation studies for the extraction efficiency of parent and De-Xy-mandestrobin in goat and hen liver, the manufacturer responded that such studies were not possible because the radiolabelled samples had already been destroyed [Bitter and Gohre, 2017, ROA-0057].

In the metabolism studies, goat and hen liver were extracted sequentially with hexane, ethyl acetate, and acetonitrile. Hexane only extracted between 2–4% of the total radioactive residue (TRR). The ethyl acetate and acetonitrile solvents extracted 43-57% of the TRR. The remaining radioactive residues were released by more elaborate procedures (protease digestion or vigorous acidic and basic hydrolyses), procedures not normally found in residue methods.

The residue enforcement method RM-48M-1 for parent and De-Xy-mandestrobin extracted liver, egg and cream with acetone/water (7/3, v/v). According to the manufacturer, the replacement of ethyl acetate and acetonitrile with acetone in extraction solvents is a generally accepted procedure, as the physical extraction properties of acetone are similar to acetonitrile.

The manufacturer suggests analysing for parent and De-Xy-mandestrobin using the residue enforcement method, RM-48M-1 (which would extract only the organic extractable fraction of the parent and De-Xy-mandestrobin) and account for the conjugated/bound parent and De-Xy-mandestrobin by multiplying the parent and De-Xy-mandestrobin concentration determined with the enforcement method by 2× and 3× respectively. This proposal is based on an incorrect calculation as the wrong concentration values for parent and De-Xy-mandestrobin were taken from the metabolism studies. Based on a correct calculation a correction factor of 100/35 = 3× is proposed for parent (Table 76) and a correction factor of 100/51 = 2 × for De-Xy-mandestrobin (Table 77).

Table 76 Distribution of parent compound in goat and hen liver from metabolism studies

	goat liver (Ph) mg/kg	goat liver (Ph) % of total	goat liver (Bz) mg/kg	goat liver (Bz) % of total	hen liver (Ph) mg/kg	hen liver (Ph) % of total	hen liver (Bz) µg/kg	hen liver (Bz) % of total	Average extr distrib
Organic solvents [a]	0.0030	30%	0.0263	56%	0.0018	21%	ND [b]	ND [b]	35%
Aqueous extraction (1 M HCl and 1 M NH_3)	0.0022	22%	0.0025	5%	0.0024	28%	0.0022	34%	18%
Protease	0.0032	32%	0.0148	31%	0.0045	52%	0.0027	42%	34%
Acetonitrile	0.0005	5%	0.0014	3%	NA	NA	NA	NA	6%
10 M HCl extraction	0.0011	11%	0.0023	5%	ND	ND	0.0015	23%	7%
Total	0.010	100%	0.0473	100%	0.0087	100%	0.0064 [b]	100%	

NA = not analysed; ND = not detected

[a] The liver tissues were sequentially extracted with hexane, ethyl acetate, acetonitrile and acidified acetonitrile. The goat liver received an additional ethyl acetate rinse. All extractions were combined.

[b] Value taken from hen metabolism study; in radiovalidation study ROA-0057 a different (erroneous value) was used.

Table 77 Distribution of De-Xy-mandestrobin in goat and hen liver from metabolism studies

	goat liver (Bz) mg/kg	goat liver (Bz) % of total	hen liver (Bz) µg/kg	hen liver (Bz) % of total	Average extraction distribution
Organic solvents [a]	0.0160 [a]	32%	0.0256 [a]	71%	51%
Mild acid extraction	0.0070 [a]	14%	0.0035	10%	12%
Protease	0.0218 [a]	44%	0.0057	16%	30%
Acetonitrile	0.0022 [a]	4%	NA	NA	4%
Harsh Acid extraction	0.0028 [a]	6%	0.0014	4%	5%
Total	0.0498 [a]	100%	0.0362 [a]	100%	

[a] Value taken from goat or hen metabolism study; in radiovalidation study ROA-0057 a different (erroneous value) was used.

Radiovalidation for soils

Extraction efficiency for various solvents was investigated as part of a radiolabelled aerobic soil degradation study 6 [Maurer & Gohre, 2013, ROM-0051]. The Sharkey soil (50 g) was treated with the [benzyl-^{14}C]-R-isomer of mandestrobin (RB) at an application rate of 9.0 mg ai/kg dry weight. Further details are described in aerobic soil degradation study 6. Identical samples of 10 g of Sharkey soil were taken at DAT 192 and were subjected to a series of extractions where parameters such as solvent type, pH and extraction time were varied.

Method A: The 10 g soil sample was mixed with 10 mL 0.04M $NaHCO_3$ and allowed to sit for 30 minutes. After addition of 40 mL of acetone, the mixture was shaken for an hour and then centrifuged. The supernatant was collected and the remaining solids were shaken with 50 mL 80:20 acetone:0.1 M HCl, (v:v) for an hour and centrifuged. The supernatant was collected and the remaining solids were shaken with 30 mL 5:1 methanol:0.5 M HCl, (v:v) for 20 minutes and then centrifuged. The supernatant was collected.

Method B: The 10 g soil sample was shaken with 50 mL 80:20 acetone:0.05M HCl, (v:v) for an hour and then centrifuged. The supernatant was collected and the extraction was repeated on the remaining solids. The supernatant was collected and the remaining solids were shaken for 20 min with 30 mL 5:1 methanol:0.5M HCl, (v:v) and then centrifuged. The supernatant was collected.

Method C: The procedure was the same as Method A, but with acetonitrile in place of acetone.

Method D: The procedure was the same as Method B, but with acetonitrile in place of acetone.

Each of the three extracts from each method was analysed by LSC. The first two extracts from each method were rotary evaporated to dryness and reconstituted in 2 mL methanol each. Equal volume aliquots from the two concentrated extracts of each method were combined and further concentrated so that the final concentration was about the same as the extracts from the 181 DAT analysis (the closest timepoint from the aerobic degradation study). The combined concentrated extracts from each of the four methods were analysed by HPLC and compared to the 181 DAT HPLC results.

All extraction methods yielded acceptable extractabilities of 86–88% (Table 78) and the degradate distributions were similar (Table 79). Method B (two extractions with acetone: 0.05M HCl (80:20)) was the basis for LC-MS/MS method RM-48S-3.

Table 78 Soil extraction efficiency for Sharkey soil

	Extraction solvents	%TRR
DAT 192 Method A	Extract 1 (80:20 Acetone:0.04M $NaHCO_3$, v/v) Extract 2 (80:20 Acetone:0.1 M HCl) Extract 3 (5:1 Methanol:0.5M HCl) Total:	51.95 31.03 3.30 86.29
DAT 192 Method B	Extract 1 (80:20 Acetone:0.05M HCl) 75.90 Extract 2 (80:20 Acetone:0.05M HCl) 9.82 Extract 3 (5:1 Methanol:0.5M HCl) 1.58 Total:	75.90 9.82 1.58 87.30
DAT 192 Method C	Extract 1 (80:20 Acetonitrile:0.04M NaHCO3) Extract 2 (80:20 Acetonitrile:0.1 M HCl) Extract 3 (5:1 Methanol:0.5M HCl) Total	55.32 30.05 3.03 88.40
DAT 192 Method C	Extract 1 (80:20 Acetonitrile:0.05M HCl) Extract 2 (80:20 Acetonitrile:0.05M HCl) Extract 3 (5:1 Methanol:0.5M HCl) Total:	78.74 8.40 1.24 88.38
DAT 181 Aerobic soil degradation study	Extract 1 (9:1 Methanol:H2O) Extract 2 (5:1 Methanol:0.5M HCl) 25.23 Total:	61.43 25.23 86.66

Table 79 Distribution of compounds in extracts from Sharkey soil at DAT 181–192

Analyte	DAT 192 method A	DAT 192 method B	DAT 192 method C	DAT 192 method D	DAT 181 aerobic soil degradation study
mandestrobin	59.8	62.2	60.9	63.8	61.1
5-COOH-mandestrobin	5.4	4.7	5.4	5.2	5.1
2-COOH-mandestrobin	5.5	5.3	5.3	5.6	5.7
DX-CA-mandestrobin	4.8	4.7	5.5	4.7	4.8
5-$CONH_2$-mandestrobin	2.1	2.2	2.2	2.2	2.4
2-$CONH_2$-mandestrobon	1.4	1.3	1.0	1	1.2
MCBX	0.6	0.8	0.6	0.6	0.4
16 min peak	2.1	1.5	2.3	2.4	2.4

Analytical methods for enforcement in plant commodities

The Meeting received validation results for the determination of the R- and S-isomer of mandestrobin, mandestrobin, and its metabolite De-Xy-mandestrobin in plant commodities for existing multiresidue methods.

LC-MS/MS multi-residue method QuEChERS

[QuEChERS, 2007] is a multi-residue method of the Official Collection of Test Methods. The method describes the analytical procedures for the determination of pesticide residues in foods of plant origin with a low fat content such as fruits, vegetables, cereals and cereal products, herbs, spices, tea and tobacco using GC-MS and/or LC-MS/MS following acetonitrile extraction and clean-up by dispersive SPE.

The method was validated for the determination of mandestrobin in peach (watery), grape (acidic) and barley grain (dry with high in starch) [Göcer, 2012, ROA-0030].

For peaches the stones were removed; for grapes the stems were removed. Peaches (10 g), grapes (10 g) and barley grain (5 g) were homogenised. Homogenised samples of barley grain were soaked in 10 mL water for 10 min (peaches and grapes do not need water addition). Homogenised samples of peach, grapes or barley were shaken vigorously with acetonitrile for 1 min. Magnesium sulphate (to bind water), sodium chloride (to salt out the analyte), trisodium citrate and disodium hydrogen citrate (to obtain a slightly acidic buffer) were added and samples were shaken again for 1 minute. After centrifugation, an aliquot of the supernatant was transferred to a dispersive SPE clean up tube containing primary and secondary amine (PSA) exchange material and magnesium sulphate. The tube was shaken for 30 sec. After centrifugation, an aliquot of the supernatant was diluted with acetonitrile/water (1:9, v/v) and analysed for mandestrobin by HPLC-MS/MS at transitions m/z 314 to 192 for quantification and m/z 314 to 160 for confirmation. Calibration was applied with external standards for mandestrobin in solvent or matrix and using linear regression. The reported LOQ of the method was 0.01 mg/kg for mandestrobin. Validation results are shown in Table 80.

An independent method validation (ILV) for the determination of mandestrobin in food of plant origin by QuEChERS was performed in peaches, grapes and barley [Rzepka, 2012, ROA-0031]. The extraction method was performed as described in [Göcer, 2012, ROA-0030]. Validation results are shown in Table 80.

The method was used in supervised field trials on rape seed [Gemrot, 2017, ROR-0282]. Analytical samples were reduced to 5.0 g for rape seed fodder (green whole plants, BBCH 77–80, dry plants without pods, BBCH 86) and 2.5 g for rape seed (BBCH 89) and dry pods with seeds (BBCH 86). Samples were soaked for 10 min in water before extraction. Validation results are shown in Table 80.

The method was also used in supervised field trials on soya bean forage and fodder [Klimmek and Gizler, 2017, ROR-0280]. Analytical samples were reduced to 10 g soya bean forage (whole plant, BBCH 77–81) and 5 g soya bean fodder (dry pods with seeds and rest of plant, BBCH 82). Samples were not soaked before extraction. Validation results are shown in Table 80.

Modification A was used in a validation study on soya bean seeds [Ivanov and Kissmann, 2015, ROA-0047], a storage stability study on dry beans and oranges [Lindner & Grewe, 2016, ROR-0269; Lindner *et al*., 2017, ROR-0286] and supervised field trials on soya bean seeds (dry seeds BBCH 84-89) [Klimmek and Gizler, 2017, ROR-0280]. Homogenised samples of 5 g dry beans (white seeds) or 5 g soya beans (dry seeds) were soaked in 10 mL water for 10–20 min (oranges do not need water addition). Samples were shaken vigorously with acetonitrile for 1–2 min. A salt mixture containing magnesium sulphate (to bind water), sodium chloride (to salt out the analyte) and trisodium citrate/disodium hydrogen citrate (to obtain a slightly acidic buffer) was added and the mixture was shaken again for 1–2 min. After centrifugation an aliquot of the supernatant was cleaned by freezing out (2.5–3.0 hours, at -18 °C). After centrifugation, an aliquot of the supernatant was mixed with primary secondary amine (PSA) exchange material and magnesium sulphate. The PSA mixture for soya bean seeds also contained C18 sorbent. The mixture was shaken for 30 sec to 2 min. After centrifugation, an aliquot of the supernatant was diluted with acetonitrile/water (10:90 or 25:75 v/v) and analysed by HPLC-MS/MS (retention time 3.0 min) at mass transition m/z 314 to 192 (quantification) and m/z 314 to 160 (confirmation). Calibration was applied with external standards for mandestrobin using a 2nd order polynomial fit (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg for mandestrobin.

Matrix effects were investigated for oranges, peaches, grapes, dry beans, soya beans (dry seeds, BBCH 84–89), barley grains, rape seed, soya bean forage (whole plant, BBCH 77–81), rape seed fodder (green whole plants, BBCH 77–80, dry pods with seeds and rest of plants, BBCH 80–87), soya bean fodder (dry pods with seeds and rest of plant, BBCH 82) [Lindner & Grewe, 2016, ROR-0269; Lindner *et al*., 2017, ROR-0286; Göcer, 2012, ROA-0030 Rzepka, 2012, ROA-0031; Ivanov and Kissmann, 2015, ROA-0047; Gemrot, 2017, ROR-0282; Klimmek and Gizler, 2017, ROR-0280] by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions. Significant matrix effects (> 20%) were observed for whole oranges (-26% to -29%), barley

grains (-26% to -67%), rape seed (-29% to -34%) and dry rape seed pods (-19% to -23%) and matrix matched standards were used for these commodities. For barley grains, the matrix effects were reduced to -16% to -26%, when the final extract was diluted four times.

HPLC-MS/MS method QuEChERS for the determination of mandestrobin is considered:

- valid (full validation) in the range 0.01–0.10 mg/kg in peaches, grapes, dry soya bean seeds, barley grains, rape seed, green rape seed fodder (BBCH 77-80);
- valid (reduced validation) only at 0.10 mg/kg in oranges and dry beans.
- valid (reduced validation) in the range 0.01–0.10 mg/kg in dry rape seed fodder (BBCH 86).
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–12 mg/kg for soya bean forage (BBCH 77-81), 0.01–0.48 mg/kg for dry soya bean fodder (BBCH 82).
- Limited recovery experiments (n = 1–2) suggest extension of the validity to the range of 0.01–1.0 mg/kg for rape seed, 0.01–6.0 mg/kg for green rape seed fodder (BBCH 77-80), 0.01–2.0 mg/kg for dry rape seed fodder (BBCH 86)

Table 80 Validation results for mandestrobin with HPLC-MS/MS multi residue method QuEChERS

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
Quantification at m/z 314 to 192									
peach (without stone)	0.01	0.01 0.10	5 5	109 104	105-113 99-108	3% 4%	< 0.3LOQ (2)	5 standards 0.1–10 ng/mL matrix matched r> 0.999	ROA-0030 validation
peach (without stone)	0.01	0.01 0.10	5 5	92 90	81–100 87-94	7.5% 3.2%	< 0.3LOQ (2)	8 standards in solvent 0.025-10 ng/mL r> 0.99	ROA-0031 ILV
grapes (without stems)	0.01	0.01 0.10	5 5	100 100	98-103 98-101	2% 2%	< 0.3LOQ (2)	5 standards 0.1–10 ng/mL matrix matched r> 0.999	ROA-0030 validation
grapes (without stems)	0.01	0.01 0.10	5 5	94 92	81–103 90–96	8.9% 2.9%	< 0.3LOQ (2)	8 standards in solvent 0.025-10 ng/mL r> 0.99	ROA-0031 ILV
barley grains	0.01	0.01 0.10	5 5	95 97	88-100 83-105	6% 9%	< 0.3LOQ (2)	5 standards 0.1–10 ng/mL matrix matched r> 0.999	ROA-0030 validation
barley grains	0.01	0.01 0.10	5 5	88 100	76-98 92–107	12% 5.7%	< 0.3LOQ (2)	8 standards in solvent 0.025-10 ng/mL r> 0.99	ROA-0031 ILV
whole orange	0.01	0.1	8	96	87-108	8.4%	< 0.3LOQ (6)	6 standards matrix matched 0.15-7.5 ng/mL 1/× weighted r^2> 0.99	ROR-0269; ROR-0286 stor stab
dry beans	0.01	0.1	8	97	87-109	7.2%	< 0.3LOQ (6)	7 standards in solvent 0.075-7.5 ng/mL 1/× weighted r^2> 0.99	ROR-0269; ROR-0286 stor stab

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
soya bean forage (whole plant BBCH 77-81)	0.01	0.01 0.10 12	1 1 1	104 99 94	- - -	- - -	< 0.3LOQ (4)	8 standards in solvent 0.025-10 ng/mL r^2≥0.98	ROR-0280 field trial
soya bean fodder (dry pods with seeds BBCH 82)	0.01	0.01 0.1 0.24	1 1 1	87 87 85	- - -	- - -	< 0.3LOQ (4)	8 standards in solvent 0.025-10 ng/mL r2≥0.98	ROR-0280 field trial
soya bean fodder (rest of plant, BBCH 82)	0.01	0.01 0.1 0.48	1 2 1	92 82 73	- 77- 87 -	- - -	< 0.3LOQ (4)	8 standards in solvent 0.025-10 ng/mL r^2≥0.98	ROR-0280 field trial
Rape seed fodder (green whole plants, BBCH 77-80)	0.01	0.01 0.1 2.0 6.0	7 7 1 1	99 96 71 105	90–103 80–109 - -	4.8% 14% - -	< 0.3LOQ (15)	7-8 matrix matched standards 0.10–10 or 25 ng/mL 1/× weighted r^2≥0.992	ROR-0282 field trial
Rape seed fodder (dry plants without pods, BBCH 86)	0.01	0.01 0.1 2.0	3 3 1	89 90 76	83-98 84-102 -	8.7% 12% -	< 0.3LOQ (5)	7-8 matrix matched standards 0.10–10 or 25 ng/mL 1/× weighted r^2≥0.992	ROR-0282 field trial
Rape seed fodder (dry pods with seeds, (BBCH 86)	0.01	0.01 0.1 1.0	2 21	71 72 76	70–71 70–74 -	- - -	< 0.3LOQ (5)	7-8 matrix matched standards 0.10–10 or 25 ng/mL 1/× weighted r^2≥0.992	ROR-0282 field trial
rape seed	0.01	0.01 0.1 1.0	6 61	91 90 105	82–97 87-93 -	6.6% 2.4% -	< 0.3LOQ (10)	7-8 matrix matched standards 0.10–10 or 25 ng/mL 1/× weighted r^2≥0.992	ROR-0282 field trial
Confirmation at m/z 314 to 160									
peach (without stone)	0.01	0.01 0.10	5 5	110 104 108	103-112 100–	4% 3%	< 0.3LOQ (2)	m/z 314 to 160 5 standards 0.1–10 ng/mL matrix matched r> 0.999	ROA-0030 validation
peach (without stone)	0.01	0.01 0.10	5 5	92 91	80–99 88-95	7.8% 3.1%	< 0.3LOQ (2)	m/z 314 to 160 8 standards in solvent 0.025-10 ng/mL r> 0.99	ROA-0031 ILV
grapes (without stems)	0.01	0.01 0.10	5 5	97 100	94-99 98-102	3% 2%	< 0.3LOQ (2)	m/z 314 to 160 5 standards 0.1–10 ng/mL matrix matched r> 0.999	ROA-0030 validation
grapes	0.01	0.01	5	95	81–104	9.2%	< 0.3LOQ	m/z 314 to 160	ROA-0031

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
(without stems)		0.10	5	91	89–97	3.7%	(2)	8 standards in solvent 0.025-10 ng/mL r> 0.99	ILV
barley grains	0.01	0.01 0.10	5 5	93 96	89–98 81–104	4% 9%	< 0.3LOQ (2)	m/z 314 to 160 5 standards 0.1–10 ng/mL matrix matched r> 0.999	ROA-0030 validation
barley grains	0.01	0.01 0.10	5 5	84 101	75-89 93-108	7.4% 5.7%	< 0.3LOQ (2)	m/z 314 to 160 8 standards in solvent 0.025-10 ng/mL r> 0.99	ROA-0031 ILV
Rape seed fodder (green whole plants, BBCH 77-80)	0.01	0.01 0.1	3 3	96 109	94-98 108-109	2.2% 0.5%	< 0.3LOQ (2)	m/z 314 to 160 7-8 matrix matched standards 0.10–10 or 25 ng/mL 1/× weighted $r^2 \geq 0.992$	ROR-0282 field trial
rape seed	0.01	0.01 0.1	3 3	83 86	77-86 83-88	6.0% 3.1%	< 0.3LOQ (2)	m/z 314 to 160 7-8 matrix matched standards 0.10–10 or 25 ng/mL 1/× weighted $r^2 \geq 0.992$	ROR-0282 field trial
Modification A (freeze out); Quantification at m/z 314 to 192									
dry soya beans	0.01	0.01 0.10	5 5	95 96	92–97 94-98	2.2% 1.7%	< 0.3LOQ ()	m/z 314 to 192 8 standards in solvent 0.025-10 ng/mL 1/× weighted $r^2 > 0.99$	ROA-0047 validation
dry soya beans (dry seeds, BBCH 84-89)	0.01	0.01 0.10	1 1	86 88		- -	< 0.3LOQ (8)	m/z 314 to 192 8 standards in solvent 0.025-10 ng/mL $r^2 \geq 0.98$	ROR-0280 field trial
Modification A (freeze out); Confirmation at m/z 314 to 160									
dry soya beans	0.01	0.01 0.10	5 5	93 90	88-96 89–92	3.4% 1.5%	< 0.3LOQ ()	m/z 314 to 160 8 standards 0.025-10 ng/mL in solvent 1/× weighted $r^2 > 0.99$	ROA-0047 validation

GC-MS multi residue method DFG-S19

The extended and revised multi residue method [DFG-S19, 1999] describes the analytical procedures for the determination of residues of organochlorine and organophosphorus compounds, nitrogen-

containing and other pesticides in foods. This method includes all former versions of DFG-S19, but provides an extended range of applications.

The method was validated for the determination of mandestrobin in rape seed [Daneva & Zetzsch, 2012, ROA-0025].

Homogenised samples of 10 g rape seed were extracted with a mixture of cyclohexane/ethyl acetate (1/1, v/v) according to module E9 with accelerated solvent extraction (ASE) at high temperature under pressure. The extract was cleaned-up according to the module gel permeation chromatography (GPC), followed by clean-up according to module C1 with a mini silica gel column (MSC). Mandestrobin was quantified by GC-MS module D4 both after the GPC clean-up and after the MSC clean-up. Quantification was done at m/z 192 and confirmation at m/z 160 and m/z 119. Calibration was done by external matrix matched standards. The reported LOQ of the method was 0.01 mg/kg for mandestrobin. Validation results are shown in Table 81.

An independent method validation (ILV) for the determination of mandestrobin in food of plant origin by DFG S19 was performed in rape seed [Toledo, 2012, ROA-0026]. The extraction method was performed as described in [Daneva & Zetzsch, 2012, ROA-0025] but with 5 g rape seed and different extraction volumes. Validation results are shown in Table 81.

Matrix effects were investigated for rape seed [Daneva & Zetzsch, 2012, ROA-0025; Toledo, 2012, ROA-0026] by comparing the detector response of solvent standard solutions with the detector response of matrix matched standard solutions. Significant matrix effects (> 20%) were observed for both clean-up procedures and at all masses analysed (ranging from -21% to +96%). Matrix matched standards were therefore used.

GC-MS method DFG-S19 for the determination of mandestrobin is considered:

- valid (full validation) in the range 0.01–0.10 mg/kg in rape seed.

Table 81 Validation results for mandestrobin with GC-MS multi residue method DFG-S19

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	% recovery range	RSD_r	control samples mg/kg (n)	calibration	reference, method
GPC clean-up; Quantification at m/z 192									
rape seed	0.01	0.01 0.10	5 5	75 73	62–84 72–79	11% 8.3%	< 0.3LOQ (2)	5 standards matrix matched 3.0–100 ng/mL r> 0.99	ROA-0025 validation
rape seed	0.01	0.01 0.10	5 5	91 85	80–103 77-95	9.4% 9.6%	< 0.3LOQ (2)	8 standards matrix matched 2.6-160 ng/mL r> 0.999	ROA-0025 ILV
GPC and MSC clean-up; Quantification at m/z 192									
rape seed	0.01	0.01 0.10	5 5	70 70	68-76 63-78	6.4% 8.0%	< 0.3LOQ (2)	5 standards matrix matched 4.5-100 ng/mL r> 0.99	ROA-0025 validation
rape seed	0.01	0.01 0.10	5 5	82 62	62–93 54-72	16% 11%	< 0.3LOQ (2)	8 standards matrix matched 7.5-230 ng/mL r> 0.999	ROA-0025 ILV
GPC clean-up; Confirmation at m/z 160									
rape seed	0.01	0.01 0.10	5 5	91 70	78-103 66-74	13% 4.8%	< 0.3LOQ (2)	5 standards matrix matched 3.0–100 ng/mL r> 0.99	ROA-0025 validation
rape seed	0.01	0.01 0.10	5 5	93 86	86-97 78-94	4.5% 8.6%	< 0.3LOQ (2)	8 standards matrix matched 2.6-160 ng/mL r> 0.999	ROA-0025 ILV

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
GPC and MSC clean-up; Confirmation at m/z 160									
rape seed	0.01	0.01 0.10	5 5	73 71	63-84 65-77	12% 6.7%	< 0.3LOQ (2)	5 standards matrix matched 4.5-100 ng/mL r> 0.99	ROA-0025 validation
rape seed	0.01	0.01 0.10	5 5	80 62	58-93 54-72	18% 11%	< 0.3LOQ (2)	8 standards matrix matched 7.5-230 ng/mL r> 0.999	ROA-0025 ILV
GPC clean-up; Confirmation at m/z 119									
rape seed	0.01	0.01 0.10	5 5	100 100	80–116 77-124	15% 18%	< 0.3LOQ (2)	5 standards matrix matched 3.0–100 ng/mL r> 0.99	ROA-0025 validation
rape seed	0.01	0.01 0.10	5 5	94 84	91–98 75-92	2.9% 9.6%	< 0.3LOQ (2)	8 standards matrix matched 2.6-160 ng/mL r> 0.999	ROA-0025 ILV
GPC and MSC clean-up; Confirmation at m/z 119									
rape seed	0.01	0.01 0.10	5 5	93 75	76-110 73-77	15% 2.0%	< 0.3LOQ (2)	5 standards matrix matched 4.5-100 ng/mL r> 0.99	ROA-0025 validation
rape seed	0.01	0.01 0.10	5 5	78 65	58-92 55-77	18% 12%	< 0.3LOQ (2)	8 standards matrix matched 7.5-230 ng/mL r> 0.999	ROA-0025 ILV

Chiral HPLC-MS/MS method DFG-S19 for R and S-isomers of mandestrobin

The extended and revised multi residue method DFG S19 (Multi-Method L 00.00–34 of the Official Collection of Test Methods, November 1999) describes the analytical procedures for the determination of residues of organochlorine and organophosphorus compounds, nitrogen-containing and other pesticides in foods. This method includes all former versions of DFG S19, but provides an extended range of applications.

DFG S19 was adapted for determination of the R- and S-isomers of mandestrobin in various plant commodities by use of chiral HPLC-MS/MS detection method. The chiral HPLC-MS/MS method DFG-S19 was described and used in supervised field trials [Delmotte, 2011, ROR-0008; Lebrun, 2012, ROR-0198], storage stability studies [Daneva & Taeufer, 2011, ROR-0007; Daneva & Taeufer, 2011a, ROR-0009] and a rotational crop study [Roussel, 2012, ROR-0202]. The chiral HPLC-MS/MS method DFG-S19 was validated in [Daneva, 2010, ROA-0005] and [Schernikau, 2010, ROA-0007].

Homogenised samples of 25 g rape seed whole plant, lettuce, carrot leaves, carrot roots and broccoli in ROR-0009 or ROR-0202 were extracted with a mixture of acetone and water (2/1, v/v) according to module E1. E1 extracts were cleaned-up with liquid/liquid partition with ethyl acetate/cyclohexane. Homogenised samples of 10 g rape seed in ROR-0007 and 10 g barley grains or 5 g barley straw in ROR-0009 or ROR-0202 were extracted with a mixture of cyclohexane/ethyl acetate (1/1, v/v) according to module E9 with accelerated solvent extraction (ASE) at high temperature under pressure. An aliquot of each extract was evaporated to dryness and re-dissolved in acetonitrile or acetonitrile/water (1:1, v/v). Chiral HPLC-MS/MS with positive electrospray ionisation at mass transitions m/z 314 to 192 for quantification and m/z 314 to 160 for confirmation was carried out for quantification of the optical isomers of mandestrobin (R- and S-isomers). A one point calibration was applied with an external standard in solvent or in matrix (for barley grain, barley

straw, carrot roots, carrot leaves). The reported LOQ for each isomer was 0.005 mg/kg. Validation results are shown in Table 81 and Table 83.

Analysis of control samples at mass transition 314 to 192 yielded no residues of the R- and S-isomers of mandestrobin above 30% of the LOQ, indicating that no matrix interferences were present at or near the retention times corresponding to the mandestrobin isomers. An exception was observed for mandestrobin isomers in the control sample of rape seed of trial FLN-10–6268 SP01 [Roussel, 2012, ROR-0202], which residues were above 30% of the LOQ, but below the LOQ level.

Matrix effects were investigated for rape seed, lettuce, carrot roots, carrot leaves, broccoli, rape seed fodder (green whole plants, BBCH 65 or green pods and the rest of the plant, BBCH 79), barley grains and barley straw [Daneva, 2010, ROA-0005; Schernikau, 2010, ROA-0007; Daneva & Taeufer, 2011a, ROR-0009; Roussel, 2012, ROR-0202; Lebrun, 2012, ROR-0198] by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions. Significant matrix effects (>20%) were observed for barley grain (-11% to -33%), barley straw (-27% to -44%), carrot roots (-27%) and carrot leaves (-34%) and matrix matched standards were used for these commodities.

Average recoveries for mass transition 314 to 160 [Daneva, 2010, ROA-0005; Schernikau, 2010, ROA-0007] ranged from 74–106% in rape seed, barley grain, barley straw and lettuce at 0.005–0.50 mg/kg (n = 5 at each level). Control samples at this transition were below 0.3 LOQ, except in lettuce. Some lettuce samples showed interferences at the retention time for the R-isomer of mandestrobin for mass transition 314 to 160, which were not found at mass transition 314 to 192. Linearity was confirmed in the range 0.2–20 ng/L.

Isomerisation between the R- and S-isomer of mandestrobin during extraction and analysis was verified by duplicate determination of the S-isomer of mandestrobin in the samples fortified with 0.50 mg/kg of the R-isomer of mandestrobin in rape seed, lettuce, barley grain and vice versa [Daneva, 2010, ROA-0005; Schernikau, 2010, ROA-0007]. No isomerisation between the R- and S-isomer of mandestrobin was observed.

Note by the reviewer:

For the determination of mandestrobin, the concentration levels of the R- and S-isomer can be added to get the LOQ and the concentration level of mandestrobin. Chiral HPLC-MS/MS method DFG-S19 for the determination of mandestrobin is considered:

- valid (full validation) in the range 0.01–1.0 mg/kg (0.005–0.50 mg/kg for R/S each) in lettuce, rape seed, barley grains and barley straw.
- valid (reduced validation) in the range 0.01–0.1 mg/kg (0.005–0.05 mg/kg for R/S each) in carrot leaves, carrot roots and green rape fodder and only at 0.1 mg/kg (0.05 mg/kg for R/S each) in broccoli
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–2.0 mg/kg (0.005-1.0 mg/kg for R/S each) green rape seed fodder (BBCH 65, 79).

Since mandestrobin levels in the 2010 and 2011 supervised rape seed fodder trials range between < 0.01–3.4 mg/kg extension of the validation is desirable for green rape seed fodder (BBCH > 60).

Table 82 Validation results for the R-isomer of mandestrobin with chiral HPLC-MS/MS method DFG-S19

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	% recovery range	RSD_r	control samples mg/kg (n)	calibration	reference, method
DFG-S19 with extraction module E1 and chiral HPLC-MS/MS for mass transition **314 to 192**									
broccoli	0.005	0.005 0.05	2 3	88 82	83-92 78-85	- 4.3%	< 0.3LOQ (6)	7-8 standards 0.15-20 ng/L	ROR-0202 rot crop

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								in solvent 1/× weighted r> 0.99	
lettuce	0.005	0.005 0.50	5 5	78 98	68-90 96-99	12% 1.2%	< 0.3LOQ	8 standards 0.2–40 ng/L in solvent r> 0.9992	ROA-0007 validation
lettuce	0.005	0.10	7	98	84-107	7.7%	< 0.3LOQ (5)	8 standards 0.2–40 ng/L in solvent r> 0.9998	ROR-0009 stor stab
lettuce	0.005	0.005 0.05	3 3	89 88	86-93 85-91	3.9% 3.4%	< 0.3LOQ (6)	6-8 standards 0.15-20 ng/L in solvent 1/× weighted r> 0.999	ROR-0202 rot crop
carrot leaves	0.005	0.005 0.05	3 3	91 95	82–96 91–99	8.6% 4.2%	< 0.3LOQ (6)	6 standards 0.15-10 ng/L matrix matched 1/× weighted r> 0.99	ROR-0202 rot crop
carrot roots	0.005	0.005 0.05	4 3	94 93	92–96 91–96	2.2% 3.1%	< 0.3LOQ (6)	6 standards 0.15-10 ng/L matrix matched 1/× weighted r> 0.99	ROR-0202 rot crop
Rape seed fodder (green whole plants, BBCH 65)	0.005	0.005 0.05 1.0	1 1 1	82 89 91	- - -	- - -	< 0.3 LOQ (1) < 0.005 (1)	8 standards 0.2–40 ng/L in solvent 1/× weighted r> 0.999	ROR-0202 rot crop
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.005	0.005 0.05 2.0	2 2 1	82 84 109	78-85 84-85 -	- - -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.005	0.005 0.05	1 1	86 84	- -	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.005	0.005 0.05 0.15 1.5	3 2 2 2	80 87 102 96	74-87 83-90 101–104 95-97	8.1% 4.3% - -	<LOQ	-	ROR-0198 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.005	0.005 0.05	1 1	84 82	- -	- -	<LOQ	-	ROR-0198 field trial
DFG-S19 with extraction module E9 with ASE and chiral HPLC-MS/MS for mass transition **314 to 192**									
rape seed	0.005	0.005 0.50	5 5	79 81	62–86 75-87	12% 5.9%	< 0.3LOQ	7 standards 0.2–20 ng/L in solvent r> 0.999	ROA-0005 validation
rape seed	0.005	0.10	7	94	83-110	9.7%	< 0.3LOQ (5)	6-8 standards 0.2–40 ng/L in solvent	ROR-0007 stor stab

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								r> 0.999	
rape seed	0.005	0.005 0.05	1 1	101 91	- -	- -	<LOQ	-	ROR-0008 field trial
rape seed	0.005	0.005 0.05	1 2	96 80	- 71–89	- -	<LOQ	-	ROR-0198 field trial
barley grains	0.005	0.005 0.50	5 5	93 106	90–97 100–111	2.7% 3.8%	< 0.3LOQ	5 standards 0.2–20 ng/L matrix matched r> 0.99999	**ROA**-0007 validation
barley grains	0.005	0.10	7	101	86-113	9.0%	< 0.3LOQ (5)	8 standards 0.2–40 ng/L in solvent r> 0.999	ROR-0009 stor stab
barley grains	0.005	0.005 0.05	3 2	81 81	75-91 76-86	11% -	< 0.3LOQ (7)	7-8 standards 0.15-20 ng/L in solvent 1/× weighted r> 0.99	ROR-0202 rot crop
barley straw	0.005	0.005 0.50	5 5	74 79	70–81 71–88	5.7% 7.9%	< 0.3LOQ	5 standards 0.2–20 ng/L matrix matched r> 0.99999	ROA-0007 validation
barley straw	0.005	0.10	7	96	87-110	10%	< 0.3LOQ (5)	8 standards 0.2–40 ng/L in solvent r> 0.9998	ROR-0009 stor stab
barley straw	0.005	0.005 0.05	2 3	74 85	72–75 70–103	- 20%	< 0.3LOQ (6)	6 standards 0.15-20 ng/L matrix matched 1/× weighted r> 0.99	ROR-0202 rot crop

Table 832 Validation results for mandestrobin S-isomer (S-2354) with chiral HPLC-MS/MS method DFG-S19

Commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
DFG-S19 with extraction module E1 and chiral HPLC-MS/MS for mass transition **314 to 192**									
lettuce	0.005	0.005 0.50	5 5	97 104	91–105 99-110	5.2% 4.5%	< 0.3LOQ	8 standards 0.2–40 ng/L in solvent r> 0.99999	**ROA**-0007 validation
lettuce	0.005	0.10	4	94	80–119	15%	< 0.3LOQ (5)	8 standards 0.2–40 ng/L in solvent r> 0.9998	ROR-0009 stor stab
lettuce	0.005	0.005 0.05	3 2	91 99	88-95 93-105	3.8% -	< 0.3LOQ (6)	6-8 standards 0.15-20 ng/L in solvent 1/× weighted r> 0.99	ROR-0202 rot crop
carrot leaves	0.005	0.005 0.05	3 3	77 76	71–84 71–80	8.4% 6.0%	< 0.3LOQ (6)	6-8 standards 0.15-20 ng/L in solvent 1/× weighted r> 0.999	ROR-0202 rot crop
carrot roots	0.005	0.005 0.05	4 3	81 83	73-92 72–94	12% 13%	< 0.3LOQ (6)	6-8 standards 0.15-20 ng/L in solvent	ROR-0202 rot crop

Commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								1/× weighted r> 0.999	
broccoli	0.005	0.005 0.05	3 2	88 96	85-93 91–102	5.3% -	< 0.3LOQ (6)	7-8 standards 0.15-20 ng/L in solvent 1/× weighted r> 0.99	ROR-0202 rot crop
Rape seed fodder (green whole plants, BBCH 65)	0.005	0.005 0.05 1.0	1 1 1	85 91 86	- - -	- - -	< 0.3 LOQ (1) < 0.005 (1)	8 standards 0.2–40 ng/L in solvent 1/× weighted r> 0.999	ROR-0202 rot crop
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.005	0.005 0.05 2.0	2 2 1	92 90 105	91–94 89–90 -	- - -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.005	0.005 0.05	1 1	88 87	- -	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.005	0.005 0.05 0.20 2.0	3 3 2 2	90 91 88 102 103	86-93 85-94 78-98 101–	4.2% 5.4% - -	<LOQ	-	ROR-0198 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.005	0.005 0.05	1 1	90 94	- -	- -	<LOQ	-	ROR-0198 field trial
DFG-S19 with extraction module E9 with ASE and chiral HPLC-MS/MS for mass transition **314 to 192**									
rape seed	0.005	0.005 0.5	5 5	81 78	74-91 72–83	9.0% 5.9%	< 0.3LOQ	7 standards 0.2–20 ng/L in solvent r> 0.99999	ROA-0005 validation
rape seed	0.005	0.10	7	92	74-107	12%	< 0.3LOQ (5)	6-8 standards 0.2–40 ng/L in solvent r> 0.999	ROR-0007 stor stab
rape seed	0.005	0.005 0.05	1 1	96 101	- -	- -	<LOQ	-	ROR-0008 field trial
rape seed	0.005	0.005 0.05	2 2	106 109 92	107- 80–104	- -	<LOQ	-	ROR-0198 field trial
barley grains	0.005	0.005 0.50	5 5	99 105	90–106 96-112	6.5% 5.5%	< 0.3LOQ	8 standards 0.2–40 ng/L in solvent r> 0.99999	ROA-0007 validation
barley grains	0.005	0.10	7	95	87-107	9.9%	< 0.3LOQ (5)	8 standards 0.2–40 ng/L in solvent r> 0.9998	ROR-0009 stor stab
barley grains	0.005	0.005 0.05	3 3	82 84	79-86 72–98	4.6% 16%	< 0.3LOQ (7)	6-8 standards 0.15-20 ng/L in solvent	ROR-0202 rot crop

Commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								1/× weighted r> 0.99	
barley straw	0.005	0.005 0.50	5 5	88 92	81–92 80–104	4.9% 10%	< 0.3LOQ	8 standards 0.2–40 ng/L in solvent r> 0.99999	ROA-0007 validation
barley straw	0.005	0.10	7	79	73-83	5.2%	< 0.3LOQ (5)	8 standards 0.2–40 ng/L in solvent r> 0.9998	ROR-0009 stor stab
barley straw	0.005	0.005 0.05	2 3	88 80	81–94 79-83	- 2.9%	< 0.3LOQ (6)	6 standards 0.15-20 ng/L matrix matched 1/× weighted r> 0.99	ROR-0202 rot crop

Analytical methods for enforcement in animal commodities

The Meeting received the description and validation for an enforcement method for the determination of mandestrobin, and its metabolite De-Xy-mandestrobin in animal commodities. No validation results for existing multi-residue methods were submitted.

HPLC-MS/MS Method RM-48M-1 for parent and De-Xy-mandestrobin

HPLC-MS/MS Method RM-48M-1 (version 24 July 2014) determines mandestrobin (sum of R and S isomers) and De-Xy-mandestrobin in animal commodities. The method was described and validated in [Nie, 2017, ROA-0054].

Homogenised samples (5 g) were shaken with a mixture of acetone/water (7/3, v/v) with a reciprocal shaker for 1 hour. The samples were kept in the refrigerator for at least one night. After centrifugation, the extract was collected and the solids remaining after extraction were shaken again with acetone/water (7/3, v/v) with a reciprocal shaker for 1 hour. After centrifugation, the extract was collected. Both extracts were combined and an aliquot of the extract was mixed with 5% sodium chloride solution and partitioned twice with dichloromethane. The dichloromethane phases were combined and evaporated to dryness and re-dissolved in acetonitrile. The acetonitrile was partitioned twice with hexane to remove oily and fatty constituents. The acetonitrile layer was evaporated to dryness. The residue was re-dissolved in hexane/ethyl acetate (9/1, v/v) and cleaned-up with a Silica Sep Pak column. The residues were eluted with ethyl acetate. The ethyl acetate was evaporated to dryness and re-dissolved in 0.05% formic acid in methanol / 0.05% formic acid in water (1:1, v/v). Samples were analysed by HPLC-MS/MS at m/z 314 to 192 (parent) and m/z 210 to 192 (De-Xy-mandestrobin). Calibration was applied with external standards in solvent using weighted 2^{nd} order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ for each analyte was 0.01 mg/kg. Validation results are shown in Tables 84 and 85.

An independent laboratory validation (ILV) was performed on cream, eggs and beef liver [Perez, 2017, ROA-0055]. The method was slightly changed. The two acetone/water extractions were conducted within a short timeframe. Results are shown in Tables 84 and 85.

The evaluation of potential matrix effects [Perez, 2017, ROA-0055] demonstrated the matrix load in cream and egg samples final volume had no influence (<20%) on the analysis of mandestrobin or De-Xy mandestrobin. The evaluation of potential matrix effects demonstrated the matrix load in beef liver samples final volume had some influence (+17 to +18%) on the analysis of mandestrobin but none on its metabolite De-Xy-mandestrobin. A matrix matched standard was used for the determination of mandestrobin in the beef liver samples.

Average recoveries for the confirmation mass transitions 314 to 160 for parent and 210 to 192 for De-Xy-mandestrobin [Perez, 2017, ROA-0055] ranged from 70–79% for parent and 74-91% for De-Xy-mandestrobin in cream, liver and eggs at 0.01–0.10 mg/kg (n = 5 at each level). Control samples at these transitions were below 0.3 LOQ. Linearity at these transitions was confirmed in the range 0.3-10 ng/L.

HPLC-MS/MS method RM-48M-1 for the determination of mandestrobin is considered:

- valid (full validation) in the range 0.01–0.10 mg/kg in liver, eggs and cream.
- validation for muscle, fat and whole milk is desirable.

Table 84 Validation results for mandestrobin with HPLC-MS/MS methods RM-48M-1 at m/z 314 to 192

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
liver	0.01	0.01 0.10	5 5	83 79	74-87 73-91	6.5% 9.8%	-	-	ROA-0054 validation
beef liver	0.01	0.01 0.10	5 5	74 79	69-78 60–86	5.0% 14%	< 0.3LOQ (2)	0.3-10 ng/mL matrix matched 1/× weighted r> 0.999	ROA-0055 ILV
egg	0.01	0.01 0.10	5 5	86 83	80–95 77-88	7.7% 6.3%	-	-	ROA-0054 validation
eggs	0.01	0.01 0.10	5 5	73 79	71–76 75-83	2.7% 3.9%	< 0.3LOQ (2)	0.3-10 ng/mL in solvent 1/× weighted r> 0.999	ROA-0055 ILV
cream	0.01	0.01 0.10	5 5	101 82	93-113 74-86	8.0% 5.6%	-	-	ROA-0054 validation
cream	0.01	0.01 0.10	5 5	76 70	65-82 64-81	9.3% 11%	< 0.3LOQ (2)	0.3-10 ng/mL in solvent 1/× weighted r> 0.999	ROA-0055 ILV

Table 85 Validation results for De-Xy-mandestrobin with HPLC-MS/MS methods RM-48M-1 at m/z 210 to 192

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
liver	0.01	0.01 0.10	5 5	105 92	103-107 81–98	1.8% 7.3%	-	-	ROA-0054 validation
beef liver	0.01	0.01 0.10	5 5	90 81	87-92 77-87	2.2% 4.3%	< 0.3LOQ (2)	0.3-10 ng/mL in solvent 1/× weighted r> 0.999	ROA-0055 ILV
egg	0.01	0.01 0.10	5 5	88 92	83-91 87-101	3.5% 6.9%	-	-	ROA-0054 validation
eggs	0.01	0.01 0.10	5 5	78 82	75-82 79-84	3.6% 2.1%	< 0.3LOQ (2)	0.3-10 ng/mL in solvent 1/× weighted r> 0.999	ROA-0055 ILV
cream	0.01	0.01 0.10	5 5	88 86	86-90 78-91	2.2% 6.3%	-	-	ROA-0054 validation
cream	0.01	0.01 0.10	5 5	82 74	79-86 68-83	3.7% 8.5%	< 0.3LOQ (2)	0.3-10 ng/mL in solvent 1/× weighted r> 0.999	ROA-0055 ILV

Analytical methods used in study reports in plant commodities

The Meeting received the description and validation for nine analytical methods for the determination of mandestrobin, and its metabolites De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH2OH-mandestrobin, 5-CH2OH-mandestrobin.

