Volume 3

Annex B

Mandestrobin

B.7 Residue data

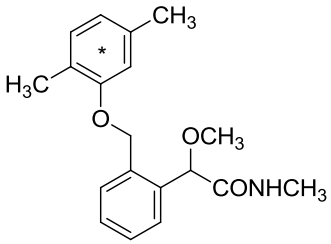
Table of contents:

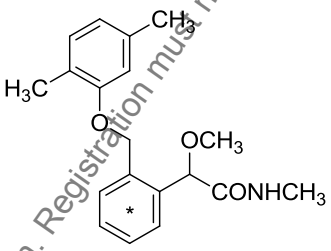
List of figures:

B.7.1 Metabolism, distribution and expression of residues in plants (Section 4, Annex IIA point 6.2.1)

S-2200 (ISO name Mandestrobin) is a new active substance and belongs to the strobilurin group of fungicides. Mandestrobin (S-2200) is a racemic mixture of two isomers: S-2200 *R*-isomer (S-2167) and S-2200 *S*-isomer (S-2354) at a rate of 50:50. S-2200 is a fungicide used for the control of white mould (Sclerotinia sclerotiorum). The envisaged use is oilseed rape. Metabolism studies have been provided on **rapeseed (oilseeds)**, **wheat (cereal crop)** and **lettuce (leafy crop)**.

Figure B.7-1: Radiolabeled Test Material



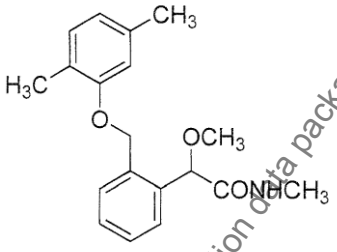


*… indicates the position of the ^{14}C-labelling

Name: **[Phenoxy-^{14}C] S-2200**
Code: CFQ40466
Radiochemical purity: 99.8%
Specific Activity: 120 mCi/mmol

Name: [**Benzyl-^{14}C] S-2200**
Code: CFQ40467
Radiochemical purity: 98.9%
Specific Activity: 123 mCi/mmol

Figure B.7-2: Non-Radiolabeled Test Material



Common Name: **S-2200 AS**
Name (IUPAC): (*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
Molecular Weight: 313.39
CAS No.: 173662-97-0
Purity: 100%

B.7.1.1 Methods

All methods used in the metabolism studies are summarised in the chapter below.

B.7.1.1.1 Combustion Analysis

Solid subsamples of dry ice ground wheat plants and post extracted solids (PES) were combusted with a Harvey Models OX-500 and OX-700 Biological Oxidizer (R. J. Harvey Instrument Corp.,Hillsdale, New Jersey) for 2 minutes. The extracted solid was dried in a hood at room temperature prior to combustion analysis. The gaseous effluent from the oxidizer was collected directly into Harvey™ oxidizer LSC cocktail (15 mL). The average LSC background using Harvey cocktail was 33 cpm and was subtracted from sample cpm. Combustion efficiency was determined with each analysis by combusting triplicate subsamples of control spiked with a known amount of [^{14}C] test substance. An identical amount of [^{14}C] test substance was added to triplicate vials of the LSC cocktail. Combustion efficiency was calculated as the ratio of the mean dpm in the spiked samples to the mean dpm in the cocktail spikes. Combustion efficiencies were 95.0% or greater. Sample dpm were corrected for combustion efficiency.

B.7.1.1.2 Liquid Scintillation Counting (LSC)

Liquid scintillation counting analysis was performed on samples to quantify radioactivity. LSC analysis was performed with a Beckman Model LS6500 Instrument (Beckman Coulter, Inc.,Fullerton, CA). Liquid samples were subsampled by volume in duplicate. Ultima Gold™ liquid scintillation cocktail (Perkin Elmer, Boston, MA) was added to each sample. Counts per min (cpm) were converted to disintegrations per min (dpm) based on a quench curve stored in the instrument's microprocessor. All quench curves were generated from NIST traceable standards. The instrument was operated in a manual background-subtract mode, therefore appropriate backgrounds were used for background subtraction.
A *detection limit* of two and one-half (2.5) times background for LSC analysis permitted detection of radioactivity in samples at approximately 0.001 µg/mL (1*00 dpm / 200,000 dpm/µg) / 0.50 mL = 0.001 µg/mL)*

B.7.1.1.3 Thin Layer Chromatography (TLC)

Reference standards (20 to 40 µg) were applied to the loading zone of the TLC plate, separately in adjacent lanes. The samples were air-dried then developed for a total distance of approximately 15 cm above the loading zone. The following equipment and conditions were used for the TLC analysis.

Bioscan AR2000 Imaging Scanner (Bioscan, Inc., Washington, D.C.)
Shortwave UV Light (Spectroline Model XX-15NF)
Plates: Whatman LK5DF, 250-µm thickness, 20 × 20 cm
Eluant 1: Chloroform:Methanol, 9:1, v:v
Eluant 2: n-Hexane:Isopropanol, 5:2, v:v
Eluant 3: Ethyl acetate:methanol:acetic acid, 18:2:1, v:v:v

Plates and Eluants used in the succeeding crop studies:

Plates: Whatman LK5DF, 250-µm thickness, 20 × 20 cm
Whatman Cellulose, 250-µm thickness, 20 × 20 cm
Eluant 1: Chloroform:Methanol, 9:1, v:v
Eluant 2: Ethyl acetate:methanol:acetic acid, 18:2:1, v:v:v
Eluent 3: n-Butanol:PRW:acetic acid, 6:1:1, v:v:v
Eluent 4: n-Butanol:acetic acid:tert-butylmethylether:PRW, 9:6:3:1, v:v:v:v
Eluent 5: n-Hexane:isopropanol, 5:2, v:v

B.7.1.1.4 High-Performance Liquid Chromatography (HPLC)

HPLC analyses were conducted on all samples using an in-line radioactivity detector (HPLC/RAM). Radioactivity was detected in the effluent using a liquid scintillation cell. The effluent was passed through the UV detector and then through the radioactivity flow detector. Percentages of radioactivity in the separated components were quantified by integrating the peaks.
For selected analyses, recovery of radioactivity from the HPLC column was determined. The amount of radioactivity injected onto the column was determined by LSC analysis of aliquots of the sample. The amount of radioactivity eluting from the column was determined by collecting the eluate from the run followed by LSC analysis. Recovery of radioactivity was calculated as the ratio of the radioactivity collected to the radioactivity injected. A *detection limit for HPLC analysis* of three times the background response in the vicinity of S-2200 permitted detection of radioactivity in samples of at least 0.011 µg/mL *(450 dpm /200,000 dpm/µg) / 0.2 mL = 0.011 µg/mL*
The following equipment and conditions were used for the HPLC analysis.
Agilent quaternary pump Series 1200 equipped with an Agilent Series 1200 variable wavelength detector
Agilent Series 1200 degasser
Agilent autosampler Series 1200, EZ Chrom Elite Version 3.2.1.31 or 3.3.2
Perkin Elmer radiochemical detector Model 625TR with Pro FSA software Version 3.4.3.36b
or
Hewlett Packard quaternary pump Series 1100 equipped with a
Hewlett Packard Series 1100 variable wavelength detector
Hewlett Packard Series 1100 degasser
Hewlett Packard autosampler Series 1100, EZ Chrome Elite Version 3.2.1.31
Packard Series 525TR radiochemical detector with FLO-ONE Software Version 3.65.

HPLC Method 1 (Typical HPLC Analysis)
Column: Zorbax Eclipse XDB-C18, 150 × 4.6 mm (Agilent Technologies, Inc.)
Flow rate: 1.0 mL/min
UV Detection: 254 nm
Solvent A: 0.1% Formic Acid in purified water
Solvent B: 0.1% Formic Acid in Acetonitrile
Radiochemical Flow: 2.0 mL/min
Cocktail type: Ultima-Flo M (Perkin Elmer)
Injection Volume: 100 µL
Detection: 500 µL Liquid Cell
Gradient table 1:

Time (min)	% A	% B
0	95	5
2	95	5
10	70	30
30	65	35
40	0	100
45	0	100
50	95	5
58	95	5

Retention time for S-2200: 39.1 min

HPLC Method 2 (Chiral Analysis)
Column: Chiralpak AD RH, 5-µm particle size, 150 × 4.6 mm (Daicel Chemical Industries)
Flow rate: 1.0 mL/min
UV Detection: 254 nm

Solvent: Purified water:Acetonitrile, 50:50, v:v (Isocratic analysis for 15 minutes)
Retention time for S-2200 isomers: 6.5 min (R-Isomer S-2167)
8.2 min (S-Isomer S-2354)

HPLC Method 3 (to profile [^{14}C]14-min region)
Column: Zorbax Eclipse XDB-C18, 150 × 4.6 mm (Agilent Technologies, Inc.)
Flow rate: 1.0 mL/min
UV Detection: 254 nm
Solvent A: 0.1% Formic Acid in purified water
Solvent B: 0.1% Formic Acid in Acetonitrile
Radiochemical Flow: 2.0 mL/min
Cocktail type: Ultima-Flo M (Perkin Elmer)
Injection Volume: 100 µL
Detection: 500 µL Liquid Cell
Gradient table 3:

Time (min)	% A	% B
0	95	5
2	95	5
10	75	25
45	75	25
48	95	5
55	95	5

HPLC Method 3 (modified to profile final harvest extracts)
Column: Zorbax Eclipse XDB-C18, 150 × 4.6 mm (Agilent Technologies, Inc.)
Flow rate: 1.0 mL/min
UV Detection: 254 nm
Solvent A: 0.1% Formic Acid in purified water
Solvent B: 0.1% Formic Acid in Acetonitrile
Radiochemical Flow: 2.0 mL/min
Cocktail type: Ultima-Flo M (Perkin Elmer)
Injection Volume: 100 µL
Detection: 500 µL Liquid Cell

Gradient table 3:

Time (min)	% A	% B
0	95	5
2	95	5
10	75	25
45	75	25
48	95	5
55	95	5

HPLC Method 4 (to profile final harvest extracts in succeeding crop studies)
Column: Phenomenex Synergi Polar RP, 5µm, 250 × 4.6 mm
Guard Column: Phenomenex Synergi Polar RP cartridge
Temperature: ambient
Flow Rate: 1.0 mL/min
UV Detection: 254 nm
Solvent A: 0.1% Trifluoroacetic Acid in purified water
Solvent B: 0.1% Trifluoroacetic Acid in Acetonitrile

Radiochemical Flow: 2.0 mL/min
Cocktail type: Ultima-Flo^{TM} M
Injection Volume: 100 - 200 µL

Detection: 500 µL Liquid Cell
Gradient table 4:

Time (min)	% A	% B
0	95	5
2	95	5
15	75	25
50	75	25
60	65	35
68	60	40
80	35	65
85	10	90
90	10	90
95	95	5
100	95	5

Retention time for S-2200: 80min

Analytical methods (HPLC and TLC) were validated with authentic standards and were shown to achieve the necessary resolution and sensitivity. HPLC column performance and chromatographic resolution were validated with authentic labeled and non-labeled standards. Radioactive detector and TLC Imaging scanner were calibrated with ^{14}C standards.

B.7.1.2 Metabolism in Rapeseed

Reference:	**Metabolism of [^{14}C]S-2200 in Rapeseed Plants**
Author(s), year:	Panthani A, Connor S. (2011)
Report/ Doc. Number:	Smithers Viscient Report no. 13048.6618 Sumitomo ref: ROM-0026
Guideline(s):	Japanese MAFF, 12-Nousan-No. 8147 US EPA OPPTS 860.1300 Nature of the Residue - Plants, Livestock; EU Document 7028/VI/95 rev. 3 (22/07/97), Appendix A OECD 501 - Guideline for the Testing of Chemicals - Metabolism in Crops
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Test Site information:
Test site: Madera, California
Testing environment: outdoor test plots
Relevant environmental characteristics: San Joaquin Valley Region
Soil characteristics:

pH	7.5
CEC (meq/100g)	10.2
OM (%)	1.2
Sand (%)	71
Silt (%)	17
Clay (%)	12

Soil classification	sandy loam

Crop information:
Crop / crop group: rapeseed/oilseeds
Variety: Phoenix Liberty Link
Test Material:
Common Name: S-2200
Name (IUPAC): (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers: S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight: 313.39
CAS No.: 173662-97-0
Purity: 100%
*Log $P_{o/w}$= 3.51 (*at 25 ± 1°C)
Radiolabel position, specific activity : [Phenoxy-^{14}C] S-2200 (120 mCi/mmol)
[Benzyl-^{14}C] S-2200 (123 mCi/mmol)
Formulation: 25% SC (suspension concentrate)
Specific activity in formulation: 200,000 dpm/µg

B.7.1.2.1 Material and Methods

The study was designed to simulate field application of [^{14}C]S-2200 to rapeseed plants to identify major components of the residue and their distribution in the plant. The study was carried out under outdoor growing conditions using two radiolabeled forms of [^{14}C]S-2200, [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 as a 25% SC formulation.
Eight outdoor plots, each consisting of above ground wooden boxes (3x3 feet) filled with sandy loam soil to a depth of approx. 1 foot were used. Each plot contained 6 rows spaced 6 inches apart. The Rapeseeds, variety "*Phoenix Liberty Link*", were planted and maintained under normal agricultural practice. The plants were overseeded and thinned later to approx. 2 inch spacing. Plastic sheeting approx. 7 feet (2 meters) high was erected all around the plots to block wind.
A set of three containers were used for each radiolabel. Each set consisted of one container treated once and two containers treated twice with [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 formualted as a 25% SC. The application rate for each application and radiolabel was equivalent to a rate of 400 g a.s./ha (2-4x the proposed GAP), using a spray volume equivalent to 400 L/ha.
The test substance was applied by manual-operated pump sprayer. The application rate was verified by LSC of the formulated test substances. After dosing, the empty spray bottles that had contained the formulation were rinsed with 10 mL of acetonitrile and duplicate 0.050 mL or 1.0 mL aliquots were assayed by LSC.
The field phase was conducted from May 2009 to August 2009. There were three treatment groups (I, II and III, IV and V), a summary of the application data is given in the table below.

Table B.7.1.2.1-1: Application details

Application number		Application details			
		Label	Application rate	Date of application	Growth stage at application
Group I (control)	Plot 1,2	na	na	na	na
Group II	Plot 3,4	[phenoxy-^{14}C] S-2200	2x 400 g a.i./ha	1st : 28.5.2009 2nd: 11.6.2009	BBCH 55-61# BBCH 66-67
Group III	Plot 5	[phenoxy-^{14}C] S-2200	1x 400 g a.i./ha	28.5.2009	BBCH 55-61#

Application number		Application details			
		Label	Application rate	Date of application	Growth stage at application
Group IV	Plot 6,7	[benzyl-^{14}C] S-2200	2x 400 g a.i./ha	1st : 28.5.2009 2nd: 11.6.2009	BBCH 55-61# BBCH 66-67
Group V	Plot 8	[benzyl-^{14}C] S-2200	1x 400 g a.i./ha	28.5.2009	BBCH 55-61#

#....54 days before final harvest

Immature plants (forage) were sampled 14 days after the 2nd application at BBCH 74 (Group I, II and IV). Forage samples were collected by cutting the plants approx. 1-2 inches above the soil line. At final harvest seed heads and mature plants (straw) were sampled 40 days (Group I, II, IV) and 54 days (Group III and V) after the last application at BBCH 89. Pods were cut from the plants with pruning shears and stored separately in Ziploc bags. The remainder of the plants (i.e. straw) was cut approx. 1-2 inches above the soil line and divided into 6 inch (15cm) segments. The mature straw and pod samples were retained, but not analysed. The sampling data are summarized in the table below:

Table B.7.1.2.1-2: Sampling details

Test Group	Plant matrices sampled	Growth stage at sampling	Time interval from last application	Harvesting method
I (control), II, IV	Immature plants (forage)	BBCH 74	14 days	cutting the plants approx. 1-2 inches above the soil line
I (control), II, IV	Mature plants (seed heads)	BBCH 89	40 days	Pods cut from the plants and separated in seeds and pods
	Mature plants (straw)			cut approx. 1-2 inches above the soil line/ divided into 6 inch (15cm) segments.
III, V	Mature plants (seed heads)	BBCH 89	54 days	Pods cut from the plants
	Mature plants (straw)			cut approx. 1-2 inches above the soil line/ divided into 6 inch (15cm) segments.

The Total Radioactive Residue (TRR) in the samples were quantitated by combustion using a biological oxidizer (R. J. Harvey Instrument Co.) followed by liquid scintillation counting and by tissue extraction followed by combustion analysis of the post-extraction solids (PES).

Immature plants

Both control and treated plant tissue of immature forage samples were surface washed with acetonitrile (3x 250 mL) and then homogenised in the presence of dry ice. Homogenised forage samples were then extracted by high speed homogenisation twice with acetone/water (80:20; v/v) and once with acetone/water/hydrochloric acid (80:20:1; v/v/v). Each sample was centrifuged to separate the extracts from the post-extraction solids (PES). The pooled extracts were concentrated using rotary evaporation. The PES of the forage samples were further characterised by weak acid hydrolysis (1M HCl, 40°C, overnight), strong acid hydrolysis (6M HCl, 80°C, 4 hours), weak base hydrolysis (0.1M NaOH, 40°C, overnight) and strong base hydrolysis (6M NaOH, 80°C, overnight).

Mature plants

Mature seed samples were homogenised without prior surface wash. Homogenised samples of rape seed were extracted by high speed homogenisation 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). Each sample was centrifuged to separate the extracts from the post-extraction solids (PES). The PES of the rape seed samples were further characterised by sequential enzyme hydrolysis with amylase and protease followed by weak acid hydrolysis (1M HCl,

40°C, overnight), strong acid hydrolysis (6M HCl, 80°C, 4 hours), weak base hydrolysis (0.1M NaOH, 40°C, overnight) and strong base hydrolysis (6M NaOH, 80°C, overnight).

The radioactivity in the surface washes and the organic extracts was determined by liquid scintillation counting (LSC). The radioactivity in the initial sample matrix and the PES were quantified by LSC after combustion. The distribution of radioactivity in oilseed rape forage and seeds was shown to be similar between the two radiolabels.
In forage, the surface wash removed 34-37% of the radioactivity. The majority of the recovered radioactivity was present in the extractable fractions, the radioactive concentration accounted for 2.01-2.19 mg/kg (55-58% TRR) in forage after two applications, 0.40- 0.64 mg/kg (85-100% TRR) for rape seed after two applications and for 0.04-0.10 mg/kg (81-91% TRR), for rape seed after one application. The un-extractable radioactivity represented 0.26-0.34 mg/kg (8% TRR) in forage after two applications, <0.01-0.07 mg/kg (0.1-15 % TRR) in rape seed after two applications and 0.01 mg/kg (9-19 % TRR) in rape seed after one application.The distribution of radioactive residues in the acetonitrile surface wash (forage only), hexane extracts (seed samples only), acetone/water (80:20; v/v) extracts, acetone/water/hydrochloric acid (80:20:1; v/v/v) extract and post-extraction solids (PES, unextractable material) are shown in the table below.

Table B.7.1.2.1-3: Distribution of [^{14}C]S-2200 residues in oilseed rape (forage and seed)

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Forage 2 x 400 g/ha PHI 14 days	Surface wash	1.47	36.74	1.18	34.16
	1st Extraction with acetone/water	1.55	38.91	1.57	45.52
	2nd Extraction with acetone /water	0.46	11.50	0.32	9.16
	Extraction with acetone /water / HCl	0.18	4.46	0.13	3.64
	Sum of extracts [1)]	3.66	91.62	3.19	92.47
	PES (post extraction solids)	0.34	8.38	0.26	7.53
	Total Radioactive Residue (TRR)	**3.99**	**100**	**3.44**	**100**
Seed 2 x 400 g/ha PHI 40 days	1st Extraction with hexane	0.10	20.70	0.10	15.67
	2nd Extraction with hexane	0.02	4.88	0.03	4.87
	3rd Extraction with hexane	<0.01	1.57	0.01	1.52
	1st Extraction with acetone/water	0.19	39.50	0.24	37.85
	2nd Extraction with acetone /water	0.06	11.69	0.16	24.66
	3rd Extraction with acetone /water	not performed		0.05	7.85
	Extraction with acetone /water / HCl	0.03	7.04	0.05	7.48
	Sum of extracts	0.40	85.37	0.64	99.89
	PES (post extraction solids)	0.07	14.63	<0.01	0.11
	Total Radioactive Residue (TRR)	**0.47**	**100**	**0.64**	**100**
Seed 1 x 400 g/ha PHI 54 days	1st Extraction with hexane	<LOQ	NA	<LOQ	NA
	2nd Extraction with hexane	<LOQ	NA	<LOQ	NA
	3rd Extraction with hexane	<LOQ	NA	<LOQ	NA
	1st Extraction with acetone/water	0.03	54.50	0.07	59.37
	2nd Extraction with acetone /water	<0.01	16.17	0.02	19.97
	Extraction with acetone /water / HCl	<0.01	10.79	0.01	11.39
	Sum of extracts	0.04	81.45	0.10	90.74
	PES (post extraction solids)	<0.01	18.55	0.01	9.26
	Total Radioactive Residue (TRR)	**0.05**	**100**	**0.11**	**100**

[1)] ...including surface wash NA= not applicable LOQ= Limit of Quantification

B.7.1.2.2 Distribution, characterisation and identification of residues

The extractable fractions were profiled separately by radio-HPLC and TLC using co-chromatography with reference standards. Chiral HPLC was employed to determine the ratio of *R*- and *S*-isomers of S-2200.

The **TRR concentrations in the [phenoxy-^{14}C]S-2200** rapeseed plant samples were 3.993 mg/kg for the forage at immature harvest, and 0.469 and 0.051 mg/kg for the Group II and Group III for the mature seeds at final harvest, respectively. The radioactivity present on the surface of the rapeseed forage samples was removed by rinsing the tissue surface with acetonitrile. The radioactive concentration in the surface rinse accounted for 1.467 mg/kg (36.74% TRR) in the forage. The majority of the recovered radioactivity was present in the extractable fractions of rapeseed samples. The radioactive concentration in the extractable portions accounted for 2.192 mg/kg (54.88% TRR) in forage and 0.401 mg/kg (85.37% TRR) and 0.041 mg/kg (81.45% TRR) for Group II (seed) and Group III (seed), respectively. The un-extractable radioactivity represented 0.335 mg/kg (8.38% TRR) in forage and 0.069 mg/kg (14.63% TRR) and 0.009 mg/kg (18.55% TRR) for Group II (seed) and Group III (seed), respectively. The results are summarized in the table below.

Table B.7.1.2.2-1: Distribution of residues in oilseed rape after application of [phenoxy-^{14}C]S-2200 in % TRR and mg as eq/kg

	Group II (Rape Forage) 2 x 400 g/ha		Group II (Rape seed) 2 x 400 g/ha		Group III (Rape seed) 1 x 400 g/ha	
Time interval from last application	14 days		40 days		54 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR - Total Radioactive residues	100	3.99	100	0.47	100	0.05
ERR - Extracted Radioactive Residues	**91.62**[1)]	**3.66**	**85.37**	**0.40**	**81.45**	**0.04**
Surface extraction (Surface wash)	36.74	1.47	n.a.	n.a.	n.a.	n.a.
Watersoluble extract radioactivity	54.88	2.19	58.23	0.27	81.45	0.04
Organosoluble extracted radioactivity	n.a.	n.a.	27,15	0,13	< LOQ	n.a.
Radioactivity released under hydrolysis conditions	8.29	0.33	13.94	0.07	n.a.	n.a.
Total identified	72.83	2.9	58.13	0.27	20.26	< 0.01
Total characterized	18.8	0.75	27.24	0.14	61.19	0.02
URR – Unextracted Radioactive Residues	**8.38**	**0.34**	**14.63**	**0.07**	**18.55**	**< 0.01**
Accountability (sum ERR and URR)	100	3.99	100	0.47	100	0.05

n.a.= not applicable
1) …..including surface wash

The **TRR concentrations in the [benzyl-^{14}C]S-2200** rapeseed plant samples were 3.44 mg/kg for the forage and 0.644 and 0.110 mg/kg for Group IV and Group V for the mature seeds at the final harvests, respectively. The radioactive concentration in the surface rinse accounted for 1.18 mg/kg (34.16% TRR) in the forage. The majority of the recovered radioactivity was present in the extractable fractions of the rapeseed samples. The radioactive concentration in the extractable portions accounted for 2.01 mg/kg (58.31% TRR) for forage and 0.643 mg/kg (99.89% TRR) and 0.100 mg/kg (90.74% TRR), for Group IV (seed) and Group V (seed), respectively. The un-extractable radioactivity represented 0.259 mg/kg (7.53% TRR), 0.001 mg/kg (0.11% TRR) and 0.010 mg/kg (9.26% TRR), for Group IV forage, Group V seed and Group V seed, respectively. The results are summarised in the table below.

Table B.7.1.2.2-2: Distribution of residues in oilseed rape after application of [benzyl-^{14}C]S-2200 in % TRR and mg as eq/kg

	Group IV (Rape Forage) 2 x 400 g/ha		Group IV (Rape seed) 2 x 400 g/ha		Group V (Rape seed) 1 x 400 g/ha	
Time interval from last application	14 days		40 days		54 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR - Total Radioactive residues	100	3,44	100	0,64	100	0,11
ERR - Extracted Radioactive Residues	**92,47[1)]**	**3,18**	**99,89**	**0,64**	**90,74**	**0,10**
Surface extraction (Surface wash)	34,16	1,18	n.a.	n.a.	n.a.	n.a.
Watersoluble extract radioactivity	58,31	2,01	77,84	0,50	90,74	0,10
Organosoluble extracted radioactivity	n.a.	n.a.	22,06	0,14	< LOQ	n.a.
Radioactivity released under hydrolysis conditions	6,04	0,21	16,8	0,04	n.a.	n.a.
Total identified	64,95	2,25	46,15	0,32	nd	nd
Total characterized	27,52	0,95	53,75	0,33	90,74	0,10
URR- Unextracted Radioactive Residues	**7,53**	**0,26**	**0,11**	**< 0,01**	**9,26**	**0,01**
Accountability (sum ERR and URR)	100	3,44	100	0,64	100	0,11

n.a.= not applicable
1) …..including surface wash

The un-extractable residues of both radiolabels were sequentially hydrolyzed with enzyme, acid and base. A portion of the immature harvest (forage) post-extraction solids (PES) was sequentially hydrolyzed with 1 M hydrochloric acid, 6 M hydrochloric acid, 1 M sodium hydroxide and 6 M sodium hydroxide at elevated temperatures. The results are summarized in the table below.

Table B.7.1.2.2-3: Distribution of radioactivity in the PES of oilseed rape forage after two applications of ^{14}C-S-2200

Fraction	Total Radioactive Residue					
	[phenoxy-^{14}C]S-2200			**[benzyl-^{14}C]S-2200**		
	% of PES[1)]	mg/kg	% TRR[2)]	% of PES[1)]	mg/kg	% TRR[2)]
Weak Acid Hydrolysis (1M HCl)	14.73	0.05	1.23	12.86	0.03	0.97
Strong Acid Hydrolysis (6M HCl)	14.40	0.05	1.21	26.21	0.07	1.97
Weak Basic Hydrolysis (1M NaOH)	35.88	0.12	3.01	26.29	0.07	1.98
Strong Basic Hydrolysis (6M NaOH)	33.91	0.11	2.84	14.85	0.04	1.12
Bound Residue	33.17	0.11	2.78	13.04	0.03	0.98
Total	132.1	0.44	11.07	93.25	0.24	7.02

*1) ..... % of PES = (Radioactivity released (dpm)) /(total dpm available) * 100*
*2) .....% TRR = (% of PES) * (% PES TRR) / 100*

A portion of the final harvest (seed) post-extraction solids (PES) was sequentially hydrolyzed with amylase and protease enzymes, 1 M hydrochloric acid, 6 M hydrochloric acid, 1 M sodium hydroxide and 6 M sodium hydroxide at elevated temperatures. The results are summarized in the table below.

Table B.7.1.2.2-4: Distribution of radioactivity in the PES of oilseed rape seed after two applications of ^{14}C-S-2200

Fraction	Total Radioactive Residue					
	[phenoxy-^{14}C]S-2200			**[benzyl-^{14}C]S-2200**		
	% of PES[1)]	mg/kg	% TRR[2)]	% of PES[1)]	mg/kg	% TRR[2)]
Amylase Hydrolysis	20.59	0.01	3.01	34.69	<0.01	6.20
Protease Hydrolysis	11.86	<0.01	1.73	15.72	<0.01	2.81
Weak Acid Hydrolysis (1M HCl)	ND	ND	ND	ND	ND	ND
Strong Acid Hydrolysis (6M HCl)	8.41	<0.01	1.23	ND	ND	ND
Weak Basic Hydrolysis (1M NaOH)	21.51	0.02	3.15	30.94	<0.01	5.53
Strong Basic Hydrolysis (6M NaOH)	32.96	0.02	4.82	12.62	<0.01	2.26
Bound Residue	8.39	<0.01	1.23	10.10	<0.01	1.81
Total	103.7	0.07	15.17	104.1	0.01	18.60

*[1)]..... % of PES = (Radioactivity released (dpm)) /(total dpm available) * 100*
*[2)].....% TRR = (% of PES) * (% PES TRR) / 100*

Characterization and identification of metabolites was made initially by comparison of HPLC/RAM sample retention times with co-injected reference standard mix. The regions or peaks of interest were then isolated from the rapeseed extracts using reverse-phase HPLC. Metabolites were then characterized by HPLC co-chromatography with reference standards and 1D-TLC co-migration with reference standards.

For confirmation of the S-2200 R:S isomer ratio the surface rinses were analyzed directly using chiral HPLC co-chromatography with S-2200 reference standard for the forage harvest samples. The ^{14}C peak corresponding to the retention time of S-2200 was isolated from the neutral extractable fraction and analyzed by Chiral HPLC co-chromatography. The S-2200 R:S isomer ratio was approximately 50:50 in the [^{14}C]S-2200 test substances and remained at approximately 50:50 in the processed samples. Isomerization of the R:S isomers of [^{14}C]S-2200 was not observed in this study.
For confirmation of S-2200 the surface rinsed samples and the [^{14}C]S-2200 isolates from the neutral extractable fractions were analyzed directly using 1D-TLC by co-spotting the isolates with authentic S-2200 standard.
It was demonstrated that [^{14}C]S-2200 isolate and S-2200 reference standard co-eluted using both chromatographic techniques. Thus the identity of [^{14}C]S-2200 was confirmed by HPLC and TLC chromatographic data.
Control samples of rapeseed forage and mature seed were fortified with [^{14}C]S-2200 at approximately 0.5 and 0.14 mg/kg, respectively to demonstrate extraction efficiency of S-2200. The fortified samples were extracted and analyzed using the same methods used for the analysis of treated samples. The results demonstrated that the radioactivity from the spiked controls could be quantitatively (>95%) extracted using the extraction method used in the study, and that parent S-2200 remained at 90% of the TRR.

Group II (Rape forage / 2x 400 g a.s./ha)
S-2200 was observed at 0.791 mg/kg (19.80% TRR) in the **[phenoxy-^{14}C]S-2200** treated rapeseed forage. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.484 mg/kg (12.13% TRR) and the conjugate of 4-OH-S-2200 was calculated to be 1.42 mg/kg (35.62% TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.20 mg/kg (5.12% TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.520 mg/kg (13.01% TRR). The largest peak in this region was accounted for 0.146 mg/kg (3.64% TRR) and characterized as multiple components by further analysis using HPLC and TLC.
Minor regions of radioactivity were observed at HPLC/RAM retention times of 11-min (Unknown 3: 0.31% TRR, 0.012 mg/kg) and 22-min (Unknown 5: 0.70% TRR, 0.028 mg/kg). 2-CH_2OH-S-2200 was observed at 0.006 mg/kg (0.16% TRR) in the forage harvest sample. Numerous minor peaks, each individual peak

accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.191 mg/kg (4.78% TRR). There were no other significant degradation products detected in the extractable fraction.

Group II (Rape seed / 2x 400 g a.s./ha)

S-2200 was observed at 0.14 mg/kg (30.68% TRR) in the **[phenoxy-^{14}C]S-2200** treated rapeseed. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which was comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.031 mg/kg (6.50% TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.07 mg/kg (14.48% TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated as 0.014 mg/kg (3.06% TRR). Another region of radioactivity at 14-min (Unknown 4) was calculated to be 0.076 mg/kg (16.14% TRR). The largest component in this region was accounted for 0.017 mg/kg (3.65% TRR) and characterized as multiple components by further analysis using HPLC and TLC. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.89% TRR, 0.004 mg/kg) and 11-min (Unknown 3: 1.06% TRR, 0.005 mg/kg). 5-COOH-S-2200 was observed at 0.016 mg/kg (3.41% TRR) in the rape seed final harvest sample. Numerous minor peaks, each individual peak accounting for 1% TRR or less, designated as others are included in the distribution table which together accounted for 0.043 mg/kg (9.15% TRR). There were no other significant degradation products detected in the extractable fraction.

Group III (Rape seed / 1x 400 g a.s./ha)

S-2200 was not detected in any extractable fraction of the **[phenoxy-^{14}C]S-2200** treated rapeseed.
The hexane and acid extractable fractions were not quantified by HPLC/RAM analysis because radioactive residues were less than 0.010 mg/kg.
An area of radioactivity was observed in the HPLC/RAM at 16-minutes which was comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.002 mg/kg (3.58% TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.004 mg/kg (7.98% TRR). Another region of radioactivity at 14-min (Unknown 4) was calculated to be 0.021 mg/kg (40.46% TRR). This area was characterized by the comparison with that of Group II, and as a result, it was considered to consist of multiple components. The largest component in this region was calculated to be 0.001 mg/kg (2.88%TRR). 5-COOH-S-2200 was observed at 0.004 mg/kg (8.70% TRR) in the rape seed final harvest sample. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 6.17% TRR, 0.003 mg/kg) and 22-min (Unknown 5: 3.77% TRR, 0.002 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.005 mg/kg (10.79% TRR). There were no other significant degradation products detected in the extractable fraction.
The results are summarized in the table below.

Table B.7.1.2.2-5: Identification and characterisation of residues in oilseed rape after application of [phenoxy-^{14}C]S-2200 in % TRR and mg as eq/kg

	Group II (Rape Forage) 2 x 400 g/ha		**Group II (Rape seed) 2 x 400 g/ha**		**Group III (Rape seed) 1 x 400 g/ha**	
Time interval from last application	14 days		40 days		54 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR – Total Radioactive residues	100	3.99	100	0.47	100	0.05
S-2200	**19.8**	**0.79**	**30.68**	**0.14**	**nd**	**nd**
Surface wash	16.39	0.65	n.a	n.a	n.a	n.a
Hexane extracts	n.a	n.a	27.15	0.13	nd	nd
Acetone/water extracts	3.08	0.12	3.53	0.02	nd	nd

Time interval from last application	Group II (Rape Forage) 2 x 400 g/ha		Group II (Rape seed) 2 x 400 g/ha		Group III (Rape seed) 1 x 400 g/ha	
	14 days		40 days		54 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
Acetone/water/HCL extracts	0.34	0.01	nd	nd	nd	nd
2-CH_2OH-S-2200 (conjugated, 16min)	**12.13**[2)]	**0.48**	**6.50**[5)]	**0.03**	**3.58**[7)]	**< 0.01**
Surface wash	4.81	0.19	n.a	n.a	n.a	n.a
Hexane extracts	n.a	n.a	nd	nd	nd	nd
Acetone/water extracts	8.05	0.32	5.76	0.03	3.58	< 0.01
Acetone/water/HCL extracts	0.43	0.02	0.74	< 0.01	nd	nd
4-OH-S-2200 (conjugated, 16min)	**35.62**[2)]	**1.42**	**14.48**[5)]	**0.07**	**7.98**[7)]	**< 0.01**
Surface wash	9.55	0.38	n.a	n.a	n.a	n.a
Hexane extracts	n.a	n.a	nd	nd	nd	nd
Acetone/water extracts	23.65	0.94	12.83	0.06	7.98	< 0.01
Acetone/water/HCL extracts	1.26	0.05	1.65	< 0.01	nd	nd
5-CH_2OH-S-2200 (conjugated, 19min)	**5.12**[3)]	**0.20**	**3.06**[3)]	**0.01**	**nd**	**nd**
2-CH_2OH-S-2200	**0.16**	**< 0.01**	**nd**	**nd**	**nd**	**nd**
5-COOH-S-2200	nd	nd	**3.41**	**0.02**	**8.70**	**< 0.01**
Total identified	**72.83**	**2.9**	**58.13**	**0.27**	**20.26**	**< 0.01**
Unknown 1 (2min)	nd	nd	0.89	< 0.01	6.17	< 0.01
Unknown 3 (11min)	0.31	0.01	1.06	< 0.01	nd	nd
Unknown 4 (14min)	13.01[4)]	0.52	16.14[6)]	0.08	40.46[8)]	0.02
Unknown 5 (22min)	0.70	0.03	nd	nd	3.77	< 0.01
Others	4.78	0.19	9.15	0.04	10.79	< 0.01
Total characterized	**18.8**	**0.75**	**27.24**	**0.14**	**61.19**	**0.02**
ERR - Extracted Radioactive Residues	91.62[1)]	3.66	85.37	0.40	81.45	0.03
URR – Unextracted Radioactive Residues	8.38	0.34	14.63	0.07	18.55	< 0.01

n.a.= not applicable nd= not detected

1) .....including surface wash

2).....Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at ratios of 33.5:66.5 for surface rinse and 25.4:74.6 for extract.

3).....Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200.

4).....Comprising of multiple compounds, largest component 3.64% TRR, 0.146 mg/kg was further separated into multiple components by HPLC and TLC

5).....The neutral extract 16 minute region was analysed by HPLC method 4 to separate the conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 31.0:69.0

6).....Comprising of multiple compounds, largest component 3.65% TRR, 0.017 mg/kg.

7).....The neutral extract 16 minute region was analysed by HPLC method 4 to separate the conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 (31.0:69.0).

8).....Comprising of multiple compounds, largest component 2.88% TRR, 0.001 mg/kg.

Group IV (Rape forage / 2x 400 g a.s./ha)

S-2200 was observed at 0.77 mg/kg (22.42% TRR) in the **[benzyl-^{14}C]S-2200** treated rapeseed forage. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.43 mg/kg (12.43% TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.93 mg/kg (27.03% TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.096 mg/kg (2.80% TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.402 mg/kg (11.68% TRR). The largest peak in this region was accounted for 0.158 mg/kg (4.60% TRR) and characterized as multiple components as shown in Group II by further analysis using HPLC and TLC. Minor regions of radioactivity were observed at HPLC/RAM retention times of 9-min (Unknown 2: 4.63%

TRR, 0.159 mg/kg), 11-min (Unknown 3: 2.27% TRR, 0.078 mg/kg). In the forage harvest sample 2-CH2OH-S-was observed at 0.004 mg/kg (0.11% TRR) and MCBX (0.16% TRR, < 0.01 mg/kg) was detected as minor metabolite demethylation of the methoxy group of the side chain. Numerous minor peaks designated as others are included in the distribution table which together accounted for 0.285 mg/kg (8.28% TRR). There were no other significant degradation products detected in the extractable fraction.

Group IV (Rape seed / 2x 400 g a.s./ha)

S-2200 was observed at 0.162 mg/kg (25.13% TRR) in the **[benzyl-^{14}C]S-2200** treated rapeseed. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which was comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.033 mg/kg (5.08% TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.071 mg/kg (11.05% TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated as 0.023 mg/kg (3.62% TRR). Another region of radioactivity at 14-min (Unknown 4) was calculated to be 0.114 mg/kg (17.74% TRR). The largest component in this region accounted for 0.014 mg/kg (2.23% TRR) and characterized as multiple components by further analysis using HPLC and TLC. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 1.84% TRR, 0.012 mg/kg), 9-min (Unknown 2: 6.52% TRR, 0.042 mg/kg) and 11-min (Unknown 3: 6.84% TRR, 0.044 mg/kg). 5-COOH-S-2200 was observed at 0.008 mg/kg (1.27% TRR) in the rape seed final harvest sample. Numerous minor peaks, each individual peak accounting for 1% TRR or less, designated as others are included in the distribution table which together accounted for 0.134 mg/kg (20.81% TRR). There were no other significant degradation products detected in the extractable fraction.

Group V (Rape seed / 1x 400 g a.s./ha)

S-2200 was not detected in any extractable fraction of the **[benzyl-^{14}C]S-2200** treated rapeseed.
The acid extractable fraction was not quantified by HPLC/RAM analysis because radioactive residues were less than 0.010 mg/kg.
Polar areas of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 36.71% TRR, 0.040 mg/kg), 9-min (Unknown 2: 5.35% TRR, 0.006 mg/kg) and 11-min (Unknown 5: 9.69% TRR, 0.011 mg/kg). The 1D-TLC analysis of the neutral extract showed that these polar peaks were each composed of numerous minor components. Peaks at 9-min and 11-min were quantified separately, while the other remaining individual peaks each account for less than 0.010 mg/kg. These minor components are designated as others, which together accounted for 0.043 mg/kg (38.99% TRR). There were no other significant degradation products detected in the extractable fraction.

The results are summarised in the table below.

Table B.7.1.2.2-6: Identification and characterisation of residues in oilseed rape after application of [benzyl-^{14}C]S-2200 in % TRR and mg as eq/kg

	Rape Forage 2 x 400 g/ha		**Rape seed** 2 x 400 g/ha		**Rape seed** 1 x 400 g/ha	
Time interval from last application	14 days		40 days		54 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR – Total Radioactive residues	100	3,44	100	0,64	100	0,11
S-2200	22,42	0,77	25,13	0,16	nd	nd
Surface wash	17,35	0,60	n.a.	n.a.	n.a	n.a
Hexane extracts	n.a.	n.a.	20,33	0,13	nd	nd
Acetone/water extracts	4,79	0,17	4,8	0,03	nd	nd
Acetone/water/HCL extracts	0,28	0,01	nd	nd	nd	nd
2-CH_2OH-S-2200 (conjugated, 16min)	12,43[2)]	0,43	5,08[5)]	0,03	nd	nd
Surface wash	3,31	0,11	n.a.	n.a.	n.a	n.a
Hexane extracts	n.a.	n.a.	nd	Nd	nd	nd

Time interval from last application	Rape Forage 2 x 400 g/ha 14 days		Rape seed 2 x 400 g/ha 40 days		Rape seed 1 x 400 g/ha 54 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
Acetone/water extracts	8,63	0,30	4,67	0,03	nd	nd
Acetone/water/HCL extracts	0,49	0,02	0,40	< 0,01	nd	nd
4-OH-S-2200 (conjugated, 16min)	27,03[2)]	0,93	11,05[5)]	0,07	nd	nd
Surface wash	7,19	0,25	n.a.	n.a.	n.a	n.a
Hexane extracts	n.a.	n.a.	nd	Nd	nd	nd
Acetone/water extracts	18,77	0,65	10,17	0,07	nd	nd
Acetone/water/HCL extracts	1,07	0,04	0,87	< 0,01	nd	nd
5-CH_2OH-S-2200 (conjugated, 19min)	2,80[3)]	0,10	3,62[3)]	0,02	nd	nd
2-CH_2OH-S-2200	0,11	< 0,01	nd	nd	nd	nd
5-COOH-S-2200	nd	nd	1,27	< 0,01	nd	nd
MCBX	0,16	< 0,01	nd	nd	nd	nd
Total identified	64,95	2,25	46,15	0,29	nd	nd
Unknown 1 (2min)	nd	nd	1,84	0,01	36,71	0,04
Unknown 2 (9min)	4,63	0,16	6,52[6)]	0,04	5,35	< 0,01
Unknown 3 (11min)	2,27	0,08	6,84[6)]	0,04	9,69	0,01
Unknown 4 (14min)	11,68[4)]	0,40	17,74[6)]	0,11	nd	nd
Unknown 5 (22min)	0,66	0,02	nd	nd	nd	nd
Others	8,28	0,29	20,81[7)]	0,13	38,99[7)]	0,04
Total characterized	**27,52**	**0,95**	**53,75**	**0,33**	**90,74**	**0,10**
ERR - Extracted Radioactive Residues	92,47[1)]	3,19	99,89	0,64	90,74	0,01
URR – Unextracted Radioactive Residues	7,53	0,26	0,11	< 0,01	9,26	0,01

n.a.= not applicable nd= not detected

1) …..including surface wash

2) …..Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 31.5:68.5

3) …..Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200

4) …..Comprising of multiple compounds, largest component 4.60% TRR, 0.158 mg/kg was further separated into multiple components by HPLC and TLC.

5) …..The neutral extract 16 minute region was analysed by HPLC method 4 to separate the conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 (31.5:68.5).

6) …..Comprising of multiple compounds. Largest component of the 14 minute region accounted for 2.23% TRR, 0.014 mg/kg

7) …..Comprising numerous minor peaks, each peak accounted for or less than 1%TRR.

A representative [phenoxy-^{14}C]S-2200 rapeseed forage sample was extracted and analyzed after approximately 7 months of **freezer storage**. Representative [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 treated final harvest samples were extracted and analyzed after approximately 7, 11 and 9 months of freezer storage, for Group II, Group III and Group V, respectively. The extracts were chromatographed by HPLC and compared to the initial profile to verify the stability of metabolites in the matrices during storage. The comparison of distributions of major ^{14}C residues in the [phenoxy-^{14}C]S-2200 treated rapeseed plants for the forage and seed extractable fractions are given in the tables below.