GC-MS Method RM-48C-1 for mandestrobin

GC-MS Method RM-48C-1 (version 20 June 2011) determines mandestrobin (sum of R and S isomers) in rape seed. The method was described, used and validated in a supervised field trial on rape seed [Green, 2013, ROR-0238].

Homogenised samples of 5 g rape seed were extracted twice with a mixture of acetone and water (7/3, v/v) by shaking for 1 hour. Extracts were combined and the acetone was evaporated using a rotary-evaporator at 40 °C. The remaining aqueous phase was mixed with 5% sodium chloride solution and partitioned with dichloromethane. The dichloromethane phase was evaporated to dryness and re-dissolved in acetonitrile. The extracted was cleaned-up by partitioning with hexane to remove oils. The remaining acetonitrile phase from this step was evaporated to dryness, re-dissolved in hexane/ethyl acetate (3/1; v/v) and cleaned up with a Silica Gel (SPE) column cartridge. The eluate was evaporated to dryness and dissolved in toluene. Mandestrobin was quantified by GC-MS with selective ion monitoring. Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg. Validation results are shown in Table 86.

GC-MS method RM-48C-1 for the determination of mandestrobin is considered:

- valid (full validation) in the range 0.01–0.1 mg/kg in soya bean seeds and 0.01–0.2 mg/kg in rape seed.

Since mandestrobin levels in the supervised trials on rape seed range between < 0.01–0.54 mg/kg, method RM-48C-1 is not considered fit for purpose for levels between 0.2–0.6 mg/kg. However, since the trials selected for MRL derivation have levels below 0.2 mg/kg, this has no impact on the selection of the trials for MRL derivation.

Table 86 Validation results for mandestrobin (sum of R and S-isomers) with GC-MS method RM-48C-1

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	% recovery range	RSD_r	control samples mg/kg (n)	calibration	reference, method
soya bean seeds	0.01	0.01 0.1	6 7	115 101	106-119 91–110	4.4% 5.9%	-	-	ROR-0238 appendix 3 validation
rape seed	0.01	0.01 0.2	4 4	114 108	104-120 96-120	6.0% 9.0%	< 0.3LOQ (4)	5 standards in solvent 0.01–0.5 mg/L 1/× weighted $r^2 > 0.99$	ROR-238 field trial

HPLC-MS/MS Method RM-48C-2 and RM-48C-2B for parent and De-Xy-mandestrobin

HPLC-MS/MS Method RM-48C-2 (version 26 August 2013) determines mandestrobin (sum of R and S isomers) and De-Xy-mandestrobin in rape seed and rape seed processed commodities. The method was described and used in a field rotational crop study, supervised field trials, storage stability and processing studies on rape seed [Green, 2013, ROR-0240; Green, 2013, ROR-0238 (including addendum); Green, 2013a, ROR-0239; Green, 2015, ROR-0258].

Homogenised samples of 5 g rape seed were extracted twice with a mixture of acetone and water (7/3, v/v) by shaking for 1 hour. Extracts were combined. An aliquot of the extract was mixed

with 5% sodium chloride solution and partitioned with dichloromethane. The dichloromethane phase was evaporated to dryness, re-dissolved in hexane/ethyl acetate (9/1, v/v) and cleaned up on a SPE Silica Sep Pak cartridge. The eluate was evaporated to dryness and re-dissolved in 0.05% formic acid in methanol/0.05% formic acid in water (1:1, v/v). Samples were analysed by HPLC-MS/MS at m/z 314 to 192 (parent) and m/z 210 to 192 (De-Xy-mandestrobin). Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ for each analyte was 0.02 mg/kg. Validation results are shown in Tables 87 and 88.

An independent laboratory validation of RM-48C-2 for rape seed and grapes is described in [Chen, 2013, ROA-0040] and summarized in Tables 87 and 88. The method was successfully validated for grapes in the first attempt, but for rape seed three attempts were required. The SPE extraction method, the glassware and the HPLC-MS/MS optimisation were the critical factors. The method was not successfully validated for De-Xy-mandestrobin in rape seed, where the average recovery at 0.2 mg/kg was below 70%. However, since the De-Xy-mandestrobin concentration in rape seed was at or below 0.02 mg/kg, this will not affect the outcome of the supervised residue trials.

In [Green, 2013, ROR-0240] HPLC-MS/MS method RM-48C-2 was slightly modified by a reduction in sample size and an increase in aliquot size. For wheat straw, a reduction in the concentrations of the calibration standards was also done. Validation results are shown in Tables 87 and 88. HPLC-MS/MS method RM-48C-2A (version 3 December 2013) is an updated method for RM-48C-2 and is described in [Bitter, 2013, ROA-0038]. The method only contains some textual changes. The method was not used anywhere.

HPLC-MS/MS Method RM-48C-2B (version 23 January 2014) is an updated method for RM-48C-2A and contains some textual changes and additional validation results for maize fodder. The method was described and used in storage stability studies [Green, 2015a, ROR-0259; Green, 2016, ROR-0274]. Validation results are shown in Tables 87 and 88.

An independent laboratory validation of RM-48C-2B for soya bean is described in [Perez, 2017b, ROA-0062] and summarized in Tables 87 and 88.

In [Green, 2016, ROR-0274] HPLC-MS/MS method RM-48C-2B was slightly modified. The sample preparation steps were identical, but the final extract was filtered using a 0.2 µm filter. The concentrations of the calibration standards were ten times higher than the original method specified. Validation results are shown in Tables 87 and 88.

HPLC-MS/MS method RM-48C-2 and RM-48C2B for the determination of mandestrobin and De-Xy-mandestrobin is considered:

- valid (full validation) in the range 0.02–0.2 mg/kg in grapes, rape seed, rape seed oil, rape seed meal and 0.01–0.1 mg/kg in soya bean seeds
- valid (reduced validation) only at 0.02 mg/kg in spinach leaves, red beet roots, red beet leaves, maize fodder, wheat forage, wheat hay, wheat grain, wheat straw, sorghum forage (parent only), sorghum grain, sorghum fodder.
- not valid for the determination of De-Xy-mandestrobin in sorghum forage

Since mandestrobin levels in the supervised rape seed trials range between < 0.01–0.54 mg/kg, method RM-48C-2 is not considered fit for purpose for levels between 0.2–0.6 mg/kg.

Table 87 Validation results for mandestrobin with HPLC-MS/MS methods RM-48C-2 and RM-48C-2B

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
HPLC-MS/MS method RM-48C-2									
grapes	0.02	0.02 0.2	5 5	81 74	76-89 69-77	6.2% 5.1%	< 0.3LOQ (2)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.999$	ROA-0040 ILV
spinach leaves	0.02	0.02	3	88	85-90	3.2%	<LOQ (3)	-	ROR-0240 field rotation
red beet roots	0.02	0.02	3	91	90–92	1.5%	<LOQ (3)	-	ROR-0240 field rotation
red beet leaves	0.02	0.02	3	99	93-104	5.5%	<LOQ (3)	-	ROR-0240 field rotation
wheat forage	0.02	0.02	5	92	88-98	4.6%	<LOQ (3)	-	ROR-0240 field rotation
wheat hay	0.02	0.02	3	75	74-77	2.1%	<LOQ (3)	-	ROR-0240 field rotation
wheat grain	0.02	0.02	5	83	73-96	11%	<LOQ (3)	-	ROR-0240 field rotation
wheat straw	0.02	0.02	4	78	68-89	12%	<LOQ (3)	-	ROR-0240 field rotation
sorghum forage	0.02	0.02	3	70	66-74	6.0%	<LOQ (3)	-	ROR-0240 field rotation
sorghum grain	0.02	0.02	3	84	79–92	7.7%	<LOQ (3)	-	ROR-0240 field rotation
sorghum fodder	0.02	0.02	3	88	86-91	3.1%	<LOQ (3)	-	ROR-0240 field rotation
rape seed	0.02	0.02 0.2	5 5	100 99	94-111 94-107	6.9% 5.2%	-	-	ROR-0238 ROR-0239 validation
rape seed	0.02	0.02 0.2	5 5	114 78	108-123 70–86	5.3% 8.7%	< 0.3LOQ (2)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99999$	ROA-0040 ILV
rape seed	0.02	0.020 0.10 0.20	3 3 2	80 88 90	71–88 84-91 89–91	11% 4.3% -	< 0.3LOQ (6)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99$	ROR-0238 field trial
rape seed	0.02	0.20	4	71	61–80	13%	< 0.3LOQ (2); 0.022 [a]	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.999$	ROR-0238 add ROR-0258 stor stab
rape seed	0.02	0.02 0.10	8 7	82 80	70–91 70–89	9.3% 8.2%	< 0.3LOQ (13) 0.007 (1) [b]	5 standards in solvent 0.5-10 µg /L 1/× weighted $r^2 > 0.99$	ROR-0239 field trial
rape seed oil	0.02	0.02 0.20	3 1	77 92	76-78 -	13% -	< 0.3LOQ (3)	5 standards in solvent 0.5-10 µg /L 1/× weighted $r^2 > 0.999$	ROR-0238 processing
rape seed meal	0.02	0.02 0.20	3 1	84 77	78-88 -	6.0% -	< 0.3LOQ (3)	5 standards in solvent 0.5-10 µg /L 1/× weighted	ROR-0238 processing

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								$r^2 > 0.9999$	
Method RM-48C-2B									
maize fodder	0.02	0.02	4	96	94-99	3.1%	-	-	ROR-0259; ROR-0274 validation
rape seed	0.02	0.02	1	86	-	-	< 0.3LOQ (1)	5 standards in solvent 0.5-10 µg /L 1/× weighted $r^2 > 0.999$	ROR-0259 stor stab
rape seed	0.02	0.02	1	113	-	-	< 0.3LOQ (1)	5 standards in solvent 5-100 µg /L 1/× weighted $r^2 > 0.999$	ROR-0274 stor stab
soya bean seeds	0.01	0.01 1.0	5 5	102 94	100–105 89–98	2.1% 3.8%	< 0.5LOQ (1)	6 standards in solvent 0.5-25 µg/L r≥0.999	ROA-0062 ILV

[a] Control sample with 0.022 mg/kg mandestrobin for day 314 of storage in [ROR-0258]

[b] Control sample with 0.007 mg/kg mandestrobin for trial N, Kipp, Alberta [ROR-239].

Table 883 Validation results for De-Xy-mandestrobin with HPLC-MS/MS method RM-48C-2 and RM-48C-2B

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
Method RM-48C-2									
grapes	0.02	0.02 0.2	5 5	89 74	74-103 67-80	13% 7.3%	< 0.3LOQ (2)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.999$	ROA-0040 ILV
spinach leaves	0.02	0.02	3	84	84-85	0.8%	<LOQ (3)	-	ROR-0240 field rotation
red beet roots	0.02	0.02	3	73	67-77	6.9%	<LOQ (3)	-	ROR-0240 field rotation
red beet leaves	0.02	0.02	4	90	80–98	8.4%	<LOQ (3)	-	ROR-0240 field rotation
wheat forage	0.02	0.02	3	95	89-100	5.6%	<LOQ (3)	-	ROR-0240 field rotation
wheat hay	0.02	0.02	3	84	82–87	3.3%	<LOQ (3)	-	ROR-0240 field rotation
wheat grain	0.02	0.02	3	86	71–94	15%	<LOQ (3)	-	ROR-0240 field rotation
wheat straw	0.02	0.02	4	85	76-92	9.3%	<LOQ (3)	-	ROR-0240 field rotation
sorghum forage	0.02	0.02	3	69	67-74	5.8%	<LOQ (3)	-	ROR-0240 field rotation
sorghum grain	0.02	0.02	3	80	78-84	3.5%	<LOQ (3)	-	ROR-0240 field rotation
sorghum fodder	0.02	0.02	3	79	74-83	5.5%	<LOQ (3)	-	ROR-0240 field rotation
rape seed	0.02	0.02 0.2	5 5	90 97	85-96 93-102	4.9% 3.7%	-	-	ROR-0238 ROR-0239 validation
rape seed	0.02	0.02 0.2	5 5	82 67	73-97 61–80	12% 11%	< 0.3LOQ (2)	5 standards in solvent	ROA-0040 ILV

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								0.5-10 µg/L 1/× weighted $r^2 > 0.99999$	
rape seed	0.02	0.020 0.10 0.20	5 5 2	89 92 96	83-100 87-99 95-98	8.0% 5.7% -	< 0.3LOQ (10)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.999$	ROR-0238 field trial
rape seed	0.02	0.20	4	83	72–93	11%	< 0.3LOQ (3)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99999$	ROR-0238 add ROR-0258 stor stab
rape seed	0.02	0.02 0.10	8 7	93 95	86-98 90–97	4.9% 3.2%	< 0.3LOQ (14)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99$	ROR-0239 field trial
rape seed oil	0.02	0.02 0.20	3 1	86 103	77-93 -	9.1% -	< 0.3LOQ (3)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99999$	ROR-0238 processing
rape seed meal	0.02	0.02 0.20	3 1	76 83	74-80 -	4.2% -	< 0.3LOQ (3)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99999$	ROR-0238 processing
Method RM-48C-2B									
maize fodder	0.02	0.02	3	79	72–84	7.8%	-	-	ROR-0259; ROR-0274 validation
rape seed	0.02	0.02	1	98	-	-	< 0.3LOQ (1)	5 standards in solvent 0.5-10 µg/L 1/× weighted $r^2 > 0.99$	ROR-0259 stor stab
rape seed	0.02	0.02	1	107	-	-	< 0.3LOQ (1)	5 standards in solvent 5-100 µg/L 1/× weighted $r^2 > 0.99$	ROR-0274 stor stab
soya bean	0.01	0.01 1.0	5 5	106 95	103-108 89–99	2.0% 4.1%	< 0.5LOQ (1)	6 standards in solvent 0.5-25 µg/L $r \geq 0.999$	ROA-0062 ILV

HPLC-MS/MS Method RM-48-G for mandestrobin and De-Xy-mandestrobin

HPLC-MS/MS Method RM-48-G (version 27 August 2013) determines mandestrobin (sum of R and S isomers) and De-Xy-mandestrobin in grapes. The method was described and used in supervised field trials and storage stability studies on grapes and strawberries and processing studies on grapes [Bitter *et al*., 2013, ROR-0234 (including addendum); Bitter, 2013, ROR-0235; Bitter *et al*., 2013, ROR-0236 (including addendum); Bitter, 2013a, ROR-0237; Bitter, 2015a, ROR-0260; Bitter, 2015, ROR-0261].

Homogenised samples of 2.5 g grapes were extracted twice with a mixture of acetone and water (7/3, v/v) by shaking for 1 hour. Extracts were combined and the acetone was evaporated off

using a rotary evaporator at 40 °C. The remaining aqueous phase was mixed with 5% sodium chloride solution and partitioned with dichloromethane. The dichloromethane phase was evaporated to dryness and re-dissolved in 0.05% formic acid in methanol/0.05% formic acid in water (1:1, v/v). Samples were analysed by HPLC-MS/MS at m/z 314 to 192 (parent) and m/z 210 and 232 (De-Xy-mandestrobin). Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ for each analyte was 0.02 mg/kg. Validation results are shown in Tables 89 and 90.

Control samples were below 0.3LOQ, except in two cases. One control sample for grapes in trial E, Kerman, CA [Bitter *et al*., 2013, ROR-0234], contained 0.0080 mg/kg mandestrobin; and one control sample for strawberries in trial F, Porterville, CA [Bitter *et al*., 2013, ROR-0236], contained 0.0083 mg/kg mandestrobin.

HPLC-MS/MS method RM-48-G for the determination of mandestrobin and De-Xy-mandestrobin is considered:

- valid (full validation) in the range 0.02–0.2 mg/kg in grapes, strawberries,
- valid (reduced validation) at only 0.02 mg/kg in grape juice and grape raisins,
- Limited recovery experiments (n = 1–2) suggest extension of the validity for both analytes to 15 mg/kg for grapes, 20 mg/kg for grape juice, 30 mg/kg for grape raisins and 5 mg/kg for strawberries.

Table 89 Validation results for mandestrobin with HPLC-MS/MS methods RM-48G

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.02	0.02 0.2	5 5	102 92	96-105 91–94	3.4% 1.3%	-	-	ROR-0234; ROR-0235; ROR-0236; ROR-0237; ROR-0260 ROR-0261 validation
grapes	0.02	0.020 0.20 5 10 15	11 9 1 1 1	103 95 86 80 86	90–121 86-104 - - -	9.3% 7.5% - - -	< 0.3LOQ (12); 0.0080 (1) [a]	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 field trial
grapes	0.02	0.02 0.20 2.0	2 2 2	114 90 91	113-116 89–91 88-94	- - -	< 0.3LOQ (4)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0235 field trial
grapes	0.02	0.02 0.1 2.0	1 1 1	100 72 88	- - -	-	< 0.3LOQ (3)	5 standards in solvent 0.5-50 µg/L r^2> 0.999	ROR-0234 add ROR-0261 stor stab
grape juice	0.02	0.02 15 20	3 1 1	93 110 84	84-100 - -	8.8% - -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 processing
grape juice	0.02	0.02 10 15	2 1 1	97 105 110	94-100 - -	- - -	< 0.3LOQ (4)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 add stor stab
grape raisins	0.02	0.02 15 20 30	3 1 1 1	98 95 94 110	81–120 - - -	20% - - -	< 0.3LOQ (5)	5 standards in solvent 0.5-50 µg/L r^2> 0.999	ROR-0234 processing
grape raisins	0.02	0.02 15	3 1	98 95	81–120 -	20% -	< 0.3LOQ (5)	5 standards in solvent	ROR-0234 add ROR-0261

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
		20 30	1 2	101 94	- 91–97	-		0.5-50 µg/L r^2> 0.999	stor stab
strawberries	0.02	0.02 0.20 5.0	7 7 1	100 97 88	76-116 74-113 -	14% 18% -	< 0.3LOQ (10); 0.0083 (1) [b]	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0236 field trial
strawberries	0.02	0.02 0.2 2.0	1 1 1	109 101 89	- - -	- - -	< 0.3LOQ (3)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0237 field trial
strawberries	0.02	0.02 0.1 0.2 2.0 2.5	1 1 1 2 1	97 88 102 94 105	- - - 91–97 -	- - - - -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0236 add ROR-0260 stor stab

[a] control sample for trial E, Kerman, CA, contained 0.0080 mg/kg mandestrobin;[ROR-0234]

[b] control sample for trial F, Porterville, CA, contained 0.0083 mg/kg mandestrobin [ROR-0236].

Table 90 Validation results for De-Xy-mandestrobin with HPLC-MS/MS method RM-48G

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.02	0.02 0.2	5 5	103 93	96-110 90–95	4.5% 2.3%	-	-	ROR-0234; ROR-0235; ROR-0236; ROR-0237; ROR-0260 validation
grapes	0.02	0.020 0.20 5 10 15	12 9 1 1 1	106 99 97 84 85	92–114 92–107 - - -	6.0% 4.6% - - -	< 0.3LOQ (13)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 field trial
grapes	0.02	0.02 0.20 2.0	2 2 1	104 100 106	100–107 99-100 -	- - -	< 0.3LOQ (4)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0235 field trial
grapes	0.02	0.02 0.1 2.0	1 1 1	92 98 90	- - -	-	< 0.3LOQ (3)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 add ROR-0261 stor stab
grape juice	0.02	0.02 15 20	3 1 1	106 112 79	103-109 - -	3.0% - -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L r^2> 0.999	ROR-0234 processing
grape juice	0.02	0.02 10 15	3 1 1	106 115 112	103-109 - -	3.0% - -	< 0.3LOQ (4)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 add ROR-0261 stor stab
grape raisins	0.02	0.02 15 20 30	3 1 1 1	108 94 97 91	106-110 - - -	1.9% - - -	< 0.3LOQ (5)	5 standards in solvent 0.5-50 µg/L r^2> 0.999	ROR-0234 processing
grape raisins	0.02	0.02 0.1 15 20	4 1 1 1	108 92 94 106	106-110 - - -	1.8% - - -	< 0.3LOQ (5)	5 standards in solvent 0.5-50 µg/L r^2> 0.99	ROR-0234 add ROR-0261 stor stab
strawberries	0.02	0.02	8	105	81–122	12%	< 0.3LOQ (11)	5 standards	ROR-0236

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
		0.20 5.0	6 1	98 99	89-102 -	5.4% -		in solvent 0.5-50 µg/L r^2> 0.999	field trial
strawberries	0.02	0.02 0.2 2.0	1 1 1	100 102 97	- - -	- - -	< 0.3LOQ (3)	5 standards in solvent 0.5-50 µg/L r^2> 0.999	ROR-0237 field trial
strawberries	0.02	0.02 0.1 0.2 2.0 2.5	1 1 1 1 1	112 102 102 96 108	- - - - -	- - - - -	< 0.3LOQ (5)	5 standards in solvent 0.5-50 µg/L r^2> 0.999	ROR-0236 add ROR-0260 stor stab

HPLC-MS/MS method SUM-1023V for De-Xy mandestrobin

HPLC-MS/MS Method SUM-1023V (version 28 February 2011) determines De-Xy-mandestrobin in various crops. The method was described and used in supervised field trials [Delmotte, 2011, ROR-0008; Lebrun, 2012, ROR-0198; Gemrot, 2017, ROR-0282; Klimmek and Gizler, 2017, ROR-0280], a storage stability on lettuce, rape seed, barley grain and straw [Daneva & Taeufer, 2012, ROR-0011] and dry beans and orange [Lindner & Grewe, 2016, ROR-0269; Lindner *et al*., 2017, ROR-0286] and validated by [Daneva *et al*., 2011, ROA-0010].

Homogenised samples of 20 g lettuce, 20 g rape seed commodities (seeds, fodder, pods), 20 g soya bean commodities (dry seeds, forage, fodder), 20 g barley grain or 5 barley straw were extracted twice with a mixture of acetone and water (80:20, v/v) during 10 min on a reciprocal shaker. Filtered extracts were combined and an aliquot of the extract was evaporated at 40 °C to evaporate the acetone. The extract was cleaned-up using a Chem Elute cartridge with dichloromethane. The eluate was evaporated to dryness, re-dissolved in hexane/acetone (8/1, v/v) and further cleaned-up by SPE using a MegaBond Elut SI cartridge. The eluate was evaporated to dryness and re-dissolved in acetonitrile/water (1/2, v/v). Residues were analysed by HPLC-MS/MS using mass transition 210 to 132 for quantification and 210 to 192 for confirmation. Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg for De-Xy-mandestrobin. Validation results are shown in Table 91.

Matrix effects were investigated for rape seed, lettuce, soya beans (dry seeds, BBCH 84-89), barley grains, soya bean forage (whole plant, BBCH 77-81), soya bean fodder (dry pods with seeds and rest of plant, BBCH 82), rape seed fodder (green whole plants, BBCH 65, 77-80, green pods and the rest of the plant, BBCH 79; dry pods with seeds and rest of plants, BBCH 80–87); and barley straw [Daneva & Taeufer, 2012, ROR-0011; Daneva *et al*., 2011, ROA-0010; Lindner & Grewe, 2016, ROR-0269; Lindner *et al*., 2017, ROR-0286, Lebrun, 2012, ROR-0198; Gemrot, 2017, ROR-0282; Klimmek and Gizler, 2017, ROR-0280] by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions. No significant matrix effects (> 20%) were observed and standards in solvent are considered appropriate.

Average recoveries for mass transition 210 to 192 [Daneva *et al*., 2011, ROA-0010] ranged from 86-92% in rape seed, barley grain, barley straw and lettuce at 0.01–0.10 mg/kg (n = 5 at each level). Control samples at this transition were below 0.3 LOQ. Linearity was confirmed using 7 standards in solvent in the range 0.25-20 ng/L with 1/× weighted regression. ROR-0269 and ROR-0286 used the mass transition 210 to 192 for quantification.

Method ER-MT-1009 is an earlier version of SUM-1023V for determination of De-Xy-mandestrobin in grapes [Tabuchi, *et al*., 2010, ROA-0058]. The analytical method is the same as SUM-1023V, but describes two clean-up variants before application to the SPE MegaBond cartridge.

In the first variant, the acetone/water extract was diluted with 5% NaCl solution and then partitioned with dichloromethane. The dichloromethane phase is dried over anhydrous sodium sulphate and then concentrated to dryness. In the second variant, the acetone/water extract was evaporated at 40 °C to evaporate the acetone. The extract was cleaned-up using a Chem Elute cartridge with dichloromethane and the eluate was evaporated to dryness. The overall recovery was 83% (RSD 4.6%) for the method with partition and 94% (RSD 3.3%) for the method with evaporation. The clean-up with evaporation was used in the SUM-1023V method. Validation results for grapes, using the clean-up with evaporation are shown in Table 91.

HPLC-MS/MS method SUM-1023V for the determination of De-Xy-mandestrobin is considered:

- valid (full validation) in the range 0.01–0.1 mg/kg in oranges, lettuce, rape seed, barley grains, barley straw, green rape seed fodder (BBCH 65, 77-80).
- valid (reduced validation) in the range 0.01–0.1 mg/kg for grapes and only at 0.1 mg/kg in dry beans
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–0.10 mg/kg for dry soya bean seeds (BBCH 84-89), green soya bean forage (BBCH 77-81), dry soya bean fodder (BBCH 82) and dry rape seed fodder (BBCH 86)
- Limited recovery experiments (n = 1–2) suggest extension of the validity to the range of 0.01–1.0 mg/kg for rape seed and green rape seed fodder (BBCH 65, 77-80).

Table 91 Validation results for De-Xy mandestrobin with HPLC-MS/MS method SUM-1023V

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSDr	control samples mg/kg (n)	calibration	reference, method
whole orange	0.01	0.01 0.1	5 8	90 86	85-93 76-97	3.4% 8.0%	< 0.3LOQ (6)	m/z 210 to 192 7 standards in solvent 0.25-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab
grapes	0.01	0.01 0.1	3 3	95 92	93-97 89–95	2.2% 3.3%	< 0.3LOQ (1)	m/z 210 to 192 5 standards in solvent 0.5-20 ng/mL r2> 0.9999	ROA-0058 validation (ChemElut)
lettuce	0.01	0.01 0.1	5 5	86 86	76-93 85-88	8.3% 1.4%	< 0.3LOQ (2)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROA-0010 validation
lettuce	0.01	0.1	7	83	70–96	11%	< 0.3LOQ (5)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROR-0011 stor stab
soya bean forage (whole plant BBCH 77-81)	0.01	0.01 0.10	1 1	90 85	- -	- -	< 0.3LOQ (4)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r2 > 0.98	ROR-0280 field trial
soya bean fodder (dry pods with seeds BBCH 82)	0.01	0.01 0.10	1 1	86 77	- -	-	< 0.3LOQ (4)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL	ROR-0280 field trial

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	% recovery range	RSDr	control samples mg/kg (n)	calibration	reference, method
								1/× weighted r2 > 0.98	
soya bean fodder (rest of plant, BBCH 82)	0.01	0.01 0.10	1 1	94 70	- -	-	< 0.3LOQ (4)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r2 > 0.98	ROR-0280 field trial
dry soya beans (dry seeds, BBCH 84-89)	0.01	0.01 0.10	1 1	70 78	- -	-	< 0.3LOQ (8)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r2 > 0.98	ROR-0280 field trial
dry beans	0.01	0.1	8	84	72–94	10%	< 0.3LOQ (6)	m/z 210 to 192 7 standards in solvent 0.25-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab
rape seed	0.01	0.01 0.1	5 5	86 87	84-90 86-87	3.1% 0.6%	< 0.3LOQ (2)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROA-0010 validation
rape seed	0.01	0.01 0.1 1.0	6 6 1	90 88 100 96	77-107 80– -	14% 8.6% -	< 0.3LOQ (10)	m/z 210 to 132 7 matrix standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
rape seed	0.01	0.1	7	88	80–95	6.6%	< 0.3LOQ (5)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROR-0011 stor stab
Rape seed fodder (green whole plants, BBCH 77-80)	0.01	0.01 0.1 1.0	6 6 1	88 91 107	79-108 85-94 -	12% 3.6% -	< 0.3LOQ (15)	m/z 210 to 132 7 matrix standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (dry plants without pods, BBCH 86)	0.01	0.01 0.1 1.0	2 2 1	89 88 98	88-89 84-91 -	- - -	< 0.3LOQ (5)	m/z 210 to 132 7 matrix standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (dry pods with seeds, BBCH 86)	0.01	0.01 0.1 1.0	2 2 1	77 89 82	76-78 86-92 -	- - -	< 0.3LOQ (5)	m/z 210 to 132 7 matrix standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.01	0.01 0.10	2 3	80 74	79-81 70–80	- 6.9%	<LOQ	-	ROR-0008 field trial

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSDr	control samples mg/kg (n)	calibration	reference, method
Rape seed fodder (green pods with seeds, BBCH 79)	0.01	0.01 0.10	1 1	72 74	- -	- -	<LOQ	-	ROR-0008 field trial
rape seed	0.01	0.01 0.10	1 1	70 71	- -	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.01	0.01 0.10	2 2	84 84	78-89 83-84	- -	<LOQ	-	ROR-0198 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.01	0.01 0.10	1 1	78 85	- -	- -	<LOQ	-	ROR-0198 field trial
rape seed	0.01	0.01 0.10	2 2	80 80	74-85 76-85	- -	<LOQ	-	ROR-0198 field trial
barley grains	0.01	0.01 0.1	5 5	91 88	88-96 83-90	3.2% 3.3%	< 0.3LOQ (2)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROA-0010 validation
barley grains	0.01	0.1	7	89	80–99	8.2%	< 0.3LOQ (5)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROR-0011 stor stab
barley straw	0.01	0.01 0.1	5 5	87 88	85-88 79–94	1.9% 6.8%	< 0.3LOQ (2)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROA-0010 validation
barley straw	0.01	0.1	7	78	72–98	11%	< 0.3LOQ (5)	m/z 210 to 132 7 standards in solvent 0.25-20 ng/mL 1/× weighted r> 0.99	ROR-0011 stor stab

HPLC-MS/MS method SUM-1021V for 4-OH-mandestrobin and its (malonyl)glucosides

HPLC-MS/MS method SUM-1021V (version 15 April 2011) was used to determine 4-OH-mandestrobin and its (malonyl)glucosides in lettuce, rape seed and barley (grain, straw). The method was described and used in supervised field trials [Delmotte, 2011, ROR-0008; Lebrun, 2012, ROR-0198; Gemrot, 2017, ROR-0282], a storage stability study on lettuce, rape seed, barley grain and barley straw [Daneva & Taeufer, 2012a, ROR-0012] and validated in [Daneva & Taeufer, 2011, ROA-0011].

Homogenised samples 20 g lettuce, rape seed commodities (seeds, fodder, dry pods) or barley grains or 5 g barley straw were extracted twice with a mixture of acetone and water (80:20, v/v) for 10 min. An aliquot of the combined extracts was evaporated at 40 °C to remove the acetone. The remaining aqueous phase was adjusted to pH 11 by the addition of 0.1 M NaOH. The extract was hydrolysed for 1 hour at room temperature to cleave any malonyl groups off. The hydrolysate was adjusted to pH = 7 by the addition of 0.1 M HCl and adjusted to pH 5 by the addition of 10 mM sodium acetate. After addition of 250 units of beta-glycosidase, extracts were shaken at 100 rpm for 3 hrs at 37 °C to cleave any glucose groups off. Clean-up of the hydrolysate was performed using a

Chem Elut cartridge with ethyl acetate. The ethyl acetate eluate was evaporated to dryness, re-dissolved in water, followed by further clean-up by SPE with an Oasis HLB cartridge with acetonitrile/water (1:1, v/v). The final extract was analysed for total 4-OH-mandestrobin by HPLC-MS/MS (retention time 8.4-8.8 min) using the transition from m/z 328 to 136 for quantification and the transition from m/z 330 to 192 for confirmation. Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg for 4-OH-mandestrobin.

Recovery experiments were conducted with free 4-OH mandestrobin, since standards for 4-OH-mandestrobin conjugates were not available. Validation results are shown in Table 92.

In ROR-0012 the day 0 and 1 month storage stability samples were cleaned with an in-house packed cartridge containing Hydromatrix diatomaceous earth instead of the pre-packed Chem Elut cartridge These samples needed an additional ethyl acetate /methanol (90:10, v/v) elution to satisfactorily recover the 4-OH-mandestrobin from the cartridge.

Modification A was used in supervised field trials on soya bean commodities [Klimmek and Gizler, 2017, ROR-0280]. Homogenised samples of 10 g soya bean forage (whole plant, BBCH 77-81), 10 g soya bean seeds (dry seeds, BBCH 84-89) or 10 g soya bean fodder (dry pods with seeds and rest of plant, BBCH 82) were shaken with acetone/1M sodium ascorbate/water (50:4:12, v/v) for 10 min. Samples were left overnight (16 hours) at ambient temperature. Samples were filtered in the presence of Celite and the remaining solids were extracted again with acetone/1M sodium ascorbate/water (50:4:12, v/v) for 10 min. Extracts were combined and further worked-up following the original procedure. Quantification was at m/z = 330 to 192, while confirmation was at m/z = 328 to 136.

Matrix effects at m/z 328 to 136 were investigated for rape seed, lettuce, soya beans (dry seeds, BBCH 84-89), rape seed (seeds, forage, pods), barley grains, soya bean forage (whole plant, BBCH 77-81), rape seed fodder (green whole plants, BBCH 65, 77-80, green pods and the rest of the plant, BBCH 79; dry pods with seeds and rest of plants, BBCH 80–87); soya bean fodder (dry pods with seeds and rest of plant, BBCH 82) and barley straw [Daneva & Taeufer, 2012a, ROR-0012; Daneva & Taeufer, 2011, ROA-0011; Gemrot, 2017, ROR-0282; Lebrun, 2012, ROR-0198; Klimmek and Gizler, 2017, ROR-0280] by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions. Matrix effects (>20%) were observed for soya bean forage (-50%), soya bean fodder (-30%) and barley straw (-39%) and matrix matched standards were used for these matrices.

Both mass transitions showed similar accuracy and precision [Daneva & Taeufer, 2012a, ROR-0012; Daneva & Taeufer, 2011, ROA-0011]. The use of two characteristic mass transitions ensures a high level of specificity and therefore an additional confirmation method is not considered necessary.

Method ER-MT-1010 is an earlier version of SUM-1021V for determination of 4-OH-mandestrobin and its (malonyl)glucosides in grapes [Tabuchi, *et al*., 2010, ROA-0059]. The analytical method is the same as SUM-1021V. Validation results are shown in Table 92.

HPLC-MS/MS method SUM-1021V for the determination of 4-OH-mandestrobin and its (malonyl)glucosides is considered

- valid (full validation) for the determination of 4-OH-mandestrobin in the range 0.01–0.1 mg/kg in lettuce, rape seed, dry soya beans, barley grains, green rape seed fodder (BBCH 77-80), barley straw;
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–0.1 mg/kg for grapes, soya bean forage (BBCH 77-81), dry soya bean fodder (BBCH 82), and dry rape seed fodder (BBCH 86).
- Limited recovery experiments (n = 1–2) suggest extension of the validity to the range of 0.01–1.0 mg/kg for rape seed, 0.01–1.0 mg/kg for green rape seed fodder (BBCH 65, 77-80)

- A radiovalidation study on green rape fodder showed sufficient hydrolysis efficiency for the (malonyl)glucoside conjugates of hydroxylated mandestrobin metabolites.

Table 92 Validation results for 4-OH mandestrobin with HPLC-MS/MS method SUM-1021V

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.01	0.01 F 0.1 F	2 2	98 97-100 97 96-98	- -	< 0.3LOQ (1)	m/z 328 to 136 5 standards in solvent 0.5-20 ng/mL r2> 0.9999	ROA-0059 validation
lettuce	0.01	0.01 F 0.1 F	5 5	84 76-91 87 83-87	6.7% 5.2%	< 0.3LOQ (2)	m/z 328 to 136 5-7 standards in solvents 0.25-20 ng/mL weighted 1/× r> 0.99	ROA-0011 validation
lettuce	0.01	0.1 F	7	85 71–101	12%	< 0.3LOQ (5)	m/z 328 to 136 5-7 standards in solvents 0.25-20 ng/mL weighted 1/× r> 0.99	ROR-0012 stor stab
rape seed	0.01	0.01 F 0.1 F	5 5	77 71–83 86 82–89	6.4% 3.2%	< 0.3LOQ (2)	m/z 328 to 136 5-7 standards in solvents 0.25-20 ng/mL weighted 1/× r> 0.99	ROA-0011 validation
rape seed	0.01	0.01 F 0.1 F 1.0 F	6 6 1	96 90–97 90 74-102 87 -	2.9% 14% -	< 0.3LOQ (10)	m/z 328 to136 7 standards in solvent 0.30–15 ng/mL 1.0–30 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
rape seed	0.01	0.1 F	7	81 71–97	12%	< 0.3LOQ (5)	m/z 328 to 136 5-7 standards in solvents 0.25-20 ng/mL weighted 1/× r> 0.99	ROR-0012 stor stab
rape seed	0.01	0.01 0.10	1 1	96 - 81 -	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green whole plants, BBCH 77-80)	0.01	0.01 0.1 1.0	6 6 1	82 67-88 87 72–99 102 -	9.9% 13% -	< 0.3LOQ (15)	m/z 328 to136 7 standards in solvent 0.30–15 ng/mL 1.0–30 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.01	0.01 0.10	2 4	79 78-80 73 70–75	- 3.0%	<LOQ	-	ROR-0198 field trial
Rape seed	0.01	0.01 0.10	2 2	84 73-95 79 78-80	- -	<LOQ	-	ROR-0198 field trial
Rape seed fodder (dry plants without pods, BBCH 86)	0.01	0.01 0.1 1.0	2 2 1	81 80–82 76 73-79 79 -	- - -	< 0.3LOQ (5)	m/z 328 to136 7 standards in solvent 0.30–15 ng/mL	ROR-0282 field trial

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery meanrange	RSD_r	control samples mg/kg (n)	calibration	reference, method
							1.0–30 ng/mL 1/× weighted r2> 0.990	
Rape seed fodder (dry pods with seeds, BBCH 86)	0.01	0.01 0.1 1.0	2 2 1	87 85-89 83 83-83 85 -	- - -	< 0.3LOQ (5)	m/z 328 to136 7 standards in solvent 0.30–15 ng/mL 1.0–30 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.01	0.01 0.10	2 2	72 70–74 80 72–87	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.01	0.01 0.10	1 1	80 - 92 -	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.01	0.01 0.10	2 2	86 76-95 90 80–100	- -	<LOQ	-	ROR-0198 field trial
barley grain	0.01	0.01 F 0.1 F	5 5	99 94-107 91 81–99	5.4% 8.1%	< 0.3LOQ (2)	m/z 328 to 136 5-7 standards matrix matched 0.25-20 ng/mL weighted 1/× r> 0.99	ROA-0011 validation
barley grain	0.01	0.1 F	7	83 71–98	12%	< 0.3LOQ (5)	m/z 328 to 136 5-7 standards matrix matched 0.25-20 ng/mL weighted 1/× r> 0.99	ROR-0012 stor stab
barley straw	0.01	0.01 F 0.1 F	5 5	101 82–112 98 69-117	12% 19%	< 0.3LOQ (2)	m/z 328 to 136 5-7 standards matrix matched 0.25-20 ng/mL weighted 1/× r> 0.99	ROA-0011 validation
barley straw	0.01	0.1 F	7	87 70–102	12%	< 0.3LOQ (5)	m/z 328 to 136 5-7 standards matrix matched 0.25-20 ng/mL weighted 1/× r> 0.99	ROR-0012 stor stab
Modification A (extraction with sodium ascorbate)								
soya bean forage (whole plant BBCH 77-81)	0.01	0.01 F 0.10 F	6 6	80 71–84 80 77-86	6.2% 5.4%	< 0.3LOQ (4)	m/z 330 to 192 6-7 standards matrix matched 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	ROR-0280 field trial
soya bean fodder (dry pods with seeds BBCH 82)	0.01	0.01 F 0.10 F	1 1	90 - 95 -	- -	< 0.3LOQ (4)	m/z 330 to 192 6-7 standards matrix matched 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	ROR-0280 field trial
soya bean fodder (rest of plant, BBCH 82)	0.01	0.01 F 0.10 F	1 1	75 - 74 -	- -	< 0.3LOQ (4)	m/z 330 to 192 6-7 standards matrix matched	ROR-0280 field trial

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery meanrange	RSD_r	control samples mg/kg (n)	calibration	reference, method
							0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	
dry soya beans (dry seeds, BBCH 84-89)	0.01	0.01 F 0.10 F	6 6	72 67-79 73 69-86	5.6% 9.0%	< 0.3LOQ (8)	m/z 330 to 192 6-7 standards matrix matched 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	ROR-0280 field trial

F Added as free 4-OH mandestrobin

HPLC-MS/MS method SUM-1022V for 2-CH_2OH-mandestrobin and its (malonyl)glucosides

HPLC-MS/MS method SUM-1022V (version 3 May 2011) was used to determine 2-CH_2OH-mandestrobin and its (malonyl)glucosides in lettuce, rape seed and barley (grain, straw). The method was described and used in supervised field trials [Delmotte, 2011, ROR-0008; Lebrun, 2012, ROR-0198], a storage stability study on lettuce, rape seed and barley grains and barley straw [Daneva & Zetzsch, 2012, ROR-0013] and validated in [Daneva *et al*., 2011, ROA-0012].

HPLC-MS/MS method SUM-1022V for the determination of 2-CH_2OH-mandestrobin and it's (The extraction method for HPLC-MS/MS method SUM-1022V is exactly the same as for HPLC-MS/MS method SUM-1021V. The final extract was analysed for total 2-CH_2OH-mandestrobin by HPLC-MS/MS (retention time 7.8-8.0 min) using the transition from m/z 328 to 137 for quantification and m/z 330 to 192 for confirmation. Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg for 2-CH_2OH-mandestrobin.

Recovery experiments were conducted with free 2-CH_2OH-mandestrobin, since standards for 2-CH_2OH-mandestrobin conjugates were not available. Validation results are shown in Table 93.

Modification A was used in supervised field trials on soya bean forage (whole plant, BBCH 77-81), soya bean seeds (dry seeds, BBCH 84-89) and soya bean fodder (dry pods with seeds and rest of plant, BBCH 82) [Klimmek and Gizler, 2017, ROR-0280]. The method is exactly the same as modification A for HPLC-MS/MS method SUM-1021V.

Modification B was used in the 2015 supervised field trials on rape seed commodities [Gemrot, 2017, ROR-0282] The method is exactly the same as HPLC-MS/MS method SUM-1021V, except that the final extract was analysed for total 2-CH_2OH-mandestrobin by HPLC-MS/MS (retention time 7.8-8.0 min) using the transition from m/z 330 to 192 for quantification and m/z 330 to 160 for confirmation.

Matrix effects at m/z 328 to 137 were investigated for rape seed, lettuce, soya beans (dry seeds, BBCH 84-89), barley grains, soya bean forage (whole plant, BBCH 77-81), rape seed fodder (green whole plants, BBCH 65, 77-80, green pods and the rest of the plant, BBCH 79; dry pods with seeds and rest of plants, BBCH 80–87); soya bean fodder (dry pods with seeds and rest of plant, BBCH 82) and barley straw [Daneva & Zetzsch, 2012, ROR-0013; Daneva *et al*., 2011, ROA-0012; Gemrot, 2017, ROR-0282; Klimmek and Gizler, 2017, ROR-0280] by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions. Matrix effects (> 20%) were observed for rape seed (-43%), barley grain, rape seed fodder (+34%) soya bean forage (-19%), soya bean fodder (-21%) and barley straw (-66%) and matrix matched standards were used for these matrices.

Both mass transitions showed similar accuracy and precision [Daneva *et al*., 2011, ROA-0012]. The use of two characteristic mass transitions ensures a high level of specificity and therefore an additional confirmation method is not considered necessary.

Method ER-MT-1011 is an earlier version of SUM-1022V for determination of 2-CH_2OH-mandestrobin and its (malonyl)glucosides in grapes [Tabuchi, *et al*., 2010, ROA-0060]. The analytical method is the same as SUM-1022V. Validation results are shown in Table .