Table B.7.1.2.2-7: Comparison of ^{14}C Residues (Neutral extrables) in Rape forage and Rape seed following 7 to 11 month frozen storage

	[phenoxy-^{14}C] S-2200		[phenoxy-^{14}C] S-2200		[phenoxy-^{14}C] S-2200		[benzyl-^{14}C] S-2200	
	Group II (Rape Forage) 2 x 400 g/ha		**Group II** (Rape seed) 2 x 400 g/ha		**Group III** (Rape seed) 1 x 400 g/ha		**Group V** (Rape seed) 1 x 400 g/ha	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
	Neutral extractable (Acetone : water = 80 : 20)[1)2)]		**Neutral extractable (Hexane and Acetone : water = 80 : 20)**					
Original analysis	50,41	2,013	78,33	0,367	70,66	0,036	79,34	0,088
Seven month frozen storage	68,01	2,094	72,64	0,380	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	67,16	0,034	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	70,96	0,086
S-2200								
Original analysis	3,08	0,123	30,68	0,144	nd	nd	nd	nd
Seven month frozen storage	2,95	0,091	27,91	0,146	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	0,85	< MDL	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	10,66	0,013
Unknown 4 (14min)								
Original analysis	8,69	0,347	13,06	0,061	40,46	0,021	nd	nd
Seven month frozen storage	25,47	0,784	13,59	0,071	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	34,06	0,017	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2,41	0,003
Conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 (16min)								
Original analysis	31,70	1,226	18,59	0,087	11,56	0,006	nd	nd
Seven month frozen storage	31,65	0,974	14,43	0,075	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	11,53	0,006	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	nd	nd
Conjugates of 5-CH_2OH-S-2200 (19min)								
Original analysis	3,40	0,136	3,06	0,014	nd	nd	nd	nd
Seven month frozen storage	3,36	0,103	nd	nd	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	nd	nd	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	nd	nd
5-COOH-S-2200								
Original analysis	nd	nd	3,41	0,016	8,70	0,004	nd	nd
Seven month frozen storage	nd	nd	1,72	0,009	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	9,35	0,005	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	nd	nd
Others								
Original analysis	2,65	0,106	8,47	0,040	nd	nd	27,59	0,030
Seven month frozen storage	3,58	0,110	8,25	0,043	n.a.	n.a.	n.a.	n.a.
Eleven month frozen storage	n.a.	n.a.	n.a.	n.a.	1,04	0,001	n.a.	n.a.
nine month frozen storage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6,85	0,008

MDL= minimum dectectable limit

1) *…..Rapeseed forage TRR was determined by combustion analysis at harvest collection to be 2.850 mg/kg. Recovery of original final harvest samples is based on this TRR value.*

2) *…..Rapeseed forage TRR was determined by combustion analysis at 7-months frozen storage to be 3.151 mg/kg. Recovery of 7-month stability samples is based on this TRR valu*

Table B.7.1.2.2-8: Comparison of ^{14}C Residues (Acid extrables) in Rape forage and Rape seed following 7 month frozen storage

	Group II (Rape Forage) 2 x 400 g/ha		Group II (Rape seed) 2 x 400 g/ha	
	TRR (%)	mg/kg	TRR (%)	mg/kg
	Acid extractable (Acetone : water : HCL=80:20:1)			
Original analysis	4,46	0,178	7,04	0,033
Seven month frozen storage	9,56	0,294	8,05	0,042
S-2200				
Original analysis	0,34	0,013	nd	nd
Seven month frozen storage	0,78	0,024	nd	nd
Unknown 4 (14min)				
Original analysis	1,41	0,057	3,07	0,014
Seven month frozen storage	3,74	0,115	2,83	0,015
Conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 (16min)				
Original analysis	1,69	0,067	2,39	0,011
Seven month frozen storage	4,21	0,130	2,22	0,012
Conjugates of 5-CH_2OH-S-2200 (19min)				
Original analysis	0,17	0,007	nd	nd
Seven month frozen storage	0,24	0,007	nd	nd
Others				
Original analysis	0,75	0,030	0,69	0,003
Seven month frozen storage	0,59	0,018	nd	nd

The metabolite profiles from the two analyses (the initial and final analyses) for the [phenoxy-^{14}C]S-2200 treated rapeseed plants (forage and seeds) were similar, indicating that [^{14}C]S-2200 and its metabolites were stable in the rapeseed samples under freezer storage conditions. Minor differences were observed in profiles from Group V [benzyl-^{14}C]S-2200 treated rapeseed final harvest samples after 9 months of freezer storage.

B.7.1.2.3 Conclusions and metabolic pathway of S-2200 in Rapeseed

The metabolism of [^{14}C]S-2200 was studied in rapeseed plants to determine the total [^{14}C] residues in rapeseed forage and mature seed and to identify and characterize the nature of the major [^{14}C] residues. The study was carried out under outdoor growing conditions using two radiolabeled forms of [^{14}C]S-2200, [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 as a 25% SC formulation.

Table B.7.1.2.3-1: Distribution of [^{14}C]S-2200 residues in oilseed rape (forage and seed)

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Forage 2 x 400 g/ha PHI 14 days	Surface wash	1.47	36.74	1.18	34.16
	Sum of extracts [1)]	2.19	54.87	2.02	58.32
	PES (post extraction solids)	0.34	8.38	0.26	7.53
	Total Radioactive Residue (TRR)	**3.99**	**100**	**3.44**	**100**
Seed 2 x 400 g/ha PHI 40 days	Surface wash	not performed			
	Sum of extracts	0.40	85.37	0.64	99.89
	PES (post extraction solids)	0.07	14.63	<0.01	0.11
	Total Radioactive Residue (TRR)	**0.47**	**100**	**0.64**	**100**
Seed	Surface wash	not performed			

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
1 x 400 g/ha PHI 54 days	Sum of extracts	0.04	81.45	0.10	90.74
	PES (post extraction solids)	<0.01	18.55	0.01	9.26
	Total Radioactive Residue (TRR)	**0.05**	**100**	**0.11**	**100**

1) …..excluding surface wash

The major [^{14}C] residues identified in the rapeseed forage and seed samples included [^{14}C]S-2200, [^{14}C] glycoside conjugates of 2-CH2OH-S-2200 and 4-OH-S-2200 with some minor metabolites such as [^{14}C]5-COOH-S-2200, [^{14}C] conjugate of 5-CH_2OH-S-2200 and some unknown (1-5) metabolites.

<u>[phenoxy-^{14}C]S-2200</u>
Following two applications at 400 g a.s./ha on rapeseed plants, the TRR declined from 3.99 mg/kg S-2200 equivalents in rape forage (PHI 14 days) to 0.47 mg/kg in rape seed harvested at maturity (PHI 40 days).
In *forage* (Group II) S-2200 accounted for 19.8% of the TRR (0.79 mg/kg). The surface washes of forage contained 36.7% of the TRR (1.5 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 12% TRR (0.48 mg/kg), 35.6% TRR (1.42 mg/kg) and 5.12% TRR (0.20 mg/kg), respectively. Free 2-CH_2OH-S-2200 was found at 0.16% TRR (<0.01 mg/kg), MCBX was not detected.
In *seeds after two applications* (Group II) S-2200 accounted for 30.7% of the TRR (0.14 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 6.5% TRR (0.03 mg/kg), 14.5% TRR (0.07 mg/kg) and 3.1% TRR (0.01 mg/kg), respectively. 5-COOH-S-2200 accounted for 3.4% of the TRR 0.02 mg/kg) and MCBX was not detected.
Following a single application at 400 g a.s./ha on rapeseed plants, the TRR in mature seed (Group III) was 0.05 mg/kg S-2200 equivalents (PHI 54 days). S-2200 was not detected. The glycosides of 2-CH_2OH-S-2200 and 4-OH-S-2200 were found at 3.6% TRR (<0.01 mg/kg) and 8 % TRR (<0.01 mg/kg), respectively. 5-COOH-S-2200 accounted for 8.7% of the TRR (<0.01 mg/kg), the conjugate of 5-CH_2OH-S-2200 and MCBX were not detected.

<u>[benzyl-^{14}C]S-2200</u>
Following two applications at 400 g a.s./ha on rapeseed plants, the TRR declined from 3.44 mg/kg S-2200 equivalents in rape forage (PHI 14 days) to 0.64 mg/kg in rape seed harvested at maturity (PHI 40 days).
In *forage* S-2200 accounted for 22.4% of the TRR (0.77 mg/kg). The surface washes of forage contained 34.1% of the TRR (1.2 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 12.4% TRR (0.43 mg/kg), 27% TRR (0.93 mg/kg) and 2.8% TRR (0.10 mg/kg), respectively. Free 2-CH_2OH-S-2200 was found at 0.11% TRR (<0.01 mg/kg), MCBX up to 0.2% (< 0.01 mg/kg).
In *seeds after two applications* S-2200 accounted for 25.1% of the TRR (0.16 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 5.1% TRR (0.03 mg/kg), 11.05% TRR (0.07 mg/kg) and 3.6% TRR (0.02 mg/kg), respectively. 5-COOH-S-2200 accounted for 1.3% of the TRR < 0.01 mg/kg) and MCBX was not detected.
Following a single application at 400 g a.s./ha on rapeseed plants, the TRR in mature seed (Group V) was 0.11 mg/kg S-2200 equivalents (PHI 54 days). S-2200 was not detected. The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200, of 5-CH_2OH-S-2200, the free form of 5-COOH-S-2200, the conjugate as well as MCBX were not detected in seeds after a single application.

All metabolites (4-OH-S-2200, 5-COOH-S-2200, 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and MCBX) were also found in the rat. The metabolites 4-OH-S-2200 and 5-COOH-S-2200 were present at >10% of dose in the rat. The *R*/*S* ratio of S-2200 remained approximately 1:1, indicating no *R*/*S* isomerization.

The [^{14}C]S-2200 that absorbed into rapeseed plants was metabolized to a number of polar products.
<u>The major metabolism pathway</u> included

- ✓ hydroxylation of the dimethylphenoxy ring to form 4-OH-S-2200 and subsequent formation of glycoside conjugate

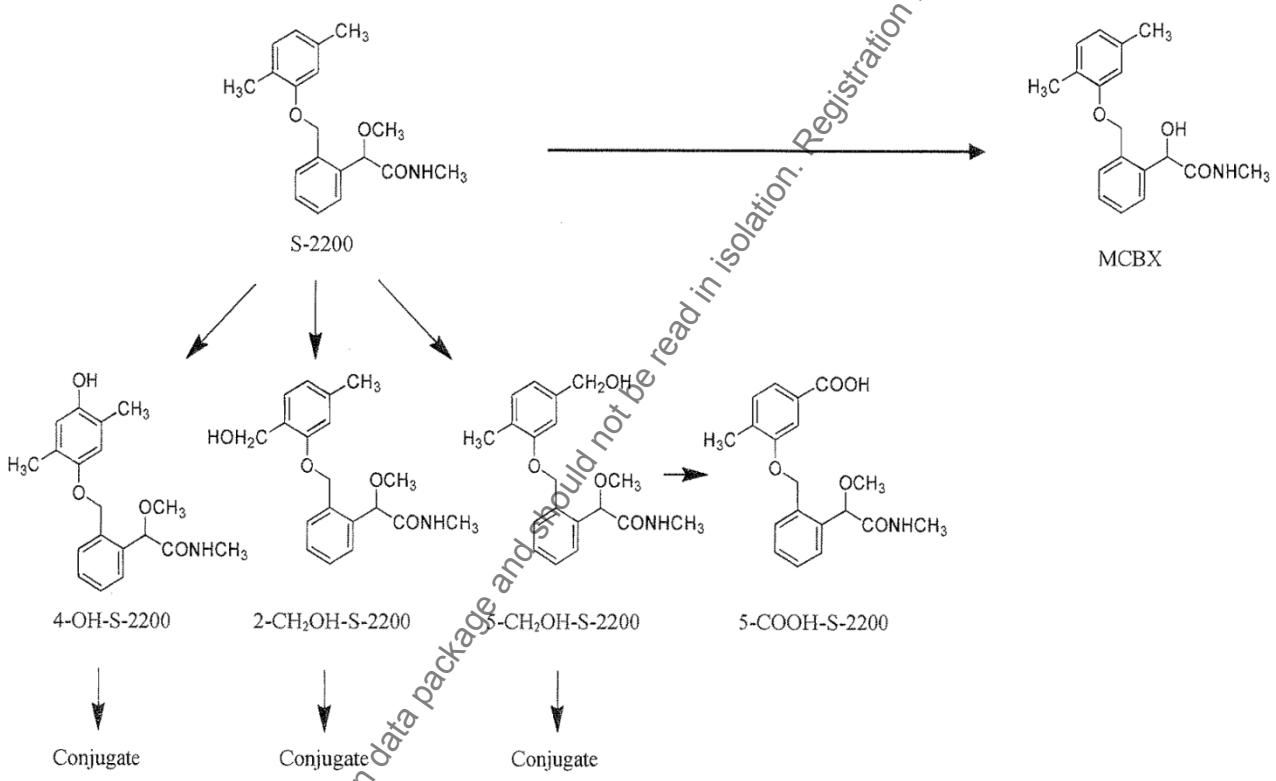
- ✓ oxidation of the methyl group attached to the 2-position of the dimethylphenoxy ring to form 2-CH_2OH-S-2200 and the corresponding glycoside conjugate.

Minor metabolic pathways included

- ✓ the demethylation of the methoxy group of the side chain to form MCBX
- ✓ oxidation of the methyl group at the 5-position of the dimethylphenoxy ring to form 5-CH_2OH-S-2200 and subsequent conjugation or
- ✓ further oxidation of 5-CH_2OH-S-2200 to form 5-COOH-S-2200

Further metabolism occurred to form other minor metabolites and polar products.

Figure B.7.1.2-1: Proposed metabolic pathway of Mandestrobin (S-2200) in oilseed rape



Code Name	Molecular Weight	Chemical Name
S-2200	313.39	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
MCBX	299.36	(RS)-2-hydroxy-N-methyl-2-[α-(2,5- xylyloxy)-o-tolyl]acetamide
4-OH-S-2200	329.39	(RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide
2-CH_2OH-S-2200	329.39	(RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-CH_2OH-S-2200	329.39	(RS)-2-[2-(5-hydroxymethyl-2- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-COOH-S-2200	343.38	(RS)-3-{2-[1-methoxy-1-(N-methylcarbamoyl) methyl]benzyloxy}-4-methylbenzoic acid

B.7.1.3 Metabolism in Wheat

Reference:	**Metabolism of [^{14}C]S-2200 in Wheat**
Author(s), year:	Panthani A, Lentz N.R. (2010a)
Report/ Doc. Number:	Springborn Smithers Laboratories Report no. 13048.6619 Sumitomo ref: ROM-0009
Guideline(s):	Japanese MAFF, 12-Nousan-No. 8147, Part 2-4-1, 24. November 2000 US EPA OPPTS 860.1300 Nature of the Residue - Plants, Livestock EU Document 7028/VI/95 rev. 3 (22/07/97), Appendix A - Metabolism and Distribution in Plants OECD 501 - Guideline for the Testing of Chemicals - Metabolism in Crops
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	yes
Comment (RMS):	The study is acceptable

Test Site information:
Test site: Sprinborn Smithers Laboratories (Wareham, Massachusetts)
Testing environment: greenhouse
Soil characteristics: Mixture of Rochester natural loamy sand (USDA classification) and commercial potting soil (9:1 w/w)

pH	6.2
CEC (meq/100g)	10.2
OM (%)	8.3
Sand (%)	71
Silt (%)	22
Clay (%)	7
Soil classification	sandy loam

Crop information:
Crop/crop group: Wheat/Cereals
Variety: Promontory
Test Material:
Common Name: S-2200
Name (IUPAC): (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers: S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight: 313.39
CAS No.: 173662-97-0
Purity: 100%
*Log $P_{o/w}$= 3.51 (*at 25 ± 1°C)
Radiolabel position, specific activity: [Phenoxy-^{14}C] S-2200 (120 mCi/mmol)
[Benzyl-^{14}C] S-2200 (123 mCi/mmol
Formulation: 25% SC (suspension concentrate)
Specific activity in formulation: 200,000 dpm/µg

B.7.1.3.1 Material and Methods

The study was designed to simulate field application of [^{14}C]S-2200 to wheat plants to identify major components of the residue and their distribution in the plant. The study was carried out under greenhouse conditions fitted with automatic controls to maintain air temperature (28°C day/21°C night, relative humidity 19-85%). Wheat seeds were planted in 11-inch pots with a seeding density of approximately 30 seeds per pot (15th April 2009). Group II (9 pots) and Group III (9 pots) were treated with [phenoxy-^{14}C]S-2200 and

[benzyl-^{14}C]S-2200 (1.89 mg a.i./pot), respectively, as a spray application with a 25% SC formulation. The application rate for each application and radiolabel was equivalent to 300 g a.s./ha, with a spray volume of 1000 L/ha. Nine pots were used for the control (group I) group.

The test substance was applied by manual-operated pump sprayer and each treatment pot was placed in an enclosed wooden frame (1.5ft x 1.5ft x 4ft) lined with plastic. A single slit was cut into one side just above the top of the plants and large enough to allow application. The plastic was taped shut around a shoulder-length glove and the application of one-half of the test material was conducted. The process was then repeated using a slit on the opposite side of the enclosure to ensure uniform coverage of the plants. Following the application, the plastic covering the top of the frame was removed to allow the plants to dry for approximately 15-minutes. The plants were then transferred to a permanent location in the greenhouse positioned in a configuration where spray-treatment plants were placed downwind from control plants to prevent cross-contamination between treatment groups.

The application rate was verified by LSC of the formulated test substances. The purity of the radiolabelled test substances was confirmed by HPLC before and after application. The control pots were maintained in the same manner as the treated ones and were kept at the opposite end of the greenhouse nearest to the exhaust fans. A summary of the application details is given in the table below.

Table B.7.1.3.1-1: Application details

Application number	Application details			
	Label	Application rate	Date of application	Growth stage at application
Group I (control)	n.a.	n.a.	n.a.	n.a.
Group II	[phenoxy-^{14}C]S-2200	1x 300 g a.i./ha	22.05.2009	BBCH 32
Group III	[benzyl-^{14}C]S-2200			

n.a.= not applicable

Wheat plants from 3 pots of each group (I, II, III) were harvested at each sampling time. Wheat forage and wheat hay were sampled 7 and 14 days after the application, respectively by cutting the stem above the soil surface. Mature wheat was sampled at final harvest 104 days after the treatment by cutting the stem above the soil surface. The mature plants were separated into straw and grain. The samples were placed in sample bags made of flexible plastic (e.g., Ziplok® freezer bags) and fresh weights for all samples were determined at the time of harvest. The sampling data are summarized in the table below:

Table B.7.1.3.1-2: Sampling details

Test group	Plant matrices sampled	Growth stage at sampling	Time interval after application	Date of sampling
I (Control), II, III	Wheat forage	BBCH 37	7	29.05.2009
	Wheat hay		14	05.06.2009
	Wheat straw & grain	BBCH 92	104	03.09.2009

n.a.= not applicable

Both control and treated plant tissue of wheat forage, hay and straw samples were surface washed with acetonitrile (approximately 500 mL). Wheat grain was not surface washed. The radioactivity in the surface rinse was quantified by LSC (2 x 1.0 mL) and analyzed by HPLC/RAM. The entire samples for treated and control tissue were ground with dry ice, five replicates (approximately 0.4 g each) of each sample was analyzed by combustion analysis.

Homogenised samples of wheat forage, hay, grain and straw were extracted twice by high speed homogenisation with acetone/water (80:20; v/v) and once with acetone/water/hydrochloric acid (80:20:1; v/v/v). Each sample was centrifuged to separate the extracts from the post-extraction solids (PES).

The Total Radioactive Residue (TRR) in the samples were quantitated by combustion of approximately 0.25 g subsamples using a biological oxidizer (R. J. Harvey Instrument Co.) followed by liquid scintillation counting and by tissue extraction followed by combustion analysis of the post-extraction solids (PES). The radioactivity in the liquid fraction was measured by direct LSC. The TRR in wheat was calculated as the sum of contributions from the surface rinse, extract and PES.
The distribution of radioactivity in wheat samples (forage, hay, straw and grain) was shown to be similar between the two radiolabels.
The surface wash removed 34-41 % of the radioactivity in wheat forage with decreasing amounts of 19-23 % in wheat hay and 2.8-3.7 % in wheat straw. The amount of extractable radioactivity (after surface washing) remained approximately constant and accounted for 53-61 % TRR in wheat forage, 66-73 % TRR in wheat hay, 59-65 % in wheat straw and 67-73 % TRR in wheat grain. However an increasing proportion of the TRR remained un-extractable with increasing PHI representing amounts of 5-6 % TRR in wheat forage, 8-11 % TRR in wheat hay, 33-38 % TRR in wheat straw and 27-33 % TRR in wheat grain.
The distribution of radioactive residues in wheat samples in the acetonitrile surface wash (except wheat grain), acetone/water (80:20; v/v) extracts, acetone/water/hydrochloric acid (80:20:1; v/v/v) extract and post-extraction solids (PES, un-extractable material) are shown in the table below.

Table B.7.1.3.1-3: Distribution of [^{14}C]S-2200 residues in wheat (forage, hay, straw and grain)

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Wheat forage 1 x 300 g/ha PHI 7 days	Surface wash	4.57	41.01	3.54	33.94
	1st Extraction with acetone/water	4.49	40.33	5.11	48.93
	2nd Extraction with acetone /water	1.08	9.71	1.00	9.62
	Extraction with acetone /water / HCl	0.36	3.19	0.22	2.06
	Sum of extracts [1)]	5.93	53.23	6.33	60.61
	PES (post extraction solids)	0.64	5.76	0.57	5.45
	Total Radioactive Residue (TRR)	**11.14**	**100**	**10.44**	**100**
Wheat hay 1 x 300 g/ha PHI 14 days	Surface wash	1.45	23.34	1.73	19.13
	1st Extraction with acetone/water	3.00	48.32	5.45	60.34
	2nd Extraction with acetone /water	0.79	12.73	0.93	10.26
	Extraction with acetone /water / HCl	0.29	4.71	0.20	2.15
	Sum of extracts [1)]	4.09	65.76	6.58	72.75
	PES (post extraction solids)	0.68	10.90	0.73	8.12
	Total Radioactive Residue (TRR)	**6.21**	**100**	**9.04**	**100**
Wheat straw 1 x 300 g/ha PHI 104 days	Surface wash	0.07	3.72	0.07	2.79
	1st Extraction with acetone/water	0.34	18.57	0.48	19.08
	2nd Extraction with acetone /water	0.41	22.30	0.69	27.59
	Extraction with acetone /water / HCl	0.33	17.84	0.45	18.03
	Sum of extracts [1)]	1.09	58.71	1.61	64.70
	PES (post extraction solids)	0.70	37.58	0.81	32.51
	Total Radioactive Residue (TRR)	**1.85**	**100**	**2.49**	**100**
Wheat grain 1 x 300 g/ha PHI 104 days	Surface wash	Not performed		Not performed	
	1st Extraction with acetone/water	<0.01	35.96	0.02	26.59
	2nd Extraction with acetone /water	<0.01	18.44	0.02	26.70
	Extraction with acetone /water / HCl	<0.01	12.57	0.02	19.38
	Sum of extracts [1)]	<0.01	66.97	0.07	72.67
	PES (post extraction solids)	<0.01	33.03	0.02	27.33
	Total Radioactive Residue (TRR)	**0.01**	**100**	**0.09**	**100**

1) …..excluding surface wash

B.7.1.3.2 Distribution, characterisation and identification of residues

The extractable fractions were profiled separately by radio-HPLC and TLC using co-chromatography with reference standards. Chiral HPLC was employed to determine the ratio of *R*- and *S*-isomers of S-2200.

The TRR concentrations in the **[phenoxy-^{14}C]S-2200** wheat samples were 11.14 mg/kg in wheat forage, 6.21 mg/kg in wheat hay, 1.85 mg/kg in wheat straw and 0.012 mg/kg in wheat grain. The radioactive concentration in the surface rinse accounted for 4.57 mg/kg (41.01% TRR) in wheat forage, 1.45 mg/kg (23.34 % TRR) in wheat hay and 0.069 mg/kg (3.72 % TRR) in wheat straw. The majority of the recovered radioactivity was present in the extractable fractions of the surface-rinsed wheat samples. The radioactive concentration in the extractable portions accounted for 5.93 mg/kg (53.23 % TRR) in wheat forage, for 4.086 mg/kg (65.76 % TRR) in wheat hay, for 1.087 mg/kg (58.71 % TRR) in wheat straw and 0.008 mg/kg (66.97 % TRR) in wheat grain. The un-extractable radioactivity represented 0.64 mg/kg (5.76% TRR) in wheat forage, 0.68 mg/kg (10.90 % TRR) in wheat hay, 0.696 mg/kg (37.58 % TRR) in wheat straw and 0.004 mg/kg (33.03 % TRR) in wheat grain. The results are summarized in the table below.

Table B.7.1.3.2-1: Distribution of residues wheat samples after application of [phenoxy-^{14}C]S-2200 in % TRR and mg as eq/kg

	Wheat forage 1 x 300 g/ha		Wheat hay 1 x 300 g/ha		Wheat straw 1 x 300 g/ha		Wheat grain 1 x 300 g/ha	
Time interval from last application	7 days		14 days		104 days		104 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR -Total Radioactive residues	100	11.14	100	6.21	100	1.85	100	0.012
ERR - Extracted Radioactive Residues	**94.24**[1)]	**10.50**	**89.10**[1)]	**5.54**	**62.42**[1)]	**1.16**	**66.97**	**< 0.01**
Surface extraction (Surface wash)	41.01	4.57	23.34	1.45	3.72	0.07	n.a.	n.a.
Watersoluble extract radioactivity	53.23	5.93	65.76	4.09	58.71	1.09	66.97	< 0.01
Radioactivity released under hydrolysis conditions	n.a.	n.a.	8.74	0.54	38.71	0.71	n.a.	n.a.
Total identified	71.39	7.96	58.85	3.66	17.72	0.33	n.a.	n.a.
Total characterized	22.84	2.55	30.22	1.89	44.69	0.83	66.97	< 0.01
URR – Unextracted Radioactive Residues	**5.76**	**0.64**	**10.90**	**0.68**	**37.58**	**0.70**	**33.03**	**< 0.01**
Accountability (sum ERR and URR)	100	11.14	100	6.22	100	1.87	100	0.01

n.a.= not applicable
1) …..including surface wash

The TRR concentrations in the **[benzyl-^{14}C]S-2200** wheat samples were 10.435 mg/kg in wheat forage, 9.04 mg/kg in wheat hay, 2.49 mg/kg in wheat straw and 0.089 mg/kg in wheat grain. The radioactive concentration in the surface rinse accounted for 3.54 mg/kg (33.94 % TRR) in wheat forage, 1.729 mg/kg (19.13 % TRR) in wheat hay and 0.07 mg/kg (2.79 % TRR) in wheat straw. The majority of the recovered radioactivity was present in the extractable fractions of the surface-rinsed wheat samples. The radioactive concentration in the extractable portions accounted for 6.325 mg/kg (60.61 % TRR) in wheat forage, for 6.576 mg/kg (72.75 % TRR) in wheat hay, for 1.613 mg/kg (64.70 % TRR) in wheat straw and 0.065 mg/kg (72.67 % TRR) in wheat grain. The un-extractable radioactivity represented 0.569 mg/kg (5.45 % TRR) in wheat forage 0.734 mg/kg (8.12 % TRR) in wheat hay, 0.810 mg/kg (32.51 % TRR) in wheat straw and 0.024 mg/kg (27.33 % TRR) in wheat grain. The results are summarized in the table below.

Table B.7.1.3.2-2: Distribution of residues in wheat samples after application of [benzyl-^{14}C]S-2200 in % TRR and mg as eq/kg

Time interval from last application	Wheat forage 1 x 300 g/ha		Wheat hay 1 x 300 g/ha		Wheat straw 1 x 300 g/ha		Wheat grain 1 x 300 g/ha	
	7 days		14 days		104 days		104 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR - Total Radioactive residues	100	10.44	100	9.04	100	2.49	100	0.09
ERR - Extracted Radioactive Residues	**94.55[1)]**	**9.87**	**91.88[1)]**	**8.31**	**67.49[1)]**	**1.68**	**72.67**	**0.07**
Surface extraction (Surface wash)	33.94	3.54	19.13	1.73	2.79	0.07	n.a.	n.a.
Watersoluble extract radioactivity	60.61	6.33	72.75	6.58	64.70	1.61	72.67	0.07
Radioactivity released under hydrolysis conditions	n.a.	n.a.	7.29	0.66	29.62	0.74	n.a.	n.a.
Total identified	81,32	8,48	50,86	4,61	29,63	0,73	66,37	0,06
Total characterized	13,23	1,39	40,92	3,71	37,87	0,96	9,0	0,01
URR – Unextracted Radioactive Residues	**5.45**	**0.57**	**8.12**	**0.73**	**32.51**	**0.81**	**27.33**	**0.02**
Accountability (sum ERR and URR)	100	10.44	100	9.04	100	2.49	100	0.09

n.a.= not applicable
1) …..including surface wash

The un-extractable residues of both radiolabels were sequentially hydrolyzed with enzyme, acid and base. A portion of the post-extraction solids (PES) of the wheat hay samples were further characterised by sequential enzyme hydrolysis with Driselase (enzyme mixture of fungal carbohydrolases), mild acid hydrolysis (0.1M HCl, 40°C overnight) and mild base hydrolysis (0.1M NaOH, 40°C overnight). The results are summarized in the table below.

Table B.7.1.3.2-3: Distribution of radioactivity in the PES of wheat hay after one application of ^{14}C-S-2200

Fraction	Total Radioactive Residue					
	[phenoxy-^{14}C]S-2200			[benzyl-^{14}C]S-2200		
	% of PES[1)]	mg/kg	% TRR[2)]	% of PES[1)]	mg/kg	% TRR[2)]
Enzyme Hydrolysis	13.45	0.09	1.47	43.38	0.32	3.52
Weak Acid Hydrolysis (0.1M HCL)	13.25	0.09	1.44	18.13	0.13	1.47
Weak Base Hydrolysis (0.1M NaOH)	53.46	0.36	5.83	28.36	0.21	2.30
Bound Residue	12.01	0.08	1.31	5.08	0.04	0.41
Total	92.18	0.62	10.05	94.96	0.70	7.72

*1) ..... % of PES = (Radioactivity released (dpm)) /(total dpm available) * 100*
*2) .....% TRR = (% of PES) * (% PES TRR) / 100*

A portion of the post-extraction solids (PES) of the wheat straw samples were further characterised by sequential mild acid hydrolysis (0.1M HCl, 40°C overnight), strong acid hydrolysis (6M HCl, 80°C, 4 hours), mild base hydrolysis (0.1M NaOH, 40°C overnight) and strong base hydrolysis (6M NaOH, 80°C overnight). The results are summarized in the table below.

Table B.7.1.3.2-4: Distribution of radioactivity in the PES of wheat straw after one application of ^{14}C-S-2200

Fraction	Total Radioactive Residue					
	[phenoxy-^{14}C]S-2200			[benzyl-^{14}C]S-2200		
	% of PES[1)]	mg/kg	% TRR[2)]	% of PES[1)]	mg/kg	% TRR[2)]
Weak Acid Hydrolysis (0.1M HCL)	15.73	0.11	5.91	15.21	0.12	4.94
Strong Acid Hydrolysis (6M HCL)	13.49	0.09	5.07	9.58	0.08	3.11
Weak Base Hydrolysis (0.1M NaOH)	30.12	0.21	11.32	31.66	0.26	10.29
Strong Base Hydrolysis (6M NaOH	43.68	0.30	16.41	34.70	0.28	11.28
Bound Residue	2.94	0.02	1.11	1.76	0.01	0.57
Total	105.97	0.74	39.82	92.91	0.75	30.21

*[1)]..... % of PES = (Radioactivity released (dpm)) /(total dpm available) * 100*
*[2)].....% TRR = (% of PES) * (% PES TRR) / 100*

Characterization and identification of metabolites was made initially by comparison of HPLC/RAM sample retention times with co-injected reference standard mix. The regions or peaks of interest were then isolated from the wheat extracts using reverse-phase HPLC. Metabolites were then characterized by HPLC co-chromatography with reference standards and 1D-TLC co-migration with reference standards.

For confirmation of the S-2200 R:S isomer ratio the surface rinses were analyzed directly using chiral HPLC co-chromatography with S-2200 reference standard for the forage and hay harvest samples. The ^{14}C peak corresponding to the retention time of S-2200 was isolated from the neutral extractable fraction and analyzed by chiral HPLC co-chromatography. The S-2200 R:S isomer ratio was approximately 50:50 in the [^{14}C]S-2200 test substances and remained at approximately 50:50 in the processed samples. Isomerization of the R:S isomers of [^{14}C]S-2200 was not observed in this study.
For confirmation of S-2200 the surface rinsed samples and the [^{14}C]S-2200 isolates from the neutral extractable fractions were analyzed directly using 1D-TLC by co-spotting the isolates with authentic S-2200 standard.
It was demonstrated that [^{14}C]S-2200 isolate and S-2200 reference standard co-eluted using both chromatographic techniques. Thus the identity of [^{14}C]S-2200 was confirmed by HPLC and TLC chromatographic data.
Control samples of homogenized wheat grain and wheat forage were fortified with [^{14}C]S-2200 at approximately 0.5 and 7.5 mg/kg, respectively to demonstrate extraction efficiency of S-2200. The fortified samples were extracted and analyzed using the same methods used for the analysis of treated samples. Control tissue was spiked with [^{14}C]S-2200, and the fortified control samples were extracted and analyzed once during the study.The results demonstrated that the radioactivity from the spiked controls could be quantitatively (>95%) extracted, and that parent S-2200 remained at 90% of the TRR.

Wheat forage (Group II)
S-2200 was observed at 5.684 mg/kg (51.01 % TRR) in the **[phenoxy-^{14}C]S-2200** treated wheat forage. MCBX was observed at 0.033 mg/kg (0.30% TRR). An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 1.177 mg/kg (10.57 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.375 mg/kg (3.36 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.686 mg/kg (6.15 % TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.615 mg/kg (5.52% TRR). The largest peak in this region was accounted for 0.064 mg/kg (0.57 % TRR) and characterized as multiple components by further analysis using HPLC and TLC. Minor regions of radioactivity was observed at HPLC/RAM retention times of 2-min (Unknown 1: 1.60 % TRR, 0.179 mg/kg), of 9-min (Unknown 2: 1.19 % TRR, 0.133 mg/kg), 11-min (Unknown 3: 1.02 % TRR, 0.114 mg/kg) and 32-min (Unknown 7: 3.0 % TRR, 0.335 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table

which together accounted for 1.093 mg/kg (9.81 % TRR). There were no other significant degradation products detected in the extractable fraction.

Wheat hay (Group II)

S-2200 was observed at 1.630 mg/kg (26.24 % TRR) in the **[phenoxy-^{14}C]S-2200** treated wheat hay. MCBX was observed at 0.046 mg/kg (0.74% TRR). An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.697 mg/kg (11.22 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.813 mg/kg (13.09 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.389 mg/kg (6.26 % TRR). Another area of radioactivity was observed at 0.081 mg/kg (1.30% TRR) and was characterized as 2-CH_2OH-S2200. An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.748 mg/kg (12.04% TRR). The largest peak in this region accounted for 0.099 mg/kg (1.59 % TRR) and was characterized as multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 1.20 % TRR, 0.075 mg/kg), of 9-min (Unknown 2: 0.59 % TRR, 0.037 mg/kg), 11-min (Unknown 3: 1.10 % TRR, 0.068 mg/kg), 31-min (Unknown 6: 0.66 % TRR, 0.041 mg/kg) and 32-min (Unknown 7: 1.97 % TRR, 0.123 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.742 mg/kg (11.94 % TRR). There were no other significant degradation products detected in the extractable fraction.

Wheat straw (Group II)

S-2200 was observed at 0.026 mg/kg (1.38 % TRR) in the **[phenoxy-^{14}C]S-2200** treated wheat straw. MCBX was observed at 0.012 mg/kg (0.66 % TRR). An area of radioactivity accounted for 0.176 mg/kg (9.50 % TRR) was characterized as 2-CH_2OH-S2200. Free forms of 5-CH_2OH-S2200, 5-COOH-S-2200 and 4-OH-S-2200 were observed at levels of 0.039 mg/kg (2.08 % TRR), 0.053 mg/kg (2.87 % TRR) and 0.023 mg/kg (1.23 % TRR), respectively. An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.388 mg/kg (20.96 % TRR). The largest peak in this region was accounted for 0.135 mg/kg (7.28 % TRR) and characterized as multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.73 % TRR, 0.014 mg/kg), of 9-min (Unknown 2: 0.98 % TRR, 0.018 mg/kg), 11-min (Unknown 3: 1.37 % TRR, 0.025 mg/kg), 16-min (Unknown 9: 4.05 % TRR, 0.075 mg/kg), 19-min (Unknown 10: 1.18 % TRR, 0.022 mg/kg) and 22-min (Unknown 5: 1.70 % TRR, 0.032 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.254 mg/kg (13.72 % TRR). There were no other significant degradation products detected in the extractable fraction.

Wheat grain (Group II)

S-2200 was not detected in any extractable fraction of the **[phenoxy-^{14}C]S-2200** treated wheat grain. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 18.42 % TRR, 0.002 mg/kg), 11-min (Unknown 3: 25.61 % TRR, 0.003 mg/kg) and 14-min (Unknown 4: 22.94 % TRR, 0.003 mg/kg). There were no other significant degradation products detected in the extractable fraction of wheat grain.

The results are summarized in the table below.

Table B.7.1.3.2-5: Identification and characterisation of residues in wheat samples after application of [phenoxy-^{14}C]S-2200 in % TRR and mg as eq/kg

Time interval from last application	Wheat forage 1 x 300 g/ha		Wheat hay 1 x 300 g/ha		Wheat straw 1 x 300 g/ha		Wheat grain 1 x 300 g/ha	
	7 days		14 days		104 days		104 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR – Total Radioactive residues	**100**	**11.14**	**100**	**6.21**	**100**	**1.85**	**100**	**0.012**
S-2200	51.01	5.68	26.24	1.63	1.38	0.03	nd	nd
Surface wash	38.49	4.29	20.96	1.30	0.27	< 0.01	nd	nd
Acetone/water extracts	11.94	1.33	4.88	0.30	0.67	0.01	nd	nd
Acetone/water/HCL extracts	0.58	0.07	0.40	0.03	0.44	< 0.01	nd	nd
2-CH_2OH-S-2200 (conjugated. 16min)	10.57[2)]	1.18	11.22[5)]	0.70	nd	nd	nd	nd
Surface wash	0.32	0.04	0.15	< 0.01	nd	nd	nd	nd
Acetone/water extracts	9.68	1.08	10.60	0.66	nd	nd	nd	nd
Acetone/water/HCL extracts	0.57	0.06	0.47	0.03	nd	nd	nd	nd
4-OH-S-2200 (conjugated. 16min)	3.36[2)]	0.38	13.09[5)]	0.81	nd	nd	nd	nd
5-CH_2OH-S-2200 (conjugated. 19min)	6.15[3)]	0.69	6.26[6)]	0.39	nd	nd	nd	nd
2-CH_2OH-S-2200	nd	nd	1.30	0.08	9.50	0.18	nd	nd
MCBX	0.30	0.03	0.74	0.05	0.66	0.01	nd	nd
5- CH_2OH-S-2200	nd	nd	nd	nd	2.08	0.04	nd	nd
5-COOH-S-2200	nd	nd	nd	nd	2.87	0.05	nd	nd
4-OH-S-2200	nd	nd	nd	nd	1.23	0.02	nd	nd
Total identified	**71.39**	**7.96**	**58.85**	**3.66**	**17.72**	**0.33**	**n.a.**	**n.a.**
Unknown 1 (2min)	1.60	0.18	1.20	0.08	0.73	0.01	18.42	< 0.01
Unknown 2 (9min)	1.19	0.13	0.59	0.04	0.98	0.02	nd	nd
Unknown 3 (11min)	1.02	0.11	1.10	0.07	1.37	0.03	25.61	< 0.01
Unknown 4 (14min)	5.52[4)]	0.62	12.04[7)]	0.75	20.96[9)]	0.39	22.94	< 0.01
Unknown 5 (22min)	nd	nd	nd	nd	1.70	0.03	nd	nd
Unknown 6 (31 min)	nd	nd	0.66	0.04	nd	nd	nd	nd
Unknown 7 (32 min)	3.0	0.34	1.97	0.12	nd	nd	nd	nd
Unknown 8 (37.8 min)	0.70	0.08	0.72	0.05	nd	nd	nd	nd
Unknown 9 (16 min)	n.a.	n.a.	n.a.	n.a.	4.05	0.08	nd	nd
Unknown 10 (19 min)	n.a.	n.a.	n.a.	n.a.	1.18	0.02	nd	nd
Others	9.81	1.09	11.94[8)]	0.74	13.72[8)]	0.25	nd	nd
Total characterized	**22.84**	**2.55**	**30.22**	**1.89**	**44.69**	**0.83**	**66.97**	**< 0.01**
ERR - Extracted Radioactive Residues	**94.24[1)]**	**10.50**	**89.10[1)]**	**5.54**	**62.42[1)]**	**1.16**	**66.97**	**< 0.01**
URR – Unextracted Radioactive Residues	5.76	0.64	10.90	0.68	37.58	0.70	33.03	< 0.01

n.a.= not applicable nd= not detected

1) .....including surface wash
2) .....Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 75.8:24.2
3) .....Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200
4) .....Comprising of multiple compounds. largest component 0.57% TRR. 0.064 mg/kg.
5) .....Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 46.2:53.8
6) .....Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200.
7) .....Comprising of multiple compounds. largest component 1.59% TRR. 0.099 mg/kg.
8) .....Comprising of numerous minor peaks. each peak accounted for or less than 1%TRR
9) .....Comprising of multiple compounds. largest component 7.28% TRR. 0.135 mg/kg.

Wheat forage (Group III)

S-2200 was observed at 6.254 mg/kg (59.93 % TRR) in the **[benzyl-^{14}C]S-2200** treated wheat forage. MCBX and De-Xy-S-2200 were observed at 0.297 mg/kg (2.84% TRR) and 0.334 mg/kg (3.20% TRR), respectively. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.570 mg/kg (5.47 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.563 mg/kg (5.40 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.445 mg/kg (4.26 % TRR). Another area of radioactivity was observed at 0.022 mg/kg (0.22 % TRR) and was characterized as 2-CH_2OH-S2200. An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.248 mg/kg (2.37% TRR). The largest peak in this region was accounted for 0.050 mg/kg (0.48 % TRR) comprising of multiple components.Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.71 % TRR, 0.075 mg/kg), of 9-min (Unknown 2: 3.32 % TRR, 0.346 mg/kg) and 37.8-min (Unknown 8: 0.47 % TRR, 0.049 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.663 mg/kg (6.36 % TRR). There were no other significant degradation products detected in the extractable fraction.

Wheat hay (Group III)

S-2200 was observed at 2.051 mg/kg (22.68 % TRR) in the **[benzyl-^{14}C]S-2200** treated wheat hay. MCBX and De-Xy-S-2200 were observed at 0.075 mg/kg (0.83% TRR) and 0.137 mg/kg (1.52 % TRR), respectively. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 1.137 mg/kg (12.58 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.496 mg/kg (5.49 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.619 mg/kg (6.85 % TRR). Another area of radioactivity was observed at 0.083 mg/kg (0.91% TRR) and was characterized as 2-CH_2OH-S2200. An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.933 mg/kg (10.32 % TRR). The largest peak in this region was accounted for 0.120 mg/kg (1.33 % TRR) comprising of multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.03 % TRR, 0.002 mg/kg), of 9-min (Unknown 2: 4.43 % TRR, 0.400 mg/kg), 22-min (Unknown 5: 0.92 % TRR, 0.084 mg/kg), 31-min (Unknown 6: 0.92 % TRR, 0.084 mg/kg) and 32-min (Unknown 7: 2.68 % TRR, 0.242 mg/kg) and 37.8-min (Unknown 8: 0.53 % TRR, 0.048 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 1.971 mg/kg (21.80 % TRR). There were no other significant degradation products detected in the extractable fraction.

Wheat straw (Group III)

S-2200 was observed at 0.050 mg/kg (1.99 % TRR) in the **[benzyl-^{14}C]S-2200** treated wheat straw. MCBX and De-Xy-S-2200 were observed at 0.010 mg/kg (0.42% TRR) and 0.294 mg/kg (11.78 % TRR), respectively. An area of radioactivity accounted for 0.160 mg/kg (6.42 % TRR) was characterized as 2-CH_2OH-S2200. Free forms of 5-CH_2OH-S2200, 5-COOH-S-2200 and 4-OH-S-2200 were observed at 0.073 mg/kg (2.93 % TRR), 0.114 mg/kg (4.58 % TRR) and 0.038 mg/kg (1.51 % TRR), respectively. An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.302 mg/kg (12.10 % TRR). The largest peak in this region was accounted for 0.065 mg/kg (2.61 % TRR) comprising of multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.07 % TRR, 0.002 mg/kg), of 9-min (Unknown 2: 3.95 % TRR, 0.098 mg/kg), 16-min (Unknown 9: 2.80 % TRR, 0.070 mg/kg), 19-min (Unknown 10: 0.03 % TRR, 0.001 mg/kg) and 22-min (Unknown 5: 1.11 % TRR, 0.028 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.444 mg/kg (17.81 % TRR). There were no other significant degradation products detected in the extractable fraction.

Wheat grain (Group III)

S-2200 was not detected in any extractable fraction of the **[benzyl-^{14}C]S-2200** treated wheat grain. De-Xy-S-2200 and 2-CH_2OH-S-2200 were observed at 0.054 mg/kg (60.55% TRR) and 0.003 mg/kg (3.12% TRR), respectively. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 4.21 % TRR, 0.004 mg/kg), 16-min (Unknown 9: 1.29 % TRR, 0.001 mg/kg), 19-min (Unknown 10: 0.23 % TRR, < 0.001 mg/kg) and 22-min (Unknown 5: 2.13 % TRR, 0.002 mg/kg). There were no other significant degradation products detected in the extractable fraction of wheat grain.

The results are summarized in the table below.