HPLC-MS/MS method SUM-1022V for the determination of 2-CH_2OH-mandestrobin and its (malonyl)glucosides is considered:

- valid (full validation) for the determination of 2-CH_2OH-mandestrobin in the range 0.01–0.1 mg/kg in lettuce, dry soya bean seeds (BBCH 84-89), rape seed, barley grains, soya bean forage (BBCH 77-81), green rape seed fodder (BBCH 65, 77-80), and barley straw;
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–0.1 mg/kg for grapes, dry soya bean fodder (BBCH 82) and dry rape seed fodder (BBCH 86).
- Limited recovery experiments (n = 1–2) suggest extension of the validity to the range of 0.01–1.0 mg/kg for rape seed, 0.01–1.0 mg/kg for green rape seed fodder (BBCH 65, 77-80).
- A radiovalidation study on green rape fodder showed sufficient hydrolysis efficiency for the (malonyl)glucoside conjugates of hydroxylated mandestrobin metabolites.

Table 93 Validation for 2-CH_2OH-mandestrobin with HPLC-MS/MS method SUM-1022V at m/z 328 to 137

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.01	0.01 F 0.1 F	2 2	100 95	99-101 93-96	- -	< 0.3LOQ (1)	m/z 328 to 137 5 standards in solvent 0.5-20 ng/mL R^2> 0.9999	ROA-0060 validation
lettuce	0.01	0.01 F 0.1 F	5 5	89 93	79–98 84-100	8.8% 7.5%	< 0.3LOQ (2)	m/z 328 to 137 6-8 standards in solvents 0.30–40 ng/mL weighted 1/× r> 0.99	ROA-0012 validation
lettuce	0.01	0.1 F	7	81	70–103	15%	< 0.3LOQ (5)	m/z 328 to 137 6-8 standards in solvents 0.30–25 ng/mL weighted 1/× r> 0.99	ROR-0013 stor stab
rape seed	0.01	0.01 F 0.1 F	5 5	92 89	83-102 83-97	9.2% 7.0%	< 0.3LOQ (2)	m/z 328 to 137 6-8 standards matrix matched 0.30–40 ng/mL weighted 1/× r> 0.99	ROA-0012 validation
rape seed	0.01	0.1 F	7	77	70–87	8.6%	< 0.3LOQ (5)	m/z 328 to 137 6-8 standards matrix matched 0.30–25 ng/mL weighted 1/× r> 0.99	ROR-0013 stor stab
rape seed	0.01	0.01 0.1 1.0	6 6 1	93 78 104	78-105 70–91 -	11% 10% -	< 0.3LOQ (10)	m/z 330 to 192 7 standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (green whole plants, BBCH 77-	0.01	0.01 0.1 1.0	6 6 1	93 104 88	81– 75-99	9.2% 11% -	< 0.3LOQ (15)	m/z 330 to 192 7 standards in solvent	ROR-0282 field trial

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
80)				99	-			0.30–15 ng/mL 1/× weighted r2> 0.990	
Rape seed fodder (dry plants without pods, BBCH 86)	0.01	0.01 0.1 1.0	2 2 1	84 80 92	76-91 79-81 -	- - -	< 0.3LOQ (5)	m/z 330 to 192 7 standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (dry pods with seeds, BBCH 86)	0.01	0.01 0.1 1.0	2 2 1	76 76 93	75-77 70–81 -	- - -	< 0.3LOQ (5)	m/z 330 to 192 7 standards in solvent 0.30–15 ng/mL 1/× weighted r2> 0.990	ROR-0282 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.01	0.01 0.10	2 2	74 74	71–78 73-76	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.01	0.01 0.10	1 1	72 72	- -	- -	<LOQ	-	ROR-0008 field trial
rape seed	0.01	0.01 0.10	1 1	74 72	- -	- -	<LOQ	-	ROR-0008 field trial
Rape seed fodder (green whole plants, BBCH 65 or plants without pods, BBCH 79)	0.01	0.01 0.10	2 3	92 88	84-99 86-90	- 2.3%	<LOQ	-	ROR-0198 field trial
Rape seed fodder (green pods with seeds, BBCH 79)	0.01	0.01 0.10	1 1	85 72	- -	- -	<LOQ	-	ROR-0198 field trial
rape seed	0.01	0.01 0.10	2 2	86 80	81–92 77-83	- -	<LOQ	-	ROR-0198 field trial
barley grain	0.01	0.01 F 0.1 F	5 5	79 78	73-83 69-84	4.8% 7.2%	< 0.3LOQ (2)	m/z 328 to 137 6-8 standards matrix matched 0.30–40 ng/mL weighted 1/× r> 0.99	ROA-0012 validation
barley grain	0.01	0.1 F	7	88	82–98	6.5%	< 0.3LOQ (5)	m/z 328 to 137 6-8 standards matrix matched 0.30–25 ng/mL weighted 1/× r> 0.99	ROR-0013 stor stab
barley straw	0.01	0.01 F 0.1 F	5 5	81 79	75-93 76-84	8.8% 3.9%	< 0.3LOQ (2)	m/z 328 to 137 6-8 standards matrix matched 0.30–40 ng/mL weighted 1/× r> 0.99	ROA-0012 validation
barley straw	0.01	0.1 F	7	83	70–105	16%	< 0.3LOQ (5)	m/z 328 to 137 6-8 standards matrix matched 0.30–25 ng/mL weighted 1/× r> 0.99	ROR-0013 stor stab
Modification A (extraction in the presence of sodium ascorbate)									
soya bean forage	0.01	0.01 F	6	91	82–	11%	< 0.3LOQ	m/z 330 to 192	ROR-0280

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean range	RSD_r	control samples mg/kg (n)	calibration	reference, method
(whole plant BBCH 77-81)		0.10 F	6	109 96 89-102	5.3%	(4)	6-7 standards matrix matched 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	field trial
soya bean fodder (dry pods with seeds BBCH 82)	0.01	0.01 F 0.10 F	1 1	97 - 89 -	- -	< 0.3LOQ (4)	m/z 330 to 192 6-7 standards matrix matched 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	ROR-0280 field trial
soya bean fodder (rest of plant, BBCH 82)	0.01	0.01 F 0.10 F	1 1	94 - 98 -	- -	< 0.3LOQ (4)	m/z 330 to 192 6-7 standards matrix matched 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	ROR-0280 field trial
dry soya beans (dry seeds, BBCH 84-89)	0.01	0.01 F 0.10 F	6 6	77 71–84 81 74-92	7.7% 8.3%	< 0.3LOQ (8)	m/z 330 to 192 8 standards in solvent 0.30–25 mg/L 1/× weighted $R^2 \geq 0.98$	ROR-0280 field trial

F Added as free 2-CH_2OH-mandestrobin.

HPLC-MS/MS method SUM-1027V for 5-CH_2OH-mandestrobin and its (malonyl)glucosides

HPLC-MS/MS method SUM-1027V (version 6 May 2011) was used to determine 5-CH_2OH-mandestrobin and its (malonyl)glucosides in lettuce and barley (grain, straw). The method was described and used in a storage stability study on lettuce and barley [Daneva & Zetzsch, 2012a, ROR-0014] and validated in [Daneva & Taeufer, 2011, ROA-0013].

The extraction method for HPLC-MS/MS method SUM-1027V is exactly the same as for HPLC-MS/MS method SUM-1021V. The final extract was analysed for total 5-CH_2OH-mandestrobin by HPLC-MS/MS (retention time 8.0–8.8 min) using the transition from m/z 328 to 137 for quantification and m/z 330 to 192 for confirmation. Calibration was applied with external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg for 2-CH_2OH-mandestrobin.

Recovery experiments were conducted with free 5-CH_2OH-mandestrobin, since standards for 5-CH_2OH-mandestrobin conjugates were not available. Both the recovery of the Chem Elut and SPE cartridge should be checked. When recoveries are unsatisfactory, the volume or the ratio of the solutions used for washing or elution of the cartridges need to be slightly modified to obtain adequate recoveries. Validation results are shown in Table 94.

Matrix effects at m/z 328 to 137 were investigated for lettuce, rape seed, barley grains and barley straw [Daneva & Zetzsch, 2012a, ROR-0014; Daneva & Taeufer, 2011, ROA-0013] by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions. No significant matrix effects were observed for lettuce and barley grain (+1.0% to +4.4%) and standards in solvent are considered appropriate for these matrices. Matrix effects were observed for barley straw (+61%) and matrix matched standards were therefore used for this matrix.

Both mass transitions showed similar accuracy and precision [Daneva & Taeufer, 2011, ROA-0013]. The use of two characteristic mass transitions ensures a high level of specificity and therefore an additional confirmation method is not considered necessary.

Method ER-MT-1012 is an earlier version of SUM-1027V for determination of 5-CH_2OH-mandestrobin and its (malonyl)glucosides in grapes [Tabuchi, *et al*., 2010, ROA-0061]. The analytical method is the same as SUM-1027V. Validation results are shown in Table 94.

HPLC-MS/MS method SUM-1027V for the determination of 5-CH_2OH-mandestrobin and its (malonyl)glucosides is considered:

- valid (full validation) for the determination of 5-CH_2OH-mandestrobin in the range 0.01–0.1 mg/kg in lettuce, barley grains, and barley straw;
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–0.1 mg/kg for grapes.
- A radiovalidation study on green rape fodder showed sufficient hydrolysis efficiency for the (malonyl)glucoside conjugates of hydroxylated mandestrobin metabolites.

Table 944 Validation for 5-CH_2OH-mandestrobin with HPLC-MS/MS method SUM-1027V

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.01	0.01 F 0.1 F	2 2	102 98	97-106 97-99	- -	< 0.3LOQ (1)	m/z 328 to 137 5 standards in solvent 0.5-20 ng/mL R^2> 0.9999	ROA-0061 validation
lettuce	0.01	0.01 F 0.1 F	5 5	76 87	72–80 85-91	4.0% 3.1%	< 0.3LOQ (2)	m/z 328 to 137 7 standards in solvents 0.30–25 ng/mL weighted 1/× r> 0.99	ROA-0013 validation
lettuce	0.01	0.1 F	7	83	70–93	9.9%	< 0.3LOQ (5)	m/z 328 to 137 7-8 standards in solvents 0.30–25 ng/mL weighted 1/× r> 0.999	ROR-0014 stor stab
barley grain	0.01	0.01 F 0.1 F	5 5	73 82	70–77 80–89	4.0% 7.9%	< 0.3LOQ (2)	m/z 328 to 137 7 standards in solvents 0.30–25 ng/mL weighted 1/× r> 0.99	ROA-0013 validation
barley grain	0.01	0.1 F	7	82	74-90	7.8%	< 0.3LOQ (5)	m/z 328 to 137 7-8 standards in solvent 0.30–25 ng/mL weighted 1/× r> 0.999	ROR-0014 stor stab
barley straw	0.01	0.01 F 0.1 F	5 5	79 79	72–85 71–89	5.9% 11%	< 0.3LOQ (2)	m/z 328 to 137 7 standards matrix matched 0.30–25 ng/mL weighted 1/× r> 0.99	ROA-0013 validation
barley straw	0.01	0.1 F	7	80	76-87	5.0%	< 0.3LOQ (5)	m/z 328 to 137 7-8 standards matrix matched 0.30–25 ng/mL weighted 1/× r> 0.999	ROR-0014 stor stab

F Added as free 5-CH_2OH-mandestrobin

HPLC-MS/MS method S10−02011 for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates

HPLC-MS/MS method S10–02011 (13 August 2012) was used to determine 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates in plant commodities. The method was described, used and validated in a rotational crop study [Roussel, 2012, ROR-0202] and in a storage stability study on dry beans and oranges [Lindner & Grewe, 2016, ROR-0269; Lindner *et al*, 2017, ROR-0286].

The extraction method for HPLC-MS/MS method S10–2011 is exactly the same as for HPLC-MS/MS methods SUM-1021V, SUM-1022V and SUM-1027V and combines the three methods to a single method. The final extract was analysed for total 4-OH-mandestrobin, total 2-CH_2OH-mandestrobin and total 5-CH_2OH-mandestrobin by HPLC-MS/MS. The retention times were 7.5 min for 2-CH_2OH-mandestrobin, 8.0 min for 5-CH_2OH-mandestrobin and 8.3 min for 4-OH-mandestrobin. The transition m/z 328 to 136 was used for quantification of 4-OH-mandestrobin. The transition m/z 328 to 137 was used for quantification of 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. The transition of m/z 330 to 192 was used for confirmation of all three compounds. Calibration was applied with mixed external standards in solvent using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ was 0.01 mg/kg for each analyte.

Recovery experiments were conducted with free 4-OH mandestrobin, free 2-CH_2OH-mandestrobin and free 5-CH_2OH-mandestrobin, since standards for the conjugates were not available. Validation results are shown in Table 95, 96 and 97.

Some samples were cleaned-up with an in-house packed cartridge containing Hydromatrix diatomaceous earth instead of the pre-packed Chem Elut cartridge These samples needed an additional ethyl acetate /methanol (90:10, v/v) elution to satisfactory recover the analytes from the cartridge.

Matrix effects at all mass transitions were investigated by comparing peak areas of solvent standard solutions with peak areas of matrix matched standard solutions [Roussel, 2012, ROR-0202; Lindner & Grewe, 2016, ROR-0269; Lindner *et al*, 2017, ROR-0286]. No significant matrix effects were observed for orange, green rape fodder, lettuce, carrot roots, carrot leaves, broccoli, dry beans and barley grain (ranging from -22% to +14%) and standards in solvent are considered appropriate for these matrices. Matrix effects were observed for barley straw (-32% to -40% for 2-CH_2OH-mandestrobin; +12 to -39% for 5-CH_2OH-mandestrobin) and matrix matched standards were therefore used for this matrix.

In some samples, matrix interferences were found with m/z 328 to 137 for 2-CH_2OH-mandestrobin in orange, dry beans, lettuce, carrot leaves, barley grain and barley straw and for 5-CH_2OH-mandestrobin in carrot roots, carrot leaves and barley straw. In these cases, the transition of m/z 330 to 192 was used for quantification.

The extraction was modified for the dry bean samples with storage intervals of 3 months and longer in ROR-0269 and ROR-0286. Samples of 20 g dried beans (white seeds) were mixed with 4 mL 1 M sodium ascorbate, 12 mL water. Acetone (50 mL) was added and the mixture was shaken with a reciprocal shaker for 10 min and then left overnight at room temperature. The solids were extracted again with 1 M sodium ascorbate/water/acetone (4:12:50, v/v) on a reciprocal shaker for 10 min. Filtered extracts were combined. Hydrolysis and further clean-up was conducted as in the original method. The modified version was validated and results were compared to recoveries obtained by parallel analysis according to the initial method. The comparison showed that the change of the method slightly improved the analytical performance.

HPLC-MS/MS method S10–02011 for the determination of 4-OH-mandestrobin; 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates is considered:

- valid (full validation) for the determination of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in the range 0.01–0.1 mg/kg in oranges;
- valid (reduced validation) for the determination of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in the range 0.01–0.1 mg/kg in dry beans and barley grains, carrot roots, carrot leaves and barley straw.
- Limited recovery experiments (n = 1–2 per level) suggest validity in the range 0.01–0.1 mg/kg for broccoli and lettuce.
- A radiovalidation study on green rape fodder showed sufficient hydrolysis efficiency for the (malonyl)glucoside conjugates of hydroxylated mandestrobin metabolites.

Table 95 Validation results for 4-OH-mandestrobin with HPLC-MS/MS method S10–02011

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	% recovery range	RSD_r	control samples mg/kg (n)	calibration	reference, method
whole orange	0.01	0.01 0.1	5 8	88 91	83-93 74-107	5.5% 11%	< 0.3LOQ (7)	m/z 328 to 136 7 standards in solvent 0.3-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab
broccoli	0.01	0.01 0.10	2 2	72 82	70–73 73-91	- -	< 0.3LOQ (6)	m/z 328 to 136 8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.999	ROR-0202 rotational
lettuce	0.01	0.01 0.10	1 1	106 70	- -	- -	< 0.3LOQ (6)	m/z 328 to 136 8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.999	ROR-0202 rotational
dry beans	0.01	0.1	6	72	61–81	11%	< 0.3LOQ (1)	m/z 328 to 136 7 standards in solvent 0.3-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab
carrot roots	0.01	0.01 0.10	4 3	95 96	87-110 80–110	11% 16%	< 0.3LOQ (6)	m/z 328 to 136 5-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.999	ROR-0202 rotational
carrot leaves	0.01	0.01 0.10	4 2	77 81	74-85 80–82	7.2% -	< 0.3LOQ (6)	m/z 328 to 136 8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.999	ROR-0202 rotational
barley grain	0.01	0.01 0.10	3 3	85 86	72–92 80–91	14% 6.6%	< 0.3LOQ (8)	m/z 328 to 136 6-8 standards 0.25-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
barley straw	0.01	0.01 0.10	3 2	87 82	71–103 77-86	13% 15%	< 0.3LOQ (6)	m/z 328 to 136 6-7 standards 0.25-50 ng/mL matrix matched	ROR-0202 rotational

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								1/× weighted r> 0.999	
Extraction with sodium ascorbate/water/acetone									
dry beans	0.01	0.01 0.1	3 6	96 87	91–100 79-101	4.8% 9.8%	< 0.3LOQ (4)	m/z 328 to 136 7 standards in solvent 0.3-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab

Table 96 Validation results for 2-CH_2OH-mandestrobin with HPLC-MS/MS method S10–02011

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
whole orange	0.01	0.01 0.1	5 8	101 89	98-106 80–94	3.2% 4.5%	< 0.3LOQ (7)	m/z 330 to 192 7 standards in solvent 0.3-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab
broccoli	0.01	0.01 0.10	1 1	72 98	- -	- -	< 0.3LOQ (6)	m/z 328 to 137 6-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
lettuce	0.01	0.01 0.10	2 2	92 95	83-102 90–100	- -	< 0.3LOQ (7)	m/z 330 to 192 6-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
dry beans	0.01	0.1	6	94	86-98	4.5%	< 0.3LOQ (1)	m/z 330 to 192 7 standards in solvent 0.3-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab
carrot roots	0.01	0.01 0.10	2 4	94 92	92–97 85-106	- 10%	< 0.3LOQ (6)	m/z 328 to 137 5-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
carrot leaves	0.01	0.01 0.10	3 3	92 91	79-100 78-106	13% 15%	< 0.3LOQ (6)	m/z 328 to 137 m/z 330 to 192 5-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
barley grain	0.01	0.01 0.10	4 4	94 86	87-101 78-94	6.1% 7.8%	< 0.3LOQ (7)	m/z 328 to 137 m/z 330 to 192 8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
barley straw	0.01	0.01	2	83	76-90	-	< 0.3LOQ (6)	m/z 330 to 192	ROR-0202

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
		0.10	2	79	70–88	-		5-7 standards 0.3-25 ng/mL matrix matched 1/× weighted r> 0.99	rotational
Extraction with sodium ascorbate/water/acetone									
dry beans	0.01	0.01 0.1	3 6	90 90	83-96 80–96	7.3% 7.5%	< 0.3LOQ (4)	m/z 330 to 192 7 standards in solvent 0.3-20 ng/mL 1/× weighted r2> 0.98	ROR-0269; ROR-0286; stor stab

Table 97 Validation results for 5-CH_2OH-mandestrobin with HPLC-MS/MS method S10–02011

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
broccoli	0.01	0.01 0.10	2 1	77 86	71–83 -	- -	< 0.3LOQ (6)	m/z 328 to 137 7-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
lettuce	0.01	0.01 0.10	2 2	88 90	87-90 88-92	- -	< 0.3LOQ (6)	m/z 328 to 137 7-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.999	ROR-0202 rotational
carrot roots	0.01	0.01 0.10	4 4	92 82	81–100 79-84	8.7% 3.2%	< 0.3LOQ (6)	m/z 328 to 137 m/z 330 to 192 7-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
carrot leaves	0.01	0.01 0.10	4 4	84 75	74-93 71–79	13% 4.4%	< 0.3LOQ (6)	m/z 328 to 137 m/z 330 to 192 7-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
barley grain	0.01	0.01 0.10	3 3	85 75	76-95 71–79	11% 5.4%	< 0.3LOQ (8)	m/z 328 to 137 7-8 standards 0.3-25 ng/mL in solvent 1/× weighted r> 0.99	ROR-0202 rotational
barley straw	0.01	0.01 0.10	1 2	80 78	- 71–86	- 14%	< 0.3LOQ (6)	m/z 330 to 192 6-8 standards 0.3-25 ng/mL matrix matched 1/× weighted r> 0.99	ROR-0202 rotational

HPLC-MS/MS method RM-48A for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates

HPLC-MS/MS method RM-48A (version 5 November, 2013) was used to determine 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates in plant commodities. The method was described, used and validated in a field rotational crop study, supervised trials on grapes, strawberries and rape seed and processing studies on grapes [Green, 2013, ROR-0240; Bitter *et al*., 2013, ROR-0234; Bitter, 2013, ROR-0235; Bitter *et al*., 2013, ROR-0236; Green, 2013, ROR-0238].

Homogenised samples of 2.5 g plant commodities were extracted twice with a mixture of acetone and water (80:20, v/v) in a reciprocal shaker for 1 h. An aliquot of the combined extracts (equivalent to 0.5 g sample) was evaporated at 35 °C to remove the acetone. Acetonitrile was added. For oily crops (rape seed) the mixture was partitioned twice with hexane to remove the oils into the hexane layer. The aqueous acetonitrile mixture (from all crops) was evaporated to near dryness. The residue was re-dissolved in 0.06 M NaOH at pH 11. The mixture was allowed to hydrolyse for 2 hours at room temperature to cleave off any malonyl groups. The hydrolysate was adjusted to pH = 5 by the addition of 0.1 M HCl and 0.01 M sodium acetate/acetic acid buffer. After addition of 2500 units of beta-glucosidase, the samples underwent enzyme hydrolysis for 3 hrs at 37 °C while shaking at 100 rpm in a reciprocal shaker bath to cleave off any glucose groups. Sodium chloride was added and the hydrolysate was adjusted to pH 9 by the addition of 0.2 M sodium carbonate/sodium bicarbonate buffer. The mixture was partitioned four times with dichloromethane on a reciprocal shaker for 1 min. The combined dichloromethane phases were evaporated to dryness and residues were re-dissolved in methanol. Aliquots of the final extract were diluted with water as necessary, filtered and analysed for total 4-OH-mandestrobin, total 2-CH_2OH-mandestrobin and total 5-CH_2OH-mandestrobin by two HPLC-MS/MS conditions. Condition 1 consisted of an Kinetex PFP HPLC column with retention times of 10.3 min for 2-CH_2OH-mandestrobin; 12.3 min for 4-OH-mandestrobin and 12.8 min for 5-CH_2OH-mandestrobin and MS-MS transitions from m/z 328.2 to 135.8 for 4-OH-mandestrobin and m/z 328.2 to 136.8 for both the 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. Condition 2 consisted of an Eclipse XDB C8 HPLC column with retention times of 4.2 min for 4-OH-mandestrobin and 5.4 min for 5-CH_2OH-mandestrobin (2-CH_2OH-mandestrobin was not analysed) and MS-MS transitions from m/z 330.0 to 192.0 for both compounds. Calibration was applied with mixed external standards for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in solvent using either a linear fit with a non-zero intercept or a 2nd order polynomial fit (weighted relative to 1/standard concentration). The reported LOQ was 0.02 mg/kg for each analyte.

Initially the method was developed using ethyl acetate/hexane (20:80, v/v) instead of dichloromethane as clean-up for the post-hydrolysed samples. The solution lacked the ability to consequently remove the target analytes from the matrix. The method was initially modified by increasing the pH of the post-hydrolysed samples to 9 by the addition of 0.06 M NaOH and partitioning with dichloromethane. This modification worked well with strawberries and grapes, but it did not perform well in other matrices. When the post-hydrolysed samples were adjusted to pH 9 using a buffered solution of 0.2 M sodium carbonate/sodium bicarbonate, performance was good for all tested matrices. Validation experiments might have been done with each of these clean-up methods.

Alternate chromatographic conditions (HPLC-MS/MS condition 2) became necessary for 4-OH-mandestrobin in various matrices due to matrix suppression. Although the alternate chromatographic conditions are suitable for analysis of 4-OH-mandestrobin and 5-CH_2OH-mandestrobin, they were used exclusively for the analysis of 4-OH-mandestrobin in wheat forage.

Recovery experiments were conducted with free 4-OH mandestrobin, free 2-CH_2OH-mandestrobin and free 5-CH_2OH-mandestrobin, since standards for their conjugates were not available. Validation results are shown in Tables 98, 99 and 100.

HPLC-MS/MS method RM-48A for the determination of 4-OH-mandestrobin; 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates is considered:

- valid (full validation) for the determination of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in the range 0.02–0.2 mg/kg in grapes, rape seed and wheat forage;
- valid (reduced validation) for the determination of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin, only at 0.02 mg/kg in grape juice, grape raisins, strawberries, spinach leaves, red beet roots, red beet leaves, wheat hay, wheat grain, wheat straw, sorghum forage, sorghum grain, sorghum fodder,
- Limited recovery experiments (n = 1–2 per level) suggest extension of the validity to 0.02–0.2 mg/kg for grape juice, grape raisins, strawberries, red beet roots, red beet leaves, wheat hay, wheat grain, wheat straw, sorghum forage, sorghum fodder.
- A radiovalidation study on green rape fodder showed sufficient hydrolysis efficiency for the (malonyl)glucoside conjugates of hydroxylated mandestrobin metabolites

Table 98 Validation results for 4-OH-mandestrobin with HPLC-MS/MS method RM-48A

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.02	0.02 0.20	6 6	105 79	91–122 73-84	9.9% 5.9%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
grapes	0.02	0.02 0.20	12 9	98 91	74-118 77-108	12% 11%	< 0.3LOQ (53)	5 standards in solvent 0.5-50 µg/L 0.125-5 µg/L 1/× weighted r2> 0.999	ROR-0234 field trial
grapes	0.02	0.02 0.20	3 1	96 90	92–99 -	3.5% -	< 0.3LOQ (16)	5 standards in solvent 0.125-5 µg/L 1/× weighted r2> 0.999	ROR-0235 field trial
grape juice	0.02	0.02 0.20	3 1	78 82	77-84 -	4.5% -	< 0.3LOQ (3)	5 standards in solvent 0.5-50 µg/L 0.125-5 µg/L 1/× weighted r2> 0.999	ROR-0234 processing
grape raisins	0.02	0.02 0.20	3 1	90 89	89–91 -	1.6% -	-	5 standards in solvent 0.5-50 µg/L 0.125-5 µg/L 1/× weighted r2> 0.999	ROR-0234 processing
strawberries	0.02	0.02 0.20	4 2	86 82	74-92 81–84	9.8% -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0236 field trial
spinach leaves	0.02	0.02 0.20	3 1	87 86	84-90 -	3.7%	<LOQ (3)	-	ROR-0240 field rotation
red beet roots	0.02	0.02 0.20	3 1	90 80	88-96 -	4.9% -	<LOQ (3)	-	ROR-0240 field rotation
red beet leaves	0.02	0.02	3	94	89–99	5.2%	<LOQ (3)	-	ROR-0240

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
		0.20	1	91	-	-			field rotation
wheat forage	0.02	0.02 0.20	6 6	76 76	74-78 69-83	2.4% 5.9%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
wheat forage	0.02	0.02 0.20	3 1	88 80	85-92 -	4.3%	<LOQ (3)	-	ROR-0240 field rotation
wheat hay	0.02	0.02 0.20	4 1	77 73	72–84 -	7.0%	<LOQ (3)	-	ROR-0240 field rotation
wheat grain	0.02	0.02 0.20	3 1	70 -	66-74	5.8%	<LOQ (3)	-	ROR-0240 field rotation
wheat straw	0.02	0.02 0.20	3 1	88 81	86-90 -	1.8%	<LOQ (3)	-	ROR-0240 field rotation
sorghum forage	0.02	0.02 0.20	3 1	86 76	66-104 -	23% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum grain	0.02	0.02 0.20	3 1	100 96	97-102 -	2.7% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum fodder	0.02	0.02 0.20	3 1	69 67	64-78 -	11% -	<LOQ (3)	-	ROR-0240 field rotation
rape seed	0.02	0.02 0.20	6 6	101 80	94-107 72–86	4.8% 5.6%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
rape seed	0.02	0.02 0.20	3 1	75 85	74-75 -	0.5% -	< 0.3LOQ (9)	5 standards in solvent 0.125-5 µg/L 1/× weighted r2> 0.999	ROR-0238 field trial
rape seed	0.02	0.02 0.20	3 1	94 92	93-95 -	1.1% -	< 0.3LOQ (6)	5 standards in solvent 0.125-5 µg/L 1/× weighted r2> 0.99	ROR-0239 field trial

Table 99 Validation results for 2-CH_2OH-mandestrobin with HPLC-MS/MS method RM-48A

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.02	0.02 0.20	6 6	102 82	95-120 80–86	9.4% 2.9%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
grapes	0.02	0.02 0.20	16 10	102 102	78-124 90–121	11% 9.1%	< 0.3LOQ (53)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0234 field trial
grapes	0.02	0.02 0.20	3 1	101 97	98-104 -	2.6% -	< 0.3LOQ (16)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0235 field trial
grape juice	0.02	0.02	3	96	92–100	4.7%	< 0.3LOQ (3)	5 standards	ROR-0234

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
		0.20	1	93	-	-		in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	processing
grape raisins	0.02	0.02 0.20	3 1	99 93	96-103 -	4.0%	-	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0234 processing
strawberries	0.02	0.02 0.20	4 2	105 100	102–108 98-102	2.3% -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0236 field trial
spinach leaves	0.02	0.02 0.20	3 1	98 98	96-100 -	2.4% -	<LOQ (3)	-	ROR-0240 field rotation
red beet roots	0.02	0.02 0.20	3 1	106 100	104-110 -	2.8% -	<LOQ (3)	-	ROR-0240 field rotation
red beet leaves	0.02	0.02 0.20	3 1	82 94	78-84 -	4.1% -	<LOQ (3)	-	ROR-0240 field rotation
wheat forage	0.02	0.02 0.20	3 1	112 110	110–115 -	2.6% -	<LOQ (3)	-	ROR-0240 field rotation
wheat hay	0.02	0.02 0.20	3 1	111 115	105-118 -	4.8% -	<LOQ (3)	-	ROR-0240 field rotation
wheat grain	0.02	0.02 0.20	3 1	109 108	107-112 -	2.5% -	<LOQ (3)	-	ROR-0240 field rotation
wheat straw	0.02	0.02 0.20	3 1	101 98	98-106 -	4.2% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum forage	0.02	0.02 0.20	3 1	90 97	90–92 -	1.4% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum grain	0.02	0.02 0.20	3 1	98 94	96-98 -	1.2% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum fodder	0.02	0.02 0.20	3 1	89 94	86-91 -	3.0% -	<LOQ (3)	-	ROR-0240 field rotation
rape seed	0.02	0.02 0.20	6 6	82 74	73-88 67-81	6.4% 6.3%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
rape seed	0.02	0.02 0.20	3 1	98 83	93-105 -	6.2% -	< 0.3LOQ (9)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0238 field trial
rape seed	0.02	0.02 0.20	3 1	96 96	89-102 -	7.1% -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.99	ROR-0239 field trial

Table 100 Validation results for 5-CH_2OH-mandestrobin with HPLC-MS/MS method RM-48A

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
grapes	0.02	0.02 0.20	6 6	101 106	96-104 102–109	3.1% 2.8%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
									ROR-0239 validation
grapes	0.02	0.02 0.20	16 10	102 101	86-125 90–122	11% 9.0%	< 0.3LOQ (53)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0234 field trial
grapes	0.02	0.02 0.20	3 1	96 96	94-98 -	2.1% -	< 0.3LOQ (16)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0235 field trial
grape juice	0.02	0.02 0.20	3 1	95 93	92–97 -	2.8% -	< 0.3LOQ (3)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0234 processing
grape raisins	0.02	0.02 0.20	3 1	95 92	93-99 -	3.3% -	-	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0234 processing
strawberries	0.02	0.02 0.20	4 2	106 100	102–110 98-102	2.6% -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.999	ROR-0236 field trial
spinach leaves	0.02	0.02 0.20	3 1	95 96	91–97 -	3.5% -	<LOQ (3)	-	ROR-0240 field rotation
red beet roots	0.02	0.02 0.20	3 1	108 100	103-114 -	5.0% -	<LOQ (3)	-	ROR-0240 field rotation
red beet leaves	0.02	0.02 0.20	3 1	81 91	77-84 -	5.2% -	<LOQ (3)	-	ROR-0240 field rotation
wheat forage	0.02	0.02 0.20	6 6	101 104	94-104 99-108	3.9% 2.7%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
wheat forage	0.02	0.02 0.20	3 1	115 107	114-117 -	1.7% -	<LOQ (3)	-	ROR-0240 field rotation
wheat hay	0.02	0.02 0.20	4 1	111 116	104-121 -	7.0% -	<LOQ (3)	-	ROR-0240 field rotation
wheat grain	0.02	0.02 0.20	3 1	106 111	105-107 -	1.3% -	<LOQ (3)	-	ROR-0240 field rotation
wheat straw	0.02	0.02 0.20	3 1	98 95	96-99 -	1.8% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum forage	0.02	0.02 0.20	3 1	86 93	84-89 -	3.2% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum grain	0.02	0.02 0.20	3 1	92 92	89–96 -	4.1% -	<LOQ (3)	-	ROR-0240 field rotation
sorghum fodder	0.02	0.02 0.20	3 1	89 91	86-92 -	3.3% -	<LOQ (3)	-	ROR-0240 field rotation
rape seed	0.02	0.02 0.20	6 6	91 87	87-95 81–92	3.5% 5.0%	< 0.3LOQ (2)	-	ROR-0234 ROR-0235 ROR-0236 ROR-0238 ROR-0239 validation
rape seed	0.02	0.02 0.20	3 1	103 83	101–106 -	2.7% -	< 0.3LOQ (9)	5 standards in solvent	ROR-0238 field trial

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
								0.5-50 µg/L 1/× weighted r2> 0.999	
rape seed	0.02	0.02 0.20	3 1	94 98	88-99 -	6.2% -	< 0.3LOQ (6)	5 standards in solvent 0.5-50 µg/L 1/× weighted r2> 0.99	ROR-0239 field trial

Analytical methods used in study reports in animal commodities

The Meeting received the description and validation for an analytical method for the determination of parent compound in animal commodities. No analytical methods are presented for the free and conjugated forms of De-Xy-mandestrobin and the hydroxylated and carboxylated mandestrobin metabolites in animal commodities.

HPLC-MS/MS Method 130509 for mandestrobin

HPLC-MS/MS Method 130509, version 10 October 2013, determines mandestrobin (sum of R and S isomers) in animal commodities. The method is an earlier version of method RM-48M-1. A method description and validation are available in [Dale and Chambers, 2018; ROR-0290; Schoenau, 2013, ROA-0041]. The method was used in a cow feeding and storage stability study [Dale and Chambers, 2018; ROR-0290].

Samples of animal tissue (2.5 g) were extracted by shaking with acetonitrile for 1 hr, followed by extraction with hexane for 30 min. The acetonitrile and hexane extracts were combined and vigorously shaken for 1 min. The phases were allowed to separate and the upper hexane layer was discarded. The acetonitrile layer was again partitioned with hexane and the hexane layer was discarded. The acetonitrile layer was evaporated to dryness, re-dissolved in dichloromethane and partitioned with 5% NaCl in water. The dichloromethane layer was collected. The remaining water phase was partitioned with dichloromethane for another two times. All three dichloromethane layers were combined and the dichloromethane was evaporated to dryness. The residues were dissolved in methanol and diluted further with methanol:water (30:50 or 50:50, v/v). Samples were analysed by HPLC-MS/MS (retention time 2.4 min) at m/z 314.3 to 192.0 (quantification) and 314.3 to 160.0 (confirmation). Calibration was applied with external standards in solvent (for milk) and matrix-matched standards (for tissues) using weighted 2nd order polynomial curves (weighted relative to 1/standard concentration). The reported LOQ for each analyte was 0.02 mg/kg. Validation results are shown in Table 101.

An independent laboratory validation (ILV) was performed on chicken breast tissue [Schoenau, 2013, ROA-0041]. Samples were analysed by HPLC-MS/MS (retention time 12 min) at m/z 314.2 to 192.2 (quantification) and 314.2 to 150.2 (confirmation). Validation results are shown in Table 101. Average recoveries for the confirmation mass transitions 314 to 150 for parent were 79-80% at 0.02–0.20 mg/kg (n = 5 at each level). Control samples at these transitions were below 0.5LOQ (n = 2). Linearity at these transitions was confirmed in the range 0.5-10 ng/L.

In the cow feeding and storage stability study [Dale and Chambers, 2018; ROR-0290], the water/dichloromethane partition step was omitted. Validation results are shown in Table 68.

Note by the reviewer:

HPLC-MS/MS method 130509 for the determination of mandestrobin is considered:

- valid (full validation) for the determination of the free form of mandestrobin in the range 0.02–0.2 mg/kg in bovine muscle, chicken muscle, bovine fat, bovine liver, bovine kidney and cow milk.

Table 101 Validation results for mandestrobin with HPLC-MS/MS methods 130509 at m/z 314 to 192

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
bovine muscle	0.02	0.021 0.21	5 5	94 94	90–100 91–99	4.2% 3.4%	< 0.3LOQ (1)	7 standards matrix matched 0.05-20 µg/L 1/× weighted r2> 0.99	ROR-0290 validation
bovine muscle	0.02	0.021 0.21	1 1	98 103	- -	- -	< 0.3LOQ (2)	-	ROR-0290 feeding
bovine muscle	0.02	0.21	1	91	-	-	< 0.3LOQ (1)	-	ROR-0290 stor stab
bovine fat	0.02	0.021 0.21	5 5	97 99	91–101 93-104	3.7% 4.3%	< 0.3LOQ (1)	7 standards matrix matched 0.05-20 µg/L 1/× weighted r2> 0.99	ROR-0290 validation
bovine fat	0.02	0.021 0.21	1 1	84 91	- -	- -	< 0.3LOQ (2)	-	ROR-0290 feeding
bovine fat	0.02	0.21	1	91	-	-	< 0.3LOQ (1)	-	ROR-0290 stor stab
bovine liver	0.02	0.021 0.21	5 5	106 97	104-109 88-104	2.0% 6.3%	< 0.3LOQ (1)	7 standards matrix matched 0.05-20 µg/L 1/× weighted r2> 0.99	ROR-0290 validation
bovine liver	0.02	0.021 0.21	1 1	104 99	- -	-	< 0.3LOQ (2)	-	ROR-0290 feeding
bovine liver	0.02	0.21	1	109	-	-	< 0.3LOQ (1)	-	ROR-0290 stor stab
bovine kidney	0.02	0.021 0.21	5 5	86 100	80–91 93-103	5.1% 3.9%	< 0.3LOQ (1)	7 standards matrix matched 0.05-20 µg/L 1/× weighted r2> 0.99	ROR-0290 validation
bovine kidney	0.02	0.021 0.21	1 1	97 90	- -	- -	< 0.3LOQ (2)	-	ROR-0290 feeding
bovine kidney	0.02	0.21	1	99	-	-	< 0.3LOQ (1)	-	ROR-0290 stor stab
whole milk	0.02	0.021 0.021	5 5	95 98	91–99 96-99	3.6% 1.4%	< 0.3LOQ (1)	7 standards in solvent 0.05-20 µg/L 1/× weighted r2> 0.99	ROR-0290 validation
whole milk	0.02	0.021 0.21	8 8	92 95	77-103 89-100	8.4% 4.3%	< 0.3LOQ (8)	-	ROR-0290 feeding stor stab
whole milk	0.02	0.021	1	97	-	-	< 0.3LOQ (1)	-	ROR-0290 stor stab
cream	0.02	0.021 0.21	1 1	113 112	- -	- -	-	-	ROR-0290 feeding
skim milk	0.02	0.021 0.21	1 1	101 97	- -	- -	-	-	ROR-0290 feeding stor stab
chicken breast	0.02	0.021 0.22	5 5	82 78	80–84 77-82	1.9% 3.7%	< 0.5LOQ (2)	5 standards in solvent 0.5-10 ng/mL r2> 0.99999	ROA-0041 ILV

Analytical methods used in study reports on soils

The Meeting received the description and validation for two analytical methods for the determination of the R- and S-isomers of mandestrobin, mandestrobin, and its metabolites 2-COOH-mandestrobin, 5-COOH-mandestrobin, De-Xy-mandestrobin, DX-CA-mandestrobin, 2-$CONH_2$-mandestrobin, 5-$CONH_2$-mandestrobin in soil.

LC-MS/MS method CLE 8213772–01V for mandestrobin and its metabolites

LC-MS/MS method CLE 8213772–01V, not dated, [Leslie, 2011b, ROA-0014] determines the R- and S-isomer of mandestrobin, De-Xy-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin in soil. The method was used in a field dissipation study on European soils [Lewis, 2012, ROR-0010] and a storage stability study [Leslie, 2011a, ROR-0006].

Soil samples (10 g) were extracted twice for 10 minutes with acetone: 0.1 M HCl (5:1, v/v), followed by filtration through Celite. The filtrate was adjusted to pH 2–3 with 5 M HCl and partitioned twice with dichloromethane in the presence of 5% sodium chloride. The dichloromethane phase was evaporated to dryness and reconstituted in water:MeOH (1:1, v/v). The extract was diluted further with the same solvent, if necessary. The extract was analysed against external standards by LC-MS/MS. Parent compound was analysed as its isomeric constituents with separation achieved using a Chiralpak column (retention time 5.0 and 5.8 min for the R- and S-isomer, respectively), whilst the metabolites were separated using a C18 column with retention times of 1.2 min for De-Xy-mandestrobin, 2.7 min for 2-COOH-mandestrobin and 3.2 min for 5-COOH-mandestrobin. Detection was at m/z 314.1 to 192.0 (quantification) and 132.1 (confirmation) for the R-and S-isomers of mandestrobin, 210.2 to 192.0 (quantification) and 132.0 (confirmation) for De-Xy-mandestrobin and 344.1 to 192.0 (quantification) and 160.0 (confirmation) for 2-COOH-mandestrobin and 5-COOH-mandestrobin. Residues were expressed as mg/kg dry weight basis by correction for the percentage moisture in the soil. The reported LOQ was 0.0025 mg/kg dry weight for the R- and S-isomer of mandestrobin and 0.005 mg/kg dry weight for each of the metabolites. The mandestrobin concentration was obtained by summing the concentrations for the R- and S-isomers of mandestrobin. The validation results for the determination of each of the analytes in soil are summarized in Table 102.

Confirmation of the method [Leslie, 2011b, ROA-0014] was demonstrated as the recovery and precision for the transitions used for confirmation for each of the isomers and associated metabolites were within the acceptance criteria (recovery 70–120% and RSD < 20%).

Note by the reviewer:

LC-MS/MS method CLE 8213772–01V for the determination of mandestrobin, De-Xy-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin in soil is considered:

- Valid for the determination of De-Xy-mandestrobin and 2-COOH-mandestrobin in the range 0.005–0.5 mg/kg.
- Valid for the determination of the R- and S-isomer of mandestrobin in the range 0.1–0.25 mg/kg. The method is not valid at the reported LOQ of 0.0025 mg/kg as the precision at this level is not acceptable (RSD > 20%) and some of the control samples showed residues up to 0.0024 mg/kg (> 0.3LOQ) and 0.0056 mg/kg. The next possible LOQ levels of 0.005–0.010 mg/kg were not validated.
- Not valid for the determination of 5-COOH-mandestrobin as both the average recovery (> 120%) and the precision (> 20%) at 0.005–0.5 mg/kg is not acceptable. For some batches in the field dissipation study and in the storage stability study the recoveries were unacceptably high for unknown reasons.

Table 102 Validation results for LC-MS/MS method CLE 8213772–01V

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
R-isomer of mandestrobin	0.0025	0.0025 0.25	14 14	90 97	81–108 91–101	7.6% 2.7%	< 0.3LOQ (n = 8)	8 standards in solvent 0.05-4 ng/mL linear r> 0.999	ROA-0014 method validation
R-isomer of mandestrobin	0.0025	0.0025 0.25	49 49	95 91	70–168 77-105	**22%** 6.1%	< 0.3LOQ 0.00080; 0.0014; 0.0016 0.0024 (n = 73)	8 standards in solvent 0.05-4 ng/mL linear r> 0.99	ROR-0010 field dissipation
R-isomer of mandestrobin	0.0025	0.1	5	96	86-110	11%	< 0.3LOQ 0.0020 (n = 5)	8 standards in solvent 0.05-4 ng/mL linear r> 0.99	ROR-0006 storage stability
S-isomer of mandestrobin	0.0025	0.0025 0.25	14 14	101 97	94-115 93-101	5.5% 2.7%	< 0.3LOQ (n = 8)	8 standards in solvent 0.05-4 ng/mL linear r> 0.999	ROA-0014 method validation
S-isomer of mandestrobin	0.0025	0.0025 0.25	46 46	96 92	77-224 80–109	**27%** 7.8%	< 0.3LOQ; 0.00076; 0.0015; 0.0024 (n = 70)	8 standards in solvent 0.05-4 ng/mL linear r> 0.999	ROR-0010 field dissipation
S-isomer of mandestrobin	0.0025	0.1	5	98	88-104	6.5%	< 0.3LOQ 0.0056 (n = 5)	8 standards in solvent 0.05-4 ng/mL linear r> 0.999	ROR-0006 storage stability
De-Xy-mandestrobin	0.005	0.005 0.50	14 14	78 86	72–86 82–95	5.7% 4.7%	< 0.3LOQ (n = 8)	8 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROA-0014 method validation
De-Xy-mandestrobin	0.005	0.005 0.50	39 41	84 82	68-106 72–89	11% 5.1%	< 0.3LOQ (n = 63)	8 standards in solvent 0.1–8 ng/mL linear r> 0.9999	ROR-0010 field dissipation
De-Xy-mandestrobin	0.005	0.1	5	81	77-89	5.9%	< 0.3LOQ (n = 5)	8 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROR-0006 storage stability
2-COOH-mandestrobin	0.005	0.005 0.50	14 15	93 99	88-101 94-105	4.3% 3.3%	< 0.3LOQ (n = 8)	8 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROA-0014 method validation
2-COOH-mandestrobin	0.005	0.005 0.50	40 42	95 95	73-120 76-106	13% 6.7%	< 0.3LOQ 0.0015 (n = 64)	8 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROR-0010 field dissipation
2-COOH-mandestrobin	0.005	0.1	5	99	91–110	8.9%	< 0.3LOQ; 0.0036; 0.0078	8 standards in solvent 0.1–8 ng/mL	ROR-0006 Storage stability

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD_r	control samples mg/kg (n)	calibration	reference, method
							(n = 5)	linear r> 0.999	
5-COOH-mandestrobin	0.005 0.50	0.005 0.50	14 14	89 98	83-95 93-103	4.1% 3.2%	< 0.3LOQ (n = 8)	6 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROA-0014 method validation
5-COOH-mandestrobin	0.005	0.005 0.50	43 45	**126** **138**	73-321 83-305	**54%** **55%**	< 0.3LOQ 0.0028 0.0043 (n = 67)	8 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROR-0010 field dissipation
5-COOH-mandestrobin	0.005	0.1	5	**138**	86-309	**69%**	< 0.3LOQ 0.0049 (n = 5)	8 standards in solvent 0.1–8 ng/mL linear r> 0.999	ROR-0006 storage stability

LC-MS/MS method RM-48S-3 for mandestrobin and its metabolites

LC-MS/MS method RM-48S-3, version 11 October 2012, [Bitter, 2013 d-h, ROR-0245, ROR-0246, ROR-0247, ROR-0248, ROR-0249] determines mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin in soil. The method was used in field dissipation and storage stability studies on Canadian and USA soils [Bitter, 2012 a-e, ROR-0245, ROR-0246, ROR-0247, ROR-0248, and ROR-0249; Green, 2013, MRID 49068568].

Soil samples (2.5 g) were extracted twice with acetone/0.05 M HCl (80:20, v/v) for one hour and then centrifuged. The supernatant was partitioned twice with dichloromethane in the presence of 5% sodium chloride. The dichloromethane phase was evaporated to dryness and reconstituted in methanol/0.05% formic acid in water (1:1, v/v). The extract was analysed against external standards by LC-MS/MS. Analytes were separated on a C18 column and detected using m/z 314 to 192 for mandestrobin, m/z 344 to 192 for 2-COOH-mandestrobin and 5-COOH-mandestrobin, m/z 343 to 192 for 2-$CONH_2$-mandestrobin and 5-$CONH_2$-mandestrobin, and m/z 224 to 146 for DX-CA-mandestrobin. Residues were expressed as mg/kg dry weight basis by correction for the percentage moisture in the soil. The reported LOQ was 0.02 mg/kg dry weight for each of the analytes. The validation results are summarized in Table 103.

An independent laboratory validation was performed using method RM-48S-3 with no major modifications [Pernell, 2013, MRID 49068638]. The first attempt was rejected because the recovery of the parent compound was far outside the 120% range. Reasons for this deviation were not given. The second attempt was successful. Validation results are summarized in Table 103.

LC-MS/MS method RM-48S-3 is considered valid for the determination of mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-CONH2-mandestrobin and 5-CONH2-mandestrobin in soil in the range 0.02–1.0 mg/kg.

Table 103 Validation results for LC-MS/MS Method RM-48S-3

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD	control samples mg/kg	calibration	reference
mandestrobin	0.02	0.02 0.10 1.0	29 16 13	94 92 89	71–112 70–108 79–98	9.6% 10% 7.1%	< 0.01 (35)	6 standards in solvent 1.25-50 ng/mL 1/× weighted	ROR-0245 field dissipation.

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD	control samples mg/kg	calibration	reference
								linear r^2> 0.99	
	0.02	0.02 0.1 0.5 1.0	29 14 6 8	87 89 93 89	70–115 72–108 72–102 76-95	12% 11% 12% 6.9%	< 0.01 (30)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0246 field dissipation
	0.02	0.02 0.2	42 37	99 96	77-118 70–115	11% 13%	< 0.0084 (59)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0247 field dissipation
	0.02	0.02 0.2	27 25	95 90	73-116 72–107	13% 12%	< 0.01 (57)	6 standards in solvent 0.15-10 ng/mL 1/× weighted linear r> 0.9999	ROR-0248 field dissipation
	0.02	0.02 0.1 1.0	21 20 1	102 103 102	93-110 93-111 -	3.5% 4.8% -	< 0.01 (54)	6 standards in solvent 10–400 ng/g 1/× weighted linear r> 0.999	ROR-0249 field dissipation
	0.02	0.02 0.20	5 5	107 113 101	101– 99-103	4.4% 1.8%	< 0.3LOQ (2)	6 standards in solvent 1.25-50 ng/mL 1/× weighted r2> 0.999	MRID 49068638; ILV
DX-CA-mandestrobin	0.02	0.02 0.10 1.0	22 12 10	114 116 104	99-121 109-120 94-113	5.2% 3.3% 5.8%	< 0.01 (29)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0245 field dissipation
	0.02	0.02 0.1 0.5 1.0	27 13 5 8	103 114 105 95	81–119 103-126 89-125 85-107	12% 6.2% 16% 9.0%	< 0.01 (30)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0246 field dissipation
	0.02	0.02 0.2	38 31	101 103	76-120 78-120	13% 12%	< 0.0084 (59)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0247 field dissipation
	0.02	0.02 0.2	26 22	95 96	70–120 73-111	14% 11%	< 0.01 (57	6 standards in solvent 0.15-10 ng/mL 1/× weighted linear r> 0.9999	ROR-0248 field dissipation
	0.02	0.02 0.1 1.0	21 20 1	106 108 112	74-116 97-120 -	9.8% 5.4% -	< 0.01 (54)	6 standards in solvent 10–400 ng/g 1/× weighted linear r> 0.999	ROR-0249 field dissipation

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD	control samples mg/kg	calibration	reference
	0.02	0.02 0.20	5 5	104 101	98-108 98-103	3.9% 1.8%	< 0.3LOQ (2)	6 standards in solvent 1.25-50 ng/mL 1/× weighted r2> 0.9999	MRID 49068638; ILV
2-COOH-mandestrobin	0.02	0.02 0.10 1.0	25 14 11	94 92 89	73-114 70–107 72–107	12% 11% 10%	< 0.01 (31)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0245 field dissipation
	0.02	0.02 0.1 0.5 1.0	27 13 6 8	87 89 89 86	71–114 79-107 77-99 72–102	13% 9.0% 7.9% 12%	< 0.01 (30)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0246 field dissipation
	0.02	0.02 0.2	39 32	104 103	71–120 77-119	12% 10%	< 0.0084 (59)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0247 field dissipation
	0.02	0.02 0.2	28 28	100 98	82–116 76-118	8.9% 11%	< 0.01 (57	6 standards in solvent 0.15-10 ng/mL 1/× weighted linear r> 0.9999	ROR-0248 field dissipation
	0.02	0.02 0.1 1.0	21 20 1	99 97 106	90–106 90–109 -	3.7% 5.7% -	< 0.01 (54)	6 standards in solvent 10–400 ng/g 1/× weighted linear r> 0.999	ROR-0249 field dissipation
	0.02	0.02 0.20	5 5	111 102 105	105-117 100–	5.2% 2.0%	< 0.3LOQ (2)	6 standards in solvent 1.25-50 ng/mL 1/× weighted r2> 0.999	MRID 49068638; ILV
5-COOH-mandestrobin	0.02	0.02 0.10 1.0	24 13 11	94 95 90	75-113 74-106 80–99	10% 8.9% 7.0%	< 0.01 (31)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0245 field dissipation
	0.02	0.02 0.1 0.5 1.0	27 13 6 8	88 91 97 84	71–113 73-106 71–109 71–91	14% 14% 14% 7.3%	< 0.01 (30)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0246 field dissipation
	0.02	0.02 0.2	41 35	102 102	71–116 71–120	12% 12%	< 0.0084 (59)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0247 field dissipation

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD	control samples mg/kg	calibration	reference
	0.02	0.02 0.2	28 26	98 98	81–116 73-113	9.0% 10%	< 0.01 (57	6 standards in solvent 0.15-10 ng/mL 1/× weighted linear r> 0.999	ROR-0248 field dissipation
	0.02	0.02 0.1 1.0	21 20 1	99 98 100	94-106 89-110 -	3.4% 5.5% -	< 0.01 (54)	6 standards in solvent 10–400 ng/g 1/× weighted linear r> 0.999	ROR-0249 field dissipation
	0.02	0.02 0.20	5 5	108 103 105	103-113 101–	3.3% 1.4%	< 0.3LOQ (2)	6 standards in solvent 1.25-50 ng/mL 1/× weighted r2> 0.999	MRID 49068638; ILV
2-$CONH_2$-mandestrobin	0.02	0.02 0.10 1.0	24 13 11	93 91 89	72–116 73-109 72–112	12% 8.6% 12%	< 0.01 (31)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0245 field dissipation
	0.02	0.02 0.1 0.5 1.0	28 13 6 8	86 90 90 91	70–114 78-110 86-94 70–108	13% 9.9% 3.3% 11%	< 0.01 (30)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0246 field dissipation
	0.02	0.02 0.2	38 33	106 104	80–120 76-120	11% 11%	< 0.01 (59)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0247 field dissipation
	0.02	0.02 0.2	27 28	100 98	84-117 76-118	9.2% 11%	< 0.01 (57	6 standards in solvent 0.15-10 ng/mL 1/× weighted linear r> 0.9999	ROR-0248 field dissipation
	0.02	0.02 0.1 1.0	21 20 1	100 97 101	93-105 88-105 -	3.9% 4.5% -	< 0.01 (54)	6 standards 10–400 ng/g 1/× weighted linear r> 0.999	ROR-0249 field dissipation
	0.02	0.02 0.20	5 5	110 102 105	103-115 100–	5.2% 1.8%	< 0.3LOQ (2)	6 standards in solvent 1.25-50 ng/mL 1/× weighted r2> 0.9999	MRID 49068638; ILV
5-$CONH_2$-mandestrobin	0.02	0.02 0.10 1.0	24 13 11	91 90 87	70–112 72–101 77-96	9.9% 9.9% 6.8%	< 0.01 (31)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0245 field dissipation
	0.02	0.02 0.1 0.5 1.0	28 14 6 8	88 91 95 86	70–110 78-104 80–101 78-95	11% 10% 8.0% 7.7%	< 0.01 (30)	6 standards in solvent 1.25-50 ng/mL 1/× weighted	ROR-0246 field dissipation

commodity	reported LOQ mg/kg	spike level mg/kg	n	% recovery mean	range	RSD	control samples mg/kg	calibration	reference
								linear r^2> 0.99	
	0.02	0.02 0.2	40 30	106 103	72–120 78-118	11% 9.6%	< 0.01 (59)	6 standards in solvent 1.25-50 ng/mL 1/× weighted linear r^2> 0.99	ROR-0247 field dissipation
	0.02	0.02 0.2	27 28	100 97	82–115 74-117	7.8% 11%	< 0.01 (57	6 standards in solvent 0.15-10 ng/mL 1/× weighted linear r> 0.999	ROR-0248 field dissipation
	0.02	0.02 0.1 1.0	21 20 1	99 97 103	90–102 77-104 -	3.5% 6.6% -	< 0.01 (54)	6 standards in solvent 10–400 ng/g 1/× weighted linear r> 0.999	ROR-0249 field dissipation
	0.02	0.02 0.20	5 5	111 103	104-116 104-105	5.3% 1.5%	< 0.3LOQ (2)	6 standards in solvent 1.25-50 ng/mL 1/× weighted r2> 0.999	MRID 49068638; ILV

STABILITY OF PESTICIDE RESIDUES IN STORED ANALYTICAL SAMPLES

The Meeting received information on the storage stability of parent, De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 4-OH- mandestrobin, 5-CH_2OH-mandestrobin in raw and processed plant commodities and on the storage stability of parent in animal tissues and milk. In addition, the Meeting received information on the storage stability of parent, De-Xy-mandestrobin, 2-COOH- mandestrobin and 5-COOH-mandestrobin in different soils.

Storage stability of spiked residues in plant commodities

Study 1 (R- and S- isomers of mandestrobin)

Storage stability was investigated by **spiking** rape (seeds) with 0.10 mg/kg of the R- and S-isomers of mandestrobin, separately [Daneva & Taeufer, 2011, ROR-0007]. Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various intervals. The R- and S-isomers of mandestrobin were quantified separately by chiral HPLC-MS/MS method DFG S19. The LOQ of the method was 0.005 mg/kg for each isomer. Average concurrent fresh recoveries were 92–94% for the R- and S-isomer of mandestrobin at 0.10 mg/kg and control samples had residues below the 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for the R- and S-isomers of mandestrobin are shown in Table 104.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes).

Study 2 (R-and S-isomers of mandestrobin)

Storage stability was investigated by **spiking** high water (lettuce) and dry crops (barley) with 0.10 mg/kg of the R- and S-isomers of mandestrobin, separately [Daneva & Taeufer, 2011a, ROR-0009]. Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various

intervals. The R- and S-isomers of mandestrobin were quantified separately by chiral HPLC-MS/MS method DFG S19. The LOQ of the method was 0.005 mg/kg for each isomer. Average concurrent fresh recoveries in lettuce, barley grain and barley straw ranged between 79-101% for the R- and S-isomer of mandestrobin at 0.10 mg/kg and control samples had residues below the 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for parent mandestrobin are shown in Table 104.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes).

Study 3 (mandestrobin, De-Xy-mandestrobin, 4-OH- and 2-CH_2OH-mandestrobin)

Storage stability was investigated by **spiking** homogenised dry beans (white seeds) and orange fruit (whole fruit) with a mixture of 0.10 mg/kg of mandestrobin and its metabolites De-Xy-mandestrobin, 4-OH-mandestrobin and 2-CH_2OH-mandestrobin [Lindner *et al*, 2017, ROR-0286]. The results include data from an interim report [Lindner & Grewe, 2016, ROR-0269].

Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various intervals. The storage stability of 4-OH-mandestrobin and 2-CH_2OH-mandestrobin in orange whole fruit was also determined after storage at -18 °C for 26 months. Mandestrobin was quantified by HPLC-MS/MS method QuEChERS. Metabolite De-Xy-mandestrobin was quantified by HPLC-MS/MS method SUM 1023V. The metabolites 4-OH-mandestrobin and 2-CH_2OH-mandestrobin were quantified by HPLC-MS/MS method S10–02011. The LOQ was 0.01 mg/kg for each analyte.

Average concurrent fresh recoveries in dried beans and oranges ranged between 96-97% for parent mandestrobin at 0.1 mg/kg and between 72–101% for De-Xy-mandestrobin, 4-OH-mandestrobin and 2-CH_2OH-mandestrobin at 0.01–0.1 mg/kg and control samples had residues below the 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for parent mandestrobin are shown in Table 105. The results for metabolites De-Xy-mandestrobin, 4-OH-mandestrobin and 2-CH_2OH-mandestrobin are shown in Tables 106 and 107.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes). Storage stability for 5-CH_2OH-mandestrobin was not evaluated in this study.

Study 4 (mandestrobin and De-Xy-mandestrobin)

Storage stability was investigated by **spiking** untreated rape seed samples with 0.20 mg/kg of mandestrobin or its metabolite De-Xy-mandestrobin [Green, 2016, ROR-0274]. This study includes the data previously published [Green, 2015, ROR-0258; Green, 2015a, ROR-0259; Green, 2013, ROR-0238 (including addendum)]. Samples were stored for 38 months at -18 ºC and were analysed in duplicate at various intervals.

Mandestrobin and De-Xy-mandestrobin were quantified by HPLC-MS/MS method RM-48C-2 (0–608 day storage samples) and RM-48C-2B method (806 day storage samples) and modified RM-48C-2B method (1162 day storage samples). The LOQ of the method was 0.02 mg/kg for each analyte.

Average concurrent fresh recoveries in rape seed were 71–113% for mandestrobin and De-Xy-mandestrobin at 0.02–0.20 mg/kg. Control samples had residues below 0.3LOQ, except the control sample for day 314 with a residue of 0.022 mg/kg mandestrobin.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for parent mandestrobin are shown in Table 105. The results for metabolite De-Xy-mandestrobin are shown in Table 106.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes), but did not show adequate performance at day 314. Nevertheless, the stability of mandestrobin and De-Xy-mandestrobin was shown to be acceptable for 38 months of frozen storage.

Study 5 (De-Xy-mandestrobin)

Storage stability was investigated by **spiking** lettuce (high water), rape seed (high oil content) and barley grains (dry crops) and barley straw with 0.10 mg/kg of the metabolite De-Xy-mandestrobin [Daneva & Taeufer, 2012, ROR-0011]. Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various intervals.

De-Xy-mandestrobin was quantified by HPLC-MS/MS method SUM-1023V. The LOQ was 0.01 mg/kg. Average concurrent fresh recoveries of De-Xy-mandestrobin in lettuce, rape seed, barley straw and barley grains ranged between 78-89% at 0.10 mg/kg and control samples had residues below 0.3 LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for De-Xy-mandestrobin are shown in Table 106.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes).

Study 6 (4-OH-mandestrobin)

Storage stability was investigated by **spiking** lettuce (high water), rape seed (high oil content) and barley grains (dry crops) and barley straw with 0.10 mg/kg of the metabolite 4-OH-mandestrobin [Daneva & Taeufer, 2012, ROR-0012]. Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various intervals.

4-OH-mandestrobin was quantified by HPLC-MS/MS method SUM-1021V. The LOQ was 0.01 mg/kg. Average concurrent fresh recoveries of 4-OH-mandestrobin for lettuce, rape seed, barley grains and barley straw ranged between 81–87% at 0.10 mg/kg and control samples had residues below 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for 4-OH-mandestrobin are shown in Table 106.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes).

Study 7 (2-CH_2OH-mandestrobin)

Storage stability was investigated by **spiking** lettuce (high water), seeds of oil seed rape (high oil content) and barley (dry crops) with 0.10 mg/kg of the mandestrobin metabolite 2-CH_2OH-mandestrobin [Daneva & Zetzsch, 2012, ROR-0013]. Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various intervals.

2-CH_2OH-mandestrobin was quantified by HPLC-MS/MS method SUM-1022V. The LOQ was 0.01 mg/kg. Average concurrent fresh recoveries of 2-CH_2OH-mandestrobin for lettuce, rape seed, barley grain and barley straw ranged from 77-88% at 0.10 mg/kg and control samples had residues below 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for 2-CH_2OH-mandestrobin are shown in Table 106.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes).

Study 8 (5-CH_2OH-mandestrobin)

Storage stability was investigated by **spiking** lettuce (high water), and barley (dry crops) with 0.10 mg/kg of the mandestrobin metabolite 5-CH_2OH- mandestrobin [Daneva & Zetzsch, 2012a, ROR-0014]. Samples were stored for 12 months at -18 ºC and were analysed in duplicate at various intervals.

5-CH_2OH-mandestrobin was quantified by HPLC-MS/MS method SUM-1027V. The LOQ of the method was 0.01 mg/kg. Average concurrent fresh recoveries of 5-CH_2OH-mandestrobin for lettuce, barley grain and barley straw ranged from 80–83% at 0.10 mg/kg and control samples had residues below 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for 5-CH_2OH-mandestrobin are shown in Table 106.

Note by the reviewer: The analytical method is valid for the purpose of this study (commodity type and concentration level of the analytes). Storage stability for 5-CH_2OH-mandestrobin in rape seed has not been investigated in this study.

Table 104 Storage stability, at ≤ -18 °C, in commodities spiked with 0.1 mg/kg of the R- or S-isomer of mandestrobin

Matrix	Storage period	R-isomer of mande strobin mean %	R-isomer of mande strobin Mean % remaining [a]	R-isomer of mandestrobin Mean concurrent recovery (%)	S-isomer of mande strobin mean %	S-isomer of mandestrobin Mean % remaining [a]	S-isomer of mande strobin Mean concurrent recovery (%)	Reference
lettuce (head)	0	105 [b]	100	-	84 [b]	100	-	[ROR-0009]
	1 month	89	85	98	113	135	119	
	3 months	84	80	84	110	131	109	
	6 months	96	91	96	104	124	92	
	12 months	97	93	96	93	111	89	
barley (grain)	0	109 [b]	100	-	105 [b]	100	-	[ROR-0009]
	1 month	82	75	99	94	89	88	
	3 months	87	79	86	89	84	89	
	6 months	93	85	102	93	88	87	
	12 months	90	82	96	89	84	87	
barley (straw)	0	106 [b]	100	-	82 [b]	100	-	[ROR-0009],
	1 month	83	78	87	72	88	73	
	3 months	77	73	88	76	92	74	
	6 months	101	95	92	79	97	79	
	12 months	82	77	88	87	106	83	
rape seed (seed)	0	88 [b]	100	-	82 [b]	100	-	[ROR-0007]
	31 days	95	108	103	103	126	93	
	91 days	83	94	91	98	120	103	
	182 days	92	105	93	100	122	94	
	364 days	105	119	110	105	128	107	

[a] The % remaining is indicated as the percentage of the initial, which is calculated by dividing the mean recovery of stored samples at each interval by the mean recovery at day 0. The values are not corrected with the concurrent recoveries.

[b] Mean of three samples.

Table 105 Storage stability at ≤ -18 °C in commodities spiked with mandestrobin (racemic mixture)

Matrix	Spike level mg/kg	Storage period (days)	parent mean %	parent Mean % remaining [a]	parent Mean concurrent recovery (%)	Reference
orange	0.10	0	89 [b]	100	-	[ROR-0286], [ROR-

Matrix	Spike level mg/kg	Storage period (days)	parent mean %	parent Mean % remaining [a]	parent Mean concurrent recovery (%)	Reference
whole fruit [d]		31	97	109	97	0269]
		95	87	98	92	
		183	93	104	101	
		280	102	115	106	
		379	106	119	108	
dried beans (white seed) [c]	0.10	0	92 [b]	100	-	[ROR-0286] [ROR-0269]
		31	98	107	95	
		95	106	115	109	
		183	85	92	95	
		280	84	91	97	
		379	97	105	106	
rape seed [c]	0.20	0	81 [c]	100	80	[ROR-0274]
		314	55 [c]	68 [c]	64	
		466	47 [c]	58 [c]	61	
		608	56 [c]	68 [c]	77	
		806	82 [c]	101 [c]	86	
		1162	90 [c]	111 [c]	113	

[a] The % remaining is indicated as the percentage of the initial, which is calculated by dividing the mean recovery of stored samples at each interval by the mean recovery at day 0. The values are not corrected with the concurrent recoveries.

[b] Mean of three samples.

[c] Values calculated by evaluator based on mean of two values. Percentage remaining is also calculated by evaluator according to [a].

Table 106 Storage stability at ≤-18 °C in commodities spiked with 0.1 mg/kg De-Xy-mandestrobin or 4-OH-mandestrobin

Matrix & spike level	Storage period (days)	De-Xy-M mean %	De-Xy-M mean % remaining [a]	De-Xy-M mean concurrent recovery (%)	Storage period (days)	4-OH-M mean %	4-OH-M mean % remaining [a]	4-OH-M mean concurrent recovery (%)	Reference
orange (whole fruit) 0.1 mg/kg	0	87 [b]	100	-	0	81 [b]	100	-	[ROR-0286], [ROR-0269]
	33	75	86	78	33	[c]	[c]	[c]	
	97	78	90	83	96	86	106	89	
	201	102	117	76	200	83	102	97	
	278	110	126	97	277	86	106	107	
	375	106	122	90	375	75	92	90	
					796	92	113	100	
lettuce (head) 0.1 mg/kg	0	77 [b]	100	-	0	92 [b]	100	-	ROR-0011] & [ROR-0012]
	28	81	106	81	45	97	105	90	
	97	85	111	78	90	88	96	83	
	180	94	122	93	181	71	77	71	
	368	90	117	96	365	71	77	73	
dried beans (white seed) 0.1 mg/kg	0	88 [b]	100	-	0	71 [b]	100	-	[ROR-0286], [ROR-0269]
	33	74	84	78	33	[c]	[c]	[c]	
	97	75	85	73	116	77	108	81 [b]	
	201	90	102	72	199	86	121	85	
	278	112	127	94	276	82	115	94	
	375	108	122	90	379	82	115	101	
barley (grain) 0.1 mg/kg	0	90 [b]	100	-	0	89	100	-	ROR-0011] & [ROR-0012]
	27	82	91	81	29	77	86	71	
	98	100	112	92	89	91	102	86	
	177	90	100	99	180	69	77	71	
	363	86	96	88	363	78	88	84	
barley (straw)	0	75 [b]	100	-	0	90	100	-	ROR-0011]
	27	77	103	77	31	76	85	70	

Matrix & spike level	Storage period (days)	De-Xy-M mean %	De-Xy-M mean % remaining [a]	De-Xy-M mean concurrent recovery (%)	Storage period (days)	4-OH-M mean %	4-OH-M mean % remaining [a]	4-OH-M mean concurrent recovery (%)	Reference
0.1 mg/kg	97	77	102	76	91	82	91	81	& [ROR-0012]
	180	91	121	98	183	88	98	102	
	367	72	96	72	366	84	93	84	
rape seed 0.1 mg/kg	0	89 [b]	100	-	0	86	100	-	ROR-0011] & [ROR-0012]
	27	83	93	80	28	81	94	71	
	94	80	89	82	91	79	92	76	
	177	86	96	95	179	74	86	75	
	363	86	96	94	363	83	97	88	
rape seed 0.2 mg/kg	0	75 [d]	100	80					[ROR-0274]
	314	88 [d]	117 [d]	93					
	466	59 [d]	78 [d]	72					
	608	74 [d]	98 [d]	88					
	806	91 [d]	121 [d]	98					
	1162	101 [d]	134 [d]	107					

M= mandestrobin; n.r. = not reported

[a] The % remaining is indicated as the percentage of the initial, which is calculated by dividing the mean recovery of stored samples at each interval by the mean recovery at day 0. The values are not corrected with the concurrent recoveries.

[b] Mean of three samples.

[c] Because of a mistake in the lab procedural recoveries were outside the acceptance criteria and one month data cannot be used for assessment of storage stability.

[d] Values calculated by evaluator based on mean of two values. Percentage remaining is also calculated by evaluator according to [a].

[e] The selected day 0 represent 118 (grapes), 218 (raisins; juice) and 346 (strawberries) days after harvest

Table 107 Storage stability at ≤ -18 °C in commodities spiked with 2-CH_2OH- or 5-CH_2OH-mandestrobin

Matrix and spike level	Storage period (days)	2-CH_2OH-M mean %	2-CH_2OH-M mean % remaining [a]	2-CH_2OH-M mean concurrent recovery (%)	Storage period (days)	5-CH_2OH-M mean %	5-CH_2OH-M mean % remaining [a]	5-CH_2OH-M mean concurrent recovery (%)	Reference
orange fruit (whole fruit) 0.1 mg/kg	0	91 [b]	100	-					ROR-0286] & [ROR-0269]
	33	[c]	[c]	[c]					
	96	84	92	80					
	200	87	95	89					
	277	80	88	90					
	375	92	101	90					
	796	94	103	90					
lettuce (head) 0.1 mg/kg	0	75 [b]	100	-	0	79 [b]	100	-	[ROR-0013] & [ROR-0014]
	26	82	110	77	31	87	110	93	
	87	82	109	72	92	81	101	76	
	180	101	135	94	182	83	104	82	
	362	89	119	103	365	95	119	91	
dried beans (white seed) 0.1 mg/kg	0	96 [b]	100	-					[ROR-0286] & [ROR-0269]
	33	[c]	[c]	[c]					
	116	90	94	90 [b]					
	199	99	103	96					
	276	89	93	80					
	374	89	92	94					
barley	0	85 [b]	100	-	0	78 [b]	100	-	[ROR-

Matrix and spike level	Storage period (days)	2-CH_2OH-M mean %	2-CH_2OH-M mean % remaining [a]	2-CH_2OH-M mean concurrent recovery (%)	Storage period (days)	5-CH_2OH-M mean %	5-CH_2OH-M mean % remaining [a]	5-CH_2OH-M mean concurrent recovery (%)	Reference
(grain)	31	82	96	86	28	75	95	90	0013] & [ROR-0014]
	87	87	102	94	90	82	104	79	
0.1 mg/kg	182	93	110	86	182	71	91	78	
	365	91	107	98	364	72	91	90	
barley (straw)	0	72 [b]	100	-	0	81 [b]	100	-	[ROR-0013] & [ROR-0014]
	31	71	98	76	29	81	100	80	
	92	78	108	89	91	74	91	76	
0.1 mg/kg	183	88	122	93	182	73	90	87	
	366	92	128	105	364	71	88	77	
oil seed rape (seed)	0	73 [b]	100	-					[ROR-0013] & [ROR-0014]
	26	81	111	70					
	89	72	99	87					
	180	88	120	83					
0.1 mg/kg	363	81	110	80					

[a] The % remaining is indicated as the percentage of the initial, which is calculated by dividing the mean recovery of stored samples at each interval by the mean recovery at day 0. The values are not corrected with the concurrent recoveries.

[b] Mean of three samples.

[c] Because of a mistake in the lab procedural recoveries were outside the acceptance criteria and one month data cannot be used for assessment of storage stability. Storage stability was shown at later time points.

Summary for studies with spiked commodities

Residues of the R- and S-isomers of mandestrobin are stable for at least 12 months in crop commodities representative of the high water (lettuce), high acid (orange, strawberry, grapes), high starch (barley grain), high protein (dry bean seeds), high oil (rape seed) commodity groups as well as in barley straw when stored at or below -18 °C. No conversion between R- and S-isomers was detected.

Mandestrobin (racemic mixture) and metabolite De-Xy- mandestrobin are stable for at least 38 months in crop commodities representative of high oil content (rape seed) and 12 months in crop commodities representative of high water (lettuce), high acid (orange, strawberry, grapes), high starch (barley grain) and high protein (dry bean seed) content as well as in barley straw when stored at or below -18 °C.

Metabolites 4-OH-mandestrobin and 2-CH_2OH-mandestrobin are stable for at least 26 months in crop commodities representative of high acid content (orange) and at least 12 months in crop commodities representative of high water (lettuce), high starch (barley grain), high protein (dry bean seed) and high oil (rape seed) content as well as in barley straw when stored at or below -18 °C.

Metabolite 5-CH_2OH-mandestrobin is stable for at least 12 months in crop commodities representative of high water (lettuce) and high protein (dried bean seed) content as well as in barley straw when stored at or below -18 °C.

Storage stability of mandestrobin and metabolites De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH in crop commodities with high acid content can be extrapolated to grape juice and storage stability in crop commodities with high oil content can be extrapolated to rape seed oil and rape seed meal. Storage stability of 5-CH_2OH-mandestrobin is not demonstrated in these matrices. Storage stability studies in grape raisins (high sugar content) were not submitted.

Storage stability of incurred residues in plant commodities

Study 1 (mandestrobin and De-Xy-mandestrobin)

Treated grapes from trial V-38175-A and a treated grape juice and grape raisin sample from trial V-31875-M were used to determine stability of **incurred** residues of mandestrobin and De-Xy-mandestrobin [Bitter, 2015, ROR-0261; Bitter *et al*., 2013, ROR-0234 (including addendum)]. At periodic intervals samples were removed from storage and analysed along with an untreated control and a freshly fortified sample. Samples were stored at -20 ºC and were analysed in duplicate at various intervals.

Mandestrobin and De-Xy-mandestrobin were quantified by HPLC-MS/MS method RM-48G. The LOQ was 0.02 mg/kg for each analyte. Average concurrent fresh recoveries in grapes, juice and raisins ranged between 72–115% for parent and De-Xy-mandestrobin at 0.02–20 mg/kg. Control samples had residues below 0.01 mg/kg.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for parent mandestrobin are shown in Table 108. The results for metabolite De-Xy-mandestrobin are shown in Table 106.

Notes by the reviewer:

- The methods used are considered valid for the purpose of this study (commodity type and concentration level of the analytes).
- The samples were already stored in the freezer for 118-218 days, when the day 0 sample for the storage stability analysis was taken. Since studies with spiked residues show that degradation of mandestrobin or De-Xy-mandestrobin is not to be expected for up to a period of 12 months for crop commodities with high acid content (grapes, grape juice) and various other crop commodity types, the studies with incurred residues suggest that storage stability can be extended to 538 days for grapes (and grape juice) and 385 days for grape raisins.

Study 2 (mandestrobin and De-Xy-mandestrobin)

Treated strawberries from a field residue trial V-38182-B [Bitter *et al*, 2013, ROR-0236] were used to determine stability of **incurred** residues of mandestrobin and De-Xy-mandestrobin [Bitter *et al*, 2013, ROR-0236 (including addendum); Bitter, 2015a, ROR-0260]. At periodic intervals samples were removed from storage and analysed along with an untreated control and a freshly fortified sample. Samples were stored at -20ºC and were analysed in duplicate at various intervals.

Mandestrobin and De-Xy-mandestrobin were quantified by HPLC-MS/MS method RM-48G. The LOQ was 0.02 mg/kg for each analyte. Concurrent fresh recoveries in strawberries ranged from 88-112% for parent mandestrobin and De-Xy-mandestrobin at 0.02–2.5 mg/kg. Control samples had residues below 0.3LOQ.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries for parent mandestrobin are shown in Table 108. The results for metabolite De-Xy-mandestrobin are shown in Table 109.

Notes by the reviewer:

- The method used is considered valid for the purpose of this study (commodity type and concentration level of the analytes).
- The samples were already stored in the freezer for 346 days, when the day 0 sample for the storage stability analysis was taken. Since studies with spiked residues show that degradation of mandestrobin or De-Xy-mandestrobin is not to be expected for up to a period of 12 months for crop commodities with high acid content (strawberries) and various other crop commodity

types, the studies with incurred residues suggest that storage stability can be extended to 571 days for strawberries.

Table 108 Storage stability at ≤ -18 °C of mandestrobin (racemic mixture) in crop commodities with incurred residues

Matrix	Storage period (days)	parent mean residue (mg/kg)	parent Mean % remaining [a]	parent Mean concurrent recovery (%)	Reference
grapes [a]	0 [b]	2.43	100	n.r.	[ROR-0261] [ROR-0234]
	232	2.37	98	n.r.	
	538	2.33	96	n.r.	
raisins [a]	0 [b]	22 [c]	100	n.r.	
	143	26 [c]	114	n.r.	
	385	23	104	n.r.	
grapes juice	0 [b]	16 [c]	100	n.r.	[ROR-0234]
	217	21	128	n.r.	
strawberry	0 [b]	2.05	100	n.r.	[ROR-0260]
	160	2.60	127	n.r.	
	466	2.17	106	n.r.	
	571	1.95	95	n.r.	

[a] Values calculated by evaluator based on mean of two values. Percentage remaining is also calculated by evaluator according to [d]

[b] Mean of three samples.

[c] The selected day 0 represent 118 (grapes), 218 (raisins; juice) and 346 (strawberries) days after harvest

Table 109 Storage stability of De-Xy-mandestrobin in crop commodities with incurred residues stored at ≤-18 °C

Matrix	Storage period (days)	De-Xy- M mean residue (mg/kg)	De-Xy-M Mean % remaining [a]	De-Xy-M Mean concurrent recovery (%)	Reference
grapes	0 [b]	0.016	100	n.r.	[ROR-0261] [ROR-0234]
	232	0.014	88	n.r.	
	538	0.015	94	n.r.	
raisins	0[b]	0.24 [c]	100	n.r.	
	219	0.53 [c]	106	n.r.	
	545	0.33	137	n.r.	
grape juice	0	0.064 [c]	100	n.r.	[ROR-0234]
	217	0.082	128	n.r.	
strawberry	0	0.015	100	n.r.	[ROR-0260]
	160	0.014	93	n.r.	
	466	0.014	93	n.r.	
	571	0.016	107	n.r.	

M= mandestrobin; n.r. = not reported

[a] The % remaining is indicated as the percentage of the initial, which is calculated by dividing the mean recovery of stored samples at each interval by the mean recovery at day 0. The values are not corrected with the concurrent recoveries.

[b] The selected day 0 represent 118 (grapes), 218 (raisins; grape juice) and 346 (strawberries) days after harvest

[c] Mean of three samples.

Storage stability in of spiked residues in animal commodities

The Meeting received storage stability studies for mandestrobin in animal tissues and milk.

In a bovine residue transfer study (feeding study) [Dale and Chambers, 2018, ROR-0290] the storage stability of mandestrobin in animal tissues and milk was determined by spiking untreated

samples with 0.21 mg/kg mandestrobin just after sampling for the duration of the study. Three recoveries per matrix were spiked at the 10 times the LOQ and stored at -18 °C for 62–93 days. Each triplicate set of recoveries was analysed concurrently with a freshly spiked recovery and an untreated control sample for each matrix.

Samples were analysed for mandestrobin using HPLC-MS/MS method 130509 with an LOQ of 0.02 mg/kg. Average concurrent fresh recoveries in liver, kidney, muscle, fat and milk ranged between 91–109% for mandestrobin at 0.02–0.2 mg/kg. Control samples had residues below 0.3LOQ.

The results are summarized in Table . The results provided are the results of three replicate samples.

Notes by the reviewer:

- The analytical method used is considered valid for the purpose of this study (commodity type and concentration level of the analytes).
- The results from the storage stability investigations demonstrated that mandestrobin was stable in animal matrices for at least 62 days in milk, 78 days in fat and 93 days in liver, kidney, and muscle.

Table 110 Storage stability of 0.21 mg/kg mandestrobin in liver, kidney, muscle, fat and milk stored at -18 °C

Matrix	storage time (days)	mandestrobin (mg/kg)	mandestrobin % remaining	concurrent recovery as mg/kg and (% recovery)
liver	93	0.2083, 0.2135, 0.2111	99, 101, 100 (mean 100)	0.2296 (109)
kidney	93	0.2280, 0.2096, 0.2209	108, 100, 105 (mean 104)	0.2084 (99)
muscle	93	0.1799, 0.1314, 0.1319	85, 62, 63 (mean 70)	0.1918 (91)
fat	78	0.2297, 0.2154, 0.2056	109, 102, 98 (mean 103)	0.2084 (99)
milk	62	0.2050, 0.2341, 0.2058	97, 111, 98 (mean 102)	0.02043 (97) [a]

[a] Spike level was 0.021 instead of 0.21 mg/kg.

Storage stability of spiked residues in soils

The Meeting received information on the storage stability of the R- and S-isomers of mandestrobin, mandestrobin, De-Xy-mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin and 2-$CONH_2$-mandestrobin in different soils.

Study 1

Storage stability of the R- and S-isomer of mandestrobin, De-Xy-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin was investigated in a German soil from a field dissipation study [Leslie, 2011a, ROR-0006]. Soil samples were fortified with a mixture of the R- and S-isomer of mandestrobin and its metabolites at 0.1 mg/kg for each analyte and stored at -10 °C or lower. Duplicate or triplicate samples were removed from the freezer at various time points up to 12 months after fortification. One untreated control sample and one freshly fortified samples were analysed concurrently with each storage stability sample. Samples were analysed with LC-MS/MS method CLE 8213772–01V with reported LOQs of 0.0025 mg/kg for the R- and S-isomer of mandestrobin and 0.005 mg/kg for the other analytes.

The percentage remaining residues after storage (uncorrected for concurrent recoveries) and concurrent recoveries are shown in Tables 111 and 112. The results demonstrate that mandestrobin and its metabolites De-Xy-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin are stable in soil when stored at -10 °C or lower for a period of at least 12 months.

Note by the reviewer:

The analytical method is considered valid at the level of 0.1 mg/kg for each analyte, except for 5-COOH-mandestrobin (see analytical method section). Control samples at each timepoint had residues below 0.3LOQ, except for the samples at the 3 and 6 month time points (0.0020 mg/kg for the R-isomer of mandestrobin; 0.0056 mg/kg for the S-isomer of mandestrobin, 0.0036–0.0078 mg/kg for 2-COOH-mandestrobin, 0.0049 mg/kg for 5-COOH-mandestrobin). As the fortification levels were at 0.1 mg/kg, this did not affect the storage stability result. The very high recovery of 5-COOH-mandestrobin was only found in one batch and the other batches allowed conclusions on the storage stability for this compound.

Table 111 Storage stability at -10 °C of the R- and S-isomer of mandestrobin, De-Xy-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin in soil spiked with 0.1 mg/kg of each analyte

Storage period	R-isomer of mandestrobin		S-isomer of mandestrobin		De-Xy -mandestrobin		2-COOH -mandestrobin		5-COOH -mandestrobin	
(months)	% rem	concurrent rec (%)	% rem	concurrent rec (%)	% rem	concurrent rec (%)	% rem	concurrent rec (%)	% rem	concurrent rec (%)
0	80	88	84	88	73	78	98	93	88	86
1	85	86	91	97	83	82	94	91	89	97
3	81	104	100	104	83	80	100	107	94	104
6	97	110	95	103	80	89	100	110	271 [a]	309 [a]
12	83	93	92	97	76	77	88	94	90	96

% rem = percentage remaining (i.e. storage stability);

concurrent rec= concurrent recovery of freshly fortified sample

[a] the high percentage remaining was caused by technical problems of the analytical method. As high recoveries were also observed during validation of the method, the method was considered not suitable for 5-COOH-mandestrobin.

Studies 2–5:

Storage stability of mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin was investigated in Canadian and US soils [Bitter, 2013 d-h, ROR-0245, ROR-0246, ROR-0247, ROR-0248, ROR-0249; Bitter, 2015 a-d, ROR-0263, ROR-0264, ROR-0265, ROR-0267, Green, 2013, MRID 49068568]. Soil samples were fortified with a mixture of mandestrobin and its metabolites at 0.1 mg/kg for each analyte and stored at -20 °C or lower. Duplicate samples were removed from the freezer at various time points up to 741–874 days after fortification. An untreated control sample and a freshly fortified sample were analysed concurrently with each storage stability sample. Samples were analysed with LC-MS/MS method RM-48S-3 with an LOQ of 0.02 mg/kg for each analyte.

Control samples at each timepoint had residues below 0.3LOQ. The % remaining residues after storage (uncorrected for concurrent recoveries) and concurrent recoveries are shown in Table .

Note by the reviewer:

- The method is considered fit for the purpose of this study.
- The results demonstrate that mandestrobin and its metabolites DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin are stable in soil when stored at -20 °C or lower for a period of at least 874 days.

Table 112 Storage stability at -20 °C of mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin in soil spiked with 0.1 mg/kg of each analyte

Storage period	mandestrobin		DX-CA-mandestrobin		2-$CONH_2$-mandestrobin		2-COOH-mandestrobin		5-$CONH_2$-mandestrobin		5-COOH-mandestrobin	
(days)	% remaining	concurrent recovery (%)	% remaining	concurrent recovery. (%)	% remaining	concurrent recovery (%)	% remaining	concurrent recovery (%)	% remaining	concurrent recovery (%)	% remaining	concurrent recovery (%)
ROR-0245; ROR-0263; Saskatoon, Saskatchewan, CAN												
0	80	83	92	93	87	81	92	83	93	88	84	85
352	86	85	114	109	77	77	75	78	75	79	73	71
741	78	93	94	96	85	97	91	93	73	94	86	96
ROR-0246; ROR-0264, Branchton, Ontario, CAN												
0	79	82	85	88	81	88	77	87	82	98	80	79
175	77	87	86	97	79	85	76	87	77	86	71	84
335	68	80	81	91	71	77	73	79	76	78	73	80
874	64	90	116	138	62	89	79	89	67	88	70	91
ROR-0247, ROR-0265, Tift County, Georgia, USA												
0	89	78	86	83	91	77	87	81	85	76	84	74
178	70	91	76	96	66	82	66	87	65	83	70	90
339	85	93	78	97	96	100	96	102	102	105	96	102
744	84	88	97	100	89	96	103	96	86	99	94	97
ROR-0248, ROR-0267, Northwood, North Dakota, USA												
0	77	71	86	88	78	74	77	73	81	76	76	76
319	76	83	85	92	81	84	82	86	81	80	86	86
741	76	83	86	74	72	75	82	77	76	79	81	82
MRID 49068568, Madera, California, USA (n = 2 for stored samples)												
0	92; 104	100	96, 101	101	94; 101	99	101; 103	99	97; 102	100	98; 99	103
175	68; 70	89	87; 87	104	71; 71	88	72; 73	90	72; 73	92	68; 72	91
336	67; 68	84	86; 88	86	73; 75	79	77; 78	80	78; 79	79	74; 76	82

Study 6

A storage stability study was performed as part of radiolabelled aerobic soil degradation study 6 [Maurer & Gohre, 2013, ROM-0051]. The soil (50 g) was treated with the [benzyl-^{14}C]-R-isomer of mandestrobin (RB), the [benzyl-^{14}C]-S-isomer of mandestrobin (SB) or the [phenoxy-^{14}C]-R-isomer of mandestrobin (RP), each at an application rate of 9.0 mg ai/kg dry weight. Further details are described in aerobic soil degradation study 6. On 366 DAT (= day 0 of storage) the remaining treated soil samples were removed from the incubation chambers and placed in the freezer at -5 °C or lower. Aliquots of soil were taken out after 406, 545 and 711–712 days of storage.