Table B.7.1.3.2-6: Identification and characterisation of residues in wheat samples after application of [benzyl-^{14}C]S-2200 in % TRR and mg as eq/kg

Time interval from last application	Wheat forage 1 x 300 g/ha		Wheat hay 1 x 300 g/ha		Wheat straw 1 x 300 g/ha		Wheat grain 1 x 300 g/ha	
	7 days		14 days		104 days		104 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR – Total Radioactive residues	100	10.44	100	9.04	100	2.49	100	0.09
S-2200	59,93	6,25	22,68	2,05	1,99	0,05	nd	nd
Surface wash	30,31	3,16	16,35	1,48	0,49	0,01	n.a.	n.a.
Acetone/water extracts	29,34	3,06	6,09	0,55	0,97	0,02	nd	nd
Acetone/water/HCL extracts	0,28	0,03	0,25	0,02	0,53	0,01	nd	nd
De-Xy-S-2200	3,20	0,33	1,52	0,14	11,78	0,29	60,55	0,05
Surface wash	0,69	0,07	nd	Nd	0,48	0,01	n.a.	n.a.
Acetone/water extracts	2,38	0,25	1,48	0,13	8,04	0,20	46,92	0,04
Acetone/water/HCL extracts	0,13	0,01	0,04	< 0,01	3,26	0,08	13,63	0,01
2-CH_2OH-S-2200 (conjugated. 16min)	5,47[2)]	0,57	12,58[5)]	1,14	nd	nd	nd	nd
Surface wash	0,23	0,02	0,31	0,03	nd	nd	n.a.	n.a.
Acetone/water extracts	5,02	0,52	12,05	1,09	nd	nd	nd	nd
Acetone/water/HCL extracts	0,22	0,02	0,22	0,02	nd	nd	nd	nd
4-OH-S-2200 (conjugated. 16min)	5,40[2)]	0,56	5,49[5)]	0,50	nd	nd	nd	nd
5-CH_2OH-S-2200 (conjugated. 19min)	4,26[3)]	0,45	6,85[6)]	0,62	nd	nd	nd	nd
2-CH_2OH-S-2200	0,22	0,02	0,91	0,08	6,42	0,16	3,12	< 0,01
MCBX	2,84	0,30	0,83	0,08	0,42	0,01	nd	nd
5- CH_2OH-S-2200	nd	nd	nd	nd	2,93	0,07	nd	nd
5-COOH-S-2200	nd	nd	nd	nd	4,58	0,11	nd	nd
4-OH-S-2200	nd	nd	nd	nd	1,51	0,04	nd	nd
Total identified	**81,32**	**8,48**	**50,86**	**4,61**	**29,63**	**0,73**	**3.67**	**0,06**
Unknown 1 (2min)	0,71	0,08	0,03	< 0,01	0,07	< 0,01	4,21	<0,01
Unknown 2 (9min)	3,32	0,35	4,43	0,40	3,95	0,10	nd	nd
Unknown 4 (14min)	2,37[4)]	0,25	10,32[7)]	0,93	12,10[9)]	0,30	nd	nd
Surface wash	0,24	0,03	0,45	0,04	0,15	< 0,01	n.a.	n.a.
Acetone/water extracts	1,98	0,21	9,71	0,88	8,06	0,20	nd	nd
Acetone/water/HCL extracts	0,15	0,02	0,16	0,02	3,89	0,10	nd	nd
Unknown 5 (22min)	nd	nd	0,31	0,03	1,11	0,03	2,13	< 0,01
Unknown 6 (31 min)	nd	nd	0,92	0,08	nd	nd	nd	nd
Unknown 7 (32 min)	nd	nd	2,68	0,24	nd	nd	nd	nd
Unknown 8 (37.8 min)	0,47	0,05	0,53	0,05	nd	nd	nd	nd
Unknown 9 (16 min)	nd	nd	nd	nd	2,80	0,07	1,29	<0,01

Time interval from last application	Wheat forage 1 x 300 g/ha		Wheat hay 1 x 300 g/ha		Wheat straw 1 x 300 g/ha		Wheat grain 1 x 300 g/ha	
	7 days		14 days		104 days		104 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
Unknown 10 (19 min)	nd	nd	nd	nd	0,03	< 0,01	0,23	< 0,01
Others	6,36	0,66	21,80[8)]	1,97	17,81[8)]	0,44	1,14	< 0,01
Surface wash	1,21	0,13	0,35	0,03	0,61	0,02	n.a.	n.a.
Acetone/water extracts	4,68	0,49	20,70	1,87	12,40	0,31	0,87	< 0,01
Acetone/water/HCL extracts	0,47	0,05	0,75	0,07	4,80	0,12	0,27	< 0,01
Total characterized	**13,23**	**1,39**	**41.02**	**3,71**	**37,87**	**0,96**	**9,0**	**0,01**
ERR - Extracted Radioactive Residues	**94,55[1)]**	**9,87**	**91,88**	**8,31**	**67,49**	**1,68**	**72,67**	**0,07**
URR – Unextracted Radioactive Residues	5,45	0,57	8,12	0,73	32,51	0,81	27,33	0,024

n.a.= not applicable nd= not detected
1)…..including surface wash
2)……Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 50.3:49.7.
3)……Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200
4)……Comprising of multiple compounds, largest component 0.48% TRR, 0.050 mg/kg.
5)…… Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 69.6:30.4
6)……Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200
7)……Comprising of multiple compounds, largest component 1.33% TRR, 0.120 mg/kg
8)……Comprising of numerous minor peaks, each peak accounted for or less than 1%TRR.
9)……Comprising of multiple compounds, largest component 7.28% TRR, 0.135 mg/kg.

Representative [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 treated final harvest samples were extracted and analyzed after approximately 9 and 2 month, respectively. The extracts were chromatographed by HPLC and compared to the initial profile to verify the stability of metabolites in the matrices during storage. The comparison of distributions of major ^{14}C residues in the [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C] S-2200 wheat plants for the forage and hay extractable fractions are given in the tables below.

Table B.7.1.3.2-7: Comparison of ^{14}C Residues (Neutral and acid extrables) in Wheat forage following 9 month frozen storage and in Wheat hay following 2 month frozen storage

	[phenoxy-^{14}C] S-2200				[benzyl-^{14}C] S-2200			
	Wheat forage (Group II)				Wheat hay (Group III)			
	Neutral extractable[1)2)] (Acetone : water = 80 : 20)		Acid extractable[1)2)] (Acetone : water : acid= 80 : 20:1)		Neutral extractable (Acetone : water = 80 : 20)		Acid extractable (Acetone : water : acid= 80 : 20:1)	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
Original analysis	82.08	5.576	5.23	0.355	86.62	6.381	2.64	0.195
nine month frozen storage	79.45	6.826	4.28	0.368	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	74.15	5.463	5.61	0.413
S-2200								
Original analysis	11.94	1.330	0.58	0.065	6.09	0.550	0.25	0.023
nine month frozen storage	9.34	1.186	0.68	0.087	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	6.53	0.554	0.29	0.025
De-Xy-S2200								
Original analysis	nd	nd	nd	nd	1.48	0.133	0.04	0.004

	[phenoxy-^{14}C] S-2200				[benzyl-^{14}C] S-2200			
	Wheat forage (Group II)				**Wheat hay** (Group III)			
nine month frozen storage	nd	nd	nd	nd	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	nd	nd	nd	nd	1.31	0.111	0.09	0.007
Unknown 4 (14min)								
Original analysis	5.02	0.559	0.33	0.037	9.71	0.877	0.16	0.015
nine month frozen storage	12.91	1.638	0.71	0.090	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	17.09	1.452	1.39	0.118
2-CH_2OH-S-2200 (conjugated. 16min)								
Original analysis	9.68	1.079	0.57	0.063	12.05	1.089	0.22	0.019
nine month frozen storage	12.36	1.569	0.56	0.071	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	11.36	0.965	0.51	0.043
4-OH-S-2200 (conjugated. 16min)								
Original analysis	3.08	0.344	0.18	0.020	5.26	0.475	0.09	0.009
nine month frozen storage	3.94	0.500	0.18	0.023	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	4.96	0.421	0.22	0.019
5-CH_2OH-S-2200 (conjugated. 19min)								
Original analysis	5.93	0.661	0.22	0.024	6.67	0.603	0.18	0.016
nine month frozen storage	7.30	0.926	0.25	0.032	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	6.89	0.586	0.36	0.031
2-CH_2OH-S-2200								
Original analysis	nd	nd	nd	nd	0.68	0.062	0.23	0.021
nine month frozen storage	0.24	0.031	0.03	0.003	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	0.15	0.013	0.43	0.037
Others								
Original analysis	7.81	0.870	0.88	0.098	20.71	1.871	0.75	0.068
nine month frozen storage	1.45	0.184	0.30	0.039	n.a.	n.a.	n.a.	n.a.
Two month frozen storage	n.a.	n.a.	n.a.	n.a.	10.09	0.857	1.09	0.092

[1] *…..wheat forage TRR was determined by combustion analysis at harvest collection to be 6.793 mg/kg. Recovery of original final harvest samples is based on this TRR value.*

[2] *…..wheat forage TRR was determined by combustion analysis at 9-months frozen storage to be 8.593 mg/kg. Recovery of 7-month stability samples is based on this TRR valu*

The metabolite profiles from the two analyses (the initial and final analyses) were very similar indicating that [^{14}C]S-2200 metabolites were stable in the wheat samples under freezer storage conditions.

B.7.1.3.3 Conclusions and metabolic pathway of S-2200 in Wheat

The metabolism of [^{14}C]S-2200 was studied in wheat to determine the total [^{14}C] residues wheat forage, hay, straw and mature grain to identify and characterize the nature of the major [^{14}C] residues. The study was carried out under greenhouse conditions using two radiolabeled forms of [^{14}C]S-2200. [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 as a 25% SC formulation.

Table B.7.1.3.3-1: Distribution of [^{14}C]S-2200 residues in wheat (forage, hay, straw and seed)

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Wheat Forage 1 x 300 g/ha PHI 7 days	Surface wash	4.57	41.01	3.54	33.94
	Sum of extracts [1)]	5.93	53.23	6.33	60.61
	PES (post extraction solids)	0.64	5.76	0.57	5.45
	Total Radioactive Residue (TRR)	**11.14**	**100**	**10.44**	**100**
Wheat hay 1 x 300 g/ha PHI 14 days	Surface wash	1.45	23.34	1.73	19.13
	Sum of extracts [1)]	4.09	65.76	6.58	72.75
	PES (post extraction solids)	0.68	10.90	0.73	8.12
	Total Radioactive Residue (TRR)	**6.21**	**100**	**9.04**	**100**
Wheat straw 1 x 300 g/ha PHI 104 days	Surface wash	0.07	3.72	0.07	2.79
	Sum of extracts [1)]	1.09	58.71	1.61	64.70
	PES (post extraction solids)	0.70	37.58	0.81	32.51
	Total Radioactive Residue (TRR)	**1.85**	**100**	**2.49**	**100**
Wheat grain 1 x 300 g/ha PHI 104 days	Surface wash	Not performed			
	Sum of extracts	<0.01	66.97	0.07	72.67
	PES (post extraction solids)	<0.01	33.03	0.02	27.33
	Total Radioactive Residue (TRR)	**0.01**	**100**	**0.09**	**100**

[1)]…..excluding surface wash

The major residues identified in the wheat forage, hay, straw and grain samples included S-2200, De-Xy-S-2200, 2-CH_2OH-S-2200 (conjugated) and 4-OH-S-2200 (conjugated) with some minor metabolites such as [^{14}C] conjugate of 5-CH_2OH-S-2200, MCBX and some unknown (1-5) metabolites.

[phenoxy-^{14}C]S-2200 treatment

Following one application at 300 g a.s./ha on wheat plants, the TRR declined from 11.1 mg/kg S-2200 equivalents in wheat forage (PHI 7 days) to 6.2 mg/kg in hay (PHI 14 days), 1.85 mg/kg in straw and 0.01 mg/kg in wheat grain harvested at maturity (PHI 104 days).

In *wheat forage* S-2200 accounted for 5.7 mg/kg (51 % TRR). The surface washes of forage contained 38.5 % of the TRR (4.3 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 10.6 % TRR (1.2 mg/kg), 3.4 % TRR (0.4 mg/kg) and 6.2 % TRR (0.7 mg/kg), respectively. MCBX accounted for 0.3 % TRR (0.03 mg/kg). Free forms of 2-CH_2OH-S-2200, 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 were not detected in wheat forage.

In *wheat hay* S-2200 accounted for 1.6 mg/kg (26.2 % TRR). The surface washes of hay contained 21 % of the TRR (1.3 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 11.2 % TRR (0.7 mg/kg), 13.1 % TRR (0.81 mg/kg) and 6.3 % TRR (0.4 mg/kg), respectively. MCBX accounted for 0.7 % TRR (0.05 mg/kg). Free 2-CH_2OH-S-2200 accounted for for 1.3 % TRR (0.08 mg/kg), free forms of 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 were not detected in wheat hay.

In *wheat straw* S-2200 accounted for 0.03 mg/kg (1.38 % TRR). The surface washes of straw samples contained 1.3 % of the TRR (0.3 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were were not detected. MCBX accounted for 0.7 % TRR (0.01 mg/kg). Free 2-CH_2OH-S-2200 accounted for 9.5 % TRR (0.02 mg/kg), free forms of 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 accounted for 2.1 % TRR (0.04 mg/kg), 2.9 % TRR (0.05 mg/kg) and 1.2 % TRR (0.02 mg/kg), respectively. S-2200, glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 as well as free forms 2-CH_2OH-S-2200, 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 were not detected *wheat grain* samples after a single application of 300 g as/ha. Minor radioactivity was observed at HPLC/RAM retention times of 2-min (Unknown 1: 18.42 % TRR, 0.002 mg/kg), 11-min (Unknown 3: 25.61 % TRR, 0.003 mg/kg) and 14-min (Unknown 4: 22.94 % TRR, 0.003 mg/kg). There were no other significant degradation products detected in the extractable fraction of wheat grain.

De-Xy-S-2200 was not detected in any of the [phenoxy-^{14}C]S-2200 treated wheat plants.

[benzyl-^{14}C]S-2200 treatment

Following one application at 300 g a.s./ha on wheat plants, the TRR declined from 10.4 mg/kg S-2200 equivalents in wheat forage (PHI 7 days) to 9.0 mg/kg in hay (PHI 14 days), 2.49 mg/kg in straw and 0.09 mg/kg in wheat grain harvested at maturity (PHI 104 days).

In *wheat forage* S-2200 accounted for 6.25 mg/kg (59.9 % TRR). The surface washes of forage contained 30.3 % of the TRR (3.2 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 5.5 % TRR (0.6 mg/kg), 5.4 % TRR (0.6 mg/kg) and 4.3 % TRR (0.45 mg/kg), respectively. De-Xy-S-2200 and MCBX accounted for 3.2 % TRR (0.3 mg/kg) and 2.8 % TRR (0.3 mg/kg), respectively. Free 2-CH_2OH-S-2200 accounted for 0.2 % TRR (0.02 mg/kg) whereas free forms of 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 were not detected in wheat forage.

In *wheat hay* S-2200 accounted for 2.05 mg/kg (22.7 % TRR). The surface washes of hay contained 16.4 % of the TRR (1.5 mg/kg). The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 12.6 % TRR (1.1 mg/kg), 5.5 % TRR (0.5 mg/kg) and 6.9 % TRR (0.6 mg/kg), respectively. De-Xy-S-2200 and MCBX accounted for 1.5 % TRR (0.1 mg/kg) and 0.8 % TRR (0.08 mg/kg), respectively. Free 2-CH_2OH-S-2200 accounted for 0.9 % TRR (0.08 mg/kg), free forms of 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 were not detected in wheat hay.

In *wheat straw* S-2200 accounted for 0.05 mg/kg (2.0 % TRR). The surface washes of straw samples contained 0.49 % of the TRR (0.01 mg/kg). De-Xy-S-2200 and MCBX accounted for 11.8 % TRR (0.3 mg/kg) and 0.42 % TRR (0.01 mg/kg), respectively.The glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 were were not detected in wheat straw smples. Free 2-CH_2OH-S-2200 accounted for 6.4 % TRR (0.02 mg/kg), free forms of 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 accounted for 2.9 % TRR (0.07 mg/kg), 4.6 % TRR (0.1 mg/kg) and 1.5 % TRR (0.04 mg/kg), respectively.

S-2200, glycosides of 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200 as well as free forms of 5-CH_2OH-S-2200, 5-COOH-S-2200 and 4-OH-S-2200 were not detected *wheat grain* samples after a single application of 300 g as/ha. De-Xy-S-2200 accounted for 60.6 % TRR (0.05 mg/kg), MCBX was not detected in wheat grain. Free 2-CH_2OH-S-2200 accounted for 3.1 % TRR (< 0.01 mg/kg). There were no other significant degradation products detected in the extractable fraction of wheat grain.

All metabolites (De-Xy-S-2200, 4-OH-S-2200, 5-COOH-S-2200, 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and MCBX) were also found in the rat. The metabolites 4-OH-S-2200 and 5-COOH-S-2200 were present in the rat at >10% of dose.

The *R*/*S* ratio of [^{14}C]S-2200 remained approximately 1:1 indicating no *R*/*S* isomerization.

S-2200, when applied to wheat plants, a significant percentage of the applied material remained on the surface of the plants. The surface rinse contained mainly [^{14}C]S-2200. The [^{14}C]S-2200 that absorbed into wheat plants was metabolized to a number of polar products.

The major metabolism pathway included

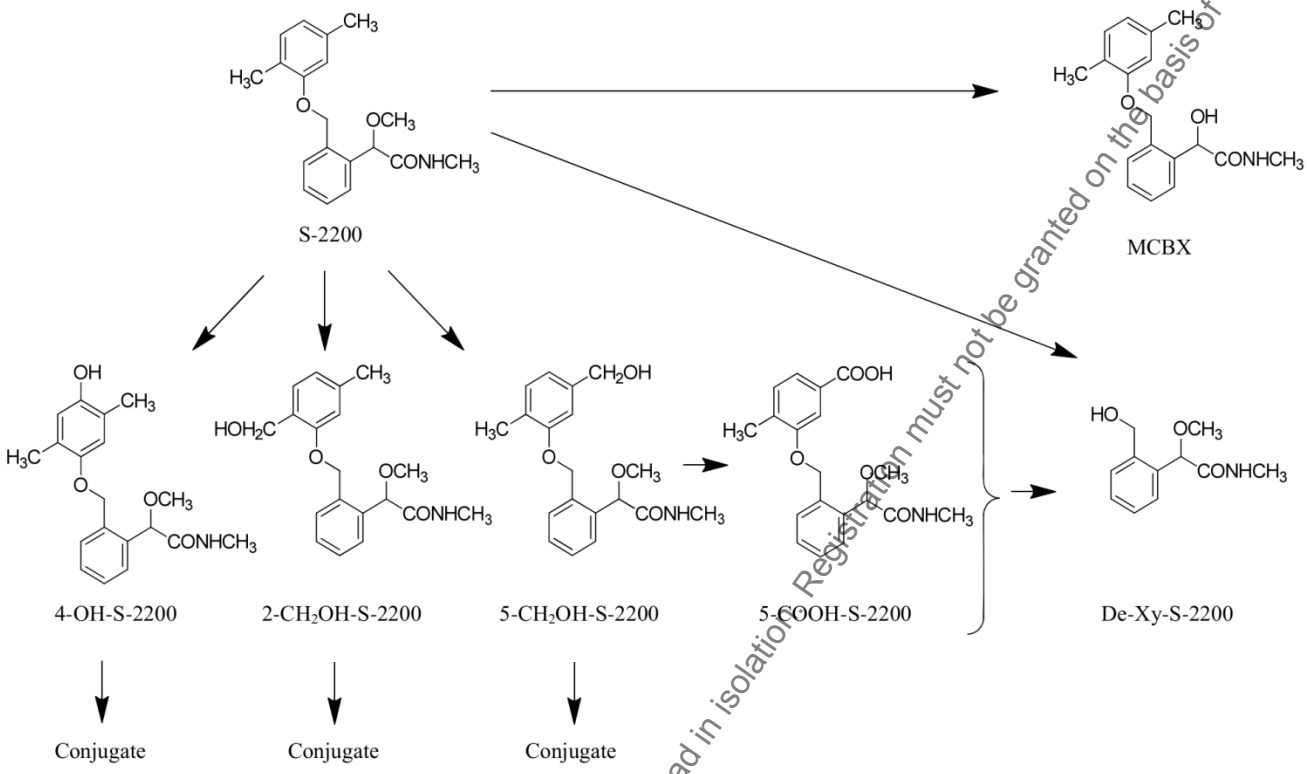
- ✓ monohydroxylation of the dimethylphenoxy ring to form 4-OH-S-2200 and subsequent formulation of glycoside conjugate
- ✓ oxidation of the methyl groups attached to the 2- and 5- positions on the dimethylphenoxy ring to form 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 and subsequent formation of the corresponding glycoside conjugates.
- ✓ cleavage of the ether linkage to form De-Xy-S-2200 [its counterpart 2,5-dimethylphenol (*p*-xylenol) was further metabolized to numerous polar components and then incorporated into plant constituents in wheat].

Minor metabolic pathways included

- ✓ *O*-demethylation of the methoxy group of the side chain to form MCBX
- ✓ oxidation of 5-CH_2OH-S-2200 to form 5-COOH-S-2200

Further metabolism occurred to form other minor metabolites and polar products.

Figure B.7.1.3-1: Proposed metabolic pathway of Mandestrobin (S-2200) in wheat



Code Name	Molecular Weight	Chemical Name
S-2200	313.39	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
MCBX	299.36	(RS)-2-hydroxy-N-methyl-2-[α-(2,5- xylyloxy)-o-tolyl]acetamide
De-Xy-S-2200	209.24	(*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide
4-OH-S-2200	329.39	(RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide
2-CH_2OH-S-2200	329.39	(RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-CH_2OH-S-2200	329.39	(RS)-2-[2-(5-hydroxymethyl-2- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-COOH-S-2200	343.38	(RS)-3-{2-[1-methoxy-1-(N-methylcarbamoyl) methyl]benzyloxy}-4-methylbenzoic acid

B.7.1.4 Metabolism in Lettuce

Reference:	**Metabolism of [^{14}C]S-2200 in lettuce plants**
Author(s), year:	Panthani A, Lentz N.R. (2010b)
Report/ Doc. Number:	Springborn Smithers Laboratories Report no. 13048.6631 Sumitomo ref: ROM-0008
Guideline(s):	Japanese MAFF, 12-Nousan-No. 8147, Part 2-4-1, 24.November 2000 US EPA OPPTS 860.1300 Nature of the Residue - Plants, Livestock EU Document 7028/VI/95 rev. 3 (22/07/97), Appendix A - Metabolism and Distribution in Plants OECD 501 - Guideline for the Testing of Chemicals - Metabolism in Crops
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	yes
Comment (RMS):	The study is acceptable

Test Site information:
Test site: Sprinborn Smithers Laboratories (Wareham, Massachusetts)
Testing environment: greenhouse
Soil characteristics: Mixture of Rochester natural loamy sand (USDA classification) and commercial potting soil (9:1 w/w)

pH	6.2
CEC (meq/100g)	10.2
OM (%)	8.3
Sand (%)	71
Silt (%)	22
Clay (%)	7
Soil classification	sandy loam

Crop information:
Crop/crop group: Lettuce/leaf vegetables
Variety/botanical name: Buttercrunch/Lactuca sativa
Test Material:
Common Name: S-2200
Name (IUPAC): (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers: S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight: 313.39
CAS No.: 173662-97-0
Purity: 100%
*Log $P_{o/w}$= 3.51 (*at 25 ± 1°C)
Radiolabel position, specific activity: [Phenoxy-^{14}C] S-2200 (120 mCi/mmol)
[Benzyl-^{14}C] S-2200 (123 mCi/mmol
Formulation: 25% SC (suspension concentrate)
Specific activity in formulation: 200,000 dpm/µg

B.7.1.4.1 Material and Methods

The study was designed to simulate field application of [^{14}C]S-2200 to lettuce plants to identify major components of the residue and their distribution in the plant. The study was carried out under greenhouse conditions fitted with automatic controls to maintain air temperature (28°C day/21°C night, relative humidity 19-65%). Lettuce seeds were planted in 13 pots (11-inch diameter)

with a seeding density of approximately 10 seeds per pot (15th April 2009). Group II (5 pots) and Group III (5 pots) were treated with [phenoxy-^{14}C]S-2200
and [benzyl-^{14}C]S-2200 (5.04 mg a.i./pot), respectively, as a spray application with a 25% SC formulation. The application rate for each application and radiolabel was equivalent to 800 g a.s./ha, with a spray volume of 1000 L/ha. Three pots were used for the control (group I) group.
The test substance was applied by manual-operated pump sprayer and each treatment pot was placed in an enclosed wooden frame (1.5ft x 1.5ft x 4ft) lined with plastic. A single slit was cut into one side just above the top of the plants and large enough to allow application. The plastic was taped shut around a shoulder-length glove and the application of one-half of the test material was conducted. The process was then repeated using a slit on the opposite side of the enclosure to ensure uniform coverage of the plants. Following the application, the plastic covering the top of the frame was removed to allow the plants to dry for approximately 15-minutes. The plants were then transferred to a permanent location in the greenhouse positioned in a configuration where spray-treatment plants were placed downwind from control plants to prevent cross-contamination between treatment groups.
The application rate was verified by LSC of the formulated test substances. The purity of the radiolabelled test substances was confirmed by HPLC before and after application. The control pots were maintained in the same manner as the treated ones and were kept at the opposite end of the greenhouse nearest to the exhaust fans. A summary of the application details is given in the table below.