Soil samples were extracted twice with methanol:water (9:1, v/v) and twice with methanol:0.5M HCl (5:1, v/v) and extracts and post-extracted solids were analysed by (combustion) LSC. The Atwater, Sharkey and KD Manning soils had acceptable mass balance recoveries of 95-113%.

The neutral extract was evaporated to dryness and redissolved in methanol. The acidic extract was rotary evaporated to remove the methanol and the remaining aqueous phase was partitioned with ethyl acetate. The ethyl acetate phase was evaporated to dryness and redissolved in methanol. The final methanol extracts of the neutral and acidic extracts were analysed separately by HPLC with LSC and UV detection against external standards. The results were summed and are shown in Table 113.

Note by the reviewer:

The data indicate that mandestrobin and its soil degradation products 2-COOH-mandestrobin and 5-COOH-mandestrobin are stable over the two year freezer storage period. DX-CA-mandestrobin was stable in the KD Manning soil, but declined by 33%-38% after 406 days of freezer storage in the Atwater soil and Sharkey soil and remained stable thereafter. No conclusions on other minor degradation products were possible.

Table 113 Storage stability at -5 °C for mandestrobin, De-Xy-mandestrobin, DX-CA-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 2-$CONH_2$-mandestrobin, and 5-$CONH_2$-mandestrobin in soils with incurred residues [a]

Storage time (days)	0	406	545	711	406	545	711
Atwater soil [a]	mean (n = 2); %AR	mean (n = 2); %AR	mean (n = 2); %AR	mean (n = 2); %AR	% remaining	% remaining	% remaining
Mandestrobin	46.1	50.3	49.1	51.8	109%	107%	112%
5-COOH-M	6.8	6.6	6.8	7.1	97%	100%	104%
2-COOH-M	5.6	6.5	7.0	7.0	116%	125%	125%
DX-CA-M	5.2	3.5	3.1	3.7	67%	60%	71%
De-Xy-M	0.0	0.0	0.0	0.0	-	-	-
2-CONH2-M	0.4	0.4	0.0	0.0	-	-	-
5-CONH2-M	0.6	0.0	0.0	0.0	-	-	-
MCBX	0.4	0.5	0.0	0.0	-	-	-%
Sharkey soil [b]							
Mandestrobin	51.4	40.9	47.2	50.4	80%	92%	98%
5-COOH-M	6.1	4.4	5.7	5.8	72%	93%	95%
2-COOH-M	6.9	8.7	7.3	7.8	126%	106%	113%
DX-CA-M	5.2	3.2	2.9	3.5	62%	56%	67%
De-Xy-M	0.0	1.4	0.0	0.0	-	-	-
2-CONH2-M	1.5	2.5	2.9	2.5	-	-	-
5-CONH2-M	2.9	1.0	3.5	4.8	-	-	-
MCBX	0.6	0.6	0.0	0.8	-	-	-
KD Manning soil							
Mandestrobin	51.4	45.3	48.9	52.8	88%	95%	103%
5-COOH-M	10.1	9.8	11	11.1	97%	109%	110%
2-COOH-M	6.6	7.1	8.3	8.1	108%	126%	123%
DX-CA-M	7.6	7.6	8.5	8.7	100%	112%	114%
De-Xy-M	0.0	0.4	0.0	0.0	-	-	-
2-CONH2-M	0.6	1.0	0.0	0.5	-	-	-
5-CONH2-M	0.7	0.0	0.0	0.0	-	-	-
MCBX	0.8	0.9	0	0.6	-	-	-

[a] sum of residues in the neutral extracts and the ethyl acetate fraction of the acidic extracts

[b] day zero of storage is 364 days after treatment of the soil (aerobic soil study 6, ROM-0051)

USE PATTERN

Mandestrobin is used as a fungicide on grapes, strawberries, rape seed and turf grass (Annual Bluegrass, Bentgrass, Fescue, Kentucky Bluegrass, Perennial Ryegrass) grown under field conditions by foliar spray application for the control of Botrytis cinerea and Sclerotinia sclerotiorum. Residue trials were not submitted for turf grass. The use pattern is summarized in Table 114.

At the time of preparation of the dossier, JMPR received information on registrations of mandestrobin in Canada and the USA on grapes, strawberries, rape seed and turfgrass. A rotational interval of 4 months is stated on the Canadian and the US label. No grazing restrictions for rape seed apply.

The compound was also registered in:

- Australia: stone fruits;
- Canada and the USA: small fruit vining group (excluding fuzzy kiwifruit), low growing berries (excluding cranberry) as well as seed treatments on beans, peas, soya beans (excluding cowpeas and field peas), maize (sweetcorn, field corn, popcorn) and rape seed;
- Japan: apple, pear, persimmon, cherry, peach, nectarine, apricot, Japanese apricot, plum, grape, cucumber, melon, watermelon, tomato (including cherry tomato), eggplant, lettuce (head and leaf), Brassica leafy vegetables, legume vegetables, pulses and tea;
- , watermelon, sweet pepper, chili pepper, lettuce (head and leaf) and ginseng;
- New Zealand: onions and beans;
- South Korea: apple, pear, persimmon, peach, Japanese apricot, grape, garlic, onion

In the EU, the representative use for EU approval was on rape seed and product authorisation evaluations are ongoing at Member State level for use on stone fruits.

Table 114 Registered pre-harvest uses of mandestrobin

Crop	Field(F)/ Green house [g]	Country	Form	Application				PHI, days
				Method	Rate kg ai/ha	Spray conc, kg ai/hL	Number (Interval in days)	
Grape	F	Canada	SC [h]	Foliar spray by air blast sprayer (ground)	0.21–0.42 (total 1.26)	0.21 – 0.42 [a]	3-4 (10–14)	10
	F	USA	SC [h]	Foliar spray (ground) [e f]	0.21 (total 0.63)	0.23 [g]	3 (≥20)	10
Strawberries	F	Canada	SC [h]	Foliar spray by field sprayer (ground) [c]	0.21–0.42 (total 1.68)	0.21–0.42 [a]	4-5 (7-14)	0
	F	USA	SC [h]	Foliar spray (ground) [c f]	0.21 (total 0.42)	0.23 [g]	2 (≥14)	0
Rape seed (canola)	F	Canada	SC [h]	Foliar spray by field sprayer (ground)	0.21–0.42 (total 0.42)	0.21–0.42 [a]	1 (365)	35 [d]
	F	Canada	SC [h]	Foliar spray by air blast sprayer (aerial)	0.21–0.42 (total 0.42)	0.42–0.84 [b]	1 (365)	35 [d]

[a] Based on a minimum application volume of 100 L/ha for ground application

[b] Based on a minimum application volume of 50 L/ha for aerial application

[c] Strawberries: begin applications at 10% bloom, or prior to infection. Do not use in greenhouses

[d] Make application between 20% and 50% bloom (BBCH 62–65). No grazing or feeding restrictions apply for rape seed (canola).

[e] Begin applications prior to infection during early bloom, bunch pre-closure and veraison (i.e. change of colour of the grapes) up to 10 days before harvest.

[f] Do not apply by air.

[g] Based on minimum application volume of 10 L/ha for ground application.

[h] Any required adjuvant can be added.

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on supervised residue trials for grapes, strawberries, soya beans and rape seed using foliar sprays. Studies on soya beans were submitted to support the definition of the residue.

Crop subgroup	Commodity	Treatment	Table no.
Small fruit, vine climbing	Grapes	Foliar treatment	115
Low growing berries	Strawberries	Foliar treatment	116
Dry beans	Soya bean (dry)	Foliar treatment	117
Small seed oilseeds	Rape seed	Foliar treatment	118
Animal feed	Soya bean forage	Foliar treatment	119
Animal feed	Soya bean fodder	Foliar treatment	120
Animal feed	Rape seed fodder	Foliar treatment	121

Application rates, spray concentrations and total residues have been rounded to two figures. Residue data are recorded unadjusted for percentage recoveries or for residue values in control samples unless otherwise stated. Unquantifiable residues are shown as below the reported LOQ of 0.01 or 0.02 mg/kg.

Where multiple analyses were conducted on a single sample, the average value is reported. Where multiple samples were taken from a single plot, the individual and average values are reported. Results are therefore sometimes presented as single values or as duplicate/triplicate values with the (mean) value between brackets. Where results from separate plots with distinguishing characteristics such as different formulations, crop varieties or treatment schedules were reported, results are listed separately for each plot.

Residues from the trials conducted according to the critical GAP, which have been used for the estimation of maximum residue levels, STMR and HR values are underlined.

The residues presented in the tables Table -Table are given as mandestrobin and its metabolites De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates, expressed as the free aglycones. The total column reflects the sum of parent and its metabolites De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates, expressed as parent. The total residues are expressed as parent equivalents by applying a molecular weight conversion factor of 1.491× to De-Xy-mandestrobin, 0.951× to 4-OH-mandestrobin and 0.951× to 2-CH_2OH-mandestrobin and 0.951× to 5-CH_2OH-mandestrobin.

Berries and other small fruit-Subgroup of small fruit, vine climbing

Grapes

Four (Canada) and Twelve (USA) residue field trials were conducted on grapes in 2011 and 2012 [Bitter *et al*., 2013, ROR-0234; Bitter, 2013, ROR-0235]. In each trial, SC formulated mandestrobin was applied three times at a rate of 0.42–0.43, 0.82–0.83 or 2.1 kg ai/ha with an interval of 10 days. Grapes received a foliar spray by air blast sprayer, except trial V-38175-C by backpack sprayer. Three trials (USA) were performed as decline trials. Adjuvants were added to each spray mix in accord with the label. All USA trials used non-ionic surfactant (NIS) at 0.12–0.13 % by volume. The Canadian trials used NIS as adjuvant at 0.063–0.065% by volume (trials A, C, D) and Agral 90 adjuvant at 0.063% by volume (trials A and C). Samples were taken ten days after the last application (with the exception of trial V-38175-J at PHI = 7 days). Grapes from decline trials were harvested at PHIs of 3, 7, 10 and 14 days. Grapes were harvested at commercial maturity. Sample sizes were in accordance with the FAO manual 2016 appendix V (at least 12 bunches/sample from separate vines with a minimum weight of 1 kg/sample).

Samples were stored at -20 °C. The maximum storage interval was 267 days for mandestrobin and De-Xy-mandestrobin in grapes in the US trials and 485 days in the Canadian trials. The maximum storage interval was 431 days for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in grapes in the US trials and 778 days in the Canadian trials.

Samples were analysed for mandestrobin and De-Xy-mandestrobin using HPLC-MS/MS method RM-48G. Samples were analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates using HPLC-MS/MS method RM-48A.The LOQ was 0.02 mg/kg for each analyte. Average concurrent fresh recoveries were 80–114% for mandestrobin and De-Xy-mandestrobin at 0.02–15 mg/kg, 91–102% for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin at 0.02–0.20 mg/kg. Control samples had residues below 0.3LOQ, except for one sample. The control sample for trial E (Kerman, CA) contained 0.0080 mg/kg mandestrobin [Bitter *et al*., 2013, ROR-0234], which did not affect the outcome of any of mandestrobin levels found.

Summaries of the trial results are given in Table 115. Residues range between 0.47-12 mg/kg for mandestrobin, < 0.02–0.05 mg/kg for De-Xy mandestrobin, < 0.02–0.02 mg/kg for 2-CH_2OH-mandestrobin and it (malonyl)glucoside conjugates, < 0.02–0.06 mg/kg for 4-OH-mandestrobin and it (malonyl)glucoside conjugates, and < 0.02–0.04 mg/kg for 5-CH_2OH-mandestrobin and its (malonyl)glucoside conjugates.

Notes by the reviewer:

- The maximum storage interval of 485 days for mandestrobin and De-Xy-mandestrobin in grapes is covered by the storage stability studies on commodities with high acid content (orange, strawberries, grapes) in combination with the storage stability studies with incurred residues in grapes.
- The maximum storage interval of 778 days for 4-OH-mandestrobin and 2-CH_2OH-mandestrobin is covered by the storage stability studies on crop commodities with high acid content (orange).
- The maximum storage interval of 431 and 778 days for 5-CH_2OH-mandestrobin in the US and Canadian trials is **NOT** covered as no storage stability studies have been conducted on crop commodities with high acid content for 5-CH_2OH-mandestrobin.
- The analytical methods are valid in the range 0.02–0.2 mg/kg. Limited recovery experiments (n = 1–2) suggest extension of the validity to 15 mg/kg for mandestrobin in grapes, which covers the levels found in these trials.
- Method RM-48A is valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin.

Table 115 Residues of SC-formulated mandestrobin in grapes (fruit bunches) after foliar spray

GRAPES Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation PHI (days)	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M* mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
cGAP Canada: 3 × 0.42 kg ai/ha, interval 10 days, PHI 10 days								
Dundee, NY, USA 2012 (DeChaunac	3 × 0.42–0.43 (10)	BBCH 85 9 Sept 10	2.4 2.4 (2.4)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial A
Orefield, PA, USA 2012 (Concord)	3 × 0.42–0.43 (10)	BBCH 85 27 Aug 0	1.4 1.7 1.5)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial B
	3 × 0.42–0.43 (10)	BBCH 85 27 Aug 3	1.1 1.0 (1.1)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
	3 ×	BBCH 85	0.78	< 0.02	< 0.02	< 0.02	< 0.02	

GRAPES Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation PHI (days)	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M* mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
cGAP Canada: 3 × 0.42 kg ai/ha, interval 10 days, PHI 10 days								
	0.42–0.43 (10)	27 Aug 7	0.61 (0.70)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	
	3 × 0.42–0.43 (10)	BBCH 85 27 Aug 10	0.79 0.68 (0.74)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
	3 × 0.42–0.43 (10)	BBCH 85 27 Aug 14	0.56 0.62 (0.59)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
Ukiah, CA, USA 2012 (Zinfandel)	3 × 0.42 (10)	BBCH 85 10 Sept 10	3.7 3.2 (3.5)	0.04 0.04 (0.04)	< 0.02 < 0.02 (< 0.02)	0.04 0.04 (0.04)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial C
	3 × 0.82–0.83 (10)	BBCH 85 10 Sept 10	5.0 3.1 (4.0)	0.04 0.04 (0.04)	< 0.02 < 0.02 (< 0.02)	0.03 0.03 (0.03)	< 0.02 0.02 (< 0.02)	
Artois, CA, USA 2012 (Rubired)	3 × 0.42 (10)	BBCH 89 28 Aug 0	3.0 [b] 4.6 [b] (3.8)	0.04 [b] 0.05 [b] (0.05)	< 0.02 0.02 (0.02)	0.04 0.04 (0.04)	0.02 0.02 (< 0.02)	[ROR-0234] V38175-Trial D
	3 × 0.42 (10)	BBCH 89 28 Aug 3	0.31 0.70 (0.51)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 0.03 (0.02)	< 0.02 < 0.02 (< 0.02)	
	3 × 0.42 (10)	BBCH 89 28 Aug 7	0.59 0.43 (0.51)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	0.04 0.04 (0.04)	< 0.02 < 0.02 (< 0.02)	
	3 × 0.42 (10)	BBCH 89 28 Aug 10	2.2 1.5 (1.9)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.03 0.05 (0.04)	0.02 0.02 (0.02)	
	3 × 0.42 (10)	BBCH 89 28 Aug 14	1.2 1.4 (1.3)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.05 0.04 (0.04)	< 0.02 < 0.02 (< 0.02)	
Kerman, CA, USA 2012 (Thompson Seedless)	3 × 0.43 (10)	BBCH 86 09 Aug 0	1.7 1.3 (1.5)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial E [a]
	3 × 0.43 (10)	BBCH 86 09 Aug 3	1.2 [b] 1.6 (1.4)	< 0.02 [b] 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	0.03 0.03 (0.03)	0.02 < 0.02 (< 0.02)	
	3 × 0.43 (10)	BBCH 86 09 Aug 7	0.04 [b] 1.7 [b] (0.86)	< 0.02 [b] 0.03 [b] (0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 0.03 (0.02)	< 0.02 0.02 (< 0.02)	
	3 × 0.43 (10)	BBCH 86 09 Aug 10	1.3 1.4 (1.4)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.04 0.04 (0.04)	0.02 0.02 (0.02)	
	3 × 0.43 (10)	BBCH 86 09 Aug 14	1.4 0.84 (1.1)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.05 0.04 (0.04)	0.03 0.02 (0.025)	
Lindsay, CA, USA 2012 (Globe)	3 × 0.42–0.43 (10)	BBCH 85 27 Aug 10	1.1 1.5 (1.3)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.03 0.03 (0.03)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial F
	3 × 0.83–0.85 (10)	BBCH 85 27 Aug 10	1.8 1.6 (1.7)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.03 0.03 (0.03)	< 0.02 < 0.02 (< 0.02)	
Selma, CA, USA 2012	3 × 0.42–0.43 (10)	BBCH 89 28 Sept 10	0.89 0.69 (0.79)	0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial G

GRAPES Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation PHI (days)	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M* mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
cGAP Canada: 3 × 0.42 kg ai/ha, interval 10 days, PHI 10 days								
(Crimson)								
Madera, CA, USA 2012 (Thompson Seedless)	3 × 0.41–0.42 (10)	BBCH 85-89 31 Jul 10	1.1 1.4 (1.2)	< 0.02 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	0.02 0.03 (0.02)	< 0.02 0.02 (< 0.02)	[ROR-0234] V38175-Trial H
	3 × 0.41–0.42 (10)	BBCH 85-89 24 Aug 10	1.7 2.2 (2.0)	0.02 < 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.02 0.02 (0.02)	0.02 0.02 (0.02)	[ROR-0234] V38175-Trial L&M [c]
	3 × 2.1 (10)	BBCH 85-89 24 Aug 10	13 [b] 11 12 [b] (12)	0.05 [b] 0.04 0.04 [b] (0.04)	0.02 0.02 0.02 (0.02)	0.07 0.07 0.05 (0.06)	0.04 0.05 0.03 (0.04)	
Templeton, CA, USA 2012 (Syrah Noir)	3 × 0.42–0.44 (10)	BBCH 85 16 Sept 10	1.7 1.2 (1.4)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial I
Granger, WA, USA, 2012 (White Riesling)	3 × 0.41–0.42 (10)	BBCH 85 10 Sept 7	1.4 1.4 (1.4)	0.02 < 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial J
	3 × 0.83–0.84 (10)	BBCH 85 10 Sept 7	2.0 2.6 (2.3)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	0.02 0.02 (0.02)	< 0.02 < 0.02 (< 0.02)	
Underwood, WA, USA, 2012 (Chardonnay)	3 × 0.41–0.42 (10)	BBCH 85 30 Sept 10	1.1 0.94 (1.0)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0234] V38175-Trial K
Cambridge, ON, Canada 2011 (Concord)	3 × 0.43–0.44 (10)	BBCH 85 19 Sept 10	0.50 0.44 (0.47)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0235] V37929-Trial A
Cambridge, ON, Canada 2011 (Marechal Foch)	3 × 0.42–0.43	BBCH 79-85 29 Aug 9	1.1 0.94 (1.0)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0235] V37929-Trial B
Branchton, ON, Canada 2012 (Concord)	0.28 + 0.14, 0.45, 0.45 (10)	BBCH 85-89 17 Sept 10	0.81 0.57 (0.69)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0235] V37929-Trial C [d]
Penticton Falls, BC, Canada 2012 (Merlot)	3 × 0.43–0.45 (10)	BBCH 89 25 Oct 11	1.0 1.1 (1.1)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	[ROR-0235] V37929-Trial A [d]

GS growth stage

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its conjugates, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

N Number of treatments, I: Interval between treatments

* Residue levels expressed as mg/kg analyte

[a] Control sample for trial E, Kerman, CA, contained 0.0080 mg/kg mandestrobin;[ROR-0234] leading to an LOQ of 0.0080×10/3=0.03 mg/kg mandestrobin for this trial. As mandestrobin levels in all samples were above 0.03 mg/kg, this does not have an impact on the reported residues.

[b] Average of 2–4 analyses

[c] One sample (11.6 mg/kg parent) was used for processing and recoded as trial M

[d] Storage period was not covered by the storage stability studies.

Additional trial information:

ROR-0234 GLP. Crop growth was not affected by weather conditions. Trial A received 0.01 inch of rain 6 hrs before the third application. Trial B received rain just after the last application, when the application had already dried off. The plot size was 420–1350 ft2. The spray volume was between 919-2274 L/ha. The field sampling strategy was not described.

ROR-0235 GLP. Crop growth was not affected by weather conditions. Trial A received 0.3 mm rain 3 hrs after the third application and 19 mm of rain during the rest of the day. Trial B received 0.8 mm rain about 7 hrs after the third application. The plot size was 36-84 ft2. Spray volume ranged from 661 to 1044 L/ha. Grapes were collected from random plants avoiding edges of the plots.

Berries and other small fruit-Subgroup of low growing berries

Strawberries

Two (Canada) and eight (USA) residue field trials were conducted on strawberries in 2012 [Bitter *et al.*, 2013, ROR-0236; Bitter, 2013a, ROR-0237]. In each trial, SC formulated mandestrobin was applied four times as a foliar spray at a rate of 0.41–0.44 or 0.84–0.87 kg ai/ha with an interval of 6-9 days. Strawberries received a foliar spray by backpack sprayers, hand booms or small plot equipment. Two trials (USA) were performed as decline trials. Adjuvants were added to each spray mix in accord with the label. All USA trials used non-ionic surfactant (NIS) at 0.125% by volume, whereas the Canadian trials used NIS at 0.063% by volume. Samples were taken directly after the last application (PHI = 0 days). Strawberries from decline trials were taken on the day of the last application and at pre harvest intervals (PHI) of 1, 3, 5 and 7 days. Strawberries were harvested at commercial maturity and caps were removed. Sample sizes were in accordance with the FAO manual 2016 appendix V (at least 1 kg/sample from 12 separate areas of the plot).

Samples were stored at -20 °C. The maximum storage interval was 397 days for mandestrobin and De-Xy-mandestrobin in strawberries in the US trials and 569 days in the Canadian trials. The maximum storage interval was 455 days for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in strawberries in the US trials D and E.

Samples were analysed for mandestrobin and De-Xy-mandestrobin using HPLC-MS/MS method RM-48G. Only the samples from trials D and E [ROR-0236] were analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates using HPLC-MS/MS method RM-48A.The LOQ was 0.02 mg/kg for each analyte.

Average concurrent fresh recoveries were 88-109% for mandestrobin at 0.02–5 mg/kg, 97-105% for De-Xy-mandestrobin at 0.02–15 mg/kg and 82–106% for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin at 0.02–0.20 mg/kg. Control samples had residues below 0.3LOQ, except for one sample. The control sample for trial F (Porterville, CA) contained 0.0083 mg/kg mandestrobin [ROR-0236], which did not affect the outcome of any of mandestrobin levels found.

Summaries of the trial results are given in Table 116. Residues range between 0.38-2.0 mg/kg for mandestrobin, < 0.02–0.02 mg/kg for De-Xy mandestrobin, < 0.02 mg/kg for 2-CH_2OH-mandestrobin and it (malonyl)glucoside conjugates, < 0.02–0.02 mg/kg for 4-OH-mandestrobin and it (malonyl)glucoside conjugates, and < 0.02–0.02 mg/kg for 5-CH_2OH-mandestrobin and its (malonyl)glucoside conjugates.

Notes by the reviewer:

- The maximum storage interval of 569 days for mandestrobin and De-Xy-mandestrobin in grapes is covered by the storage stability studies on commodities with high acid content

(orange, strawberries, grapes) in combination with the storage stability studies with incurred residues in strawberries.

- The maximum storage interval of 455 days for 4-OH-mandestrobin and 2-CH_2OH-mandestrobin is covered by the storage stability studies on crop commodities with high acid content (orange).
- The maximum storage interval of 455 days for 5-CH_2OH-mandestrobin is **NOT** covered as no storage stability studies have been conducted on crop commodities with high acid content for 5-CH_2OH-mandestrobin.
- The analytical methods are valid in the range 0.02–0.2 mg/kg. Limited recovery experiments (n = 1–2) suggest extension of the validity to 5 mg/kg for mandestrobin in strawberries, which covers the levels found in these trials.
- Method RM-48A is valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin.
- Only a limited number of samples were analysed for the hydroxy compounds. Metabolism studies on lettuce at PHI = 5 days and supervised trials on grapes at PHI = 10 days indicate that levels of (conjugated) 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin are very low compared to parent compound. At the shorter PHI of 0 days, these metabolites are not expected to contribute significantly to the total residue. This hypothesis is confirmed by the few samples of strawberries that were analysed for the hydroxy compounds.

Table 116 Residues of SC formulated mandestrobin in strawberry (fruits) after foliar spray

STRAW BERRIES Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last applic PHI (days)	M mg/kg	De-Xy-M (*) mg/kg	2-CH_2OH-M (*) mg/kg	4-OH-M (*) mg/kg	5-CH_2OH-M (*) mg/kg	[Report] Trial no
cGAP Canada: 4x 0.42 kg ai/ha, interval 7 days, PHI 0 days								
Penn Yan, NY, USA 2012 (Honeoye)	4 × 0.42–0.43 (7)	BBCH 87 14 June 0	0.47 [c] 0.49 [c] (0.48)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0236, Trial V-38182-A
Oviedo, FL, USA 2012 (Camarosa)	4 × 0.42 (6-7)	BBCH 87 16 Apr 0	2.1 2.0 (2.0)	0.02 0.02 (0.02)	NA	NA	NA	ROR-0236, Trial V-38182-B
	4 × 0.42 (6-7)	BBCH 87 16 Apr 1	1.9 2.1 (2.0)	0.02 0.02 (0.02)	NA	NA	NA	
	4 × 0.42 (6-7)	BBCH 87 16 Apr 3	1.8 2.2 (2.0)	0.02 0.02 (0.02)	NA	NA	NA	
	4 × 0.42 (6-7)	BBCH 87 16 Apr 5	0.53 0.52 (0.52)	0.02 0.02 (0.02)	NA	NA	NA	
	4 × 0.42 (6-7)	BBCH 87 16 Apr 7	0.32 0.45 (0.38)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Comstock Park, MI, USA 2012 (All Star)	4 × 0.42 (7)	BBCH 85-87 05 June 0	0.68 0.72 (0.70)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0236, Trial V-38182-C
Salinas, CA, USA 2012 (San Juan)	4 × 0.42–0.43 (6-7)	BBCH 87 16 Oct 0	0.57 0.33 (0.45)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 < 0.02)	< 0.02 < 0.02 (< 0.02)	ROR-0236, Trial V-38182-D
	4 × 0.85–0.87 (6-7)	BBCH 87 16 Oct 0	0.65 1.4 [c] (1.0)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 0.02 (0.02)	< 0.02 0.02 (0.02)	
Santa Maria	4 × 0.42	BBCH 85	1.5	< 0.02	< 0.02	< 0.02	< 0.02	ROR-0236,

STRAW BERRIES Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last applic PHI (days)	M mg/kg	De-Xy-M (*) mg/kg	2-CH_2OH-M (*) mg/kg	4-OH-M (*) mg/kg	5-CH_2OH-M (*) mg/kg	[Report] Trial no
cGAP Canada: 4x 0.42 kg ai/ha, interval 7 days, PHI 0 days								
CA, USA 2012 (Albion)	(6-7)	30 May 0	0.86 (1.2)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	Trial V-38182-E
	4 × 0.84–0.85 (6-7)	BBCH 85 30 May 0	2.2 1.6 (1.9)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
Porterville CA, USA 2012 (Albion)	4 × 0.42 (6-7)	BBCH 89 14 May 0	0.91 0.60 (0.76)	0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0236, Trial V-38182-F [b]
	4 × 0.42 (6-7)	BBCH 89 14 May 1	0.93 0.70 (0.82)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	4 × 0.42 (6-7)	BBCH 89 14 May 3	0.79 0.46 (0.62)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	4 × 0.42 (6-7)	BBCH 89 14 May 5	0.45 0.40 (0.42)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	4 × 0.42 (6-7)	BBCH 89 14 May 7	0.41 0.41 (0.41)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Hillsboro, OR, USA 2012 (Tri Star)	4 × 0.42 (6-7)	BBCH 61–83 16 Aug 0	0.81 1.0 (0.92)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0236, Trial V-38182-G
Chula, GA, USA 2012 (Sweet Charlie)	4 × 0.41–0.44 (7)	BBCH 87 19 April 0	1.3 1.2 (1.2)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0236, Trial V-38182-H
Brantford, ON, Canada 2011 (Saint-Pierre)	4 × 0.42–0.44 (7)	BBCH 81–85 20 June 0	1.1 0.92 (1.0)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0237, Trial V-37869- [b]
St-Jean-Baptise, QC, Canada 2012 (Annapolis)	4 × 0.41–0.42 (6-9)	BBCH 87-89 28 June 0	0.60 0.64 (0.62)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0237, Trial V-37869-B [c]

GS: growth stage; NA = not analysed

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its conjugates, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

N: Number of treatments, I: Interval between treatments

* Residue levels expressed as mg/kg analyte.

[a] Control sample for trial F, Porterville, CA, contained 0.0083 mg/kg mandestrobin [ROR-0236], leading to an LOQ of 0.0083 ×10/3 = 0.03 mg/kg mandestrobin for this trial. As mandestrobin levels in all samples were above 0.03 mg/kg, this does not have an impact on the reported residues.

[b] Control sample for trial F, Porterville, CA, contained 0.0083 mg/kg mandestrobin [ROR-0236], leading to an LOQ of 0.0083 ×10/3 = 0.03 mg/kg mandestrobin for this trial. As mandestrobin levels in all samples were above 0.03 mg/kg, this does not have an impact on the reported residues.

[c] Storage period was not covered by the storage stability studies.

Additional trial information:

ROR-0236: GLP. Crop growth was not affected by weather conditions. Trial C received 0.5 inches of rain 10 hrs after the last application. Plot size 500–4000 ft^2. The foliar applications were done by handboom (A, H), small-plot equipment (F) or backpack sprayer (B, C, D, E, and G). Spray volume ranged from 242–434 L/ha. Strawberries were collected in an even distribution from at least from 12 separate areas of the plot.

ROR-0237: GLP. Crop growth was not affected by weather conditions. Plot size 30–45 m2 Boom sprayers (A, B). Spray volume ranged from 976 to 1053 L/ha. Strawberries were collected in an even distribution from at least from 12 separate areas of the plot.

Pulses – Subgroup of dry beans

For pulses no GAP was submitted. Studies were provided to support the definition of the residue.

Soya bean (dry)

Eight residue field trials were conducted on soya beans in 2015 in Brazil [Klimmek and Gizler, 2017, ROR-0280]. In each trial, SC formulated mandestrobin was applied with a boom sprayer. The foliar spray was applied three times at 0.26 kg ai/ha with an interval of 10 days using a spray volume of 200 L/ha for each application. No adjuvants were used. Plants were taken by hand with knife aid from at least 12 different locations within the plot. No plants were taken from the outer plants of the plots or from the area of spray overlap. After harvest and storage in polyethylene bags, plants were hit by wood stakes until the seeds came out of the pods. The seeds were collected from all the parts of the plants. Dry seeds (1.0–1.5 kg) were taken at 21–22 DALT (BBCH 84-89) and at 28 DALT (BBCH 86–89).

Samples were stored at -18 °C. The maximum storage interval for mandestrobin was 119 days, for De-Xy-mandestrobin 153 days interval and for 4-OH-mandestrobin and 2-CH_2OH-mandestrobin 297 days.

Samples were analysed for mandestrobin using the Modification A of HPLC-MS/MS multi residue method QuEChERS, for De-Xy-mandestrobin using HPLC-MS/MS method SUM-1023V, for 4-OH-mandestrobin and its (malonyl)glucosides using a modification A of HPLC-MS/MS method SUM-1021V and for 2-CH_2OH-mandestrobin and its (malonyl)glucosides using a modification A of HPLC-MS/MS method SUM-1022V. The LOQ was 0.01 mg/kg for each analyte. Average concurrent recoveries were within the range of 70–120% for mandestrobin and its metabolites at 0.01–0.10 mg/kg. Control samples had residues below 0.3LOQ.

Summaries of the trial results are given in Table 117. Residue levels in soya bean seeds ranged between < 0.01–0.01 mg/kg for the parent compound and < 0.01 mg/kg for each of the metabolites.

Note by the reviewer:

- The maximum storage interval of 119–297 days for parent and its metabolites was covered by the storage stability data on commodities with high protein (dry bean seed) and/or commodities with high oil content (rape seed).
- The analytical methods are valid in the range 0.01–0.1 mg/kg for mandestrobin, 4-OH-mandestrobin and 2-CH_2OH-mandestrobin. Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–0.10 mg/kg for De-Xy-mandestrobin, 4-OH-mandestrobin.
- Methods SUM-1021V and SUM-1022V are valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin and 2-CH_2OH-mandestrobin.
- Metabolite 5-CH_2OH-mandestrobin was not analysed but is expected to be < 0.01 mg/kg based on the results for the other two hydroxy compounds.

Table 117 Residues of SC formulated mandestrobin in soya bean seeds (dry harvested) after foliar spray

SOYA BEANS (DRY) Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation	PHI (days)	parent mg/kg	De-Xy-M (*) mg/kg	2-CH_2OH-M (*) mg/kg	4-OH-M (*) mg/kg	5-CH_2OH-M (*) mg/kg	[Report] Trial no
Mafra, SC, Brazil, 2015 (5909 RR)	3× 0.26 (10, 10)	BBCH 77 13 Feb	22 28	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	NA NA	[ROR-0280] S-14-05177-01
Ponta Grossa, PR, Brazil 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 79 16 Feb	21 28	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	NA NA	[ROR-0280] S-14-05177-02
Arapoti, PR, Brazil, 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 79 23 Feb	21 28	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	NA NA	[ROR-0280] S-14-05177-03
Engeheiro Coelho, SP, Brazil, 2015 (Valiosa RR)	3 × 0.26 (10, 11)	BBCH 78 16 Mar	21 28	0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	NA NA	[ROR-0280] S-14-05177-04
Chapadao do Sul, MS, Brazil, 2015 (Whermann 791 RR)	3 × 0.26 (10, 11)	BBCH 84 07 Feb	21	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0280] S-14-05177-05
Costa Rica, MS, Brazil 2015 (Anta 82)	3 × 0.26 (11, 10)	BBCH 78 06 Feb	21	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0280] S-14-05177-06
Chapadao do Ceu, GO, Brazil, 2015 (Anta 82)	3 × 0.26 (11, 11)	BBCH 82 07 Feb	21	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0280] S-14-05177-07
Alto Taquari, MT, Brazil, 2015 (Wehrmann 791 RR)	3 × 0.26 (11, 11)	BBCH 81 06 Feb	21	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0280] S-14-05177-08

GS = growth stage; N = Number of treatments; I = Interval between treatments; NA = not analysed

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its (malonyl)glucosides, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

(*) Residue levels expressed as mg/kg analyte

Additional trial information:

ROR-0280 GLP. Weather conditions did not affect crop growth. Most trials received between 0 to 7.0 mm rain on the day of application (trial 01, 02, 03, 06), except trial 04, 05, 07, 08 which received up to 41, 19, 19 and 14 mm rain on the day of application. Plot sizes ranged from 60–105 m^2. Soil types included sandy clay (trial 04), clay (trial 7, 8), heavy clay (trial 01, 02, 03, 05, 06).

Oilseeds-Subgroup of small seed oilseeds

Rape seed

Trials in the USA and Canada

Fourteen (Canada) and nine (USA) residue field trials were conducted on rape seed in 2010 and 2011 [Green, 2013, ROR-0238; Green, 2013a, ROR-0239]. In each trial a single foliar application of SC formulated mandestrobin was sprayed at a rate of 0.42–0.46, 0.84 or 2.0–2.1 kg ai/ha between BBCH 62–78 (20% bloom to 80% pods final size). Rape seed received as a foliar spray by plot sprayer, tractor-mounted boom, hand-held sprayer or backpack sprayer. Two trials (one in the USA and one in Canada) were decline trials. Adjuvants were added to each spray mix in accord with the label. All the trials used a non-ionic surfactant (NIS) 0.25% NIS (USA) or 0.125% NIS (Canada) or Ag-Surf (trial G, Canada). Additionally, plots V-37238-A, B, C and I were also treated with crop oil concentrate (COC) at 1.0% by volume and plots V-37284 A through F were treated with COC at 0.5% by volume. Rape seed was swath cut and allowed to dry in the field until a moisture content of 10–12%, rape seed were collected (sample weight 0.5 kg) from at least 12 separate areas of the plot.

Samples were stored at -20 °C. The maximum storage interval was 745 days (24 months) for mandestrobin in rape seed in the US trials and 1137 days (37 months) in the Canadian trials. The maximum storage interval was 1114 days (37 months) for De-Xy-mandestrobin in rape seed in the US trials and 1117 days (37 months) in the Canadian trials. The maximum storage interval was 1106-1127 days (37 months) for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in rape seed in the US trials and 719-739 days (24 months) in the Canadian trials.

Samples were analysed for mandestrobin using GC-MS method RM-48C-1 for trials A to D in ROR-0238 with an LOQ of 0.01 mg/kg and HPLC-MS/MS method RM-48C-2 for all other trials with an LOQ of 0.02 mg/kg. Samples were analysed for De-Xy-mandestrobin using HPLC-MS/MS method RM-48C-2 with an LOQ of 0.02 mg/kg. Only the samples that had received the exaggerated dose rates were analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates using HPLC-MS/MS method RM-48A with an LOQ of 0.02 mg/kg for each analyte.

Average concurrent fresh recoveries were 108-114% for mandestrobin at 0.01–0.2 mg/kg using GC-MS method RM-48C-1, 80–90% for mandestrobin at 0.02–0.2 mg/kg using HPLC-MS/MS method RM-48C-2 and 83-103% for De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin at 0.02–0.20 mg/kg. Control samples had residues below 0.3LOQ, except for one sample. The control sample for trial N (Kipp, Alberta) contained 0.0070 mg/kg mandestrobin [Green, 2013, ROR-0238].

Summaries of the trial results of the USA and Canada trials are given in Table 118. Residues range between < 0.01–0.54 mg/kg for mandestrobin and < 0.02 mg/kg for each of the metabolites.

The pre-harvest interval in the US trials was reported as the interval between application and cutting the rape seed (i.e. excluding the drying time), while the pre-harvest interval in the Canadian trials was reported as the interval between application and collecting the seeds (i.e. cutting plus drying). In Table 118, the pre-harvest interval was defined as the period between application and cutting. Where also the drying time in the field was reported, it was indicated in the table below as (DAT + drying both in days).

Notes by the reviewer:

- Sample sizes were not in accordance with the FAO manual 2016 appendix V (at least 1–2 kg/sample sampled from 12 separate areas of the plot) as only 0.5 kg was collected. Since rape seed is an extremely small seed this devotion can be accepted.

- The maximum storage interval of 37 months for mandestrobin and De-Xy-mandestrobin in rape seed is covered by the storage stability studies on crop commodities with high oil content (rape seed).
- The maximum storage interval for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in the US and Canadian trials is **NOT** covered by the storage stability studies as these have been conducted up to a period of 12 months for 4-OH-mandestrobin and 2-CH_2OH-mandestrobin and no storage stability studies have been conducted on crop commodities with high oil content for 5-CH_2OH-mandestrobin.
- The control sample for trial N (Kipp, Alberta) contained 0.0070 mg/kg mandestrobin. Since this level affects the LOQ, results from this trial cannot be used for MRL derivation.
- The analytical methods are valid in the range 0.01–0.2 mg/kg for RM-48C-1 and 0.02–0.2 mg/kg for RM-48C-2 and RM-48A. Since mandestrobin levels in the supervised rape seed trials range between < 0.01–0.54 mg/kg, method RM-48-C-1 and RM-48C-2 are not considered fit for purpose for levels between 0.2–0.6 mg/kg. However, since the trials selected for MRL derivation have levels below 0.2 mg/kg, this has no impact on the selection of the trials for MRL derivation.
- Method RM-48A is valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin.
- In most of the trials, the hydroxy compounds have not been analysed. However, since the trials containing the highest amounts of mandestrobin showed that these hydroxy compounds were below the LOQ of < 0.02 mg/kg, residue levels in the other samples can be expected to be below the LOQ of 0.02 mg/kg as well.

Trials in Northern and Southern Europe

A total of 22 supervised residue trials on spring and winter rape seed were conducted in Northern and Southern Europe in 2010, 2011 and 2015 [Delmotte, 2011, ROR-0008; Lebrun, 2012, ROR-0198, Gemrot, 2017, ROR-0282]. SC formulated mandestrobin was applied once as a foliar spray by hand carried boom sprayer or single wheel sprayer using spray volumes of 140–310 L/ha. The single application was at 0.20–0.22 kg ai/ha at growth stage BBCH 65 (flowering) in the 2010 and 2011 trials or at 0.14–0.16 kg ai/ha at growth stage BBCH 75–79 (pod formation) in the 2015 trials. No adjuvants were added. Rape seed plants were collected by hand from at least 12 separate areas of the plots. Only the central part of the plot was sampled, leaving 0.5 meter on both ends of the plot and the borders. After cutting, the rape seed was not left to dry in the field. The seeds (0.50–1.9 kg in 2010/2011 and > 0.5 kg in 2015) were collected by hand or using a small threshing machine. rape seed were collected at commercial harvest (BBCH 89) at DAT 56–91 in the 2010 and 2011 trials or at DAT 38–48 in the 2015 trials.

Samples were stored at -18 °C or lower for a maximum period of 217 days for mandestrobin, 229 days for De-Xy-mandestrobin, 272 days for 4-OH-mandestrobin and 229 days for 2-CH_2OH-mandestrobin.

Samples from the 2010 and 2011 trials were analysed for the R- and S-isomers of mandestrobin using chiral HPLC-MS/MS method DFG S19 with an LOQ of 0.005 mg/kg for each isomer. The reported mandestrobin level in Table 118 is the sum of both isomers. Samples from the 2015 trials were analysed for mandestrobin using LC-MS/MS multi-residue method QuEChERS with an LOQ of 0.01 mg/kg. Samples were analysed for De-Xy-mandestrobin using HPLC-MS/MS method SUM-1023V, for 4-OH-mandestrobin using HPLC-MS/MS method SUM-1021V and for 2-CH_2OH-mandestrobin using HPLC-MS/MS method SUM-1022V (or modification B of method SUM-1022V in the 2015 trials). Metabolites were analysed with an LOQ of 0.01 mg/kg. Average concurrent recoveries for rape seed were in the range 70–120% for the R- and S-isomers of mandestrobin (2010, 2011) at 0.005–0.05 mg/kg and for mandestrobin (2015), De-Xy-mandestrobin,

4-OH-mandestrobin, and 2-CH_2OH-mandestrobin at 0.01–0.10 mg/kg. Control samples had residues below LOQ.

Summaries of the trial results of the European trials are given in Table 118. Residue levels in rape seed ranged between < 0.01–0.04 mg/kg for the parent compound and < 0.01 mg/kg for each of the metabolites.

Notes by the reviewer:

- The maximum storage periods of 217-272 days are covered by the storage stability studies on crop commodities with high oil content (rape seed).
- The analytical methods are valid in the range 0.01–0.5 mg/kg for chiral method DFG S19 and 0.01–0.1 mg/kg for QuEChERS, SUM-1023V, SUM-1021V, SUM-1022V. The methods are considered fit for the purpose of this study.
- Methods SUM-1021V, SUM-1022V are valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin.
- Metabolite 5-CH_2OH-mandestrobin was not analysed. Since the other hydroxy compounds were found to be below the LOQ of 0.01 mg/kg, levels of 5-CH_2-OH mandestrobin are also expected to be below the LOQ of 0.01 mg/kg.