Table B.7.1.4-1: Application details

Application number	Application details			
	Label	Application rate	Date of application	Growth stage at application
Group I (control)	na	na	na	na
Group II	[phenoxy-^{14}C] S-2200	2x 800 g a.i./ha	1st : 26.5.2009	BBCH 43
Group III	[phenoxy-^{14}C] S-2200		2nd: 05.6.2009	BBCH 48

n.a.= not applicable

Lettuce plants were harvested 5 days after the 1st application (immature sampling) and five days after the 2nd application of the test item (mature harvest at a PHI of 5 days). Samples were not collected prior to the intermediate harvest or during the period between the intermediate harvest and final harvest. Lettuce plants from the control (1 pot per harvest) and each treated plant group (2 pots per intermediate harvest, 3 pots per final harvest) were harvested at intermediate and final harvest by cutting approximately 2 to 4 cm above soil level. Any adhering soil was removed from the plants by gentle shaking or brushing. Following each collection samples were placed in sample bags made of flexible plastic (e.g., Ziplok® freezer bags) and fresh weights for all samples were determined at the time of harvest. The sampling data are summarized in the table below:

Table B.7.1.4-2: Sampling details

Test group	Plant matrices sampled	Growth stage at sampling	Time interval after application (days)	Date of sampling
I (Control), II, III	Lettuce (intermediate harvest)	BBCH 45	5	31.05.2009
	Lettuce (final harvest)	BBCH 49	5	10.06.2009

n.a.= not applicable

Both control and treated leaf tissue were gently surface washed with approximately 400 mL of acetonitrile. The radioactivity in the surface wash was quantified by LSC (2 x 1.0 mL) and analyzed by HPLC/RAM. The

entire samples for treated and control tissue were ground with dry ice, five replicates (approximately 0.4 g each) of each sample was analyzed by combustion analysis.
Homogenised samples of the surface washed lettuce samples at the intermediate harvest and final harvest were extracted twice by high speed homogenisation with acetone/water (80:20; v/v) and once with acetone/water/hydrochloric acid (80:20:1; v/v/v). Each sample was centrifuged to separate the extracts from the post-extraction solids (PES).
The Total Radioactive Residue (TRR) in the samples were quantitated by combustion of approximately 0.25 g subsamples using a biological oxidizer (R. J. Harvey Instrument Co.) followed by liquid scintillation counting and by tissue extraction followed by combustion analysis of the post-extraction solids (PES). The radioactivity in the liquid fraction was measured by direct LSC. The TRR in lettuce was calculated as the sum of contributions from the surface wash, extract and PES.
The TRR values as sum of fractions (including surface wash) expressed as S-2200 equivalents in lettuce leaves collected five days after 1st application (immature sampling) and five days after the 2nd application (at harvest sampling) were 35.11 and 43.14 mg/kg, respectively, for the [phenoxy-^{14}C] label, and 27.94 and 41.59 mg/kg, respectively, for the [benzyl-^{14}C] label. The distribution of radioactivity in lettuce leaves was similar between radiolabels from each sampling event, but 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 unextractable residues (PES).
The surface wash removed 88 % of the radioactivity in immature lettuce samples taken 5 days after the 1st application and 78-82 % in mature samples taken 5 days after the 2nd application. The amount of extractable radioactivity (after surface washing) remained approximately constant and accounted for 11-12 % TRR in immature lettuce samples and 17-20 % TRR in mature lettuce samples. However an increasing proportion of the TRR remained un-extractable with increasing PHI representing amounts of 0.2 % TRR in immature lettuce and 1 % TRR in mature lettuce samples taken 5 days after the 2nd application.
The distribution of radioactive residues in lettuce samples in the acetonitrile surface wash, acetone/water (80:20; v/v) extracts, acetone/water/hydrochloric acid (80:20:1; v/v/v) extract and post-extraction solids (PES, un-extractable material) are shown in the table below.

Table B.7.1.4-3: Distribution of [^{14}C]S-2200 residues in lettuce

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		**[benzyl-^{14}C]S-2200**	
		mg/kg	%TRR	mg/kg	%TRR
Immature Lettuce 2 x 800 g/ha PHI 5 days	Surface wash	31.03	88.38	24.54	87.82
	1st Extraction with acetone/water	3.43	9.76	2.83	10.11
	2nd Extraction with acetone /water	0.49	1.38	0.44	1.58
	Extraction with acetone /water / HCl	0.10	0.27	0.08	0.27
	Sum of extracts [1)]	4.01	11.42	3.34	11.96
	PES (post extraction solids)	0.07	0.21	0.06	0.22
	Total Radioactive Residue (TRR)	**35.11**	**100**	**27.94**	**100**
Mature Lettuce 2 x 800 g/ha PHI 5 days	Surface wash	35.32	81.87	32.64	78.49
	1st Extraction with acetone/water	6.36	14.75	7.16	17.20
	2nd Extraction with acetone /water	0.73	1.69	1.00	2.41
	Extraction with acetone /water / HCl	0.24	0.54	0.34	0.83
	Sum of extracts [1)]	7.33	16.99	8.50	20.44
	PES (post extraction solids)	0.49	1.14	0.44	1.07
	Total Radioactive Residue (TRR)	**43.14**	**100**	**41.59**	**100**

[1)] … excluding surface wash

B.7.1.4.2 Distribution, characterisation and identification of residues

The extractable fractions were profiled separately by radio-HPLC and TLC using co-chromatography with reference standards. Chiral HPLC was employed to determine the ratio of *R*- and *S*-isomers of S-2200.

The TRR concentrations in the **[phenoxy-^{14}C]S-2200** lettuce samples were 35.11 mg/kg in immature lettuce, and 43.14 mg/kg in mature lettuce. The majority of the recovered radioactivity was present in the surface rinse and accounted for 31.03 mg/kg (88.38% TRR) in immature lettuce and 35.32 mg/kg (81.87% TRR) in mature lettuce. The radioactive concentration in the extractable portions accounted 4.01 mg/kg (11.42% TRR) in immature lettuce and 7.33 mg/kg (16.99% TRR) in mature lettuce. The un-extractable radioactivity represented 0.07 mg/kg (0.2 % TRR) in immature lettuce and 0.49 mg/kg (1.14 % TRR) in mature lettuce.The results are summarized in the table below.

Table B.7.1.4-4: Distribution of residues lettuce samples after application of [phenoxy-^{14}C]S-2200 in % TRR and mg as eq/kg

	Immature lettuce 2 x 800 g/ha		Mature lettuce 2 x 800 g/ha	
Time interval from last application	5 days		5 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR -Total Radioactive residues	100	35.11	100	43.14
ERR - Extracted Radioactive Residues[1)]	**99.79**	**35.04**	**98.86**	**42.64**
Surface extraction (Surface wash)	88.38	31.03	81.87	35.32
Watersoluble extract radioactivity	11.42	4.01	16.99	7.33
Radioactivity released under hydrolysis conditions	n.a.	n.a.	0.24	0.56
Total identified	97,13	34,46	96,15	41,5
Total characterized	1,65	0,59	2,7	1,17
URR – Unextracted Radioactive Residues	**0.21**	**0.07**	**1.14**	**0.49**
Accountability (sum ERR and URR)	100	35.1	100	43.1

n.a.= not applicable
1) …..including surface wash

The TRR concentrations in the **[benzyl-^{14}C]S-2200** lettuce samples were 27.94 mg/kg in immature lettuce, and 41.59 mg/kg in mature lettuce. The majority of the recovered radioactivity was present in the surface rinse and accounted for 24.5 mg/kg (87.8% TRR) in immature lettuce and 32.6 mg/kg (78.5% TRR) in mature lettuce. The radioactive concentration in the extractable portions accounted 3.34 mg/kg (11.96 % TRR) in immature lettuce and 8.50 mg/kg (20.44 % TRR) in mature lettuce. The un-extractable radioactivity represented 0.06 mg/kg (0.2 % TRR) in immature lettuce and 0.44 mg/kg (1.07 % TRR) in mature lettuce.The results are summarized in the table below.

Table B.7.1.4-5: Distribution of residues in lettuce after application of [benzyl-^{14}C]S-2200 in % TRR and mg as eq/kg

	Immature lettuce 2 x 800 g/ha		Mature lettuce 2 x 800 g/ha	
Time interval from last application	5 days		5 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR -Total Radioactive residues	100	27.94	100	41.59
ERR - Extracted Radioactive Residues[1)]	**99.78**	**27.88**	**98.93**	**41.14**
Surface extraction (Surface wash)	87.82	24.54	78.49	32.64
Watersoluble extract radioactivity	11.96	3.34	20.44	8.50

Time interval from last application	Immature lettuce 2 x 800 g/ha		Mature lettuce 2 x 800 g/ha	
	5 days		5 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg
Radioactivity released under hydrolysis conditions	n.a.	n.a.	0.73	0.30
Total identified	96.78	27.05	95.5	39.72
Total characterized	2.2	0.84	3.44	1.43
URR – Unextracted Radioactive Residues	**0.22**	**0.06**	**1.07**	**0.44**
Accountability (sum ERR and URR)	100	27.94	100	41.9

n.a.= not applicable
1)…..including surface wash

The un-extractable residues of both radiolabels were sequentially hydrolyzed with enzyme, acid and base. A portion of the post-extraction solids (PES) of the mature lettuce samples were further characterised by sequential enzyme hydrolysis with Driselase (enzyme mixture of fungal carbohydrolases), weak acid hydrolysis (0.1M HCl, 40°C overnight) and weak base hydrolysis (0.1M NaOH, 40°C overnight). The results are summarized in the table below.

Table B.7.1.4-6: Distribution of radioactivity in the PES of mature lettuce after two application of ^{14}C-S-2200

Fraction	Total Radioactive Residue					
	[phenoxy-^{14}C]S-2200			**[benzyl-^{14}C]S-2200**		
	% of PES[1)]	mg/kg	% TRR[2)]	% of PES[1)]	mg/kg	% TRR[2)]
Enzyme Hydrolysis	11.74	0.058	0.13	13.65	0.060	0.15
Weak Acid Hydrolysis (0.1M HCL)	3.96	0.020	0.05	5.36	0.024	0.06
Weak Base Hydrolysis (0.1M NaOH)	33.13	0.164	0.38	49.01	0.217	0.52
Bound Residue	52.92	0.261	0.61	26.63	0.118	0.28
Total	101.75	0.502	1.16	94.65	0.419	1.01

1)…..% of PES = (Radioactivity released (dpm)) /(total dpm available) * 100
2)…..% TRR = (% of PES) * (% PES TRR) / 100

Characterization and identification of metabolites was made initially by comparison of HPLC/RAM sample retention times with co-injected reference standard mix. The regions or peaks of interest were then isolated from the lettuce extracts using reverse-phase HPLC. Metabolites were then characterized by HPLC co-chromatography with reference standards and 1D-TLC co-migration with reference standards.

For confirmation of the S-2200 R:S isomer ratio the surface rinses were analyzed directly using chiral HPLC co-chromatography with S-2200 reference standard for the intermediate and final harvest samples. The ^{14}C peak corresponding to the retention time of S-2200 was isolated from the neutral extractable fraction and analyzed by Chiral HPLC co-chromatography. The S-2200 R:S isomer ratio was approximately 50:50 in the [^{14}C]S-2200 test substances and remained at 50:50 in the processed samples. Isomerization of the R:S isomers of [^{14}C]S-2200 was not observed in this study.
For confirmation of S-2200 the surface rinsed samples and the [^{14}C]S-2200 isolates from the neutral extractable fractions were analyzed directly using 1D-TLC by co-spotting the isolates with authentic S-2200 standard. It was demonstrated that [^{14}C]S-2200 isolate and S-2200 reference standard co-eluted using both chromatographic techniques. Thus the identity of [^{14}C]S-2200 was confirmed by HPLC and TLC chromatographic data.
Control samples of homogenized intermediate and final harvest samples were fortified with [^{14}C]S-2200 at approximately 4 and 8 mg/kg, respectively to demonstrate extraction efficiency of S-2200. The fortified samples were extracted and analyzed using the same methods used for the analysis of treated samples. The

results demonstrated that the radioactivity from the spiked controls could be quantitatively (>95%) extracted using the extraction methodology used in this study and that parent S-2200 remained at 90% of the TRR.

Immature lettuce (Group II)

S-2200 was observed at 32.97 mg/kg (93.90 % TRR) in the **[phenoxy-^{14}C]S-2200** treated immature lettuce plants. MCBX was observed at 0.239 mg/kg (0.68% TRR). An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.148 mg/kg (0.42 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.773 mg/kg (2.20 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.327 mg/kg (0.93 % TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.356 mg/kg (1.01 % TRR) and characterized as multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.16 % TRR, 0.056 mg/kg), 32-min (Unknown 7: 0.11 % TRR, 0.037 mg/kg) and 37.8-min (Unknown 8: 0.08 % TRR, 0.029 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.102 mg/kg (0.29 % TRR). There were no other significant degradation products detected in the extractable fraction.

Mature lettuce (Group II)

S-2200 was observed at 39.278 mg/kg (91.05 % TRR) in the **[phenoxy-^{14}C]S-2200** treated mature lettuce plants. MCBX was observed at 0.255 mg/kg (0.59% TRR). An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.226 mg/kg (0.52 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 1.177 mg/kg (2.73 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.545 mg/kg (1.26 % TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.736 mg/kg (1.71 % TRR) and characterized as multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.25 % TRR, 0.108 mg/kg) and 31-min (Unknown 3: 0.14 % TRR, 0.059 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.260 mg/kg (0.60 % TRR). There were no other significant degradation products detected in the extractable fraction.

The results are summarized in the table below.

Table B.7.1.4-7: Identification and characterisation of residues in lettuce leaves after application of [phenoxy-^{14}C]S-2200 in % TRR and mg as eq/kg

Time interval from last application	Immature lettuce (Group II) 2 x 800 g/ha		Mature lettuce (Group II) 2 x 800 g/ha	
	5 days		5 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR – Total Radioactive residues	**100**	**35.11**	**100**	**43.14**
S-2200	93.90	32.97	91.05	39.28
Surface wash	88.38	31.03	81.87	35.32
Acetone/water extracts	5.38	1.89	8.98	3.87
Acetone/water/HCL extracts	0.14	0.05	0.20	0.09
2-CH_2OH-S-2200 (conjugated. 16min)	0.42[2)]	0.15	0.52[5)]	0.23
4-OH-S-2200 (conjugated. 16min)	2.20[2)]	0.77	2.73[5)]	1.18
5-CH_2OH-S-2200 (conjugated. 19min)	0.93[3)]	0.33	1.26[6)]	0.55
2-CH_2OH-S-2200	nd	nd	nd	nd
MCBX[7)]	0.68	0.24	0.59	0.26

Time interval from last application	Immature lettuce (Group II) 2 x 800 g/ha 5 days		Mature lettuce (Group II) 2 x 800 g/ha 5 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg
Total identified	**98.13**	**34.46**	**96.15**	**41.5**
Unknown 1 (2min)	0.16	0.06	0.25	0.11
Unknown 2 (9min)	nd	nd	nd	nd
Unknown 3 (11min)	nd	nd	0.14	0.06
Unknown 4 (14min)	1.01[4)]	0.36	1.71[4)]	0.74
Unknown 5 (22min)	nd	nd	nd	nd
Unknown 6 (31 min)	nd	nd	nd	nd
Unknown 7 (32 min)	0.11	0.04	nd	nd
Unknown 8 (37.8 min)	0.08	0.03	nd	nd
Others	0.29	0.10	0.60	0.26
Total characterized	**1.65**	**0.59**	**2.7**	**1.17**
ERR - Extracted Radioactive Residues[1)]	**99.79**	**35.04**	**98.86**	**42.64**
URR – Unextracted Radioactive Residues	0.21	0.07	1.14	0.49

n.a.= not applicable nd= not detected

[1)]……including surface wash
[2)]……Calculated by the ratio of 2-CH_2OH-S-2200 and 4-OH-S-2200 as 16.1:83.9 from the hydrolysis results of the final harvest sample
[3)]……Confirmed by enzymatic treatment of the final harvest sample to be a conjugate of 5-CH_2OH-S-2200
[4)]……Comprising of multiple compounds
[5)]……Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 16.1:83.9.
[6)]……Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200
[7)]……Identity confirmed by HPLC and TLC co-chromatography with authentic reference standard

Immature lettuce (Group III)

S-2200 was observed at 25.95 mg/kg (92.87 % TRR) in the **[benzyl-^{14}C]S-2200** treated immature lettuce plants. MCBX and De-Xy-S-2200 were observed at 0.158 mg/kg (0.57 % TRR) and 0.0.197 mg/kg (0.71 % TRR), respectively. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.102 mg/kg (0.36 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 0.437 mg/kg (1.56 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.198 mg/kg (0.71 % TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be 0.110 mg/kg (0.40 % TRR) and characterized as multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.025 % TRR, 0.09 mg/kg), 9-min (Unknown 2: 0.90 % TRR, 0.252 mg/kg) and 32-min (Unknown 7: 0.11 % TRR, 0.037 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.421 mg/kg (1.51 % TRR). There were no other significant degradation products detected in the extractable fraction.

Mature lettuce (Group III)

S-2200 was observed at 36.996 mg/kg (88.96 % TRR) in the **[benzyl-^{14}C]S-2200** treated mature lettuce plants. MCBX and De-Xy-S-2200 were observed at 0.404 mg/kg (0.97 % TRR) and 0.268 mg/kg (0.65 % TRR), respectively. An area of radioactivity was observed in the HPLC/RAM at 16-minutes which comprised of two components. The conjugate of 2-CH_2OH-S-2200 was calculated to be 0.268 mg/kg (0.65 % TRR) and the conjugate of 4-OH-S-2200 was calculated to be 1.152 mg/kg (2.77 % TRR). An area of radioactivity at 19-minutes was characterized as a conjugate of 5-CH_2OH-S-2200 and calculated to be 0.626 mg/kg (1.50 % TRR). An additional area of radioactivity at approximately 14-minutes (Unknown 4) was calculated to be

0.312 mg/kg (0.75 % TRR) and characterized as multiple components. Minor regions of radioactivity were observed at HPLC/RAM retention times of 2-min (Unknown 1: 0.59 % TRR, 0.244 mg/kg) and 9-min (Unknown 3: 0.64 % TRR, 0.265 mg/kg). Numerous minor peaks, each individual peak accounting for less than 1% TRR, designated as others are included in the distribution table which together accounted for 0.608 mg/kg (1.46 % TRR). There were no other significant degradation products detected in the extractable fraction.

The results are summarized in the table below.

Table B.7.1.4-8: Identification and characterisation of residues in lettuce leaves after application of [benzyl-^{14}C]S-2200 in % TRR and mg as eq/kg

	Immature lettuce (Group III) 2 x 800 g/ha		**Mature lettuce (Group III) 2 x 800 g/ha**	
Time interval from last application	5 days		5 days	
	TRR (%)	mg/kg	TRR (%)	mg/kg
TRR – Total Radioactive residues	**100**	**27.94**	**100**	**41.59**
S-2200	92.87	25.95	88.96	37.0
Surface wash	86.98	24.30	78.49	32.64
Acetone/water extracts	5.75	1.61	10.13	4.21
Acetone/water/HCL extracts	0.13	0.04	0.33	0.14
De-Xy-S2200[7)]	0.71	0.20	0.65	0.27
Surface wash	0.22	0.06	nd	nd
Acetone/water extracts	0.49	0.14	0.60	0.25
Acetone/water/HCL extracts	nd	nd	0.05	0.02
2-CH_2OH-S-2200 (conjugated. 16min)	0.36[2)]	0.10	0.65[5)]	0.27
4-OH-S-2200 (conjugated. 16min)	1.56[2)]	0.44	2.77[5)]	1.15
5-CH_2OH-S-2200 (conjugated. 19min)	0.71[3)]	0.20	1.50[6)]	0.63
2-CH_2OH-S-2200	nd	nd	nd	nd
MCBX[7)]	0.57	0.16	0.97	0.40
Total identified	**96.78**	**27.05**	**95.5**	**39.72**
Unknown 1 (2min)	0.09	0.03	0.59	0.24
Unknown 2 (9min)	0.90	0.25	0.64	0.27
Unknown 3 (11min)	nd	nd	nd	nd
Unknown 4 (14min)[4)]	0.40	0.11	0.75	0.31
Unknown 5 (22min)	nd	nd	nd	nd
Unknown 6 (31 min)	nd	nd	nd	nd
Unknown 7 (32 min)	0.11	0.03	nd	nd
Unknown 8 (37.8 min)	nd	nd	nd	nd
Others	1.51	0.42	1.46	0.61
Total characterized	**2.2**	**0.84**	**3.44**	**1.43**
ERR - Extracted Radioactive Residues[1)]	**99.78**	**27.88**	**98.93**	**41.14**
URR – Unextracted Radioactive Residues	0.22	0.06	1.07	0.44

n n.a.= not applicable nd= not detected

[1)]……including surface wash
[2)]……Calculated by the ratio of 2-CH_2OH-S-2200 and 4-OH-S-2200 as 18.9:81.1 from the hydrolysis results of the final harvest sample
[3)]……Confirmed by enzymatic treatment of the final harvest sample to be a conjugate of 5-CH_2OH-S-2200.
[4)]……Comprising of multiple compounds
[5)]……Confirmed by enzymatic treatment of 16 minute region to be conjugates of 2-CH_2OH-S-2200 and 4-OH-S-2200 at a ratio of 18.9:81.1
[6)]……Confirmed by enzymatic treatment of 19 minute region to be a conjugate of 5-CH_2OH-S-2200
[7)]……Identity confirmed by HPLC and TLC co-chromatography with authentic reference standard

Representative [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 treated final harvest samples were extracted and analyzed after approximately 6 and 1 month, respectively. The extracts were chromatographed

by HPLC and compared to the initial profile to verify the stability of metabolites in the matrices during storage. The comparison of distributions of major ^{14}C residues in the [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C] S-2200 treated mature lettuce plants are given in the tables below.

Table B.7.1.4-9: Comparison of ^{14}C Residues (Neutral and acid extrables) in mature lettuce samples following 6 month frozen storage (phenoxy-^{14}C) and 1 month frozen storage (benzyl-^{14}C)

	[phenoxy-^{14}C] S-2200				[benzyl-^{14}C] S-2200			
	Mature Lettuce (Group II)				**Mature lettuce** (Group III)			
	Neutral extractable[1)2)] (Acetone : water = 80 : 20)		**Acid extractable**[1)2)] (Acetone : water : acid= 80 : 20:1)		**Neutral extractable** (Acetone : water = 80 : 20)		**Acid extractable** (Acetone : water : acid= 80 : 20:1)	
	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg	TRR (%)	mg/kg
Original analysis	16,44	7,092	0,54	0,235	19,62	8,157	0,83	0,343
6 month frozen storage	18,31	8,143	0,66	0,295	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	19,29	7,961	0,44	0,183
S-2200								
Original analysis	8,98	3,874	0,20	0,087	10,13	4,214	0,33	0,139
6 month frozen storage	10,91	4,851	0,13	0,057	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	9,15	3,778	0,12	0,049
De-Xy-S2200								
Original analysis	nd	nd	nd	nd	0,60	0,249	0,05	0,020
6 month frozen storage	nd	nd	nd	nd	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	0,82	0,339	nd	nd
2-CH_2OH-S-2200 (conjugated. 16min)								
Original analysis	0,51	0,220	0,01	0,006	0,62	0,259	0,02	0,010
6 month frozen storage	0,50	0,220	0,03	0,013	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	0,58	0,240	0,01	0,005
4-OH-S-2200 (conjugated. 16min)								
Original analysis	2,66	1,148	0,07	0,029	2,67	1,110	0,10	0,042
6 month frozen storage	2,58	1,148	0,15	0,065	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	2,49	1,028	0,06	0,023
5-CH_2OH-S-2200 (conjugated. 19min)								
Original analysis	1,23	0,530	0,03	0,015	1,44	0,600	0,06	0,026
6 month frozen storage	1,20	0,532	0,06	0,027	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	1,41	0,582	0,03	0,012
MCBX								
Original analysis	0,57	0,246	0,02	0,009	0,94	0,389	0,04	0,015
6 month frozen storage	0,73	0,324	0,02	0,008	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	0,79	0,327	0,02	0,008
Others								
Original analysis	0,52	0,225	0,08	0,036	1,39	0,580	0,07	0,028
6 month frozen storage	0,42	0,189	0,04	0,018	n.a.	n.a.	n.a.	n.a.
1 month frozen storage	n.a.	n.a.	n.a.	n.a.	2,40	0,990	0,11	0,047

1) .....Lettuce tissue TRR was determined by combustion analysis at harvest collection to be 7.219 ppm. Recovery of original final harvest samples is based on this TRR value.

2) .....Lettuce tissue TRR was determined by combustion analysis at 6-months frozen storage to be 9.279 ppm. Recovery of 6-month stability samples is based on this TRR value.

Considering the low concentration values in the mature lettuce samples the metabolite profiles from the two analyses (the initial and final analyses) were similar indicating that [^{14}C]S-2200 metabolites were stable in the mature lettuce samples under freezer storage conditions.

B.7.1.4.3 Conclusions and metabolic pathway of S-2200 in Lettuce

The metabolism of [^{14}C]S-2200 in lettuce plants was studied to identify and characterize the nature of the major [^{14}C] residues. The study was carried out under greenhouse conditions using two radiolabeled forms of [^{14}C]S-2200, [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 as a 25% SC formulation.

Table B.7.1.4-10: Distribution of [^{14}C]S-2200 residues in lettuce plants

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Immature Lettuce 2 x 800 g/ha PHI 5 days	Surface wash	31.03	88.38	24.54	87.82
	Sum of extracts [1)]	4.01	11.42	3.34	11.96
	PES (post extraction solids)	0.07	0.21	0.06	0.22
	Total Radioactive Residue (TRR)	**35.11**	**100**	**27.94**	**100**
Mature Lettuce 2 x 800 g/ha PHI 5 days	Surface wash	35.32	81.87	32.64	78.49
	Sum of extracts [1)]	7.33	16.99	8.50	20.44
	PES (post extraction solids)	0.49	1.14	0.44	1.07
	Total Radioactive Residue (TRR)	**43.14**	**100**	**41.59**	**100**

[1)].....excluding surface wash

The major residues identified in the immature and mature lettuce plants included S-2200, De-Xy-S-2200, 2-CH_2OH-S-2200 (conjugated) and 4-OH-S-2200 (conjugated) with some minor metabolites such as [^{14}C] conjugate of 5-CH_2OH-S-2200, MCBX and some unknown (1-5) metabolites.

Group II [phenoxy-^{14}C] S-2200 treatment

Following two application of S-2200 to lettuce at a rate of 800 g a.s./ha, the TRR in immature lettuce 5 days after the first application was 35.1 mg/kg S-2200 equivalents, the TRR in mature lettuce 5 days after the second application accounted for 43.1 mg/kg S-2200 equivalents. The radioactive residues remained mainly on the surface of the leaves with TRR values of 88 % and 82 % in the surface wash of immature lettuce and mature lettuce plants, respectively. De-Xy-S-2200 and free 2-CH_2OH-S-2200 were not detected in any of [phenoxy-^{14}C]S-2200 treated lettuce plants.

In *immature lettuce* S-2200 accounted for 32.97 mg/kg (94 % TRR), most of which was found in the surface washes. The glycoside conjugates of 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 were found at < 1% TRR, the glycoside conjugate of 4-OH-S-2200 accounted for 2 % TRR (0.8 mg/kg). MCBX accounted for < 1 % TRR.

In *mature lettuce* S-2200 accounted for 39.3 mg/kg (91 % TRR), most of which was found in the surface washes. The glycosides of 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 2.7 % TRR (1.2 mg/kg) and 1.3 % TRR (0.6 mg/kg), respectively. The glycoside conjugate of 2-CH_2OH-S-2200 and MCBX accounted for < 1 % TRR.

Group III [benzyl-^{14}C] S-2200 treatment

Following two application of S-2200 to lettuce at a rate of 800 g a.s./ha, the TRR in immature lettuce 5 days after the first application was 27.9 mg/kg S-2200 equivalents, the TRR in mature lettuce 5 days after the second application accounted for 41.6 mg/kg S-2200 equivalents The radioactive residues remained mainly on the surface of the leaves with TRR values of 87 % and 78 % in the surface wash of immature lettuce and mature lettuce plants, respectively. Free 2-CH_2OH-S-2200 were not detected in any of [benzyl-^{14}C]S-2200 treated lettuce plants.

In *immature lettuce* S-2200 accounted for 25.95 mg/kg (93 % TRR), most of which was found in the surface washes. De-Xy-S-2200 was found at 0.7 % TRR (0.20 mg/kg), the glycoside conjugates of 2-CH_2OH-S-2200

and 5-CH_2OH-S-2200 were found at < 1% TRR. The glycoside conjugate of 4-OH-S-2200 accounted for 1.6 % TRR (0.4 mg/kg) whereas MCBX was < 1 % TRR.
In *mature lettuce* S-2200 accounted for 37 mg/kg (89 % TRR), most of which was found in the surface washes. De-Xy-S-2200 was found at 0.7 % TRR (0.3 mg/kg). The glycoside conjugate of 2-CH_2OH-S-2200 accounted for < 1 % TRR, whereas the glycoside conjugates of 4-OH-S-2200 and 5-CH_2OH-S-2200 were found at 2.8 % TRR (1.2 mg/kg) and 1.5 % TRR (0.6 mg/kg), respectively. MCBX accounted for 1 % TRR (0.4 mg/kg).

All metabolites (De-Xy-S-2200, 4-OH-S-2200, 5-COOH-S-2200, 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and MCBX) were also found in the rat. The metabolites 4-OH-S-2200 and 5-COOH-S-2200 were present in the rat at >10% of dose.
The *R*/*S* ratio of [^{14}C]S-2200 remained approximately 1:1 indicating no *R*/*S* isomerization.

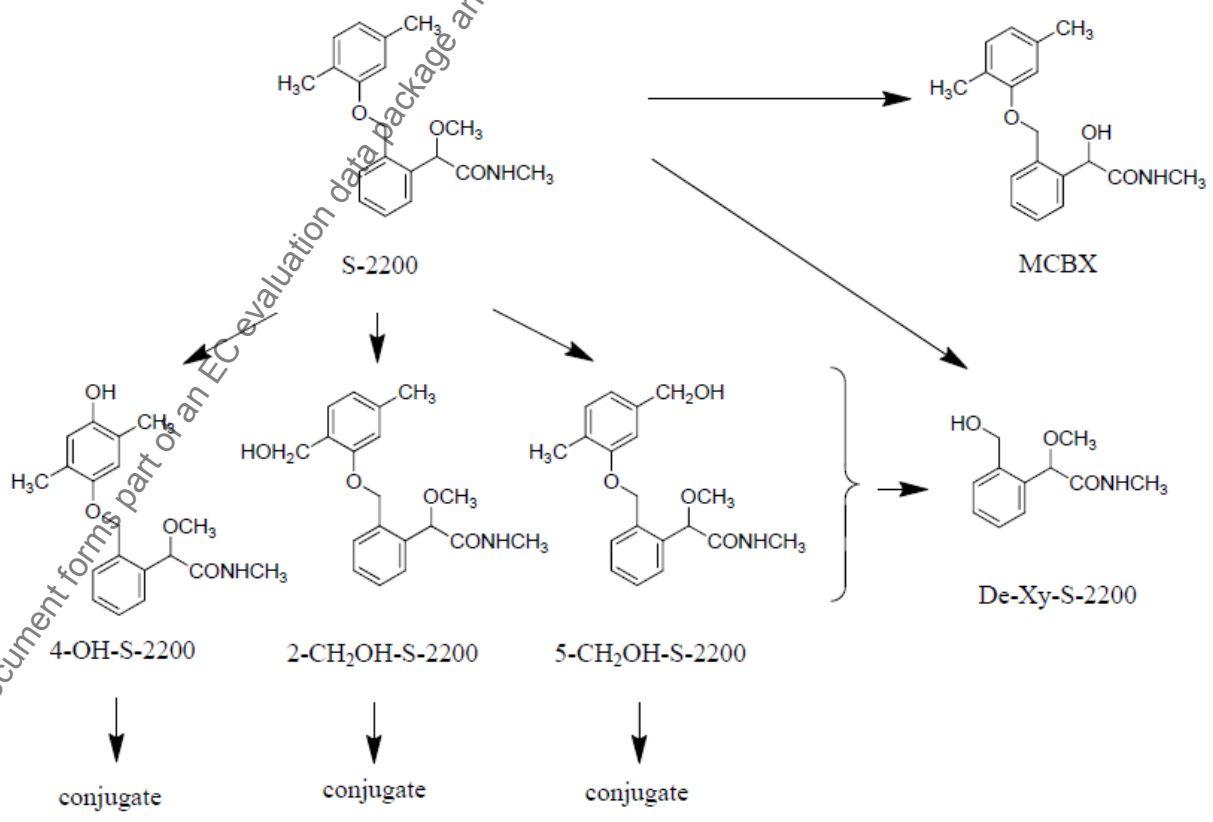
The [^{14}C]S-2200 that absorbed into lettuce plants was metabolized to a number of polar products.
The major metabolism pathway included

- ✓ hydroxylation of the phenoxy ring to form 4-OH-S-2200 and subsequent formation of the glycoside conjugate
- ✓ oxidation of the methyl group attached to the phenoxy ring to form 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 and
- ✓ subsequent formation of the corresponding glycoside conjugates.

Minor metabolic pathways included

- ✓ *O*-demethylation of the methoxy group of the side chain to form MCBX
- ✓ cleavage of the ether linkage to form De-Xy-S-2200

Figure B.7.1.4-1: Proposed metabolic pathway of Mandestrobin (S-2200) in lettuce



Code Name	Molecular Weight	Chemical Name
S-2200	313.39	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
MCBX	299.36	(RS)-2-hydroxy-N-methyl-2-[α-(2,5- xylyloxy)-o-tolyl]acetamide
De-Xy-S-2200	209.24	(*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide
4-OH-S-2200	329.39	(RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
2-CH_2OH-S-2200	329.39	(RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-CH_2OH-S-2200	329.39	(RS)-2-[2-(5-hydroxymethyl-2- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide

B.7.1.5 Overall conclusion on the metabolism in plants

The available plant metabolism studies on oilseed rape, wheat and lettuce have been conducted using two radiolabeled forms of [^{14}C]S-2200, [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200, as a 25% SC formulation. An overview is given in the table below.

Crop	Plant parts analysed	Category*	Application rate [g as/ha]	x GAP**	Comment RMS
Oilseed rape	Forage, seed	Pulses & oilseeds	2x 400 1x 400	2x	Valid
Wheat	Forage, hay, straw, grain	Cereals	1x 300	1.5x	Valid
Lettuce	Immature lettuce Mature lettuce	Leafy crops	2x 800	4x	Valid

* according Document 7028/VI/95 rev.3 (22.7.97), Appendix A, Metabolism, distribution and expression of residues in plants

** proposed GAP for oilseed rape: 1x 200 g as/ha

Mandestrobin (S-2200) is extensively metabolised in crops. The route of the metabolism of Mandestrobin (S-2200) has been shown to be similar in all three crop groups. The main route of metabolism in crops is via hydroxylations and oxidations and subsequent glycoside conjugation, to yield the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 and their conjugates. Minor metabolic pathways involved demethylation of the methoxy group of the side chain to form MCBX, and cleavage of the ether linkage to form De-Xy-S-2200.

S-2200 was a major component of the residue in all crops. The *R*/*S* ratio of [^{14}C]S-2200 remained approximately 1:1 indicating no *R*/*S* isomerization in all tested crops. The major metabolites found at levels >10% TRR were 4-OH-S-2200 (conjugated), 2-CH_2OH-S-2200 (conjugated) and De-Xy-S-2200. All the free metabolites found in crops are also found in the rat, however only 4-OH-S-2200 and 5-COOH-S-2200 are major metabolites in the rat (found at >10% of dose).

B.7.2 Metabolism, distribution and expression of residues in livestock

B.7.2.1 Metabolism in Poultry (Section 4, Annex IIA point 6.2.2)

Reference:	**[^{14}C]S-2200 - Absorption, distribution, metabolism and excretion following repeated oral administration to the laying hen**
Author(s), year:	[redacted] 2012a)
Report/ Doc. Number:	[redacted] Report no. 8227547 Sumitomo ref: ROM-0040
Guideline(s):	US EPA OPPTS 860.1300; Nature of the residue – Plants, Livestock EU Guideline 7030/VI/95 - Rev. 3, Metabolism and Distribution in Domestic Animals, 1997. OECD 503, Metabolism in Livestock
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

The absorption, distribution, metabolism and excretion of S-2200 was investigated on laying hens following daily oral administration of [benzyl-^{14}C]S-2200 or [phenoxy-^{14}C]S-2200 for 14 consecutive days at a dose level equivalent to 10 ppm in the diet.
Eggs and excreta were collected during the dosing period. Animals were sacrificed approximately 6 hours after the last dose. Muscle (breast and thigh), fat (peritoneal and perirenal), liver (whole organ) and skin (including subcutaneous fat) were sampled for analysis.

Livestock information:

Species/breed	Lohman Brown/Laying hens (Gallus domesticus)
Number of tested animals	20
Age	Female adults
Weight	
at initiation of the study	1.73 kg – 2.07 kg
at the end of the study	1.85 kg - 2.13 kg
Egg Production[1)]	
During acclimatisation	95 % - 96 %
During experimental phase	92 % - 93 %
Housing	Individually in metabolism cages designed for the separate collection of eggs and excreta
Acclimatisation period	approximately 7 weeks
Diet	commercially available ground concentrate, grit *ad libitum*
Water	Fresh tap water *ad libitum*
Feed consumption	
During acclimatisation	136 g – 144 g dry diet/day
During experimental phase	136 g – 138 g dry diet/day
Environmental conditions	
Temperature	20-23 C
Humidity	33-70 %
Air changes	at least 10 air changes/hour
Light exposure	16-hour fluorescent light/8-hour dark

[1)]….. before the beginning of exposure, defined as 100% when one egg was produced within a 24 hour interval

Test Material:

Common Name:	S-2200
Name (IUPAC):	(RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers:	S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight:	313.39
CAS No.:	173662-97-0
Purity:	100 %
Log $P_{o/w}$	3.51 (at 25 ± 1°C)
Radiolabel position (specific activity)	[phenoxy-^{14}C] S-2200 (120 mCi/mmol) [benzyl-^{14}C] S-2200 (123 mCi/mmol)
Purity	[phenoxy-^{14}C] S-2200: 98.9 % [benzyl-^{14}C] S-2200: 98.7 %
R/S isomer ratio	Formulated [phenoxy-^{14}C] S-2200: 49.45 : 49.6 Formulated [benzyl-^{14}C] S-2200: 49.1 : 48.7

B.7.2.1.1 Material and Methods

The test substance was pipetted onto carboxymethylcellulose contained in gelatine capsules (size 00) to achieve the required dose rate (10 ppm based on diet). The solvent was allowed to evaporate prior to the capsules being sealed. Prior to the preparation of the capsules for dosing, trial capsule preparations for each label were performed to measure the stability of the test substances over a 15 day period. The radiochemical purity of the encapsulated S-2200 was measured. One capsule per radiolabel, prepared at the same time as the doses, was extracted with a suitable solvent prior to the first dose and following the last dose. The total amount of radioactivity in the extract was determined and a sample of each extract was analysed by HPLC using a similar method to that used for determination of radiochemical purity.

Dosing:

Two groups of ten laying hens were orally dosed with either [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 once daily following the morning egg collection for a total of 14 days. The targeted daily dose was 10 ppm in the diet, based on food consumption during the acclimatisaton period. The observed daily dose rate of [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200, respectively, was 1.8 mg/day corresponding to a total dose of 25.2 mg/animal for the duration of the study. The average dose level of [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 was 13.24 mg/kg and 13.04 mg/kg in the diet, respectively, based on the actual feed intake during the dosing period. The experimental phase was conducted from 7th July to 20th July 2010. A summary of the dosing regime is given in the table below.

Table B.7.2.1.1-1: Dosing regime

Label	No. of hens	total feed consumption[1)] per day	Mean body weight[2)]	Dose level			
		kg dry weight	kg	mg as/kg feed/day	mg as/day	mg as/animal (total dose)	mg as/kg bw/day
[phenoxy-^{14}C] S-2200	10	136	1.73	13.24	1.8	25.2	1.04
[benzyl-^{14}C] S-2200	10	138	2.07	13.04	1.8	25.2	0.87

1)….during experimental phase (mean value)
2)….at the 1st dose (average over 10 hens)

Sample collection and handling:
Excreta were collected daily at 24 hour intervals and up to necropsy. Eggs were collected immediately before dosing and in the afternoon, 5 to 8 hours after dosing. Cage debris was removed at each excreta collection. Approximately 6 hours after the final dose, the animals were removed from the metabolism cage and terminated by cervical dislocation. At the end of the in-life phase metabolism cages were rinsed with water and then methanol to provide a final cage wash. The following tissues were collected for analysis:

• Muscle (maximum amounts of breast and thigh)
• Fat (maximum amounts of peritoneal and perirenal)
• Liver (whole organ)
• Skin (including subcutaneous fat)

Perirenal fat was not present in any animal and was therefore not collected. Excreta samples were stored at < - 10°C after collection. Following the in-life phase, excreta was thawed, mixed with the addition of water and re-frozen. Tissues were homogenised on the day of collection and stored at <-10°C. Eggs were stored between 2 and 8°C, prior to being pooled by animal over a 24 hour period and sub-sampled. The bulk egg samples were then stored frozen at <-10°C and the sub-sample stored at 2-8°C prior to LSC analysis.

Excreta samples were mixed with deionised water and fat samples were finaly chopped or macerated. Muscle and liver samples were homogenised in an appropriate volume of deionised water or finaly chopped or macerated and then solubilised prior to analysis by liquid scintillation counting (LSC). Aliquots of egg and cage wash were analysed directly for radioactivity by liquid scintillation counting (LSC).
Tissue and egg samples containing total radioactive residues (TRRs) >0.01 mg/kg were subjected to solvent extraction, protease digestion, acid and base hydrolysis. Analysis and identification of residues in the extracts was conducted by radio-HPLC using co-chromatography with authentic reference standards. Selected extracts were also analysed by LC-MS in positive ion mode for metabolite identification.

HPLC Method
Column: Waters Atlantis dC18 (150 x 4.6 mm id, 5 µm particle size)
Flow rate: 1.0 mL/min
UV Detection: 254 nm
Solvent A: 0.05% aqueous formic acid
Solvent B: methanol : acetonitrile (50:50 v/v)
Detection: 500 µL Liquid Cell
Gradient table:

Time (min)	% A	% B
0	90	10

10	85	15
30	50	50
45	45	55
55	30	70
65	30	70
65.1	90	10
75	90	10

Retention time for S-2200: 54.7 min

Where appropriate, fractions were collected over 12 second intervals into 96 well plates and subjected to LSC by TopCount NXT Microplate Scintillation Counter. Chromatograms were reconstituted from the LSC data.
For LC/MS, a ThermoFisher LTQ Orbitrap hybrid mass spectrometer was coupled to a ThermoFisher Accela HPLC system *via* an API interface. The analytical method used was as above. The eluent was split 1:10 between the mass spectrometer and the fraction collector. Fractions were collected over 12 second intervals into 96 well plates and subjected to LSC by TopCount NXT Microplate Scintillation Counter. Chromatograms were reconstituted from the LSC data. Positive ion electrospray mass spectrometric analysis was carried out with a capillary temperature of 325°C, a capillary voltage of 14 V and a spray voltage of 4kV. The scan range was m/z 100 to 700.

Egg samples from day 11 (dosed with [Phenoxy-^{14}C]S-2200) or day 12 (dosed with [Benzyl-^{14}C]S-2200), **fat** and **muscle samples** were pooled by radiolabel and used for the determination of the nature of the residues. A portion of the pooled sample was sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile. The hexane extract was partitioned against acetonitrile. The combined ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were concentrated and analysed by HPLC. Portions of the phenoxy-labelled egg extract was also analysed by LC/MS.
Liver samples were pooled by radiolabel and sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, water, 1M HCl and 1M ammonia solution. The hexane extract was partitioned against acetonitrile. The ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were combined and concentrated for analysis by HPLC. For both labels the water, 1M HCl and 1M ammonia solution extracts were combined, freeze dried, reconstituted in acetonitrile:water (3:1 v:v), methanol, acetonitrile and acetonitrile:water (1:1 v:v), reduced in volume under nitrogen and centrifuged. The top phase of the resulting biphasic extract was discarded, and the bottom phase analysed by HPLC. The unextracted residue from liver was subjected to protease digestion for 18 hours at 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. The residue remaining after protease digestion was extracted with acetonitrile and then subjected to acid hydrolysis with 10M HCl under reflux, the acid hydrolysate was then analysed by HPLC. The residue after acid hydrolysis was subjected to base hydrolysis with 10M NaOH under reflux.
Skin samples were pooled by radiolabel and sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, water, 1M HCl and 1M ammonia solution. The hexane extract was partitioned against acetonitrile. The ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were combined and concentrated for analysis by HPLC. For the phenoxy label the water, 1M HCl and 1M ammonia solution extracts were combined, freeze dried, reconstituted in acetonitrile:water (3:1 v:v), methanol, acetonitrile and acetonitrile:water (1:1 v:v), reduced in volume under nitrogen and centrifuged. The top phase of the resulting biphasic extract was discarded, and the bottom phase analysed by HPLC.

Storage stability data were not necessary since metabolite profiling was completed within six months after necropsy/sampling.