Table 118 Residues of SC formulated mandestrobin in rape seed after foliar spray

RAPE SEEDS Location, year, (variety)	kg ai/ha	GS and date at applic	PHI [a] (DAT + drying (days))	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
Sharon, ND, USA, 2010 (Invigor 8440)	0.43	BBCH 78 18 July	42 (35+7)	< 0.01 < 0.01 (< 0.01)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-A
	0.42	BBCH 78 18 July	42 (35+7)	< 0.01 < 0.01 (< 0.01)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Velva, ND, USA, 2010 (DKL52–41)	0.43	BBCH 63 25 June	45 (35+10)	< 0.01 < 0.01 (< 0.01)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-B
	0.42	BBCH 63 25 June	45 (35+10)	< 0.01 < 0.01 (< 0.01)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Velva, ND, USA, 2011 (DeKalb 52–41 RR)	0.42	BBCH 62 28 June	48 (41+7)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-G
	0.42	BBCH 64 03 July	43 (36+7)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	0.42	BBCH 67 08 July	38 (31+7)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	0.43	BBCH 69 13 July	33 (26+7)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Northwood, ND, USA, 2010 (Invigor 8440)	0.42	BBCH 65 19 July	49 (35+14)	0.13 0.12 (0.13)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-D
	2.1	BBCH 65 19 July	49 (35+14)	0.47 0.47 (0.47)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	

RAPE SEEDS Location, year, (variety)	kg ai/ha	GS and date at applic	PHI [a] (DAT + drying (days))	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
Northwood, ND, USA, 2011 (Invigor LL 8440)	0.41	BBCH 62 03 July	47 (36+11)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-F
	0.42	BBCH 64 28 July	42 (34+8)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-J&K [b]
	2.1	BBCH 64 28 July	42 (34+8)	0.15 0.12 0.23 (0.16)	< 0.02 < 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 NA (< 0.02)	< 0.02 < 0.02 NA (< 0.02)	< 0.02 < 0.02 NA (< 0.02)	
American Falls, ID, USA, 2011 (V1037)	0.41	BBCH 69 18 Aug	43 (34+9)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-H
	0.84	BBCH 69 18 Aug	43 (34+9)	< 0.02 0.020 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
Rupert, ID, USA, 2011 (46A76)	0.42	BBCH 77-78 27 July	43 (35+8)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	ROR-0238, Trial V-37238-I
	0.41	BBCH 77-78 27 July	43 (35+8)	0.027 0.024 (0.026)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Minidoka, ID, USA, 2010 (46A76)	0.40	BBCH 78-79 26 July	44 (36+8)	0.026 0.044 (0.035)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0238], Trial V-37238-C
	0.46	BBCH 78-79 26 July	44 (36+8)	0.016 0.011 (0.014)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Minto, MB, Canada, 2010 (NX4-205CL)	0.42	BBCH 67-73 19 July	38 (25+13)	0.024 0.023 (0.024)	< 0.02 0.020 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-A
	0.42	BBCH 67-73 19 July	38 (25+13)	0.043 0.036 (0.040)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Minto, MB, Canada, 2011 (73-55RR)	0.42	BBCH 67-68 21 July	41 (31+10)	0.042 0.065 (0.054)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-J
	0.42	BBCH 69 25 July	37 (27+10)	0.10 0.12 (0.11)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	0.44	BBCH 73 29 July	33 (23+10)	0.12 0.088 (0.10)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
	0.42	BBCH 79 3 Aug	28 (18+10)	0.075 0.079 (0.077)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Killarney, MB, Canada, 2010 (NX4-205CL)	0.44	BBCH 67 16 July	40 (27+13)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-B
	0.44	BBCH 67 16 July	40 (27+13)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Elgin, MB, Canada, 2010 (71–45 RR)	0.42	BBCH 67-71 19 July	39 (24+15)	0.032 0.039 (0.036)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-C
	0.43	BBCH 67-71 19 July	39 (24+15)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Elgin,	0.42	BBCH	35	< 0.02	< 0.02	NA	NA	NA	[ROR-0239],

RAPE SEEDS Location, year, (variety)	kg ai/ha	GS and date at applic	PHI [a] (DAT + drying (days))	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
MB, Canada, 2011 (5440)		69 22 July	(26+9)	< 0.02 (< 0.02)	< 0.02 (< 0.02)				Trial V-37284-H
Boissevain, MB, Canada , 2011 (71–45RR)	0.42	BBCH 67-73 29 July	39 (27+12)	0.057 0.072 (0.064)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-I
Blaine Lake, SK, Canada, 2010 (45 H 28)	0.42	BBCH 79 3 Aug	46 (24+22)	0.020 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-D
	0.42	BBCH 79 3 Aug	46 (24+22)	< 0.02 0.021 0.020	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Blaine Lake, SK, Canada , 2011 (Dekalb 71–45 RR)	0.41	BBCH 77-79 19 Aug	31 (21+10)	0.47 0.54 (0.51)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-L
Rosthern, SK, Canada, 2010 (DKLB 72–55)	0.43	BBCH 79 4 Aug	44 (20+24)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-E
	0.42	BBCH 79 4 Aug	44 (20+24)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Rosthern, SK, Canada, 2010 (45 H 28)	0.42	BBCH 69-79 4 Aug	44 (23+21)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-F
	0.42	BBCH 69-79 4 Aug	44 (23+21)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	
Rosthern, SK, Canada, 2011 (Invigor 5770)	0.41	BBCH 69-75 28 July	35 (22+13)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-K
Duck Lake, SK, Canada, 2011 (Dekalb 71–45 RR)	0.41	BBCH 77-78 17 Aug	37 (23+14)	0.037 0.042 (0.040)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-M
	0.84	BBCH 77-78 17 Aug	37 (23+14)	0.21 0.20 (0.20)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
Coalhurst, AB, Canada, 2010 (Liberty Link Invigor 5440)	0.43	BBCH 65-67 5 Aug	41 (27+14)	0.035 0.057 (0.046)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-G
Kipp, AB, Canada, 2011 (9533 RR)	0.41	BBCH 61–65 27 July	77 (52+15)	< 0.02 0.020 (< 0.02)	< 0.02 < 0.02 (< 0.02)	NA	NA	NA	[ROR-0239], Trial V-37284-N [c]
	2.0	BBCH 61–65 27 July	77 (52+15)	0.23 0.25 (0.24)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	< 0.02 < 0.02 (< 0.02)	
Epoye; N France, 2010 (Safran)	0.20	BBCH 65; 5 May	PHI 76	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0008]; Trial FLN-10–6267 FR01
Mulfingen /Railhof;	0.21	BBCH 65;	PHI 78	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0008]; Trial FLN-

RAPE SEEDS Location, year, (variety)	kg ai/ha	GS and date at applic	PHI [a] (DAT + drying (days))	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
Germany, 2010 (Visby)		3 May							10–6267 GE01
Moulsoe; UK, 2010 (DK Cabernet)	0.20	BBCH 65; 4 June	PHI 56	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0008]; Trial FLN-10–6267 UK01
Montgaillard Lauragais; S France 2010 (Cokeliko)	0.22	BBCH 65; 28 April	PHI 71	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0008]; Trial FLN-10–6267 FR02
St Pardon de Conques; S France 2010 (Standol)	0.21	BBCH 65; 26 April	PHI 59	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0008]; Trial FLN-10–6267 FR03
Paterne Racan; N France 2011 (Zeruca)	0.20	BBCH 65; 13 April	PHI 85	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-01
Brimont; N France 2011 (Ovasion)	0.21	BBCH 65; 18 April	PHI 70	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-02
Seevetal; Germany 2011 (NK Petrol)	0.20	BBCH 65; 29 April	PHI 83	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-05
Alte Noythe Germany 2011 (Visby)	0.20	BBCH 65; 28 April	PHI 91	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-06
Welles bourne UK, 2011 (DK Cabernet)	0.20	BBCH 65; 19 April	PHI 86	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-07
Pergain Tallac; S France 2011 (Mercury)	0.20	BBCH 65; 6 April	PHI 61	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-03
Gontaud de Nogaret; S France 2011 (Hybri Star)	0.20	BBCH 65; 7 April	PHI 60	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0198]; Trial 11/01898756-04
Swiecice, Mazowieckie Poland 2015 (Marcus)	0.16	BBCH 77; 8 July	PHI 40	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 PL02
Deventer, Overijssel, Netherlands 2015 (Genie)	0.15	BBCH 77-79; 5 June	PHI 41	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 NL03
Middlefart, Funen, Denmark	0.15	BBCH 75; 16	PHI 41	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013

RAPE SEEDS Location, year, (variety)	kg ai/ha	GS and date at applic	PHI [a] (DAT + drying (days))	M mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
2015 (Sy Alister)		June							DK04
Banbury, Oxfordshire, UK, 2015 (Excalibur)	0.14	BBCH 77; 19 June	PHI 48	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 UK05
Kótaj Szabolcs-Szatmárbereg, Hungary 2015 (Pioneer PT 200)	0.15	BBCH 77-78; 21 May	PHI 41	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 HU07
Maklár, Heves, Hungary 2015 (Hybrirock)	0.16	BBCH 78-79; 21 May	PHI 42	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 HU08
Prellenkirchen, Lower Austria, Austria 2015 (Remy)	0.15	BBCH 79; 30 May	38	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 AU09
Goch-Hassum, North Rhine Westphalia, Germany 2015 (Campino)	0.15	BBCH 77; 7 July	42	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 GE10
Höör, Snogeröd, Skania, Sweden, 2015 (Compass)	0.15	BBCH 78; 26 June	41	< 0.01	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 SW11
Gennes, Loire, N France 2015 (Fantasio)	0.15	BBCH 75; 7 July	38	0.04	< 0.01	< 0.01	< 0.01	NA	[ROR-0282]; Trial 15SGS013 FR12

GS: growth stage; NA= not analysed

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its (malonyl)glucosides, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

* Residue levels expressed as mg/kg analyte.

[a] The PHI is the total of the days after treatment (DAT) + the time for cutting and drying, both durations are indicated between brackets.

[b] One of the samples (parent 0.230 mg/kg) from trial J was used for processing and recoded as trial K.

[c] Control sample for trial N, Kipp, Alberta contained 0.0070 mg/kg mandestrobin [ROR-0239] leading to an LOQ of 0.0083 ×10/3=0.03 mg/kg mandestrobin for this trial. As this result affects residue levels in treated samples of the 0.42 kg ai/ha dose rate, residues from this trial cannot be selected for MRL derivation.

Additional trial information:

ROR-0238 GLP. Weather conditions did not affect crop growth. Plot size 300–4000 ft^2. Spray volume ranged from 111–177 L/ha.

ROR-0239: GLP. Weather conditions did not affect crop growth, except in trial G where seeding was delayed and harvest was late due to cool and wet weather. Plot L received 0.3 mm rain 4.5 hours after the application; plot N received a light drizzle 45 min and 6 hours after the application. Plot size 50–100 m^2. Spray volume ranged from 144-168 L/ha.

ROR-0008: GLP. Weather conditions did not affect crop growth. Plot UK01 received 0.3 mm rain within 18 hrs after application. Plot size 30–90 m^2. Spray volume ranged from 200–300 L/ha. Soil texture at trial sites was silt loam, sandy loam and clay loam.

ROR-0198: GLP. Weather conditions did not affect crop growth. Plot size 30–100 m^2. Spray volume ranged from 200–250 L/ha. Soil texture at trial sites was loamy clay, sandy loam, clay loam and sand.

ROR-0282: GLP: Weather conditions did not affect crop growth. Plot PL02 received 3.4 mm rain within 4 hrs after application; plot UK05 received 0.4 mm rain within 14 hrs after application; plot HU07 received 5 mm rain within 20 hrs after application; plot AU09 received 12 mm rain within 6 hrs after application. Plot sizes ranged from 45-600 m^2. Spray volumes ranged from 191–310 L/ha. Soil textures were sandy loam (trial 02, 09, 10) , loamy sand (trial 03), sand (trial 04), loam (trial 05, 07, 08, 11), clay loam (12).

Animal feed items

Soya bean forage

According to the OECD feed table soya bean plants may be grazed from the flowering stage to near maturity (BBCH 60–80).

Four residue field trials were conducted on soya bean forage in 2015 in Brazil [Klimmek and Gizler, 2017, ROR-0280]. In each trial, SC formulated mandestrobin was applied with a boom sprayer. The foliar spray was applied three times at 0.26 kg ai/ha with an interval of 10 days using a spray volume of 200 L/ha for each application. No adjuvants were used. A total of 12–20 plants were taken by hand with knife aid from at least 12 different locations within the plot. No plants were taken from the outer plants of the plots or from the area of spray overlap. Whole plants (1.2–2.4 kg) were taken immediately after the third treatment at DALT 0 (BBCH 77-79) and at 14 DALT (BBCH 79-81).

Samples were stored at -18 °C. The maximum storage interval for mandestrobin was 119 days, for De-Xy-mandestrobin 153 days interval and for 4-OH-mandestrobin and 2-CH_2OH-mandestrobin 297 days.

Samples were analysed for mandestrobin using HPLC-MS/MS multi residue method QuEChERS, for De-Xy-mandestrobin using HPLC-MS/MS method SUM-1023V, for 4-OH-mandestrobin and its (malonyl)glucosides using a modification A of HPLC-MS/MS method SUM-1021V and for 2-CH_2OH-mandestrobin and its (malonyl)glucosides using a modification A of HPLC-MS/MS method SUM-1022V. The LOQ was 0.01 mg/kg for each analyte. Average concurrent recoveries were within the range of 70–120% for mandestrobin at 0.01–12 mg/kg and its metabolites at 0.01–1.0 mg/kg. Control samples had residues below 0.3LOQ.

Summaries of the trial results are given in Table 117. Residue levels in soya bean forage ranged between 0.16-11 mg/kg for the parent compound, < 0.01 mg/kg for De-Xy-mandestrobin, 0.03–0.07 mg/kg for 2-CH_2OH-mandestrobin and 0.02–0.05 mg/kg for 4-OH-mandestrobin.

Note by the reviewer:

- The maximum storage intervals of 119–297 days for soya bean forage were covered by the storage stability studies on commodities with high water content (lettuce).
- Method SUM-1022 V is valid in the range 0.01–0.10 mg/kg for the determination of 2-CH_2OH-mandestrobin and its (malonyl)glucosides in soya bean forage. Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–12 mg/kg of parent and 0.01–0.10 mg/kg for each of the other metabolites in soya bean forage (BBCH 77-81).
- Methods SUM-1021V and SUM-1022V are valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin and 2-CH_2OH-mandestrobin.
- Metabolite 5-CH_2OH-mandestrobin was not analysed, but may be expected to be present at similar levels as the other hydroxy compounds.

Table 119 Residues of SC formulated mandestrobin in soya bean forage (whole plant) after foliar spray

SOYA BEAN FORAGE Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation	PHI (days)	parent mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M* mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
Mafra, SC, Brazil, 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 77 13 Feb	0 7	11 0.49	< 0.01 < 0.01	0.07 0.03	0.05 0.03	NA	[ROR-0280] S-14-05177-01
Ponta Grossa, PR, Brazil 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 79 16 Feb	0 7	3.9 0.74	< 0.01 < 0.01	0.04 0.04	0.03 0.05	NA	[ROR-0280] S-14-05177-02
Arapoti, PR, Brazil, 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 79 23 Feb	0 7	4.8 0.56	< 0.01 < 0.01	0.04 0.03	0.02 0.04	NA	[ROR-0280] S-14-05177-03
Engeheiro Coelho, SP, Brazil, 2015 (Valiosa RR)	3 × 0.26 (10, 11)	BBCH 78 16 Mar	0 7	5.9 0.16	< 0.01 < 0.01	0.04 0.03	0.03 0.03	NA	[ROR-0280] S-14-05177-04

GS = growth stage; N = Number of treatments; I = Interval between treatments; NA = not analysed

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its (malonyl)glucosides, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

* Residue levels expressed as mg/kg analyte

Additional trial information:

ROR-0280 GLP. Weather conditions did not affect crop growth. Most trials received between 0 to 7.0 mm rain on the day of application (trial 01, 02, 03), except trial 04 which received up to 41 mm rain on the day of application. Plot sizes were 102–105 m^2. Soil types included sandy clay (trial 04), and heavy clay (trial 01, 02, 03).

Soya bean fodder

Soya bean fodder (> BBCH 80) is not mentioned as a feed commodity in the OECD feed table. The trials were summarized to support the definition of the residue.

Four residue field trials were conducted on soya bean fodder in 2015 in Brazil [Klimmek and Gizler, 2017, ROR-0280]. In each trial, SC formulated mandestrobin was applied with a boom sprayer. The foliar spray was applied three times at 0.26 kg ai/ha with an interval of 10 days using a spray volume of 200 L/ha for each application. No adjuvants were used. A total of 12–20 plants were taken by hand with knife aid from at least 12 different locations within the plot. No plants were taken from the outer plants of the plots or from the area of spray overlap. Dry pods with seeds (1.0–1.3 kg) and rest of plants (1.0–1.4 kg) were taken at 14 DALT (BBCH 82).

Samples were stored at -18 °C. The maximum storage interval for mandestrobin was 119 days, for De-Xy-mandestrobin 153 days interval and for 4-OH-mandestrobin and 2-CH_2OH-mandestrobin 297 days.

Samples were analysed for mandestrobin using HPLC-MS/MS multi residue method QuEChERS, for De-Xy-mandestrobin using HPLC-MS/MS method SUM-1023V, for 4-OH-mandestrobin and its (malonyl)glucosides using a modification A of HPLC-MS/MS method SUM-1021V and for 2-CH_2OH-mandestrobin and its (malonyl)glucosides using a modification A of HPLC-MS/MS method SUM-1022V. The LOQ was 0.01 mg/kg for each analyte. Average concurrent recoveries were within the range of 70–120% for mandestrobin at 0.01–0.48 mg/kg and its metabolites at 0.01–1.0 mg/kg. Control samples had residues below 0.3LOQ.

Summaries of the trial results are given in Table 119. Residue levels in soya bean fodder (dry pods with seeds or rest of plants) ranged between 0.05–0.37 mg/kg for the parent compound, < 0.01 mg/kg for De-Xy-mandestrobin, < 0.01–0.07 mg/kg for 2-CH_2OH-mandestrobin and < 0.01–0.10 mg/kg for 4-OH-mandestrobin.

Note by the reviewer:

- The maximum storage interval for of 119-297 days is covered by the storage stability studies on barley straw.
- Limited recovery experiments (n = 1–2) suggest validity in the range 0.01–0.48 mg/kg of parent and 0.01–0.10 mg/kg for each of the other metabolites in dry soya bean fodder (BBCH 82).
- Methods SUM-1021V and SUM-1022V are valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin and 2-CH_2OH-mandestrobin.
- Metabolite 5-CH_2OH-mandestrobin was not analysed, but may be expected to be present at similar levels as the other hydroxy compounds.

Table 120 Residues of SC-formulated mandestrobin in soya bean fodder (dry pods with seeds and rest of plant) after foliar spray

SOYA BEAN FODDER Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation	PHI (d)	Matrix	parent mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M(*) mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
Mafra, SC, Brazil, 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 77 13 Feb	14	d pods d rest	0.11 0.37	< 0.01 < 0.01	< 0.01 0.07	< 0.01 0.10	NA	[ROR-0280] S-14-05177-01
Ponta Grossa, PR, Brazil 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 79 16 Feb	14	d pods d rest	0.13 0.15	< 0.01 < 0.01	< 0.01 0.02	0.01 0.02	NA	[ROR-0280] S-14-05177-02
Arapoti, PR, Brazil, 2015 (5909 RR)	3 × 0.26 (10, 10)	BBCH 79 23 Feb	14	d pods d rest	0.15 0.19	< 0.01 < 0.01	< 0.01 0.02	0.01 0.02	NA	[ROR-0280] S-14-05177-03

SOYA BEAN FODDER Location, year, (variety)	N × kg ai/ha (I in days)	GS and date at last appli-cation	PHI (d)	Matrix	parent mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M(*) mg/kg	5-CH_2OH-M * mg/kg	[Report] Trial no
Engeheiro Coelho, SP, Brazil, 2015 (Valiosa RR)	3 × 0.26 (10, 11)	BBCH 78 16 Mar	14	d pods d rest	0.05 0.09	< 0.01 < 0.01	< 0.01 0.04	< 0.01 0.07	NA	[ROR-0280] S-14-05177-04

GS = growth stage; N = Number of treatments; I = Interval between treatments; NA = not analysed

d pods = dry pods with seeds; d rest = rest of the dry plant

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its (malonyl)glucosides, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

* Residue levels expressed as mg/kg analyte

Additional trial information:

ROR-0280 GLP. Weather conditions did not affect crop growth. Most trials received between 0 to 7.0 mm rain on the day of application (trial 01, 02, 03), except trial 04 which received up to 41 mm rain on the day of application. Plot sizes were 102–105 m^2. Soil types included sandy clay (trial 04), and heavy clay (trial 01, 02, 03).

Rape seed fodder

According to the OECD feed table, rape seed forage may be grazed before the flowering stage (BBCH 60). Rape seed fodder (green whole plants or green pods with seeds and the rest of the plant, > BBCH 60) is not listed as a feed commodity. The trials were summarized to support the definition of the residue.

A total of 11 supervised residue trials on winter rape seed fodder were conducted in Northern and Southern Europe in 2010, 2011 and 2015 [Delmotte, 2011, ROR-0008; Lebrun, 2012, ROR-0198; Gemrot, 2017, ROR-0282]. SC formulated mandestrobin was applied once as a foliar spray by hand carried boom sprayer or single wheel sprayer using spray volumes of 140–310 L/ha. The single application was at 0.20–0.22 kg ai/ha at growth stage BBCH 65 (flowering) in the 2010 and 2011 trials or at 0.14–0.16 kg ai/ha at growth stage BBCH 75-79 (pod development) in the 2015 trials. No adjuvants were added. Rape seed plants were collected from at least 12 separate areas of the plots. Only the central part of the plot was sampled, leaving 0.5 meter on both ends of the plot and the borders. Green whole plants (1.0–3.6 kg, BBCH 65 in 2010/2011 and > 0.5 kg BBCH 75-81 in 2015), green pods with seeds and the rest of the plant (0.5-3.3 kg and 1.0–2.9 kg, BBCH 79 in 2010/2011) and dry pods with seeds and the rest of the plant (> 0.5 kg and > 1.0 kg, BBCH 80–87 in 2015) were harvested by hand using a knife or shears by cutting the plants at 15 cm above soil level. Rape seed plants were collected at DAT 3 hrs (whole plants, BBCH 65) and at DAT 29-50 (green pods with seeds and rest of plant, BBCH 79) in the 2010 and 2011 trials or at DAT 0, 14, 20–22 (green whole plants, BBCH 77-80) and DAT 30–33 (dry pods and rest of plant, BBCH 86),

Samples were stored at -18 °C or lower for a maximum period of 217 days for mandestrobin, 229 days for De-Xy-mandestrobin, 272 days for 4-OH-mandestrobin and 229 days for 2-CH_2OH-mandestrobin.

Samples from the 2010 and 2011 trials were analysed for the R- and S-isomers of mandestrobin using chiral HPLC-MS/MS method DFG S19 with an LOQ of 0.005 mg/kg for each isomer. The reported mandestrobin level in Table 118 is the sum of both isomers. Samples from the 2015 trials were analysed for mandestrobin using LC-MS/MS multi-residue method QuEChERS with an LOQ of 0.01 mg/kg. Samples were analysed for De-Xy-mandestrobin using HPLC-MS/MS method SUM-1023V, for 4-OH-mandestrobin using HPLC-MS/MS method SUM-1021V and for 2-

CH_2OH-mandestrobin using HPLC-MS/MS method SUM-1022V (or modification B of method SUM-1022V in the 2015 trials). Metabolites were analysed with an LOQ of 0.01 mg/kg. Average concurrent recoveries in green whole plants, green pods and rest of plants and dry pods and rest of plants were in the range 70–120% for the R- and S-isomers of mandestrobin at 0.005–2.0 mg/kg and for mandestrobin, De-Xy-mandestrobin, 4-OH-mandestrobin, and 2-CH_2OH-mandestrobin at 0.01–0.10 mg/kg. Control samples had residues below LOQ.

Summaries of the trial results of the European trials are given in Table 121. Residue levels in rape seed fodder (>BBCH 60) ranged between < 0.01–4.2 mg/kg for the parent compound, < 0.01–0.07 mg/kg for De-Xy-mandestrobin, < 0.01–0.11 mg/kg for 2-CH_2OH-mandestrobin and < 0.01–0.68 mg/kg for 4-OH-mandestrobin.

Notes by the reviewer:

- Maximum storage intervals of 217 -272 days for green or dry rape seed fodder are covered by the storage stability studies on commodities with high water content (lettuce) and barley straw.
- For Chiral HPLC-MS/MS method DFG S19, limited recovery experiments (n = 1–2) suggest validity of the determination of mandestrobin in the range 0.01–2.0 mg/kg (0.005-1.0 mg/kg for R/S each) green rape seed fodder (BBCH 65, 79). Since mandestrobin levels in the 2010–2011 supervised green rape seed fodder trials ranged between < 0.01–3.4 mg/kg extension of the validation is desirable.
- For QuEChERS, SUM-1021V and SUM-1022 V limited recovery experiments (n = 1–2) suggest validity in green rape seed fodder (BBCH 65, 77-80) up to 6.0 mg/kg mandestrobin and up to 1.0 mg/kg De-Xy-mandestrobin, 4-OH-mandestrobin, and 2-CH_2OH-mandestrobin. These levels cover the levels found in the supervised trials.
- Methods SUM-1021V and SUM-1022V are valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin and 2-CH_2OH-mandestrobin.
- Metabolite 5-CH_2OH-mandestrobin was not analysed, but may be expected to be present at similar levels as the other hydroxy compounds.

Table 121 Residues of SC formulated mandestrobin in rape seed fodder after a single foliar spray

RAPE SEED FODDER Location, year, (variety)	kg ai/ha	GS and date at applic	PHI (day)	matrix	parent mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	Report Trial no [ref]
Epoye; N France, 2010 (Safran)	0.20	BBCH 65; 5 May	0 29 29	g WP g rest g pods	2.8 0.10 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA	[ROR-0008] Trial FLN-10–6267 FR01
Mulfingen /Railhof; Germany, 2010 (Visby)	0.21	BBCH 65; 3 May	0 50 50	g WP g rest g pods	1.5 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA	[ROR-0008] Trial FLN-10–6267 GE01
Montgaillard Lauragais; S France 2010 (Cokeliko)	0.22	BBCH 65; 28 April	0 29 29	g WP g rest g pods	1.5 0.031 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA	[ROR-0008] Trial FLN-10–6267 FR02
Brimont; N France 2011 (Ovasion)	0.21	BBCH 65; 18 April	0 31 31	g WP g rest g pods	2.1 0.36 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA	[ROR-0198] Trial 11/01898756-02

RAPE SEED FODDER Location, year, (variety)	kg ai/ha	GS and date at applic	PHI (day)	matrix	parent mg/kg	De-Xy-M * mg/kg	2-CH_2OH-M * mg/kg	4-OH-M * mg/kg	5-CH_2OH-M * mg/kg	Report Trial no [ref]
Alte Noythe Germany 2011 (Visby)	0.20	BBCH 65; 28 April	0 40 40	g WP g rest g pods	2.2 0.015 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA	[ROR-0198] Trial 11/01898756-06
Gontaud de Nogaret; S France 2011 (Hybri Star)	0.20	BBCH 65; 7 April	0 32 32	g WP g rest g pods	3.4 0.10 0.029	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA	[ROR-0198] Trial 11/01898756-04
Swiecice, Mazowieckie Poland 2015 (Marcus)	0.16	BBCH 77; 8 July	0 14 21 33 33	g WP g WP g WP d rest d pods	1.2 0.02 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 0.01 0.02 0.02	NA	[ROR-0282] Trial 15SGS013 PL02
Deventer, Overijssel, Netherlands 2015 (Genie)	0.15	BBCH 77-79; 5 June	0 14 21 32 32	g WP g WP g WP d rest d pods	0.97 0.07 0.02 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.01 0.02 0.01 < 0.01 < 0.01	NA	[ROR-0282] Trial 15SGS013 NL03
Middlefart, Funen, Denmark 2015 (Sy Alister)	0.15	BBCH 75; 16 June	0 14 22 31 31	g WP g WP g WP d rest d pods	0.73 0.03 0.03 0.02 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 0.04	NA	[ROR-0282] Trial 15SGS013 DK04
Banbury, Oxfordshire, UK, 2015 (Excalibur)	0.14	BBCH 77; 19 June	0 14 20 31 31	g WP g WP g WP d rest d pods	1.5 0.09 0.07 0.04 0.03	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 0.02	NA	[ROR-0282] Trial 15SGS013 UK05
Gennes, Loire, N France 2015 (Fantasio)	0.15	BBCH 75; 7 July	0 14 22 31 31	g WP g WP g WP d rest d pods	4.2 3.1 1.6 1.6 2.8	< 0.01 0.05 0.02 0.06 0.07	< 0.01 0.10 0.07 0.07 0.11	< 0.01 0.68 0.31 0.25 0.25	NA	[ROR-0282] Trial 15SGS013 FR12

GS = growth stage; NA= not analysed;

g WP = green whole plant (BBCH 60–80); g pods = green pods with seeds (BBCH 71–80), g rest = green whole plant without pods (BBCH 71–80); d pods = dry pods with seeds (BBCH 80–89); d rest = dry whole plant without pods (BBCH 80–89);

M: mandestrobin, De-Xy-M: De-Xy-mandestrobin, 2-CH_2OH-M: 2-CH_2OH-mandestrobin and its (malonyl)glucosides, 4-OH-M: 4-OH-mandestrobin and its (malonyl)glucosides, 5-CH_2OH-M: 5-CH_2OH-mandestrobin and its (malonyl)glucosides

* Residue levels expressed as mg/kg analyte.

Additional trial information:

ROR-0008: GLP. Weather conditions did not affect crop growth. Plot UK01 received 0.3 mm rain within 18 hrs after application. Plot size 30–90 m2. Spray volume ranged from 200–300 L/ha. Soil texture at trial sites was silt loam, sandy loam and clay loam.

ROR-0198: GLP. Weather conditions did not affect crop growth. Plot size = 30–100 m2. Spray volume ranged from 200–250 L/ha. Soil texture at trial sites was loamy clay, sandy loam, clay loam and sand.

ROR-0282: GLP: Weather conditions did not affect crop growth. Plot PL02 received 3.4 mm rain within 4 hrs after application; plot UK05 received 0.4 mm rain within 14 hrs after application. Plot sizes ranged from 60–600 m^2. Spray volumes ranged from 190–300 L/ha. Soil textures were sandy loam (trial 02) , loamy sand (trial 03), sand (trial 04), loam (trial 05), clay loam (12).

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

Not applicable.

In processing

The Meeting received information on the nature of residues under conditions simulating pasteurisation, baking/brewing/boiling and sterilisation. In addition, the Meeting received processing studies on grapes (juice and raisins) and rape seed (refined oil, extracted meal).

Nature of residues processing study

The behaviour of mandestrobin was studied (Table 122) under conditions simulating pasteurisation, baking/brewing/boiling and sterilisation [Dixon and Gilbert, 2011, ROM-0027]. The radiolabelled form of [^{14}C]-mandestrobin used for this study was a 1:1 racemic mixture of the R- and S-isomer of mandestrobin and was labelled in the phenoxy ring system.

Duplicate solutions were prepared in 0.1 M sterile citrate buffers at pH 4, 5 and 6. The buffer solutions were incubated in the dark for 20 minutes at 90 °C (pH 4), 60 minutes at 100 °C (pH 5) or 20 minutes at 120 °C (pH 6). The buffer solution was then removed from each tube and assayed for radioactivity by LSC on the day of incubation. A sample was taken for analysis by HPLC, co-injected with the authentic reference compound. Selected samples were also analysed by thin-layer chromatography to confirm the presence of the test substance.

The pH of the solutions did not change throughout the course of each experiment. The mean recovery of radioactivity applied to the test systems ranged from 96.2–102.0% for hydrolysed samples after each sampling interval. Table 4 shows radioactivity in the treated samples and control samples comprised only parent (no hydrolysis products were observed).

Table 122 Recovery and identification of radioactivity in 1 mg/L ^{14}C-mandestrobin solutions under pasteurisation, baking/brewing/boiling and sterilisation simulating conditions (treated/control).

Conditions	post incubation (%TAR)	parent S-2200 (%TRR)	unknowns (%TRR)	unresolved background (%TRR)
pH 4 (90 °C, 20 min) (pasteurisation)	96/97	98/98	0.8/0.8	1.3/0.8
pH 5 (100 °C, 60 min) (baking/brewing/boiling)	101/101	99/97	0.7/1.5	0.3/1.3
pH 6 (120 °C, 20 min) (sterilisation)	102/102	98/99	1.3/1.1	1.0/0.4

Supervised processing studies

Study 1

A supervised residue field trial with grapes was conducted in the USA in 2012 [Bitter *et al*., 2013, ROR-0234]. An SC formulation containing mandestrobin (+NIS adjuvant), was applied at exaggerated rates of 3 times 0.85 kg ai/A (total seasonal rate of 2.55 kg ai/A), corresponding with 3 times 2.1 kg ai/ha (total rate 6.3 kg ai/ha) and a 10 day interval to grapes.

RAC specimens (12 bunches from 6 different vines weighing at least 1 kg for regular residue measurements) were collected from the trials at 10 days after the last application (DALA). Samples collected for fruit processing weighed at least 27 kg (untreated grapes) to 37 kg (treated grapes). Immediately before the start of processing, 1 kg samples of the raw agricultural commodities were obtained from each specimen for processing purposes. Specimens for processing of juice were placed on ice immediately after collection and processed within 48 hours of sampling. The bulk raisins that

remained were dried as per commercially acceptable standards for grape drying time (3 Sept. – 20 Sept).

Drying

From the bulk 13.9 kg was removed and placed on trays to dry for approximately 17 days in the field to make raisins. Raisin samples were then collected and stemmed by hand.

Fresh juice processing

The remainder of the bulk (27.6-13.9 kg = 13.7 kg) was placed in a Zambelli stemmer. Large stems were removed. The berries with small stems were removed and placed in a crusher/juicer to squeeze juice out of the grapes through a cheese cloth into containers and the fresh juice was placed into freezers. Yield factors were not provided.

All specimens (RAC and processed commodity specimens) were frozen (-18 °C) on the day of collection. The maximum storage interval for Trial V-38175-M was 249 days for mandestrobin and De-Xy-mandestrobin in grapes, 294 days for grape juice and 277 days for grape raisins. The maximum storage interval for Trial V-38175-M was 371 days for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin in grapes, 365 days in grape juice and 354 days in grape raisins.

Samples were analysed for mandestrobin and De-Xy-mandestrobin using HPLC-MS/MS method RM-48G with an LOQ of 0.02 mg/kg. Samples were analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates using HPLC-MS/MS method RM-48A with an LOQ of 0.02 mg/kg for each analyte.

Average concurrent fresh recoveries for grapes, grape juice and grape raisins ranged between 79-112% for mandestrobin and De-Xy-mandestrobin at 0.02–30 mg/kg and 78-102% for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin at 0.02–0.20 mg/kg. Control samples had residues below 0.3LOQ.

Residues as measured in the treated RAC and processed samples are summarized in Table 123. Residues range between 11.6-22.4 mg/kg for parent, 0.04–0.24 mg/kg for De-Xy-mandestrobin, 0.02–0.11 mg/kg for 2-CH_2OH-mandestrobin and its (malonyl)glucoside conjugates; 0.02–0.05 mg/kg for 4-OH-mandestrobin and its (malonyl)glucoside conjugates; 0.03–0.13 mg/kg for 5-CH_2OH-mandestrobin and its (malonyl)glucoside conjugates.

Notes by the reviewer:

- The maximum storage interval of 249 days for grapes, 294 days for grape juice and 277 days for grape raisins for mandestrobin and De-Xy-mandestrobin is covered by the storage stability studies on commodities with high acid content (orange, strawberries, grapes) in combination with the storage stability studies with incurred residues in grapes, grape juice and grape raisins.
- The maximum storage interval of 371 days for grapes and 365 days for grape juice for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin is covered by the storage stability studies on crop commodities with high acid content (orange, strawberries, grapes) and high water content (lettuce).
- The maximum storage interval of 354 days for grape raisins for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin is NOT covered by storage stability studies. Since storage stability is shown for at least 12 months for all representative commodities, degradation of residues during storage is not expected for grape raisins.
- The analytical methods are valid in the range 0.02–0.2 mg/kg for grapes and at 0.02 mg/kg for grape juice and grape raisins. For method RM-48G, limited recovery experiments (n = 1–2)

suggest extension of the validity for mandestrobin and De-Xy-mandestrobin to 15 mg/kg for grapes, 20 mg/kg for grape juice, 30 mg/kg for grape raisins. For method RM-48A, limited recovery experiments (n = 1–2 per level) suggest extension of the validity to 0.2 mg/kg for grape juice and grape raisins. This covers the levels found in the study.

- Method RM-48A is valid for the determination of (malonyl)glucoside conjugates of 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin.
- Considering that the nature of residues study showed that mandestrobin is stable upon heating, the processing factor in raisins (PF_{total} = 2.0) is lower than expected from the dry weights (factor 5, OECD ENV/JM/MONO(2008)). Information on the dry weight of the grapes and raisins was not provided and therefore the actual expected concentration factor for this study cannot be verified.

Table 123 Residues and processing factors for processed grape commodities

Location Year variety	Treatment	Matrix	parent mg/kg	De-Xy-M mg/kg *	2-CH_2OH-M mg/kg *	4-OH-M mg/kg *	5-CH_2 OH-M mg/kg *	PF parent [b]	[Ref] Trial
Madera, CA, USA, 2012 (Thompson Seedless)	3 × 2.1 kg ai/ha RTI = 10 days, DALT = 10 days	fruit (RAC)	11.6 [a]	0.04 [a]	0.02	0.05	0.03	-	[ROR-0234] V38175 Trial M
		fresh juice	16.2	0.06	0.03	0.02	0.03	1.4	
		raisins	22.4	0.24	0.11	0.05	0.13	1.9	

M= mandestrobin

(*) Residues are expressed as themselves, unless indicated otherwise

[a] Average of four analyses.

[b] Processed product / RAC

Study 2

A supervised residue field trial with rape seed was conducted in the USA in 2011 [Green, 2013, ROR-0238]. In a field residue studies two trial sites were treated by foliar application with one exaggerated dose application rate of nominally 2.1 kg ai/ha (0.85 kg ai/Acre). Adjuvant NIS was added to the mix. The applications were made with a spray volume of 140 L/ha. The last application was made at growth stage BBCH 64 (28 July 2011). Samples for processing were collected only from one field (V-37238-J). rape seed were harvested at normal commercial harvest, 34 days after the last application. The rape seed plants were swath cut and allowed to dry in the field. Duplicate samples were collected from separated areas of the field, placed directly into sample bags and placed on ice. The seeds were transported to the processing facility (South Navasota, Texas, USA). The rape seed were processed into refined oil and extracted meal using standardised procedures simulating commercial industrial processes.

Crude oil and presscake processing

Rape seeds (13.0181/16.1789 kg) were dried and screened *(0.41/0.64, 1.09/1.54 kg large and small screenings*), resulting in *cleaned seeds (10.75/12.66 kg)*. Aspiration was not required. For further processing steps 5.44 kg of cleaned seeds was used. Cleaned seed was flaked in an AT Ferrell flaking roll. Flakes were heated to 82–99 °C for 10–15 minutes. Flakes were pressed (expelled) using a AgOil press to mechanically remove a portion of the *crude oil (1.340/1.823 kg)* from the *presscake (2.77/2.95 kg).* Yield from cleaned seeds to crude oil 25/33% and to presscake 51/54%.

Solvent extracted meal

Press cake was further processed by solvent extraction with n-hexane, for 30 minutes at 49-60 °C. This was repeated another two times followed by washes. After draining, the extracted presscake (meal) was desolventized with warm air, resulting in solvent extracted meal (1.59/2.40 kg) and crude oil (0.673/0.384 kg). Solvent extracted crude oil was passed through a vacuum evaporator and heated to 91–96 °C for n-hexane removal. Toasted rape seed meal (1.048/1.058 kg) was the result of toasting 1.0 kg of solvent extracted meal by heating the moisture adjusted meal to 99-104 °C for 18-22 minutes. Yield from cleaned seeds to toasted meal 31/47%.

Refined oil processing

Crude oil from expelling *(1.340/1.823 kg)* and from solvent extraction *(0.673/0.384 kg)* was combined. Crude oil samples were first degummed. Crude oil was heated to 50–65 °C and a 10% w/w citric acid solution was added to the oil at a rate of 2 g citric acid to 100 g crude oil. Solution was mixed at high rpm for 10–15 minutes, followed by slow mixing and hot water (43–52 °C) addition at a rate of 1.5–2.0 g of water per 100 g of oil. After 30–35 minutes mixing, degummed oil and gums (crude lecithin) was separated by centrifugation. Degummed crude oil was vacuum filtered and dried by mixing the oil under vacuum and heating to 85–99 °C for 20–30 minutes. Gums were discarded. The percentage free fatty acid was determined and oil was placed in a water bath and pre-treated by addition of 85% phosphoric acid ad 0.01–0.05% by weight of crude oil and mixing for 29-31 minutes at 40–44 °C. After pre-treatment, 30.6/32.3 NaOH was added to *850 g of crude oil* and mixed for 19–21 minutes at 40–44 °C and then for 9-11 at 65-70 °C. Neutralised oil was decanted and centrifuged to separate the alkali *refined oi*l *(783/775 g)* and *soapstock (76/82 g)*. Refined oil was filtered. Soapstock was discarded.

During bleaching, *alkali refined oil (783/775 g)* was heated to 40–50 °C, activated bleaching earth *(15.7/15.5 g)* was added and placed under vacuum. Temperature was increased to 85–100 °C for 10–15 minutes, followed by lowering of the temperature and adding a filter aid. Spent bleaching earth and filter aid were discarded. The resulting *bleached oil (752/746 g)* was deodorized without significant fractionation by steam bathing the oil for 45–60 minutes under vacuum and temperature held between 234 and 250 °C resulting in *deodorised oil (707/672 g)* and *deodorized distillates (236/213 gs).*

All specimens (RAC and processed commodity specimens) were frozen (-18 °C) on the day of collection. The maximum storage interval for VP-37238-J was 315 days for mandestrobin and De-Xy-mandestrobin in rape seed, 273–300 days for refined rape seed oil and 263 days for rape seed meal. The maximum storage interval for rape seed, rape seed oil and rape seed meal for each analyte is covered by the storage stability studies on crop commodities with high oil content.

Samples were analysed for mandestrobin and De-Xy-mandestrobin using HPLC-MS/MS method RM-48C-2 with an LOQ of 0.02 mg/kg. Average concurrent fresh recoveries in rape seed, rape seed oil and rape seed meal ranged between 76–103% for mandestrobin and De-Xy-mandestrobin at 0.02–0.2 mg/kg. Control samples had residues below 0.3LOQ. Since residue levels exceed only slightly the highest level of 0.2 mg/kg validated, the analytical method is considered fit for purpose.

Residues as measured and processing factors as derived in the treated RAC and processed samples are summarized in Table 124. Results were not corrected for control levels or for individual concurrent method recoveries.

Notes by the reviewer:

- In this study, residues seem to decrease with processing from rape seed to rape seed oil and rape seed meal (PF for parent is 0.060 and 0.24). Since the nature of residue study showed that the parent compound is stable during processing, this result is unexpected.

- Samples of rape seed oil and rape seed meal were not analysed for 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin and their (malonyl)glucoside conjugates. The rape seed of a limited number of trials were however analysed for these metabolites (see supervised field trials) and found not to contain any of these metabolites (< 0.02 mg/kg each).

Table 124 Residues and processing factors in rape seed and processed rape seed commodities

Location Year variety	Treatment	Matrix	parent mg/kg	De-Xy-M mg/kg *	2-CH_2OH-M mg/kg *	4-OH-M mg/kg *	5-CH_2 OH-M mg/kg *	PF parent [a]	[Ref] Trial
Northwood, ND, USA, 2011 (Invigor LL 8440)	1 × 2.1 kg ai/ha (+NIS) at BBCH 64, DALT =34 days	rape seed (RAC)	0.232	< 0.02	NA	NA	NA	-	[ROR-0238] trial VP-37238-J
		refined oil	0.014	< 0.02	NA	NA	NA	0.060	
		extracted meal	0.046	< 0.02	NA	NA	NA	0.20	

M= mandestrobin; NIS = non-ionic surfactant.

* Residues are expressed as themselves, unless indicated otherwise

[a] Processed product / RAC

RESIDUES IN ANIMAL COMMODITIES

The Meeting received information on feeding studies in dairy and beef cattle.

Direct animal treatments

Not applicable.

Farm animal feeding studies

A residue feeding study in dairy and beef cattle was conducted in Australia in 2012 [Dale and Chambers, 2018; ROR-0290]. Three groups of three lactating cows (Brown Swiss and crosses; 2–10 years old) were fed mandestrobin via the diet for 27 consecutive days. Doses were administered as a powder and applied directly onto each animals bail feed during morning milking. Consumption of bail feed and applied dose were confirmed. Three groups of three to six female beef cattle animals (Hereford, Angus & Hereford, Angus Cross; 13 months old) were administered mandestrobin as an oral drench for 27 consecutive days. The oral drench was prepared by mixing tap water with the contents of a disposable syringe containing mandestrobin solution. One additional beef cattle animal served as control. The three groups of dairy and beef cattle received 25, 75 or 150 ppm diet, corresponding with a calculated mean dose of 0.97, 2.9 or 5.8 mg/kg bw/day. After 27 days of dosing, animals were kept for depuration until day 49.

No health problems were observed during the study. The cows weighed 586-724 kg (dairy cattle) and 202–288 kg (beef cattle) at the start of treatment. Dairy cows had a milk yield of 13.5-27.0 L/day (day -8), with a group mean of 20 L/day per cow.

Dairy cattle were milked twice daily. Duplicate milk samples were taken from all dairy cattle from all four quarters at day -1, 1, 3, 7, 14, 21, 28, 35, 42, 49. Subsamples from AM milk (60%) were combined with subsamples from the PM milk (40%). Furthermore, skimmed milk and cream were produced from whole milk collected at day 21.

One beef cattle animal per group was sacrificed at day 28 (24 hours after the last dose), while at day 35, 42, and 49 one beef cattle animal was sacrificed for the low and high dose group. Samples of liver (123–336 g), kidney (318–636 g), muscle (142–377 g) and perirenal fat (200–506 g) were collected from all beef cattle animals.

Milk and tissue samples were stored at -10 °C or lower until extraction (65 days). Prepared extracts were kept at 1–10 °C and were analysed within 3 days after extraction. The storage stability for mandestrobin in each matrix is sufficiently covered by the storage stability studies.

Samples were analysed for mandestrobin using HPLC-MS/MS method 130509 with an LOQ of 0.02 mg/kg. Average concurrent fresh recoveries in liver, kidney, muscle, fat, milk, cream and skim milk were 84–113% for mandestrobin at 0.02–0.2 mg/kg. Control samples had residues below 0.3 LOQ.

Analytical results in tissue samples are shown in Table 125. Mandestrobin residues in tissues above the LOQ were found only in samples of liver and fat at the 75 and 150 ppm dose levels. After 7, 14 and 21 days of depuration (day 35, 42, 49) residues of mandestrobin in liver or fat were below the LOQ of 0.02 mg/kg.

Mandestrobin levels in whole milk were below the LOQ of 0.02 mg/kg at all sampling intervals and all dose levels. The day 21 skimmed milk sample did not show residues above the LOQ of 0.02 mg/kg at all dose levels. Mandestrobin residues above the LOQ were found only in the day 21 milk cream samples (< 0.02–0.034 mg/kg) at the 150 ppm dose level but in none of the samples at lower dose levels. Results are shown in Table 125.

Table 125 Residues in tissues and milk for 25, 75 and 150 ppm cattle groups dosed with mandestrobin

Sample	Dose rate (ppm feed)	Sampling day	Parent (mg/kg) individual cows	Parent (mg/kg) average
Muscle	25	28	< 0.02, < 0.02, < 0.02	< 0.02
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
	75	28	< 0.02, < 0.02, < 0.02	< 0.02
	150	28	< 0.02, < 0.02, < 0.02	< 0.02
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
Liver	25	28	< 0.02, < 0.02, < 0.02	< 0.02
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
	75	28	0.032, 0.055, 0.057	**0.048**
	150	28	0.073, 0.12, 0.28	**0.16**
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
Kidney	25	28	< 0.02, < 0.02, < 0.02	< 0.02
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
	75	28	< 0.02, < 0.02, < 0.02	< 0.02
	150	28	< 0.02 , < 0.02, < 0.02	< 0.02
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
Fat	25	28	< 0.02, < 0.02, < 0.02	< 0.02
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
	75	28	< 0.02, < 0.02, 0.023	< 0.02 [a]
	150	28	0.021, 0.038, 0.040	**0.033**
	depuration	35	< 0.02, < 0.02, < 0.02	< 0.02
Milk cream	25	21	< 0.02, < 0.02, < 0.02	< 0.02
	75	21	< 0.02, < 0.02, < 0.02	< 0.02
	150	21	< 0.02, < 0.02, 0.034	**0.021** [a]

[a] calculated based on the levels reported below the LOQ.

NATIONAL RESIDUE DEFINITION

The national residue definitions for mandestrobin at the time of this evaluation are:

Australia/New Zealand (plants):

Residue definition for enforcement: mandestrobin

Residue definition for dietary risk assessment: mandestrobin

Canada (plants and animals):

Residue definition for enforcement:	mandestrobin
Residue definition for dietary risk assessment:	no definition

EU (plant):

Residue definition for enforcement:	mandestrobin
Residue definition for dietary risk assessment:	sum of mandestrobin, De-Xy-mandestrobin, 4-OH-mandestrobin conjugate and 2-CH_2OH-mandestrobin conjugate, expressed as mandestrobin

Japan (plant)

Residue definition for enforcement:	mandestrobin
Residue definition for dietary risk assessment:	mandestrobin
Livestock:	no MRLs and no residue definition

South Korea (plant)

Residue definition for enforcement:	mandestrobin
Residue definition for dietary risk assessment:	mandestrobin
Livestock:	no MRLs and no residue definition

USA (plant):

Residue definition for enforcement:	mandestrobin
Residue definition for dietary risk assessment:	mandestrobin

APPRAISAL

At the Forty-ninth Session of the CCPR (2017), mandestrobin was scheduled for toxicology and residue evaluation as a new compound by the 2018 JMPR. During the 2018 JMPR it appeared that soil degradation studies, field dissipation studies and additional supervised field trials were available that could aid in the definition of the residue. The residue evaluation was therefore postponed to the 2019 JMPR.

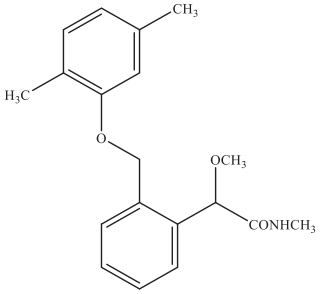
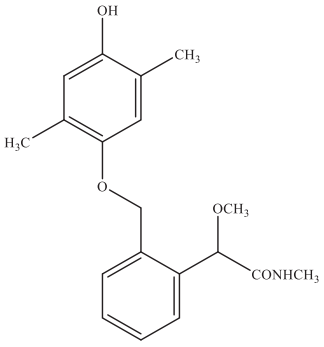
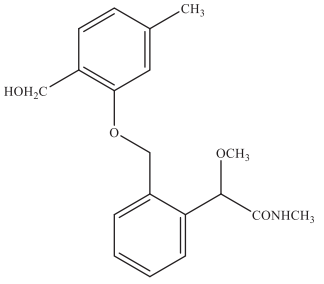
The 2018 JMPR established an ADI of 0–0.2 mg/kg bw and an ARfD of 3.0 mg/kg bw for women of childbearing age. The 2018 Meeting concluded that it was not necessary to establish an ARfD for mandestrobin for the remainder of the population.

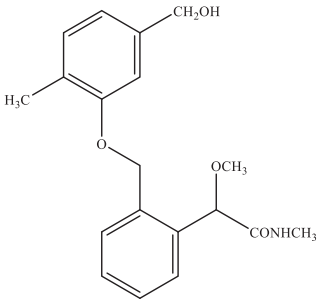
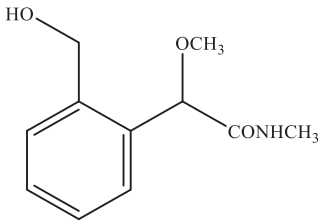
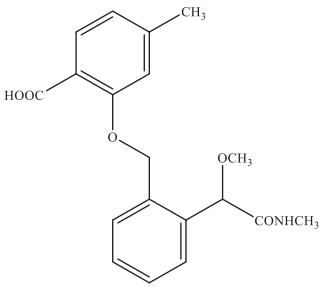
Mandestrobin is a systemic strobilurin fungicide. It acts on various fungi and also on the control of bacterial nuclear disease. Mandestrobin acts by inhibiting mitochondrial respiration. It binds at the Qo-centre on cytochrome b and blocks electron transfer between cytochrome b and cytochrome c1, disrupting the energy cycle within the fungus by halting the production of ATP.

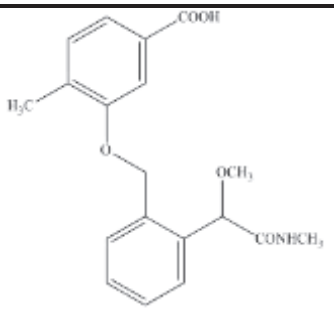
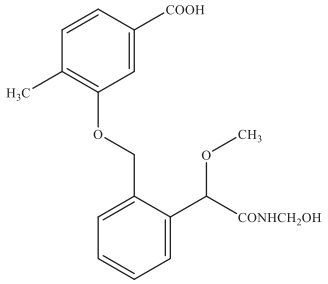
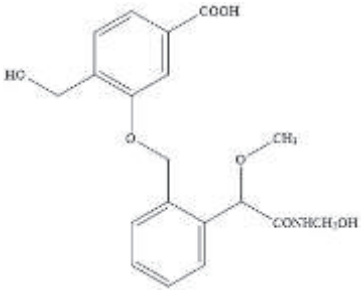
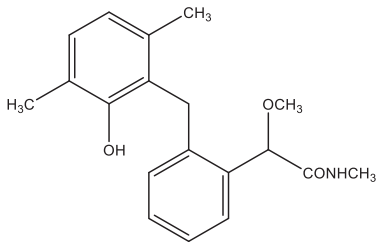
The Meeting received information from the manufacturer on identity, physical and chemical properties, metabolism in plant and livestock, confined and field rotational crop studies, soil degradation studies, field dissipation studies, residue analysis, storage stability, use patterns, supervised residue trials, fate of residues during processing and livestock feeding studies.

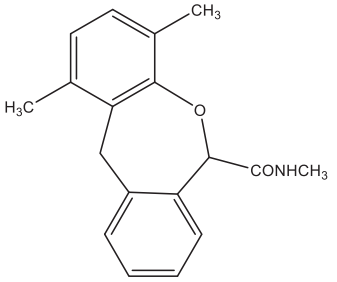
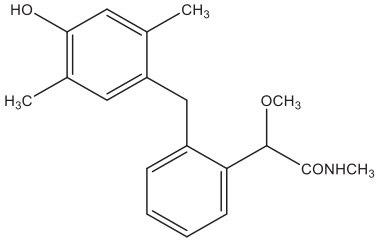
The IUPAC name of mandestrobin is (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide. The CAS name is 2-[(2,5-dimethylphenoxy)methyl]-α-methoxy-N-methylbenzeneacetamide. Mandestrobin (S-2200) is a racemic mixture of S-2167 (R-isomer) and S-2354 (S-isomer). All compounds referred to in the appraisal are listed in the table below.

Table 1 Abbreviations for the relevant compounds referred to in the appraisal

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
Mandestrobin	IUPAC: (2RS)-2-((2,5-dimethylphenoxy)methyl)phenyl)-2-methoxy-N-methylacetamide  MW = 313.39	Hydrolysis in water Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Tomato fruits; Tomato leaves Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60); Rape seed [application at bloom] Rotated wheat grain; Rotated wheat straw Rotated wheat forage; Rotated wheat hay Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Egg Poultry liver Poultry muscle Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
4-OH- mandestrobin	IUPAC: (2*RS*)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-*N*-methylacetamide  MW = 329.39	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rape seed [application before or at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat grain; Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot leaves Egg Poultry liver Poultry muscle Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney
2-CH_2OH-mandestrobin	IUPAC: (2*RS*)-2-[2-(2-hydroxymethyl-5-methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide  MW = 329.39	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Wheat grain Green rape seed fodder (BBCH > 60) Rape seed [application before or at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Poultry liver Poultry muscle Poultry skin Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
5-CH_2OH-	IUPAC: (2*RS*)-2-[2-(5-hydroxymethyl-2-	Immature lettuce; Mature lettuce

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
mandestrobin	methylphenoxymethyl)phenyl]-2-methoxy-*N*-methylacetamide  MW = 329.39	Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rape seed [application at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot leaves Milk fat Ruminant liver; Ruminant kidney
De-Xy- mandestrobin	IUPAC: (2*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide  MW = 210.253	Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Wheat grain Egg Poultry liver Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
2-COOH-mandestrobin	IUPAC: 2-({2-[(1*RS*)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid  MW = 343.38	Photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Egg Poultry liver Poultry skin Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
5-COOH-mandestrobin	IUPAC: 3-({2-[(1*RS*)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid	Photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Wheat straw Rape seed [before and at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Poultry skin Ruminant liver; Ruminant kidney Ruminant fat

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
	 MW = 343.38	
5-CA-mandestrobin-NHM	3-((2-(2-((hydroxymethyl)amino)-1-methoxy-2-oxoethyl)benzyl)oxy)-4-methylbenzoic acid  MW = 359.38	Egg Poultry skin; Poultry fat Skimmed milk Ruminant liver
5-CA-2-HM-mandestrobin-NHM	4-(hydroxymethyl)-3-((2-(2-((hydroxymethyl)amino)-1-methoxy-2-oxoethyl)benzyl)oxy)benzoic acid  MW = 375.38	Poultry skin Ruminant liver; Ruminant kidney
Mandestrobin-OR	(*RS*)-2-(2-(2-hydroxy-3,6-dimethylbenzyl)phenyl)-2-methoxy-N-methylacetamide 	Photolysis in water Soil surface photodegradation The benzyl radical recombined at the o- position of the phenoxy radical.
Mandestrobin-ORC	(*RS*)-N,1,4-trimethyl-6,11-dihydrodibenzo[b,e]oxepine-6-carboxamide	Photolysis in water Analysed in soil surface photodegradation but not detected

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
		Formed from mandestrobin-OR by cyclisation.
Mandestrobin-PR	(*RS*)-2-(2-(4-hydroxy-2,5-dimethylbenzyl)phenyl)-2-methoxy-N-methylacetamide 	Photolysis in water Analysed in soil surface photodegradation but not detected The benzyl radical recombined at the p- position of the phenoxy radical.

PHYSICAL AND CHEMICAL PROPERTIES

Mandestrobin is not volatile (3.4 × 10–5 mPa at 20 °C). Solubility in water is low (15.8 mg/L) and higher in various organic solvents (up to 522 g/L in acetone). Hydrolysis is unlikely to be a significant route of degradation in the environment, but photodegradation in water to 3% TRR was shown within 30 days.

Plant metabolism

The Meeting received plant metabolism studies for mandestrobin under greenhouse conditions (topical and soil applications on tomato plants and foliar spray applications on lettuce and wheat), under outdoor conditions (foliar application on oilseed rape), and on seed treatments of maize grains and soya bean seeds.

In all plant metabolism studies the R/S ratio of mandestrobin remained approximately 1:1.

Where two values are separated by "/", they account for the phenoxy [Ph-^{14}C]- and benzyl [Bz-^{14}C] labelled mandestrobin, respectively.

Plant metabolism after greenhouse applications

The metabolic fate of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was also studied in greenhouse grown tomato plants following 3 topical applications, using syringes, to fruits or leaves at a rate approximating 0.3 kg ai/ha each, with a 10-day interval. Plants were harvested 3 days after the last treatment. Total radioactive residues in fruit and leaves were 7.4–14 mg eq/kg and 87–199 mg eq/kg, respectively. Fruit and leaves were surface rinsed with acetonitrile and extracted with acetone/water. No residues were detected in the untreated leaves and fruits indicating no translocation from the treated fruits or leaves to the other parts of the plants. The majority of the radioactivity (95–99%) of the total radioactive residue (TRR) remained on the fruit or leaf surface, suggesting

mandestrobin is not systemic. The major compound was identified as parent: 99–100% TRR in fruits and 98–99% TRR in leaves.

The metabolic fate of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in greenhouse grown tomato plants following a single soil application at a rate of 0.9 kg ai/ha at fruit developing stage. Plants and soil were collected at 24 days after treatment. Insignificant translocation from soil to plant was observed: 0.06–0.08% of the total applied radioactivity (TAR) was found in leaves and none in fruits.

The metabolism of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in greenhouse grown lettuce following two foliar applications each at 0.82 kg ai/ha with a 10-day interval. Lettuce was treated at BBCH 43 and BBCH 48. Samples of immature and mature lettuce were taken 5 days after each treatment, respectively. Total radioactive residues in immature and mature lettuce were 35 and 43 mg eq/kg for the phenoxy label and 28 and 42 mg eq/kg for the benzyl label, respectively. A high proportion of the residue was removed by acetonitrile surface wash (78–88% TRR) and the total amount extracted with acetone/water, including the acetonitrile surface wash, was > 98% TRR. The principal component of the residue in the immature and mature lettuce plant was the parent compound (89–94% TRR).

The metabolism of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in greenhouse grown wheat following a single foliar spray application at 0.31 or 0.30 kg ai/ha, respectively. Wheat was treated at BBCH 32. Samples of wheat forage and wheat hay (each BBCH 37) were taken 7 and 14 days after treatment, respectively. Grain and straw samples were collected at maturity at 104 days after treatment. Total radioactive residues in wheat forage, hay, straw and grain were 11/10 mg eq/kg, 6.2/9.0 mg eq/kg, 1.9/2.5 mg eq/kg and 0.012/0.089 mg eq/kg, respectively.

Acetonitrile surface wash removed a total of 41/34% TRR in wheat forage, 23/19% TRR in wheat hay, and 3.7/2.8% TRR in wheat straw samples, harvested at 7, 14 and 104 days after treatment, respectively. A total of 91/92, 84/90, 45/49 and 54/53% TRR could be extracted from wheat forage, hay, straw and grain with acetone/water (including the surface wash), respectively. The remaining residues from hay and straw could be released with enzymes, acid and base until less than 2% TRR remained as solids.

In wheat grains a total of 64% TRR was identified for the benzyl label only. Parent mandestrobin was not detected and De-Xy-mandestrobin was the major component with 61% TRR (0.054 mg eq/kg). None of the ^{14}C-phenoxy labelled material could be identified, noting the low total residues.

A total of 71/81% TRR was identified in wheat forage, 59/51% TRR in wheat hay, and 18/30% TRR in wheat straw. Parent was the major component in wheat forage (51/60% TRR or 5.7–6.2 mg eq/kg) and hay (26/23% TRR or 1.6/2.1 mg eq/kg). In wheat straw, parent mandestrobin accounted only for 1.4/2.0% TRR (0.026/0.050 mg eq/kg). The residue in wheat straw consisted of several low level components with De-Xy-mandestrobin (up to 12% TRR) being the major component. De-Xy-mandestrobin accounted for 3.2% TRR in wheat forage and 1.5% TRR in wheat hay. Free and malonylglucoside conjugated 4-OH-mandestrobin was detected in wheat straw, forage and hay with levels ranging from 1.2/1.5% TRR in straw to 13/5.5% TRR in hay. Free and malonylglucoside conjugated 2-CH_2OH-mandestrobin accounted for 11/5.5% TRR in wheat forage, 11/13% TRR in hay and 9.5/6.4% TRR in straw. Other identified and unidentified metabolites accounted for less than 10% TRR (< 0.83 mg eq/kg).

Plant metabolism after outdoor applications

The metabolism of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in field grown oilseed rape following either one application at 0.39 kg ai/ha at BBCH 55–61 or following two foliar spray applications of 0.39–0.38 kg ai/ha each, applied at BBCH 55–61 (pre-bloom) and BBCH 66–67 (full bloom), with an interval of 14 days. Rape seed samples were harvested at maturity (BBCH 89) at 54 days after the single treatment or at 40 days after the double treatment. Green rape fodder samples

(BBCH > 60) were collected 14 days after the double treatment only. Total radioactive residues were 3.4–4.0 mg eq/kg in green rape fodder, 0.47–0.64 mg eq/kg in rape seed at DAT 40 (double treatment) and 0.050–0.11 mg eq/kg in rape seed at DAT 54 (single treatment).

In rape seeds (DAT 40) after two applications, hexane combined with acetone/water extracted 78/92% TRR, while 58/46% TRR was identified. Parent was the major compound with 31/25% TRR (0.14/0.16 mg eq/kg). The major metabolite was malonylglucoside conjugated 4-OH-mandestrobin, which was found at 14/11% TRR (0.068–0.071 mg eq/kg). Other identified and unidentified metabolites were below 10% TRR.

In rape seed (DAT 54) after one application, hexane combined with acetone/water extracted 72/79% TRR, while only 20/0% TRR was identified. None of the components were found at levels above 10% TRR. Parent was not detected. Malonylglucoside conjugated 4-OH-mandestrobin (8.0% TRR), malonylglucoside conjugated 2-CH_2OH-mandestrobin (3.6% TRR) and free 5-COOH-mandestrobin (8.7% TRR) were the major components identified in the phenoxy label only. None of the residues in the ^{14}C-benzyl label experiment were identified.

In green rape fodder (DAT 14) after two applications, acetone/water extracted 87/89% TRR (including 34–37% TRR that was removed with an acetonitrile surface wash). A total of 73/65% TRR was identified. The principal component of the residue was the malonylglucoside conjugated form of 4-OH-mandestrobin (36/27% TRR), followed by parent (20/22% TRR) and the free and malonylglucoside conjugated form of 2-CH_2OH-mandestrobin (12/13% TRR). Other identified and unidentified metabolites were below 10% TRR.

The Meeting noted that the two studies on rape seeds had different metabolic profiles. The single application and the first of the two applications were conducted before flowering, i.e. prior to seed formation. Since the GAP indicates that application should be conducted at flowering (BBCH 62–65), the study where the second application was conducted at BBCH 66–67 is considered more relevant for the definition of the residue.

Metabolism after seed treatment

The metabolic fate of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in maize grains and soya bean seeds following seed treatment at 9.3–12 g ai/100 kg of seeds. Seeds were planted 6 days after treatment. Harvest occurred at appropriate growth stages. The soya bean food RACs and the maize food and feed RACs did not take up mandestrobin related residues to any significant extent (< 0.005 mg eq/kg). Significant residues were found only in maize fodder (< 0.005–0.008 mg eq/kg; benzyl label only), soya bean forage (0.038–0.061 mg eq/kg) and soya bean hay (0.030–0.050 mg eq/kg).

Extraction with acetonitrile and acetonitrile/water released a total of 71% TRR from maize fodder (benzyl label only), 87/89% TRR from soya bean forage and 48/53% TRR from soya bean hay, while only 8.2, 29/7.6, 3.7/3.4% TRR was identified, respectively. The major compound identified in soya bean forage was De-Xy-mandestrobin at 5.3–12% TRR (0.005 mg eq/kg). Identified components in maize fodder and soya bean hay were all below 10% TRR. Mandestrobin was found at 1.2–1.9% TRR (soya bean hay), 2.3–7.9% TRR (soya bean forage) and 2.8% TRR (maize fodder), but never exceeded 0.003 mg eq/kg. HPLC profiles showed two major unidentified fractions which accounted for 14–29% TRR (0.001–0.022 mg eq/kg) and 9.6–22% TRR (0.002–0.016 mg eq/kg).

Metabolism in confined rotational crops

In a confined rotational crop study, [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was applied to bare soil at a single rate of 1.6 kg ai/ha. Rotational crops (lettuce, carrots and wheat) were planted at intervals (PBI) of 30, 120 and 365 days after application. Wheat forage, hay, straw and grain, lettuce, carrot roots and leaves were harvested at appropriate growth stages. Significant residues (0.019–4.5 mg eq/kg) were found in all crop commodities at all plant back intervals, except in wheat grains

and carrot roots at PBI 365 where total radioactivity did not exceed 0.01 mg eq/kg. Total radioactivity of residues in rotational crops generally declined from PBI 30 to PBI 120 and PBI 365.

Acetone/water extracted 77–87% TRR from carrot roots, 59–92% TRR from lettuce, carrot leaves and wheat forage, 45–62% TRR from wheat hay, and 34–64% TRR from wheat straw. Residues from wheat grain could not be extracted to a significant extent, with only 5.2–5.8% TRR for the phenoxy label and 31–37% TRR for the benzyl label. Only 47–65% TRR (carrot roots, benzyl label, but was higher for the phenyl-label), 6.9–53% TRR (wheat forage), 16–71% TRR (lettuce), 5.4–26% TRR (carrot leaves), 9.3–45% TRR (wheat hay), and 14–33% TRR (wheat straw) was identified.

Parent was present at low concentrations in all crop commodities, with a maximum of 78% TRR (0.040 mg eq/kg) in carrot roots at PBI 30 days or 1.8% TRR (0.045 mg eq/kg) in wheat forage at PBI 30 days. Major metabolites found in free and malonylglucoside or glucoside conjugated form were 4-OH-mandestrobin (at 2.7–34% TRR in all samples, except carrot roots), 5-CH_2OH-mandestrobin (1.7–32% TRR in all samples, except wheat grain) and to a minor extent 2-CH_2OH-mandestrobin (0–16% TRR in all samples, except wheat grain). Metabolite De-Xy-mandestrobin was not observed in the confined rotational crop study. Other identified and unidentified metabolites were below 10% TRR.

Summary and conclusion of metabolism in primary and rotational crops

Mandestrobin remains at the treated surface of the plant with very little translocation, absorption and degradation during the first 5 days after treatment under greenhouse conditions as was shown for tomato and lettuce (> 80% TRR in surface wash). With increasing time, parent compound is absorbed and metabolized as was shown by the decreasing amounts of residues found in the surface washes of field grown green rape fodder (34–37% TRR at DALT 14) and greenhouse grown wheat forage (34–41% TRR at DAT 14) and wheat straw (2.8–3.7% TRR at DAT 104).

In lettuce and tomato the TRR consisted nearly completely of unchanged parent (98–100% TRR). Parent (23–60% TRR) was also the major compound in wheat forage, wheat hay, and green rape fodder after foliar treatment, but not in wheat straw and wheat grain (DAT 104). In rotational root crop commodities (carrot roots) at PBI 30 and 120 days, parent accounted for 53–78% TRR, albeit at low absolute levels (0.015–0.040 mg eq/kg). For applications with increasing PHI and/or where uptake via the soil is possible, a larger variety of metabolites occur. De-Xy-mandestrobin was the major and only identified compound in wheat grain (61% TRR) and it was also the major compound in primary wheat straw (12% TRR). Both parent and De-Xy-mandestrobin were found at low levels (< 0.005 mg eq/kg) in seed treated feed commodities (soya bean forage, maize fodder). Compounds at levels above 10% TRR were free and (malonyl)glucoside conjugated 4-OH-mandestrobin (3.4–36% TRR), 2-CH_2OH-mandestrobin (3.6–14% TRR), and 5-CH_2OH-mandestrobin (4.3–6.8% TRR). Major compounds in the confined rotational crops were free and (malonyl)glucoside conjugated 4-OH-mandestrobin (1.7–24% TRR), 5-CH_2OH-mandestrobin (2.9–27% TRR) and 2-CH_2OH-mandestrobin (0.69–16% TRR). The identity of the residues in the wheat grain from field grown rotational crops remains unknown, with parent compound and 4-OH-mandestrobin detected at trace levels (< 0.001–0.008 mg eq/kg).

The metabolism of mandestrobin in primary and rotational crops is essentially the same, although quantitative levels of parent and metabolites differ.

The metabolic pathway of mandestrobin included monohydroxylation of the dimethylphenoxy ring to form 4-OH-mandestrobin followed by formation of the glucoside and (malonyl)glucoside conjugates. Oxidation of the methyl groups attached to the 2- and 5-positions on the dimethylphenoxy ring to form 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and 5-COOH-mandestrobin was followed by formation of the corresponding (malonyl)glucoside conjugates. Minor metabolic pathways included the O-demethylation of the methoxy-group of the side chain. Another metabolic pathway includes cleavage of the ether linkage to form De-Xy-mandestrobin, which can only be detected with the benzyl label.

Since a photolysis study in water indicates that significant photo-degradation may occur, the Meeting discussed whether the metabolism studies using foliar treatments conducted in a greenhouse (lettuce, wheat) were representative for uses in the field. Comparison of the nature and magnitude of residues found in green rape fodder (outdoor, DALT 14 days) and in wheat hay (greenhouse, DALT, 14 days) shows no accelerated degradation by photolysis, since parent compound accounted for 20/22% TRR and 26/22% TRR, respectively for green rape fodder and wheat hay. Although no photolytic degradation products were used as reference compounds in any of the metabolism studies, all unknowns in the metabolism studies were below 10% TRR or < 0.01 mg eq/kg, indicating that photolytic degradation products may be present at trace levels only. From this, the Meeting concluded that photolysis is not a significant form of degradation. Therefore, the metabolism studies in the greenhouse can be used in support of outdoor uses.

All plant metabolites identified in the primary and rotational crop studies were found in the rat metabolism study.

Animal metabolism

The Meeting received information on the fate of orally-dosed mandestrobin in rat, lactating goats and laying hens. Where two values are given divided by "/", they account for the [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin labels, respectively.

Metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2018.

Lactating goats were orally dosed with [Phenoxy-^{14}C]-mandestrobin or [Benzyl-^{14}C]-mandestrobin, equivalent to 13–14 ppm in the feed for 7 consecutive days. Goats were sacrificed 6 hrs after the last dose. The majority of the cumulative administered dose was recovered in urine and faeces at 35–40 and 38–42% TAR, respectively. Radioactivity recovered in tissues (liver, kidney, muscle and fat) accounted for a total of 0.27–0.33% TAR. Radioactivity accounted for 0.002–0.005% TAR in milk fat and 0.024–0.073% TAR in skimmed milk. The highest recovery found in edible tissues was in liver (0.22–0.29% TAR).

Steady state conditions in milk were achieved within 1 day of the first dose administration, indicating that mandestrobin is rapidly eliminated. TRR levels in milk were 0.006 and 0.035 mg eq/kg in the aqueous and fat fraction of milk, respectively. Highest TRR levels were found in liver (0.32/0.61 mg eq/kg), followed by kidney (0.17/0.41 mg eq/kg), fats (0.012–0.031 mg eq/kg) and loin and flank muscle (0.010–0.015 mg eq/kg).

Extractability of radioactivity from milk, kidney, muscle and fat with solvents (hexane, acetonitrile and ethyl acetate) ranged from 72–100% TRR, except liver (60–70% TRR). Only liver post-extracted solids were treated with enzymes, acid and base until less than 6% TRR remained as solids. Only 53/47 (milk fat), -/32 (skimmed milk); 31/36 (muscle), 65/37 (fat), 48/41 (liver) and 59/53 (kidney) as % TRR was identified.

Parent was the major compound identified in milk fat (33/32% TRR), goat muscle (23/18% TRR) and fat (50/23% TRR). In liver, kidney and skimmed milk lower levels of parent were found (1.6 to 7.7% TRR). A major metabolite identified in goat liver (20/11% TRR) and kidney (25/20% TRR) was free 5-COOH-mandestrobin. A second major metabolite identified in kidney was free and glucuronide conjugated 4-OH-mandestrobin (17/13% TRR). Free 2-CH_2OH-mandestrobin was found in muscle up to 10% TRR (0.0015 mg eq/kg). The major compound identified in skimmed milk was 5-CA-mandestrobin-NHM at 15% TRR (0.003 mg eq/kg). Several other metabolites were identified in various goat matrices but only at levels <10% TRR and below 0.01 mg eq/kg, apart from 2-CH_2OH-mandestrobin (up to 0.038 mg eq/kg in liver) and 2-COOH-mandestrobin (up to 0.014 mg eq/kg in liver). The fat extract from the benzyl label contained one region of 38% TRR (0.011 mg eq/kg) that could not be identified, despite exhaustive efforts.

Two groups of laying hens were orally dosed once daily for 14 consecutive days via capsules equivalent to 13 ppm in the feed. Hens were sacrificed 6–7 hours after dosing. The majority of the

administered dose was recovered in excreta (83/98% TAR). A minor part of the total radioactivity was recovered in eggs (0.21/0.18% TAR) and tissues (0.070/0.090% TAR). The highest residue concentration in tissues was found in the liver (0.29/0.30 mg eq/kg) followed by skin (0.048/0.054 mg eq/kg), fat (0.032/0.032 mg eq/kg) and muscle (0.014/0.024 mg eq/kg). Total radioactive residues in whole eggs achieved a plateau concentration of 0.11 mg eq/kg [Ph-^{14}C]-label after 11 days of dosing. Total radioactive residues in egg whites and egg yolk were not determined separately.

The residue after solvent extraction (hexane, ethyl acetate and acetonitrile) accounted for 86–96% TRR in fat, eggs and skin. In liver and muscle the extracted residue accounted for 63–66% TRR and 52–59% TRR, respectively. An additional 28–32% TRR was released from liver by sequential extraction with water, 1 M HCl, 1 M NH_3 and protease digestion. Only 19–23% (liver), 5.0–5.9% TRR (muscle), 42–59% TRR (eggs, fat) and 10–33% TRR (skin) was identified.

In eggs and hen fat, the main component of the radioactive residue was free parent, accounting for 51/33% TRR (0.058/0.025 mg eq/kg) and 50/34% TRR (0.016/0.011 mg eq/kg), respectively. All metabolites identified in egg and fat were present at levels < 0.01 mg eq/kg. In muscle and skin, parent mandestrobin and identified metabolites were found at trace levels (< 0.001 mg eq/kg).

In hen liver, parent mandestrobin accounted for 3.0/2.1% TRR (equivalent to 0.009/0.006 mg eq/kg). The main components in liver were free (8.6% TRR) and conjugated (3.6% TRR) De-Xy-mandestrobin (total of 12% TRR, equivalent to 0.036 mg eq/kg) with the [^{14}C-Bz]-label only and free (13% TRR/-) and conjugated (2.0/2.7% TRR) 4-OH-mandestrobin (total 15/2.7% TRR, equivalent to 0.045/< 0.001 mg eq/kg). Other metabolites were present at trace levels (< 0.01 mg eq/kg).

Summary and conclusion of metabolism in livestock

Parent was the major compound identified in milk fat, goat muscle and goat and poultry fat and eggs (18–51% TRR). In poultry muscle and skin, goat and poultry liver, goat kidney and skimmed milk lower levels of parent were found (1.3–7.7% TRR). Free 5-COOH-mandestrobin was identified as major metabolite in goat liver and kidney (11–25% TRR). A second major metabolite identified in kidney was free and glucuronide conjugated 4-OH-mandestrobin (13–17% TRR). Free 2-CH_2OH-mandestrobin was found in muscle up to 10% TRR. The major compound identified in skimmed milk was 5-CA-mandestrobin-NHM (15% TRR). Several other metabolites were identified in various goat matrices at levels <10% TRR and mostly below 0.01 mg eq/kg, apart from 2-CH_2OH-mandestrobin (up to 0.038 mg eq/kg in liver) and 2-COOH-mandestrobin (up to 0.014 mg eq/kg in liver).

In summary, the primary metabolic process observed in lactating goats and laying hens included a series of hydroxylations and oxidations, N-demethylation, O-demethylation, ether hydrolysis and (glucuronide) conjugation. Hydroxylation of the phenoxy ring gives 4-OH-mandestrobin, and hydroxylation of the methyl groups on the phenoxy ring gives 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. Further oxidation of the hydroxymethyl groups to the carboxylic acid gives 2-COOH-mandestrobin, 5-COOH- mandestrobin and 5-CA-2-HM-mandestrobin. Hydroxylation also occurs on the N-methyl group, to give 5-CA-mandestrobin-NHM and 5-CA-2-HM-mandestrobin-NHM. Mandestrobin is also subject to hydrolysis of the phenoxy ether link, yielding De-Xy-mandestrobin, N-demethylation of 5-COOH-mandestrobin, and O-demethylation. The primary metabolites are further metabolized by conjugation.

The metabolic profile of mandestrobin in ruminants and poultry is very similar to that of rats.

Environmental fate

The Meeting received information on hydrolysis and aqueous photolysis, aerobic degradation in soil under laboratory conditions, soil field dissipation and on field rotational crops.

Mandestrobin was shown to be stable to hydrolysis at pH 4–9. No change in ratio between the R- and S-isomers was observed. Hydrolysis is not a significant route of degradation.

Mandestrobin was extensively degraded under simulated sunlight in pH 7 water. The DT_{50} values ranged from 3 to 5 days. Major degradation products identified were mandestrobin-OR at 22–24% TAR, mandestrobin-ORC at 16–18% TAR and mandestrobin-PR at 9.3–9.6% TAR. Mandestrobin-OR and mandestrobin-PR degrade quickly with DT_{50} values of 5.1 and 2.5 days, respectively. Photolysis forms a significant route of degradation in water.

With DT_{50} values ranging from 49–64 days photolysis is not considered to be a significant route of degradation on the soil surface.

DT_{50} values for mandestrobin in aerobic soil (n = 10) under laboratory conditions ranged from 49–378 days, with a geomean DT_{50} of 151 days, indicating possible accumulation in soil. No isomerisation between the R- and S-isomer of mandestrobin under aerobic soil conditions was observed. Major degradation products identified were 5-COOH mandestrobin at 3.5–20% TAR, 2-COOH-mandestrobin at 1.6–8.6% TAR, 5-$CONH_2$-mandestrobin at 1.0–14% TAR and 2-$CONH_2$-mandestrobin at 0.4–14% TAR. Degradation products 5-COOH-mandestrobin and 2-COOH-mandestrobin degraded rapidly with DT_{50} values of 22–41 days and 18–26 days.

DT_{50} values for mandestrobin in soil (n = 9) from field dissipation studies ranged from 2.3–165 days, with the high value indicating possible accumulation of mandestrobin in soil and the need to consider a plateau value for evaluating residues in rotational crops.

The maximum seasonal rate of 0.42 kg ai/ha is based on a cGAP for rape seed for application at flowering (BBCH 62–65). Although the crop may intercept the full application at this stage, rape fodder is not fed to livestock at BBCH > 60 and will be ploughed into the soil after harvest of the seeds. Therefore the full application may end up in the soil and the crop interception factor is 1.

Based on the highest DT_{50} of 165 days in the field dissipation studies and using the formulas presented in OECD guidance (2018)[2], the soil accumulation factor is 0.275, leading to a plateau value of 0.12 kg ai/ha (0.275 × 0.42 kg ai/ha). The subsequent corrected maximum seasonal rate of 0.54 kg ai/ha (0.42 kg ai/ha + 0.12 kg ai/ha) can be used for assessing potential residues in rotational crops.

Field rotational crop studies

The Meeting received two field rotational crop studies. One study was conducted in France and Spain where mandestrobin was applied at a single rate of 0.21 kg ai/ha to the preceding crop oilseed rape at BBCH 65 (full bloom). Oilseed rape was crushed and incorporated into the soil 14 days after application. Follow-up crops lettuce, carrot, broccoli, and barley were planted at PBIs of 14, 120 and 365 days.

The second study was conducted in 2011–2012 in Fresno, CA, USA. Plots with primary crop leaf lettuce were treated with four foliar applications at BBCH 15, 17, 19 (i.e. 5, 7, 9 or more true leaves unfolded) and 49 (typical size, form and firmness) of mandestrobin at a rate of 0.42 kg ai/ha (each, with an interval of 7 days) and a total seasonal rate of 1.7 kg ai/ha. The leaf lettuce was removed one day after the last application. Follow-up crops spinach, red beets, wheat or sorghum were planted at PBIs of 101, 253 and 356 days.

Succeeding crops were harvested at their appropriate growth stages. No residues (< 0.01 mg/kg in EU trials; < 0.02 mg/kg in USA trials) of parent or metabolites De-Xy-mandestrobin and free and (malonyl)glucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin were found in any succeeding crop samples at any plant back interval.

The Meeting noted that in the first field rotational crop study the application rate (0.21 kg ai/ha) was below the corrected maximum seasonal rate of 0.54 kg ai/ha and that application was on

[2] GUIDANCE DOCUMENT ON RESIDUES IN ROTATIONAL CROPS, OECD Environment, Health and Safety Publications, Series on Pesticides No. 97, Series on Testing & Assessment No. 279, ENV/JM/MONO(2018)9

primary crop instead of bare soil. In the second field rotational crop study the application rate (4 × 0.42 kg ai/ha = 1.7 kg ai/ha) is higher than the corrected maximum seasonal rate of 0.54 kg ai/ha. However, the product was not applied to bare soil and the treated lettuce was removed from the plot 1 day after the last application. Though mandestrobin was not applied to bare soil, the Meeting concluded that the crop coverage at the first three applications would together have allowed for a total dose rate approximating 0.54 kg ai/ha of reaching the bare soil and concluded that residues in rotational crops are unlikely.

Methods of Analysis

The Meeting received description and validation data for analytical methods of plant and animal commodities for the determination of mandestrobin, De-Xy-mandestrobin and free and (malonyl)glucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin metabolites.

Multi-residue enforcement methods were available for plant commodities. Multi-residue method QuEChERS with HPLC-MS/MS detection was valid for the determination of mandestrobin in crops with high acid content (oranges, grapes), high water content (peaches), high oil content (soya bean seeds) and high starch content (wheat grains). Multi-residue method DFG-S19 with GC-MS detection was valid for the determination of mandestrobin in crops with high oil content (rape seeds). The limit of quantification (LOQ) was 0.01 mg/kg for each method. When the DFG-S19 method was combined with chiral HPLC-MS/MS detection, the R- and S-isomers of mandestrobin could be determined individually with an LOQ of 0.005 mg/kg for each isomer (lettuce, carrot roots and leaves, broccoli, green rape seed fodder, rape seed, barley grain and straw).

The analytical method for enforcement of animal commodities used acetone/water (7:3, v/v) for extraction. After clean-up, mandestrobin and De-Xy-mandestrobin were determined by HPLC-MS/MS with an LOQ of 0.01 mg/kg for each analyte. The method was successfully validated for free forms of mandestrobin and De-Xy-mandestrobin in liver, eggs and cream. Validation for muscle, fat and whole milk is desirable. The Meeting noted that for quantitative extraction of the residues from liver samples further treatment with 1 M HCl and protease is needed to liberate parent and De-Xy-mandestrobin.

Analytical methods used in the study reports used acetone/water (4:1, or 7:3, v/v) for extraction of residues from various plant commodities. The extracts were treated sequentially with 0.06–0.1 M NaOH (1–2 hours at room temperature) and beta-glucosidase (3 hours at 37 °C) to release the aglycones of (malonyl)glucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin metabolites. After clean-up residues were determined by HPLC-MS/MS. For all methods, final quantification is achieved using HPLC-MS/MS, with an LOQ of 0.01–0.02 mg/kg for each analyte.

Radiovalidation with ^{14}C labelled green rape seed fodder and rape seed from a metabolism study indicated that the extraction efficiency for acetone/water 4:1 and 7:3 is sufficient. Efficient hydrolysis of the (malonyl)glucoside conjugated hydroxylated mandestrobin metabolites could be demonstrated in green rape seed fodder but not in rape seed.

All methods for plant commodities were successfully validated for mandestrobin, De-Xy-mandestrobin and free 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin metabolites, demonstrating good reproducibility in the concentration range up to 0.2 mg/kg per analyte. Considering the single validation results in the different high acid matrices as a whole (n>5), would lead to acceptable validation of the levels expected in the residue trials (up to 3.6 mg/kg in grapes).

Analytical methods used in the study reports on animal commodities used acetonitrile and hexane for extraction of mandestrobin. After clean-up, mandestrobin was determined by HPLC-MS/MS with an LOQ of 0.02 mg/kg. The method was successfully validated for the free forms of mandestrobin in liver, kidney, muscle, fat and milk. The Meeting noted that for quantitative extraction

of residues from liver samples further treatment with 1 M HCl and protease is needed to liberate parent.

Stability of pesticide residues in stored analytical samples

The Meeting received storage stability studies in plant and animal commodities. No change in the ratio between R- and S-isomers was detected during storage.

The R- and S-isomers of mandestrobin and metabolite De-Xy-mandestrobin are stable for at least 12 months in crop commodities representative of high water (lettuce), high acid (orange, strawberry, grape, grape juice), high starch (barley grain), high protein (dry bean seed) commodity groups as well as in barley straw and at least 39 months in commodities with high oil content (rape seed) when stored at or below -18 °C. Storage stability studies with incurred residues suggest that storage stability for mandestrobin and De-Xy-mandestrobin can be extended to 538 days for grapes and grape juice, 385 days for grape raisins and 571 days for strawberries.

Metabolites 4-OH-mandestrobin and 2-CH_2OH-mandestrobin are stable for at least 26 months in crop commodities representative of high acid content (orange) and at least 12 months in crop commodities representative of high water (lettuce), high starch (barley grain), high protein (dry bean seed), high oil (rape seed) commodity groups as well as in barley straw when stored frozen at or below -18 °C.

Metabolite 5-CH_2OH-mandestrobin is stable for at least 12 months in crop commodities representative of the high water (lettuce) and high protein (dry bean seed) commodity groups as well as in barley straw when stored at or below -18 °C.

Mandestrobin (racemic mixture) is stable for at least 62 days in milk, 78 days in fat and 93 days in liver, kidney and muscle.

Definition of the residue

Residue definitions for plant commodities

Parent was the major compound (20–100% TRR) in the majority of crop commodities (tomatoes, lettuce, rape seed, green rape seed fodder, wheat forage, wheat hay). Parent was not detected or only at very low concentrations in wheat grain and wheat straw (DAT 104). De-Xy-mandestrobin was the major compound (12–61% TRR) in wheat grain and wheat straw. Both parent and De-Xy-mandestrobin were found at low levels in feed commodities grown from treated seed (soya bean forage, maize fodder).

No residues are expected in rotational crops and mandestrobin is stable under the processing conditions representing pasteurisation, cooking and sterilisation.

Supervised field trials on fruits (grapes, strawberries), oilseeds (rape seeds) and feed (green rape seed fodder, soya bean forage and fodder) show that parent compound is the major compound found. Residues at or below the LOQ were found in pulses (dry soya bean seeds). GAPs and trials on cereals were not submitted.

Suitable analytical methods for enforcement are available for mandestrobin.

The Meeting concluded that mandestrobin can be considered a suitable marker compound for enforcement purposes and decided to define the residue for enforcement/monitoring as mandestrobin. If uses are extended to include uses on cereals the residue definition for plant commodities may need to be revisited.

Besides parent, several compounds observed in the metabolism studies were considered for dietary risk assessment. These compounds include De-Xy-mandestrobin and free and malonylglucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. These metabolites were not observed in supervised field trials on dry soya bean seeds

and rape seeds and they were incidentally found at low levels up to 0.02–0.05 mg/kg in supervised field trials on grapes and strawberries at cGAP rates, but below 4% of parent. In the metabolism study on lettuce these metabolites were found at absolute levels between 0.10–1.2 mg eq/kg, but the sum of metabolites was below 6.3% of parent. The metabolites were only found at significant levels (>10% parent) in metabolism studies and supervised trials on feed items (such as forage/fodder crops of pulses, oilseeds and cereals).

Both the free and conjugated forms of metabolite 4-OH-mandestrobin are found in rat (free form <1% of the applied dose; conjugated form >30% of the applied dose in bile). Limited toxicity studies were available. Based on these studies and the high levels in bile, the (acute and long term) toxicity of the free and conjugated 4-OH-mandestrobin metabolite is considered to be covered by the parent compound. Furthermore, field residue trial data show that exposure from plant commodities is very low relative to parent compound, either <LOQ or approximating 1% of parent mandestrobin, with the exception of 1 trial.

Metabolite De-Xy-mandestrobin and the free form of metabolites 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin were found in rat (<1% of the applied dose). Limited toxicity studies were available leading to the conclusion that the acute toxicity of De-Xy-mandestrobin, free and conjugated 2-CH_2OH-mandestrobin is considered to be similar to that of the parent compound. No experimental data were available for 5-CH_2OH-mandestrobin. Quantitative structure-activity relationship (QSAR) analysis indicates that the metabolite is of similar toxicity to parent. Furthermore, the similarity with the chemical structure of 2-CH_2OH-mandestrobin allows for a read-across approach, also concluding its similarity with parent mandestrobin. Residue trial data show that exposure to these metabolites through the current uses is low relative to parent compound (<5%).

For long-term toxicity, the TTC approach could be applied for those three metabolites using Cramer Class III.

The estimated long-term exposure based on uses on fruits, fruiting vegetables, pulses and oilseeds and using maximum values found in the supervised residue trials resulted in the following maximum long-term dietary exposures:

De-Xy-mandestrobin	0.58 µg/kg bw per day
2-CH_2OH-mandestrobin (including conjugates)	0.29 µg/kg bw per day
5-CH_2OH-mandestrobin (including conjugates)	0.43 µg/kg bw per day

The Meeting noted that all estimated exposures are below the threshold of toxicological concern for Cramer Class III compounds (1.5 µg/kg bw per day for long-term risk assessment).

The Meeting concluded that De-Xy-mandestrobin and the hydroxy-mandestrobin compounds and their conjugates would not contribute significantly to the dietary exposure of mandestrobin and decided to define the residue for dietary risk assessment for plant commodities as mandestrobin.

The Meeting noted that the current uses do not lead to livestock exposure. For future uses, De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin (including conjugates) and 5-CH_2OH-mandestrobin (including conjugates) should be reconsidered, as these compounds may contribute significantly to the dietary burden and may contribute to residues of concern in animal commodities.

Residue definitions for commodities of animal origin

In animal metabolism studies parent was identified in all animal commodities, albeit at low levels in goat liver, kidney of goat and hen, hen muscle and skin (1.3–7.7% TRR). The Meeting concluded that mandestrobin parent (free) is therefore a suitable marker compound for enforcement. A suitable analytical method for determining free forms of mandestrobin is available.

The Meeting decided to define the residue for enforcement/monitoring in animal commodities as mandestrobin.

The log K_{ow} for mandestrobin is 3.4–3.5. The goat and hen metabolism studies showed a clear tendency of the parent compound to partition into the fat tissues, with a ratio of approximately 40:1 observed in hen fat and muscle and a ratio between fat and aqueous fraction in milk of 15:1. The effect was less pronounced in goat fat and muscle, but also showed a tendency to concentrate in the fat fraction. The Meeting concluded that the residue is fat-soluble.

Besides parent, several compounds observed in the metabolism studies were considered for dietary risk assessment. These compounds include De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 5-CA-2-HM-mandestrobin-NHM and 5-CA-mandestrobin-NHM.

Metabolite 5-CA-mandestrobin-NHM was found to be a major metabolite (15% TRR) in skimmed milk only, but found in absolute levels below 0.01 mg eq/kg. Metabolite 5-CA-2-HM-mandestrobin-NHM was found at low levels in liver and kidney (0.88–5.2% TRR), generally < 0.01 mg eq/kg, but up to 0.021 mg eq/kg in one kidney sample (one label). The mean contribution of this compound to the toxicologically significant residue is less than 10%. Both metabolites were therefore excluded from the residue definition for dietary risk assessment.

Other metabolites, De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, and their conjugates, were found at levels above 0.01 mg eq/kg in liver and/or kidney and they were found at levels that were higher than the parent compound. Their contribution relative to parent compound amounted to 105–571% for De-Xy-mandestrobin, 11–812% for 4-OH-mandestrobin, 32–252% for 2-CH_2OH-mandestrobin, 80–221% for 2-COOH-mandestrobin and 143–1250% for 5-COOH-mandestrobin.

The acute and long-term toxicity of 4-OH-mandestrobin and its conjugates is covered by the parent. The metabolite should be included in the definition for dietary risk assessment.

Metabolites De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin are found in rat (≤ 1.3% of the applied dose). Limited toxicity studies were available leading to the conclusion that the acute toxicity of metabolites De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin is considered to be similar to that of the parent compound and should be included in the residue definition for acute dietary risk assessment.

The Meeting decided to define the residue for **acute** dietary risk assessment in animal commodities as: the sum of parent and 4-OH-mandestrobin, De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin and their conjugates, expressed as parent compound.

The toxicological data were insufficient to conclude on the long-term toxicity for these compounds. For long-term dietary risk assessment, the TTC approach could be applied using Cramer Class III. Since there is no exposure to livestock based on the current uses, long-term exposure is 0 µg/kg bw per day, and thus below the threshold of toxicological concern for Cramer Class III compounds (1.5 µg/kg bw per day for long-term risk assessment).

The Meeting decided to define the residue for **long-term** dietary risk assessment in animal commodities as: the sum of parent and 4-OH-mandestrobin and its conjugates, expressed as parent compound.

The Meeting calculated conversion factors based on the results of the animal metabolism studies to be used to convert parent to the residue definitions for dietary risk assessment.

Table 2 Conversion factors for dietary risk assessment for animal commodities

	Acute dietary risk assessment	Long term dietary risk assessment
Milk fat	1.3	1.1
Skimmed milk	2.8	1.5
Mammalian muscle	1.7	-
Mammalian fat	1.3	-

Mammalian liver	8.1	1.2
Mammalian kidney	26	9.1
Poultry liver	7.5	4.1
Poultry muscle	3.3	2.9
Poultry fat	1.3	1.1
Poultry skin	6.5	3.6
Eggs	1.1	1.1

In summary

Definition of the residue for compliance with the MRL in plant and animal commodities and for dietary risk assessment in plant commodities: *mandestrobin*

The Meeting may revisit the residue definition with uses on cereals.

Definition of the residue for acute dietary risk assessment in animal commodities: *sum of parent, De-XY-mandestrobin, 4-OH-mandestrobin, 2-CH_2-OH-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin and their conjugates, expressed as parent compound*.

Definition of the residue for long-term dietary risk assessment in animal commodities: *sum of parent and 4-OH-mandestrobin and its conjugates, expressed as parent compound*.

The Meeting considers the residue *fat-soluble*.

Results of supervised residue trials on crops

The Meeting received supervised trials data for the foliar application of mandestrobin on grapes, strawberry and rape seed. Residue trial data were made available from Canada, Europe and the USA. Labels were available from Canada and the USA describing the registered uses of mandestrobin.

Grapes

The critical GAP for mandestrobin on grapes in Canada and the USA is a foliar application at a rate of 3 × 0.42 kg ai/ha with an interval of 10 days and a PHI of 10 days.

In the trials on grapes in Canada and the USA matching the US GAP an adjuvant was used. Residue levels in grapes (parent) in ranked order were (n = 11): 0.74, 0.79, 1.0, 1.0, 1.3, 1.4, 1.4, 1.9, 2.0, 2.4 and 3.5 mg/kg (highest individual value: 3.7 mg/kg).

The Meeting estimated a maximum residue level 5 mg/kg, an STMR of 1.4 mg/kg and an HR of 3.7 mg/kg for mandestrobin on grapes.

Strawberry

The critical GAP for mandestrobin on strawberries in Canada and the USA is a foliar application at a rate of 4 × 0.42 kg ai/ha with an interval of 7 days and a PHI of 0 days.

In the trials from Canada and the USA matching the US GAP, an adjuvant was used. Residue levels in strawberries (parent) in ranked order were (n = 8): 0.45, 0.48, 0.70, 0.82, 0.92, 1.2, 1.2 and 2.0 mg/kg (highest individual value: 2.2 mg/kg).

Based on the Canadian and US trials for strawberries, the Meeting estimated a maximum residue level of 3 mg/kg, an STMR of 0.87 mg/kg and an HR of 2.2 mg/kg for mandestrobin on strawberries.

Rape seed

The critical GAP for mandestrobin on rape seed in Canada and the USA is a single foliar application at a rate of 0.42 kg ai/ha with an instruction for growth stage (20–50% bloom, BBCH 62–65) and PHI

(35 days). The Meeting decided that trial data reflecting application at growth stage up to BBCH 69 with a PHI of 35 days are suitable for maximum residue level and STMR estimation.

Trials performed on rape seed from Canada and the USA matching the US GAP were selected based on BBCH 61–69 and a PHI of 35 days (-25%). Residue levels in rape seed (parent) in ranked order were (n = 9): < 0.01, < 0.02 (5), 0.046, 0,11, and 0.13 mg/kg.

The Meeting estimated a maximum residue level of 0.2 mg/kg and an STMR of 0.02 mg/kg.

Soya bean (dry)

Supervised trials on dry soya bean were submitted, but no GAP was provided or intended.

Soya bean forage and fodder

Supervised trials on soya bean forage and fodder were submitted, but no GAP was provided or intended.

Rape seed fodder

Supervised trials on rape seed fodder were submitted. As the GAP requires application beyond BBCH 60 and rape seed fodder beyond BBCH 60 is not suitable as livestock feed, the trials were not used to derive median or highest residues.

Fate of residues during processing

High temperature hydrolysis

The degradation of (Ph-^{14}C)-mandestrobin was studied under hydrolytic conditions at high temperatures (90–120 °C) in sterile aqueous buffers at pH 4, 5 and 6 to simulate common processing practice (pasteurisation, baking/brewing/boiling and sterilisation). No degradation was observed at any of the investigated pH and temperature ranges.

The Meeting concluded that mandestrobin is stable under hydrolytic conditions typically occurring during processing.

Residues in processed commodities

The Meeting received information on the fate of mandestrobin during processing in grapes (juice and raisins) and rape seed (refined oil and extracted meal). Considering the $LogK_{ow}$ of 3.4–3.5 a processing factor of 1.4 was not considered representative for juice. Noting that only one trial was available, and the Meeting decided not to calculate an STMR-P for grape juice.

Table 3 Estimated processing factors for the commodities considered at this Meeting

Raw commodity [STMR/HR]	Processed commodity	Individual processing factors	Mean or best estimate processing factor	STMR-P = $STMR_{RAC}$ × PF (mg/kg)	HR-P = HR_{RAC} × PF (mg/kg)
STMR grapes (parent): 1.4 mg/kg, HR: 3.7 mg/kg					
Grapes	Raisins	2.0	2.0	2.8	7.4
STMR rape seed (parent): 0.02 mg/kg					
Rape seed	Refined oil [a]	0.06	0.06	0.0012	-
	Extracted meal	0.20 [b]	0.20 [b]	0.004 [b]	-

[a] Refined, bleached, deodorized

[b] Based on parent. Though, according to metabolism studies metabolites could be expected in rape, no metabolites were observed in the field residue trials even when high levels of parent were found.

The Meeting estimated a maximum residue level of 10 mg/kg (MRL_{grapes} of 5 mg/kg × 2.0) for Grapes, dried.

Residues in animal commodities

Farm animal dietary burden

Grape pomace and rape seed meal are the only feed items relevant to the uses considered by the current Meeting. Based on the OECD diets listed in Appendix IX of the 2016 edition of the FAO manual, grape pomace (dry) is only a significant feed item in Australia. As there is no registration for use of mandestrobin on grapes in Australia, and import of grape pomace would not occur, the Meeting did not include grape pomace in the dietary burden for mandestrobin. Using the median value of 0.004 mg/kg for rape seed meal in the dietary burden calculator results in very low dietary burdens for livestock.

Table 4 Estimated maximum and mean dietary burdens of farm animals

	Animal dietary burden: mandestrobin, ppm of dry matter diet							
	US-Canada		EU		Australia		Japan	
	max	mean	max	mean	max	mean	max	mean
Beef cattle	0.0002	0.0002	-	-	0.0009	0.0009	-	-
Dairy cattle	0.0005	0.0005	0.0005	0.0005	0.0007	0.0007	-	-
Poultry – broiler	0.0007	0.0007	0.0008	0.0008	0.0002	0.0002	-	-
Poultry – layer	0.0007	0.0007	0.0005	0.0005	0.0002	0.0002	-	-

Farm animal feeding studies

The Meeting received a bovine feeding study (beef and dairy cattle), which provided information on likely residues resulting in animal tissues and milk from mandestrobin residues in animal diets.

Mandestrobin was fed via the diet to three to six dairy or beef cattle animals per dose group for 27 consecutive days. Animals were administered mandestrobin via bail feed (dairy cattle) or oral drench (beef cattle). The animals received 0 (1 animal), 25, 75, or 150 ppm in dry feed, corresponding to a calculated mean dose of 0, 0.97, 2.9 and 5.8 mg/kg bw per day in both dairy cattle (milk) and beef cattle (tissues). Animals were sacrificed 24 hours after the last dose, and tissues were analysed for residues of mandestrobin; metabolite residues were not analysed.

Mandestrobin residues were <LOQ (0.02 mg/kg) in whole milk, muscle or kidney at all dose levels, <LOQ in cream at the 25 and 75 ppm dose levels, and <LOQ in fat or liver at the 25 ppm dose level. At the 75 ppm dose level, mean and highest residues of 0.048 and 0.057 mg/kg were observed in liver and < 0.02 and 0.023 mg/kg in fat. At the 150 ppm dose level, mean and highest residues of 0.16 and 0.28 mg/kg were observed in liver, 0.033 and 0.040 mg/kg in fat and 0.021 and 0.034 mg/kg in milk cream.

The Meeting did not receive residue data from poultry feeding studies.

Animal commodity maximum residue levels

Since livestock is not significantly exposed (<1.0 × 10–3 ppm, based on rape seed meal) and mandestrobin was not observed in the dietary feeding study in lactating cows dosed at 25 ppm feed, residues of mandestrobin, including all toxicologically relevant metabolites, are not expected in milk, eggs, and livestock tissues. As a valid analytical method for determination of parent compound in animal commodities is available, the Meeting decided to estimate maximum residue levels for animal commodities.

The Meeting recommended a maximum residue level of 0.01(*) mg/kg and an STMR and HR of 0 mg/kg in mammalian meat (muscle, fat), mammalian fat, mammalian edible offal, poultry meat (muscle, fat), poultry fat, poultry edible offal, and eggs and a maximum residue level of 0.01(*) mg/kg and an STMR of 0 mg/kg in milk.

FURTHER WORK OR INFORMATION

Desirable:

- Validated analytical methods for all relevant metabolites and their conjugates in animal commodities.
- Feeding study, where all relevant metabolites are analysed if livestock dietary burdens become significant.

RECOMMENDATIONS

On the basis of the data obtained 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 compliance with the MRL in plant and animal commodities and for dietary risk assessment in plant commodities: *mandestrobin*.

The Meeting may revisit the residue definition with uses on cereals.

Definition of the residue for acute dietary risk assessment in animal commodities: the sum of parent, (2RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide (4-OH-mandestrobin) + (2RS)-2-(2-hydroxymethylphenyl)-2-methoxy-N-methylacetamide (De-XY-mandestrobin) + 2RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide (2-CH_2-OH-mandestrobin) + 2-({2-[(1RS)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid (2-COOH-mandestrobin), + 3-({2-[(1RS)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid (5-COOH-mandestrobin) and their conjugates, expressed as parent compound.

Definition of the residue for long-term dietary risk assessment in animal commodities: the sum of parent, (2RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide (4-OH-mandestrobin), and its conjugates, expressed as parent compound.

The residue is fat-soluble.

Table 5 Residue levels suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Commodity		Recommended maximum residue levels (mg/kg)		STMR(-P) (mg/kg)	HR(-P) (mg/kg)
CCN	Name	New	Previous		
FB 0269	Grapes	5	-	1.4	3.7
DF 0269	Grapes, dried (=Currents, Raisins and Sultanas)	10		2.8	7.4
MF 0100	Mammalian fats (except milk fats)	0.01(*)	-	0	0
ML 0106	Milks	0.01(*)	-	0	-
MM 0095	Meat (from mammals other than marine mammals)	0.01(*)	-	0 (muscle) 0 (fat)	0 (muscle) 0 (fat)
MO 0105	Edible offal (mammalian)	0.01(*)	-	0 (liver) 0 (kidney)	0 (liver) 0 (kidney)
PE 0112	Eggs	0.01(*)	-	0	0
PF 0111	Poultry fats	0.01(*)	-	0	0
PM 0110	Poultry meat	0.01(*)	-	0 (muscle) 0 (fat)	0 (muscle) 0 (fat)
PO 0111	Poultry, edible offal of	0.01(*)	-	0	0
FB 0275	Strawberry	3.0	-	0.87	2.2

SO 0495	Rape seed	0.2	-	0.02	-
OR 0495	Rape seed oil	-	-	0.0012	-

Table 6 Additional values for feed commodities used in the calculation of the dietary burdens

Commodity		Recommended MRL (mg/kg)		STMR(-P) (mg/kg)	HR(_P) (mg/kg)
CCN	Name	New	Previous		
SM 0495	Rape seed meal	-	-	0.004	-

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for mandestrobin is 0–0.2 mg/kg bw. The International Estimated Daily Intakes (IEDIs) for mandestrobin were estimated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2019 JMPR Report.

The IEDIs ranged from 0–2% of the maximum ADI. The Meeting concluded that long-term dietary exposure to residues of mandestrobin from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The 2018 JMPR established an ARfD for mandestrobin of 3.0 mg/kg bw for women of childbearing age. The 2018 Meeting concluded that it was not necessary to establish an ARfD for mandestrobin for the remainder of the population. The International Estimate of Short Term Intakes (IESTIs) for mandestrobin were calculated for the food commodities and their processed commodities for which HRs/HR-Ps or STMRs/STMR-Ps were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2019 JMPR Report.

The IESTIs varied from 0–4% of the ARfD for women of childbearing age. The Meeting concluded that acute dietary exposure to residues of mandestrobin from uses considered by the present Meeting is unlikely to present a public health concern.

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ROM-0071	Ando, D., Fukushima, M.,	2010	Definitive Identification of Major Conjugates of S-2200 in Wheat Hay, Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Hyogo, Japan, Study no: PLA2010A; Report no EF 2010 27, 28 April 2010

Code	Author	Year	Title, Institute & report number, Submitting manufacturer and report code, GLP/Non-GLP. Published/Unpublished
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ROR-0249	Bitter, J.	2013h	S-2200: Terrestrial Field Soil Dissipation on Bare Ground in California Valent Technical Center, Dublin, CA, USA, MRID 49068573; 201300324 Project ID: V-11–37969, 27 November 2013 GLP, unpublished
ROR-0263	Bitter, J.	2015a	Addendum to S-2200: Terrestrial Field Soil Dissipation on Bare Ground in Saskatchewan, Canada.(Freezer Storage Stability) Valent Technical Center, Dublin, CA, USA, MRID 49490303; 201500437 Project ID: VP-37955, 31 July 2015 GLP, unpublished
ROR-0264	Bitter, J.	2015b	Addendum to S-2200: Terrestrial Field Soil Dissipation on Bare Ground in Ontario, Canada.(Freezer Storage Stability) Valent Technical Center, Dublin, CA, USA, MRID 49490304; 201500438 Project ID: VP-37959, 31 July 2015 GLP, unpublished
ROR-0265	Bitter, J.	2015c	Addendum to S-2200: Terrestrial Field Soil Dissipation on Bare Ground in Georgia (Freezer Storage Stability) Valent Technical Center, Dublin, CA, USA, MRID 49490305; 201500439

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			Project ID: VP-37963, 31 July 2015 GLP, unpublished
ROR-0267	Bitter, J.	2015d	Addendum to S-2200: Terrestrial Field Soil Dissipation on Bare Ground in North Dakota (Analysis of Additional Samples and Freezer Storage Stability) Valent Technical Center, Dublin, CA, USA, MRID 49490306; 201500441 Project ID: VP-37966, 04 Augustus 2015 GLP, unpublished
ROR-0260	Bitter, J.	2015a	Addendum to S-2200: Magnitude of the residue on strawberries (freezer storage stability) Valent Technical Center, Dublin, CA, USA MRID 49490309; 201500430 Project ID: VP-38182 Addendum, 30 July 2015 [Addendum to ROR-0236] GLP, unpublished
ROR-0261	Bitter, J.	2015	Addendum to S-2200: Magnitude of the residue on grapes (freezer storage stability), Valent Technical Center, Dublin, CA, USA MRID 49490308; 201500435 Project ID: VP-38175 Addendum, 30 July 2015 [Addendum to ROR-0234] GLP, unpublished
ROA-0057	Bitter, J., Gohre, K.	2017	S-2200: Response for Request for Livestock Radiovalidation Study, Valent Technical Center, Dublin, CA, USA, Report No. 201700387, 11 December 2017 GLP, unpublished
ROR-0234	Bitter, J. Foster, J., Green, C.A.	2013	Determination of S-2200 and DeXy-S-2200 in Grapes; Valent Technical Center, Dublin, CA, USA, MRID 49068676 ; 201300332, Project ID V-12–38175, (including addendum on storage stability) 10 December 2013 GLP, unpublished
ROR-0236	Bitter, J., Foster, J., Green, C.A.	2013a	S-2200: Magnitude of the residue on strawberries Valent Technical Center, Dublin, CA, USA MRID 49068678; 201300329 Project ID V-12–38182, (includes addendum on storage stability) 6 December 2013 GLP, unpublished
ROA-0040	Chen, C.	2013	Independent Laboratory Validation for the Determination of S-2200 and De-Xy-S-2200 in Grapes and Canola Seed using LC-MS/MS; Critical Path Services, Garnet Valley, PA, USA, MRID 49068659; 201300348, Project ID: 13-CPS-020, 16 December 2013 GLP, unpublished
ROP-0039	Crane, M.	2012b	S-2200TG: Determination of Physical State, Colour and Odour and Relative Density, Smithers Viscient (ESG) Ltd, Harrogate, North Yorkshire, UK Study no. 8262798, November 2012 GLP, unpublished
ROR-0290	Dale, T., & Chambers, H.	2018	Determination of the tissue and milk residues profile of mandestrobin following oral administration to lactating dairy cattle and non-lactating beef cattle, Invetus Pty Ltd, Armidale Research Centre, NSW, Australia, Study No. SUMB3298, Sponsor study No. F16-002–2200-CATTLE-RES2, 25 January 2018 GLP, unpublished
ROA-0005	Daneva, E.	2010	Adaptation and Validation of Multi-Method DFG S19 for the Determination of Residues of S-2200 in Seeds of Oilseed Rape, Eurofins Dr. Specht GLP, Hamburg, Germany,

Code	Author	Year	Title, Institute & report number, Submitting manufacturer and report code, GLP/Non-GLP. Published/Unpublished
			Specht Study No: SUM-1011V Specht File Reference: G10–0061; EASSM No: S10–00854; 28 June 2010 GLP, unpublished
ROA-0010	Daneva, E., Breyer, N., Taeufer, A.	2011	Validation of an analytical method for the determination of S-2200 metabolite, De-Xy-S-2200, in seeds of oilseed rape, barley (grain and straw) and lettuce (head); Eurofins Dr. Specht GLP GmbH, Hamburg, Germany, Study Code: S10–02910, Specht Internal Code: SUM-1023V Specht File Reference: G10–0204 28 February 2011 GLP, unpublished
ROA-0012	Daneva, E., Breyer, N., Taeufer, A.	2011	Validation of an analytical method for the determination of S-2200 metabolites, 2-CH_2OH-S-2200 and its conjugates, in seeds of oilseed rape, barley (grain and straw) and lettuce (head); Eurofins Dr. Specht GLP GmbH, Hamburg, Germany, Study Code: S10–02909; Specht Internal Code SUM-1022V, Specht File Reference G10–0203 3 May 2011 GLP, unpublished
ROR-0007	Daneva, E., Taeufer, A.	2011	Freezer storage stability study of S-2200 (its optical isomers of S-2167 (*R*-isomer) and S-2354 (*S*-isomer)) in seeds of oilseed rape Eurofins Dr Specht GLP GmbH, Hamburg, Germany Specht Report no. SUM-1012, Specht File Reference, G10–0062 EASSM Code: S10–00855 1 July 2011 GLP, unpublished
ROR-0009	Daneva, E., Taeufer, A.	2011a	Freezer storage stability study of S-2200 (its optical isomers of S-2167 (*R*-isomer) and S-2354 (*S*-isomer)) in/on high-water and dry crops over 12 months, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Study code S10–01949 EAS Chem Internal Code: SUM-1017 17 November 2011 GLP, unpublished
ROA-0011	Daneva, E., Taeufer, A.	2011	Validation of an analytical method for the determination of S-2200 metabolites, 4-OH-S-2200 and its conjugates, in seeds of oilseed rape, barley (grain and straw) and lettuce (head); Eurofins Dr. Specht GLP GmbH, Hamburg, Germany, Study Code: S10–02908; Specht Internal Code: SUM-1021V, Specht File Reference: G10–0202 15 April 2011 GLP, unpublished
ROR-0011	Daneva, E., Taeufer, A.	2012	Freezer storage stability study of S-2200 metabolite, De-Xy-S2200, in lettuce (head), seeds of oilseed rape and barley (grain and straw) over 12 months, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Study code: S10–02911 EAS Chem Internal Code: SUM-1024, 30 March 2012 GLP, unpublished
ROA-0013	Daneva, E., Taeufer, A.	2011	Validation of an analytical method for the determination of S-2200 metabolites, 5-CH_2OH-S-2200 and its conjugates, in barley (grain and straw) and lettuce (head); Eurofins Dr. Specht GLP GmbH, Hamburg, Germany, Study Code S10–03385; Specht Internal Code: SUM-1027V,

Code	Author	Year	Title, Institute & report number, Submitting manufacturer and report code, GLP/Non-GLP. Published/Unpublished
			Specht File Reference: G10–0298 6 May 2011 GLP, unpublished
ROR-0012	Daneva, E., Taeufer, A.	2012a	Freezer storage stability study of S-2200 metabolite, 4-OH-S-2200, in lettuce (head), seeds of oilseed rape and barley (grain and straw) over 12 months, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany, Study code: S10–02912 EAS Chem Internal Code: SUM-1025, 30 March 2012 GLP, unpublished
ROR-0013	Daneva, E., Zetzsch, A.	2012	Freezer storage stability study of S-2200 metabolite, 2-CH_2OH-S-2200, in lettuce (head), seeds of oilseed rape and barley (grain and straw) over 12 months, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany, Study Code S10–2913 EAS Chem Internal Code. SUM-1026, 30 March 2012 GLP, unpublished
ROR-0014	Daneva, E., Zetzsch, A.	2012a	Freezer storage stability study of S-2200 metabolite, 5-CH_2OH-S-2200, in lettuce (head) and barley (grain and straw) over 12 months, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Study Code: S10–03386 EAS Chem Internal Code: SUM-1028, 30 March 2012 GLP, unpublished
ROA-0025	Daneva, E. & Zetzsch, A.	2012	Validation of Modular Multiple Analytical Method DFG S19 (Extended and Revised Version) for Determination of Residues of S-2200 in Seeds of Oilseed Rape, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany, Study Code: S10–00846; EAS Chem Internal Code: SUM-1010V, 26 March 2012 GLP, unpublished
ROR-0008	Delmotte, R.	2011	Magnitude of the Residue of S-2200 25% SC and its metabolites in Winter Rape Seed Raw Agricultural Commodity after foliar application – Northern and Southern Europe-2010 Staphyt, Inchy-en-Artois, France, Eurofins Dr. Specht GLP GmbH, Hamburg, Germany, Staphyt study no. FLN-10–6267 Eurofins Project ID S10–10724, 6 September 2011 GLP, unpublished
ROM-0027	Dixon, K., Gilbert, J.	2007	[^{14}C]S-2200: Nature of the residue (high temperature hydrolysis) study Covance Laboratories Ltd. Harrogate, North Yorkshire, UK Study no. & Report No 8239214, June 2011 GLP, unpublished
ROP-0041	Foster, B., Crane, M.	2013	Amended Final Report 1 S-2200TG: Determination of the Solvent Solubility Smithers Viscient (ESG) Ltd, Harrogate, North Yorkshire, UK Study no. 8262799 Amended Report 1 of February 2013 Replaces original report of February 2012. GLP, unpublished
ROR-0282	Gemrot, F.	2017	Magnitude of the residue of mandestrobin, tebuconazole and their metabolites in Oilseed rape (Raw Agricultural Commodity) in Northern Europe – 2015 SGS AGRI MIN, Bruguières, France Study No. 15SGS013, 30 March 2017 GLP, unpublished
ROA-0030	Göcer, M	2012	Validation of an Analytical Method for the Determination of S-2200 in Various Crop Types for Post-Registration Control and Monitoring Purpose; PTRL Europe, Ulm, Germany,

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			Study ID: P 2721 G, 25 September 2012 GLP, unpublished
ROM-0050	Gohre, K.	2013	S-2200: Degradation Under Aerobic Conditions in Soil, Valent Technical Center, Dublin, CA, USA, MRID 49068559; 201300302; Project ID: VP-37114, 13 November 2013 GLP, unpublished
ROA-0039	Gohre, K., Aston, J.	2013	Radiovalidation of Residue Methods for S-2200 and its Metabolites, Valent Technical Center, Dublin, CA, USA, MRID 49068657; 201300186; Project ID: VP-38071, 30 August 2013 GLP, unpublished
ROM-0019	Graham R., Gilbert, J.	2010	[^{14}C]S-2354 (S-2200 S-isomer): Photodegradation on a Soil Surface – Amendment 1 to Final Report Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Covance Study Number: 8200197 Amendment 1, 22 December 2010 GLP, unpublished
ROM-0020	Graham R., Gilbert, J.	2011a	[14C]S-2354 (S-2200 S-isomer): Photodegradation on a Soil Surface Amendment 1 to Final Report Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Covance Study Number: 8200202 Amendment 1, 27 January 2011 GLP, unpublished
ROM-0028	Graham, R., Gilber, J.	2011b	[^{14}C]S-2167 (S-2200 *R*-isomer): Aerobic soil metabolism and degradation Covance Laboratories Ltd,, Harrogate, North Yorkshire, UK Study Number: 8200203, Sponsor ID 1002463, September 2011 GLP, unpublished
ROM-0029	Graham, R., Gilbert, J.	2011c	[^{14}C]S-2354 (S-2200 *S*-isomer): Aerobic soil metabolism and degradation Covance Laboratories Ltd,, Harrogate, North Yorkshire, UK Study Number: 8200207, Sponsor ID 1002463, November 2011 GLP, unpublished
ROM-0030	Graham, R., Gilbert, J.	2011d	[^{14}C]S-2167 (S-2200 *R*-isomer): Aerobic degradation in two soils Covance Laboratories Ltd,, Harrogate, North Yorkshire, UK Study Number: 8229193, Sponsor ID 1002463, July 2011 GLP, unpublished
ROM-0031	Graham, R., Gilbert, J.	2011e	[^{14}C]S-2354 (S-2200 *S*-isomer): Aerobic degradation in two soils Covance Laboratories Ltd,, Harrogate, North Yorkshire, UK Study Number: 8229194, Sponsor ID 1002463, November 2011 GLP, unpublished
ROR-0238	Green, C.A.	2013	S-2200: Magnitude of the residue on canola grown in the United States Valent Technical Center, Dublin, CA, USA MRID 49068671; 201300343 Project ID: VP-37238 (including addendum on storage stability) 13 December 2013 GLP, unpublished
ROR-0239	Green, C.A	2013	S-2200: Magnitude of the residue on canola grown in Canada Valent Technical Center, Dublin, CA, USA, MRID 49068672; 201300344 Project ID VP-37284, 13 December 2013 GLP, unpublished
ROR-0240	Green, CA	2013	S-2200: Residues in rotational crops following application of S-2200 SC to leaf lettuce. Valent Technical Center, Dublin, CA, USA Project ID VP-38087 MRID 49068682; 201300345, 13 December 2013 GLP, unpublished
MRID 49068568	Green, CA	2013	S-2200: Terrestrial Field Soil Dissipation on Established Turfgrass in California, Valent Technical Center, Dublin, CA, USA, Project ID V-11–37948 MRID 49068568, 201300334, 11 December 2013

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			GLP, unpublished
ROR-0258	Green, C.A.	2015	Addendum to S-2200: Magnitude of the residue on canola grown in the United States (freezer storage stability) Valent Technical Center, Dublin, CA, USA MRID 49490307; 201500429 Project ID: VP-37238 Addendum, 29 July 2015 [Addendum to ROR-0238] GLP, unpublished
ROR-0259	Green, C.A.	2015a	Addendum to S-2200: Magnitude of the residue on canola grown in the United States (freezer storage stability) Valent Technical Center, Dublin, CA, USA MRID 49705601; 201500498 Project ID: VP-38238 Addendum, 17 November 2015 [Addendum to ROR-0238] GLP, unpublished
ROR-0274	Green, C.A.	2016	Addendum to S-2200: Magnitude of the residue on canola grown in the United States (freezer storage stability) Valent Technical Center, Dublin, CA, USA MRID 49957801, 201600578 Project ID: VP-37238 Addendum, 15 November 2016 [Addendum to ROR-0238] GLP, unpublished
ROM-0039	Hardwick, T.	2012b	Amended Final Report 2: [^{14}C]S-2200-Absorption, distribution, metabolism and excretion following repeated oral administration to the lactating ruminant, Covance Laboratories Ltd, Harrogate, United Kingdom Covance Study no. 8227546, Client identifier 1002463, Amended final report 2 of 9 September 2013 replaces amended final report 1 of 23 October 2012 and original report of 26 January 2012 GLP, unpublished
ROM-0040	Hardwick, T.	2012a	Amended Final Report 1: [^{14}C]S-2200-Absorption, distribution, metabolism and excretion following repeated oral administration to the laying hen, Covance Laboratories Ltd, Harrogate, United Kingdom Covance Study no 8227547, Client identifier 1002463, Amended final report of 23 October 2012 replaces original report of 24 January 2012 GLP, unpublished
ROM-0001	Ichise, K., Fujisawa, T., Matobo, Y., Katagi, T.	2007	Metabolism of S-2200 in Tomato (Preliminary Study). Environmental Health Science Laboratory Sumitomo Chemical Co. Ltd, Takarazuka, Hyogo, Japan. Study No. PLA2007B, 17 December 2007. non-GLP, unpublished
ROA-0047	Ivanov, E.; Kissmann H	2015	Validation of the Multi-Residue Method QuEChERS for the determination of mandestrobin (S-2200) in Soybean seeds, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany EAS Study No. S15-00287; SUM-1501V, 7 August 2015 GLP, unpublished
ROM-0055	Jalal MAF	2010	Residues in Crops Grown from Seeds Treated with RS-[Benzyl-^{14}C] S-2200 and RS- [Phenoxy-^{14}C]-S-2200. Valent Technical Center, Dublin, CA, USA Project ID VP-37088 MRID 49068641; 201000196, 13 May 2010 GLP, unpublished
ROM-0056	Jalal MAF	2013	Characterization of Radioactive Residues of [Benzyl-^{14}C]S-2200 and [Phenoxy-^{14}C]S-2200 in Corn and Soybean Samples from Study VP-37088 Valent Technical Center, Dublin, CA, USA

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			Project ID VP-38307 MRID 49068640; 201300072, 18 March 2013 GLP, unpublished
ROM-0035	Jarvis T, Callow B, Mamouni A	2012	Calculation of laboratory soil kinetics for S-2200 and its major metabolites according to FOCUS (2006) Guidance Exponent international Ltd., Harrogate, Yorkshire, UK 8 July 2012 non-GLP, Unpublished
UK0–4844	Jarvis T, Montesano V	2019	Recalculation of North American soil degradation/dissipation kinetics for mandestrobin according to FOCUS (2006, 2014) Guidance Exponent international Ltd., Harrogate, Yorkshire, UK Report 1700868.UK0–4844, 31 July 2019 non-GLP, Unpublished
ROR-0280	Klimmek S., Gizler, A.	2017	Determination of Residues of Mandestrobin in Soybean Following Three Applications of S-2200 in Brazil in 2014 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany EAS Study No. S14-05177; (SUM-1406), 06 April 2017 GLP, unpublished
ROR-0198	Lebrun, F.	2012	Magnitude of the Residue of S-2200 25% SC and its metabolites in Winter Rape Seed Raw Agricultural Commodity after foliar application – Northern and Southern Europe-2011 SGS Institut Fresenius GmbH, Taunusstein, Germany Eurofins Agroscience Services, Hamburg, Germany SGS study no. IF-11/01898756 EAS Chem Project Identity Number, S11–01242, 14 June 2012 GLP, unpublished.
ROP-0001	Lentz, N.R., Van Meter III, D.S.	2009a	Determination of the water solubility of S-2200 PAI, Springborn Smithers Laboratories, Wareham, Massachusetts, USA Study no. 13048.6606, 24 November 2009 GLP, unpublished
ROP-0002	Lentz, N.R., Van Meter III, D.S.	2009b	Determination of the water solubility of S-2167 PAI Springborn Smithers Laboratories, Wareham, Massachusetts, USA Study no. 13048.6607, 24 November 2009 GLP, unpublished
ROP-0003	Lentz, N.R., Van Meter III, D.S.	2009c	Determination of the water solubility of S-2354 PAI Springborn Smithers Laboratories, Wareham, Massachusetts, USA Study no. 13048.6608, 25 November 2009 GLP, unpublished
ROP-0014	Lentz, N.R., Van Meter III, D.S.	2011a	S-2200 PAI – Determination of the solvent solubility, Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6610, 4 February 2011 GLP, unpublished
ROP-0015	Lentz, N.R., Van Meter III, D.S.	2011b	S-2167 PAI – Determination of the solvent solubility Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6611, 4 February 2011 GLP, unpublished
ROP-0016	Lentz, N.R., Van Meter III, D.S.	2011c	S-2354 PAI – Determination of the solvent solubility Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6612, 4 February 2011 GLP, unpublished
ROP-0017	Lentz, N.R., Van Meter III, D.S.	2011e	Determination of the Thermal Explodablity of S-2200 TGAI, Smithers Viscient, Wareham, Massachusetts, USA Study No.: 13048.6616, 4 February 2011 GLP, unpublished
ROR-0006	Leslie, S.	2011a	S-2200 and metabolites: Storage stability of residues in EU soil stored deep frozen Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Study number 8202025, March 2011 GLP, unpublished
ROA-0014	Leslie, S.	2011b	S-2200 and metabolites: Validation of an analytical method for residues in EU soil Covance Laboratories Ltd, Harrogate, North Yorkshire, UK

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			Study number 8213772, March 2011 GLP, unpublished
ROM-0005	Lewis, C.J., Alderman, D	2010a	[^{14}C]S-2167 (S-2200 R-isomer): Hydrolytic stability Covance Laboratories Ltd, Harrogate, United Kingdom Covance Study no. 8200206, Report no 8200206-D2149 Sponsor ID 1002463, April 210 GLP, unpublished
ROM-0006	Lewis, C.J., Alderman, D	2010b	[^{14}C]S-2354 (S-2200 S-isomer): Hydrolytic stability Covance Laboratories Ltd, Harrogate, United Kingdom Covance Study no. 8200194, Report no 8200194-D2149 Sponsor ID 1002463, April 2010 GLP, unpublished
ROM-0011	Lewis, C.J., Alderman, D.	2010d	Amended Final Report 1 [^{14}C]S-2354 (S-2200 S-isomer): Photodegradation and quantum yield in sterile, aqueous solution. Covance Laboratories Ltd, Study no. 8200195, Sponsor ID 1002463, November 2010 Amended report of November 2010, replaces original report of October 2010 GLP, unpublished
ROM-0013	Lewis, C.J., Alderman, D.	2010c	[^{14}C]S-2167 (S-2200 *R*-isomer): Photodegradation and quantum yield in sterile, aqueous solution, Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Study no. 8200199, Sponsor ID 1002463, October 2010 GLP, unpublished
ROR-0010	Lewis, C.J.	2012	S-2200: The dissipation of residues in soil in Northern and Southern Europe, Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Study no. 8202031, Sponsor ID 1002463, January 2012 GLP, unpublished
ROM-0017	Lewis, C.J., Gilbert, J.	2010	[^{14}C]2-COOH- S-2200: Aerobic degradation in Three Soils, Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Study no. 8213033, Sponsor ID 1002463, December 2010 GLP, unpublished
ROM-0018	Lewis, C.J., Gilbert, J.	2010	[^{14}C]5-COOH- S-2200: Aerobic degradation in Three Soils, Covance Laboratories Ltd, Harrogate, North Yorkshire, UK Study no. 8213036, Sponsor ID 1002463, December 2010 GLP, unpublished
ROR-0269	Lindner, M. Grewe, D.	2016	Interim report Storage Stability of residues of mandestrobin and its metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH_2OH-S-2200 in dried beans and orange fruit, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Study Code S15-01208, EAS Chem Internal Code: SGS-1507L 6 July 2016 GLP, unpublished
ROR-0286	Lindner, M., Grewe, D., Leischow, J.	2017	Final report Storage Stability of residues of mandestrobin and its metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH_2OH-S-2200 in dried beans and orange fruit, Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Study code:. S15-01208, EAS Chem Internal Code: SGS-1507L 18 October 2017 GLP, unpublished
ROM-0051	Maurer J., Gohre, K.	2013	S-2200: Degradation Under Aerobic Conditions in Soil – Rate studies, Valent Technical Center, Dublin, CA, USA, MRID 49068560; 201300327; Project ID: VP-37202, 06 December 2013 GLP, unpublished
ROP-0029	Minamisaki, T.	2012a	Preliminary Analysis of S-2200 Technical Grade, Osaka Laboratory, Sumika Chemical Analysis Service Ltd, Osaka, Japan,

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			Study no. 12002, 31 July 2012 GLP, unpublished
ROA-0054	Nie, C.	2017	Validation of Enforcement Analytical Method for Determination of S-2200 and De-Xy-S-2200 in Animal Commodities, Valent Technical Center, Dublin, CA, USA, Report No. 20170037, Project ID. VP-41000, 6 November 2017 GLP, unpublished
ROP-0030	Onishi, T.	2012c	Stability of S-2200 Technical Grade to Normal and Elevated Temperatures, Metals and Metal Ions, Osaka Laboratory, Sumika Chemical Analysis Service Ltd, Osaka, Japan, Study no. 12001, 31 July 2012 GLP, unpublished
ROP-0048	Onishi, T.	2015	Preliminary Analysis of Mandestrobin Technical Grade (Tama Kagaku Kogyo), Sumika Chemical Analysis Service, Ltd, Study no. GP14090, 26 March 2015 GLP, unpublished
ROM-0032	Panthani, A., Connor, S., Malekani, K.	2011	Confined rotational crop study with [^{14}C]S-2200 Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6630, 18 November 2011 GLP, unpublished
ROM-0026	Panthani, A., Connor, S.	2011	Metabolism of [^{14}C]S-2200 in rape seed plants Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6618, 15 June 2011 GLP, unpublished
ROM-0008	Panthani, A., Lentz, N.R.	2010b	Metabolism of [^{14}C]S-2200 in lettuce plants, Springborn Smithers Laboratories, Wareham, Massachusetts, USA Study no 13048.6631, 1 July 2010 GLP, unpublished
ROM-0009	Panthani, A., Lentz, N.R.	2010a	Metabolism of [^{14}C]S-2200 in Wheat Springborn Smithers Laboratories, Wareham, Massachusetts, USA Study no. 13048.6619, 14 July 2010 GLP, unpublished
ROA-0055	Perez, S.	2017	Independent Laboratory Validation of Valent Analytical Method RM-48M-1: "Determination of Mandestrobin and De-Xy-S-2200 in Animal Commodities" ADPEN Laboratories, Inc., Jacksonville, FL, USA Report No. 201700378; Valent Study no: VP-41001; ADPEN Study no: 17H0104; 21 November 2017, GLP, unpublished
ROA-0062	Perez, S.	2017	Independent Laboratory Validation of Valent Analytical Method RM-48C-2B: "Determination of S-2200 and De-Xy-S-2200 in Crops" ADPEN Laboratories, Inc., Jacksonville, FL, USA Report No. 201700246; Valent Study no: VP-39377; MRID: 49957802, 18 August 2017, GLP, unpublished
MRID 49068638	Pernell M	2013	S-2200: Independent Laboratory Validation of Valent Method RM-48S-3, Determination of S-2200 and Metabolites in Soil, Critical Path Services, LLC (CPS), Garnet Valley, PA, USA Project ID 13-CPS-002; MRID 49068638, 201300037, 15 February 2015 GLP, unpublished
ROP-0021	Proctor, K.L., Lentz, N.R.,	2011a	S-2200 PAI – Determination of vapour pressure, Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6604, 18 February 2011 GLP, unpublished

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ROP-0022	Proctor, K.L., Lentz, N.R.,	2011b	S-2167 PAI – Determination of vapour pressure Smithers Viscient, Wareham, Massachusetts, USA Study no. 13048.6605, 7 March 2011 GLP, unpublished
ROR-0202	Roussel, Ch.H.	2012	Rotational field-crops residue study after application of S-2200 25 SC (25 % w/v) to winter rape seed-Northern and Southern Europe 2010–2011 STAPHYT, Inchy-en-Artois, France Staphyt Study no FLN-10–6268, EUROFINS, Dr Specht GLP GmbH, Hamburg, Germany Eurofins analytical phase no: S10–02011 CEMAS, North Ascot, Berkshire, United Kingdom CEMAS identity no: CEMS-4758 25 August 2012 GLP, unpublished
ROA-0031	Rzepka, S.	2012	Independent Laboratory Validation of an Analytical Method for the Determination of S-2200 in Peach, Grape and Barley Grain for Post-Registration Control and Monitoring Purpose, Eurofins/Dr. Specht Laboratorien, Hamburg, Germany, Study Code: SCA-1203V File Reference: M12–00288, 1 November 2012 GLP, unpublished
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ROA-0041	Schoenau E	2013	S-2200: Independent Laboratory Validation of Valent Method RM-48M-1 for the determination of S-2200 in tissue samples. Golden Pacific Laboratories, Fresno, CA, USA Project ID 130509; MRID 49068660; 201300311; 26 November 2013 GLP, Unpublished
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