B.7.2.1.2 Distribution, characterisation and identification of residues

Excretion balance and tissue distribution investigations were performed following repeated oral administrations to laying hens for 14 consecutive days at a dose level of 10 mg in the diet. Approximately 85% of the administered radioactivity was recovered from the hens dosed with [phenoxy-^{14}C]S-2200, and 100% from the hens dosed with [benzyl-^{14}C]S-2200. The majority of the administered dose was excreted since 83% of the total dose of [phenoxy-^{14}C]S-2200 and 98% of the total dose of [benzyl-^{14}C]S-2200 were recovered in the excreta. The TRR in muscle, fat, skin and liver accounted for <0.1% of the dose, the TRR in eggs accounted for 0.2% of the dose for both labels. A summary is given in the table below.

Table B.7.2.1.2-1: Total radioactive residues in eggs, tissues and excreta following administration of [^{14}C]S-2200 to laying hens at 13 mg/kg in the diet

Matrix	Total Radioactive Residue			
	[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
	mg/kg	% of dose	mg/kg	% of dose
Fat (peritoneal)	0.033	0.003	0.032	0.005
Liver	0.295	0.055	0.299	0.063
Muscle (breast)	0.013	0.007	0.025	0.014
Muscle (thigh)	0.014	0.003	0.023	0.005
Skin	0.048	0.003	0.054	0.003
Total tissues	-	0.07	-	0.09
Egg	0.051 - 0.113[1)]	0.21	0.050 - 0.081[1)]	0.18
Excreta	-	83.37	-	98.35
Cage wash	-	1.33	-	0.99
Total Recovery	**-**	**84.98**	**-**	**99.61**

[1)]……residue range for day 2-14

Radioactive residues in egg increased from 0.05 mg/kg on Day 2 to 0.11 mg/kg on Day 11 for [phenoxy-^{14}C]S-2200 and from 0.05 mg/kg on Day 2 to 0.08 mg/kg on Day 7 for [benzyl-^{14}C]S-2200. An overview is given in the following table.

Table B.7.2.1.2-2: Total radioactive residues in eggs over time following administration of [^{14}C]S-2200 to laying hens at 13 mg/kg in the diet

Sampling Time	Total Radioactive Residue			
	[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
	mg/kg	% of dose	mg/kg	% of dose
Day 2	0.051	0.010	0.050	0.013
Day 3	0.073	0.014	0.062	0.014
Day 4	0.066	0.014	0.052	0.011
Day 5	0.068	0.013	0.040	0.009
Day 6	0.053	0.010	0.051	0.010
Day 7	0.096	0.017	**0.081**	0.020
Day 8	0.088	0.014	0.072	0.012
Day 9	0.083	0.015	0.077	0.015
Day 10	0.107	0.019	0.067	0.015
Day 11	**0.113**	0.022	0.072	0.016
Day 12	0.081	0.017	0.075	0.018
Day 13	0.094	0.018	0.064	0.014
Day 14	0.099	0.019	0.068	0.013
Day 15 (Necropsy)*	0.084	0.008	0.051	0.001

*…… The eggs from Day 15 were collected at termination (6 hours after last dose), therefore the mg/kg residue values are not comparable to the other days

The dose level used in this study (13 mg/kg diet) represents a highly exaggerated rate relative to the maximum possible dietary exposure of 0.008 ppm diet; however the radioactive residue levels in egg, liver, fat, skin and muscle were low.

Following administration of **[phenoxy-^{14}C]S-2200** to hens the highest total radioactive residues were found in liver accounting for 0.295 mg/kg S-2200 equivalents. The total radioactive residues in other tissues were low and accounted for 0.014 mg/kg in muscle, 0.048 mg/kg in skin and 0.033 mg/kg in fat, respectively. Total radioactive residues in eggs reached a maximum on day 11 (0.113 mg/kg).
Residues in egg, skin and fat were readily extractable with organic and polar solvents (hexane, acetonitrile, ethyl actetate, 1% formic acid in acetonitrile) releasing 91.6 % TRR (0.104 mg/kg) in egg, 92.0 % TRR (0.043 mg/kg) in skin and 96.6 % TRR (0.032 mg/kg) in fat. In liver and muscle only 62.7% TRR (0.185 mg/kg) and 51.7 % TRR (0.007 mg/kg), respectively, were extractable with organic or polar solvents. In liver a TRR of 37.3 % (0.110 mg/kg) remained as post extraction solids, but a further 19.2% TRR (0.057 mg/kg) was released by protease hydrolysis, and 5.9% TRR (0.017 mg/kg) by strong acid followed by strong base hydrolysis.

Following administration of **[benzyl-^{14}C]S-2200** to hens the highest total radioactive residues were found in liver accounting for 0.299 mg/kg S-2200 equivalents. The total radioactive residues in other tissues were low and accounted in muscle for 0.024 mg/kg, in skin for 0.054 mg/kg and for fat 0.032 mg/kg, respectively. Total radioactive residues in eggs reached a maximum on day 7 (0.081 mg/kg).
Residues in egg, skin and fat were readily extractable with organic and polar solvents (hexane, acetonitrile, ethyl actetate, 1% formic acid in acetonitrile), releasing 88.7 % TRR (0.67 mg/kg) in egg, 86.2 % TRR (0.047 mg/kg) in skin and 91.6 % TRR (0.029 mg/kg) in fat. In liver and muscle only 65.6% TRR (0.197 mg/kg) and 58.8 % TRR (0.014 mg/kg), respectively, were extractable with organic or polar solvents. In liver a TRR of 34.4 % (0.103 mg/kg) remained as post extraction solids, but further 21.5 % TRR (0.065 mg/kg) was released by protease hydrolysis, and 7.4 % TRR (0.022 mg/kg) by strong acid followed by strong base hydrolysis.
The results are summarised in the table below.

Table B.7.2.1.2-3: Distribution of [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 residues in egg and tissues (expressed as mg/kg S-2200 equivalents and % TRR)

Sample	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Eggs	Pooled organic fraction	0.104	91.5	0.067	88.7
	Hexane fraction	<0.001	0.1	<0.001	<0.1
	PES (post extraction solids)	0.010	8.4	0.009	11.3
	Total Radioactive Residue (TRR)	**0.113**	**100**	**0.075**	**100**
Muscle	Pooled organic fraction	0.007	50.8	0.014	58.3
	Hexane fraction	<0.001	0.9	<0.001	0.5
	PES (post extraction solids)	0.007	48.3	0.010	41.2
	Total Radioactive Residue (TRR)	**0.014**	**100**	**0.024**	**100**
Skin	Pooled organic fraction	0.033	69.3	0.040	72.7
	Hexane fraction	0.001	1.4	<0.001	1.0
	Aqueous extracts	0.010	21.3	0.007	12.5
	PES (post extraction solids)	0.004	8.0	0.008	14.3
	Total Radioactive Residue (TRR)	**0.048**	**100**	**0.054**	**100**
Fat	Pooled organic fraction	0.030	90.7	0.029	88.8
	Hexane fraction	0.002	5.9	<0.001	2.8
	PES (post extraction solids)	0.001	3.4	0.003	8.4
	Total Radioactive Residue (TRR)	**0.033**	**100**	**0.032**	**100**
	Pooled organic fraction	0.147	49.8	0.149	49.7

Sample	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Liver	Hexane fraction	0.002	0.6	0.003	0.9
	Aqueous extracts	0.036	12.3	0.045	15.0
	PES (post extraction solids)	0.110	37.3	0.103	34.4
	Exhaustive extraction of post-extraction solids				
	Protease digest - Aqueous phase	0.047	15.8	0.052	17.3
	Protease digest - Organic phase	0.002	0.6	0.004	1.3
	Organic extract of protease debris	0.008	2.8	0.009	2.9
	Acid hydrolysis (10M HCl)	0.013	4.4	0.020	6.7
	Base hydrolysis (10M NaOH)	0.004	1.5	0.002	0.7
	Amount remaining unextracted	0.036	12.2	0.017	5.5
	Total Radioactive Residue (TRR)	**0.295**	**100**	**0.299**	**100**

Characterization and identification of metabolites was made HPLC co-chromatography against authentic reference standards. The identity of parent S-2200 in eggs was confirmed by LC/MS.
HPLC analysis of the organic extracts showed the presence of at least 18 peaks or diffuse regions of interest.

Following administration of **[phenoxy-^{14}C]S-2200** to hens the main component of the radioactive residue in eggs was S-2200, accounting for 0.058 mg/kg (51.2% TRR). The free metabolites 2-COOH-S-2200, 4-OH-S-2200 and MCBX were found at levels of 0.7 % TRR (< 0.001 mg/kg), 1.5 % TRR (0.002 mg/kg) and 1.3 % TRR (0.002 mg/kg), respectively. The main component of the extractable residue in liver was 4-OH-S-2200 (0.040 mg/kg, 13.6 % TRR), S-2200 was present at a level of 1.4 % TRR (0.004 mg/kg). The free metabolites 2-COOH-S-2200 and 2-CH_2OH-S-2200 accounted for 2.4 % TRR (0.007 mg/kg) and 0.9 % TRR (0.003 mg/kg), respectively. MCBX were not detected in liver. Hydrolysis of the post-extraction solids released small amounts of S-2200 (1.5 % TRR, 0.005 mg/kg), 5-CA-2-HM-S-2200 (1.1 % TRR, 0.003 mg/kg) and 4-OH-S-2200 (1.6 % TRR, 0.005 mg/kg), indicating that these were present as bound or conjugated residues. Total radioactive residue levels in muscle were low, only S-2200 was present at a level of 2.2% TRR (< 0.001 mg/kg) and the free metabolite 2-CH_2OH-S-2200 was found at 3.7 % TRR (< 0.001 mg/kg). Radioactive residues in skin contained a number minor metabolites (< 0.01 mg/kg), the most significant were 2-COOH-S-2200 (9.2 % TRR, 0.004 mg/kg) and 4-OH-S-2200 (6.1 % TRR, 0.003 mg/kg). S-2200 accounted for 3.1 % TRR (0.002 mg/kg), MCBX was not detected. The main component of the radioactive residue in fat was S-2200 accounting for 49.5 % TRR (0.016 mg/kg). The free metabolites 4-OH-S-2200, 5-CA-S-2200-NHM and MCBX were present at very low levels (≤ 0.001 mg/kg). The results are summarised in the table below.

Table B.7.2.1.2-4: Characterisation and identification of [phenoxy-^{14}C]S-2200 residues in egg and tissues

	Egg (Day 11)		Liver		Muscle		Skin		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR -Total Radioactive residues	0.113	100	0.295	100	0.014	100	0.048	100	0.033	100
ERR - Extracted Radioactive Residues	**0.104**	**91.5**	**0.183**	**62.1**	**0.007**	**50.8**	**0.043**	**90.6**	**0.030**	**90.7**
S-2200 (free)	0.058	51.2	0.004	1.4	<0.001	2.2	0.002	3.1	0.016	49.5
S-2200 (bound/conjugated)			0.005	1.5						
5-CA-HM-S-2200-NHM (free)	-	-	-	-	-	-	0.001	2.9	-	-
5-CA-2-HM-S-2200 (free)	-	-	-	-	-	-	0.002	4.6	-	-

5-CA-2-HM-S-2200 (bound/conjugated)	-	-	0.003	1.1	-	-	-	-	-	-
5-CA-MCBX-NDM (free)	-	-	-	-	-	-	<0.001	1.9	-	-
2-COOH-S-2200 (free)	<0.001	0.7	0.007	2.4	-	-	0.004	9.2		-
2-CH_2OH-S-2200 (free)	-	-	0.003	0.9	<0.001	3.7	0.001	2.7	-	-
4-OH-S-2200 (free)	0.002	1.5	0.040	13.6	-	-	0.003	6.1	0.001	4.3
4-OH-S-2200 (bound/conjugated)			0.005	1.6						
5-CA-S-2200-NHM (free)	-	-	-	-	-	-	0.001	2.1	<0.001	2.5
5-COOH-S-2200 (free)	-	-	-	-	-	-	<0.001	0.6	-	-
MCBX (free)	0.002	1.3	-	-	-	-	-	-	<0.001	2.8
Total identified	**0.062**	**54.8**	**0.067**	**22.6**	**<0.001**	**5.9**	**0.016**	**33.3**	**0.019**	**59.1**
URR – Unextracted Radioactive Residues	0.010	8.4	0.110	37.3	0.007	48.3	0.004	8.0	0.001	3.4

Following administration of **[benzyl-^{14}C]S-2200** to hens the main component of the radioactive residue in eggs was S-2200, accounting for 0.025 mg/kg (33.1 % TRR). The free metabolites 4-OH-S-2200, 5-CA-S-2200-NHM and De-Xy-S-2200 were found at levels of 4.4 % TRR (0.003 mg/kg), 3.7 % TRR (0.003 mg/kg) and 0.4 % TRR (< 0.001 mg/kg), respectively. The main component of the extractable residue in liver was De-Xy-S-2200 (0.029 mg/kg, 9.7 % TRR), S-2200 was present at a level of 0.7 % TRR (0.002 mg/kg). The free metabolites 5-CA-2-HM-MCBX, 5-CA-MCBX-NDM, 4-OH-S-2200 and 5-CA-S-2200-NDM accounted for less than 1 % TRR (< 0.01 mg/kg) each. Hydrolysis of the post-extraction solids released small amounts of S-2200 (1.4 % TRR, 0.004 mg/kg), De-Xy-S-2200 (2.4 % TRR, 0.007 mg/kg) and 4-OH-S-2200 (1.9 % TRR, 0.006 mg/kg), indicating that these were present as bound or conjugated residues. Total radioactive residue levels in muscle were low, S-2200 was present at a level of 1.3 % TRR (< 0.001 mg/kg) and the free metabolites 2-CH_2OH-S-2200 and 4-OH-S-2200 were found at 1.3 % TRR (< 0.001 mg/kg) and 2.5 % TRR (< 0.001 mg/kg), respectively. Radioactive residues in skin contained a number of minor metabolites (< 0.01 mg/kg), the most significant were 4-OH-S-2200 (4.8 % TRR, 0.003 mg/kg) and De-Xy-S-2200 (2.6 % TRR, 0.001 mg/kg). S-2200 accounted for 1.5 % TRR (< 0.001 mg/kg), the free metabolite 5-CA-2-HM-S-2200 was present at 1.3 % TRR (< 0.001 mg/kg). The main component of the radioactive residue in fat was S-2200 accounting for 33.9 % TRR (0.011 mg/kg). De-Xy-S-2200 was present at a level of 9.6 % TRR (0.003 mg/kg), the free metabolites 4-OH-S-2200 and MCBX were present at low levels accounting for 6.5 % TRR (0.002 mg/kg) and 1.6 % TRR ((< 0.001 mg/kg), respectively. The results are summarised in the table below.

Table B.7.2.1.2-5: Characterisation and identification of [benzyl-^{14}C]S-2200 residues in egg and tissues

	Egg (Day 12)		Liver		Muscle		Skin		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR -Total Radioactive residues	0.075	100	0.299	100	0.024	100	0.054	100	0.032	100
ERR - Extracted Radioactive Residues	**0.067**	**88.7**	**0.194**	**64.7**	**0.014**	**58.3**	**0.046**	**85.2**	**0.029**	**88.8**
S-2200 (free)	0.025	33.1	0.002	0.7	<0.001	1.3	<0.001	1.5	0.011	33.9
S-2200 (bound/conjugated)	-	-	0.004	1.4	-	-	-	-	-	-
De-Xy-S-2200 (free)	<0.001	0.4	0.029	9.7	-	-	0.001	2.6	0.003	9.6
De-Xy-S-2200 (bound/conjugated)	-	-	0.007	2.4	-	-	-	-	-	-

5-CA-HM-S-2200-MCBX (free)	-	-	0.002	0.8	-	-	-	-	-	-
5-CA-2-HM-S-2200 (free)	-	-	-	-	-	-	<0.001	1.3	-	-
5-CA-MCBX-NDM (free)	-	-	0.003	0.9	-	-	-	-	-	-
2-CH2OH-S-2200 (free)	-	-	-	-	<0.001	1.3	-	-	-	-
4-OH-S-2200 (free)	0.003	4.4	0.002	0.8	<0.001	2.5	0.003	4.8	0.002	6.5
4-OH-S-2200 (bound/conjugated)	-	-	0.006	1.9	-	-	-	-	-	-
5-CA-S-2200-NHM (free)	0.003	3.7	-	-	-	-	-	-	-	-
5-CA-S-2200-NDM (free)	-	-	0.001	0.4	-	-	-	-	-	-
MCBX (free)	-	-	-	-	-	-	-	-	<0.001	1.6
Total identified	**0.031**	**41.6**	**0.057**	**19.0**	**0.001**	**5.0**	**0.006**	**10.1**	**0.017**	**51.6**
URR – Unextracted Radioactive Residues	0.009	11.3	0.103	34.4	0.010	41.2	0.008	14.3	0.003	8.4

B.7.2.1.3 Conclusions and metabolic pathway of S-2200 in poultry

The absorption, distribution, metabolism and excretion of S-2200 was investigated in laying hens following daily oral administration of [phenoxyl-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 for 14 consecutive days. The average dose level was 13.24 mg/kg and 13.04 mg/kg in the diet. Eggs and excreta were collected during the dosing period. Animals were sacrificed approximately 6 hours after the last dose. Muscle (breast and thigh), fat (peritoneal and perirenal), liver (whole organ) and skin (including subcutaneous fat) were sampled for analysis.

The majority of the administered dose was excreted since 83% of the total dose of [phenoxy-^{14}C]S-2200 and 98% of the total dose of [benzyl-^{14}C]S-2200 were recovered in the excreta. The TRR in muscle, fat, skin and liver accounted for <0.1% of the dose, the TRR in eggs accounted for 0.2% of the dose for both labels.

Total radioactive residues in eggs, tissues and excreta following administration of [^{14}C]S-2200 to laying hens at 13 mg/kg in the diet

Matrix	Total Radioactive Residue			
	[Phenoxy-^{14}C]S-2200		[Benzyl-^{14}C]S-2200	
	mg/kg	% of dose	mg/kg	% of dose
Fat (peritoneal)	0.033	0.003	0.032	0.005
Liver	0.295	0.055	0.299	0.063
Muscle (breast)	0.013	0.007	0.025	0.014
Muscle (thigh)	0.014	0.003	0.023	0.005
Skin	0.048	0.003	0.054	0.003
Total tissues	-	0.07	-	0.09
Egg	0.051 - 0.113[1)]	0.21	0.050 - 0.081[1)]	0.18
Excreta	-	83.37	-	98.35
Cage wash	-	1.33	-	0.99
Total Recovery	**-**	**84.98**	**-**	**99.61**

[1)] ……residue range for day 2-14

For both levels the highest total radioactive residues were found in liver with 0.295 and 299 mg/kg, respectively. The total radioactive residues found in other tissues were rather low (0.014 up to 0.054 mg/kg). Total radioactive residues in eggs reached a maximum on day 11 (0.113 mg/kg) for the [phenoxy-^{14}C]S-2200 doses hens and on day seven (0.081 mg/kg) for [benzyl-^{14}C]S-2200 dosed animals.

The metabolism of [^{14}C]S-2200 was extensive, with individual extracts from tissues containing up to 21 peaks or diffuse regions of interest, many of which were considered to be multi-component. A number of the radioactive residues, following both more and less aggressive extraction techniques, shared chromatographic properties with parent compound and supplied metabolite standards.

[phenoxy-^{14}C]S-2200 treatment

Residues in egg, skin and fat were readily extractable with organic and polar solvents (hexane, acetonitril, ethyl actetate, 1% formic acid in acetonitrile) releasing 91.6 % TRR (0.104 mg/kg) in egg, 92.0 % TRR (0.044 mg/kg) in skin and 96.6 % TRR (0.031 mg/kg) in fat.
The main component of the radioactive residue in **eggs** was S-2200 (51.2% TRR, 0.058 mg/kg). The free metabolites 2-COOH-S-2200, 4-OH-S-2200 and MCBX were found at levels of < 0.01 mg/kg each (0.7-1.5 %). Radioactive residues in **skin** contained a number minor metabolites (< 0.01 mg/kg), the most significant were 2-COOH-S-2200 (9.2 % TRR, 0.004 mg/kg) and 4-OH-S-2200 (6.1 % TRR, 0.003 mg/kg). The main component of the radioactive residue in **fat** was S-2200 accounting for 49.5 % TRR (0.016 mg/kg). In **liver** and muscle only 62.7% TRR (0.185 mg/kg) and 51.7 % TRR (0.007 mg/kg), respectively, were extractable with organic or polar solvents. Hydrolysis of the post-extraction solids released small amounts of S-2200 (1.5 % TRR, 0.005 mg/kg), 5-CA-2-HM-S-2200 (1.1 % TRR, 0.003 mg/kg) and 4-OH-S-2200 (1.6 % TRR, 0.005 mg/kg), indicating that these were present as bound or conjugated residues. Total radioactive residue levels in **muscle** were rather low (< 0.001 mg/kg), S-2200 was present at a level of 2.2% TRR (< 0.001 mg/kg).

[benzyl-^{14}C]S-2200 treatment

Residues in egg, skin and fat were readily extractable with organic and polar solvents (hexane, acetonitril, ethyl actetate, 1% formic acid in acetonitrile), releasing 88.7 % TRR (0.67 mg/kg) in egg, 86.2 % TRR (0.047 mg/kg) in skin and 91.6 % TRR (0.030 mg/kg) in fat.
The main component of the radioactive residue in **eggs** was S-2200 (33.1 % TRR, 0.025 mg/kg). The free metabolites 4-OH-S-2200, 5-CA-S-2200-NHM and De-Xy-S-2200 were found at levels of < 0.01 mg/kg each (0.4 - 4.4 %). Radioactive residues in **skin** contained a number of minor metabolites (< 0.01 mg/kg), the most significant were 4-OH-S-2200 (4.8 % TRR) and De-Xy-S-2200 (2.6 % TRR). S-2200 accounted for 1.5 % TRR (< 0.001 mg/kg). The main component of the radioactive residue in **fat** was S-2200 (33.9% TRR, 0.011 mg/kg). De-Xy-S-2200 was present at a level of 9.6 % TRR (0.003 mg/kg), the free metabolites 4-OH-S-2200 (6.5 % TRR, 0.002 mg/kg) and MCBX (1.6 % TRR, < 0.001 mg/kg) were present only at low levels. In liver and muscle only 65.6% TRR (0.196 mg/kg) and 58.8 % TRR (0.014 mg/kg), respectively, were extractable with organic or polar solvents. The main component of the extractable residue in **liver** was De-Xy-S-2200 (0.029 mg/kg, 9.7 % TRR), S-2200 were present at a level of 0.7 % TRR (0.002 mg/kg). Hydrolysis of the post-extraction solids released small amounts of S-2200 (1.4 % TRR, 0.004 mg/kg), De-Xy-S-2200 (2.4 % TRR, 0.007 mg/kg) and 4-OH-S-2200 (1.9 % TRR, 0.006 mg/kg), indicating that these were present as bound or conjugated residues. Total radioactive residue levels in **muscle** were rather low (< 0.001 mg/kg), S-2200 was present at a level of 1.3 % TRR (< 0.001 mg/kg).

Overall residue levels were low in all matrices, the only residue present in a single matrix at levels greater than 0.05 mg/kg was S-2200 with 0.058 mg/kg in eggs ([phenoxy-^{14}C]S-2200). LC/MS confirmed the presence of S-2200 as the major residue in eggs.

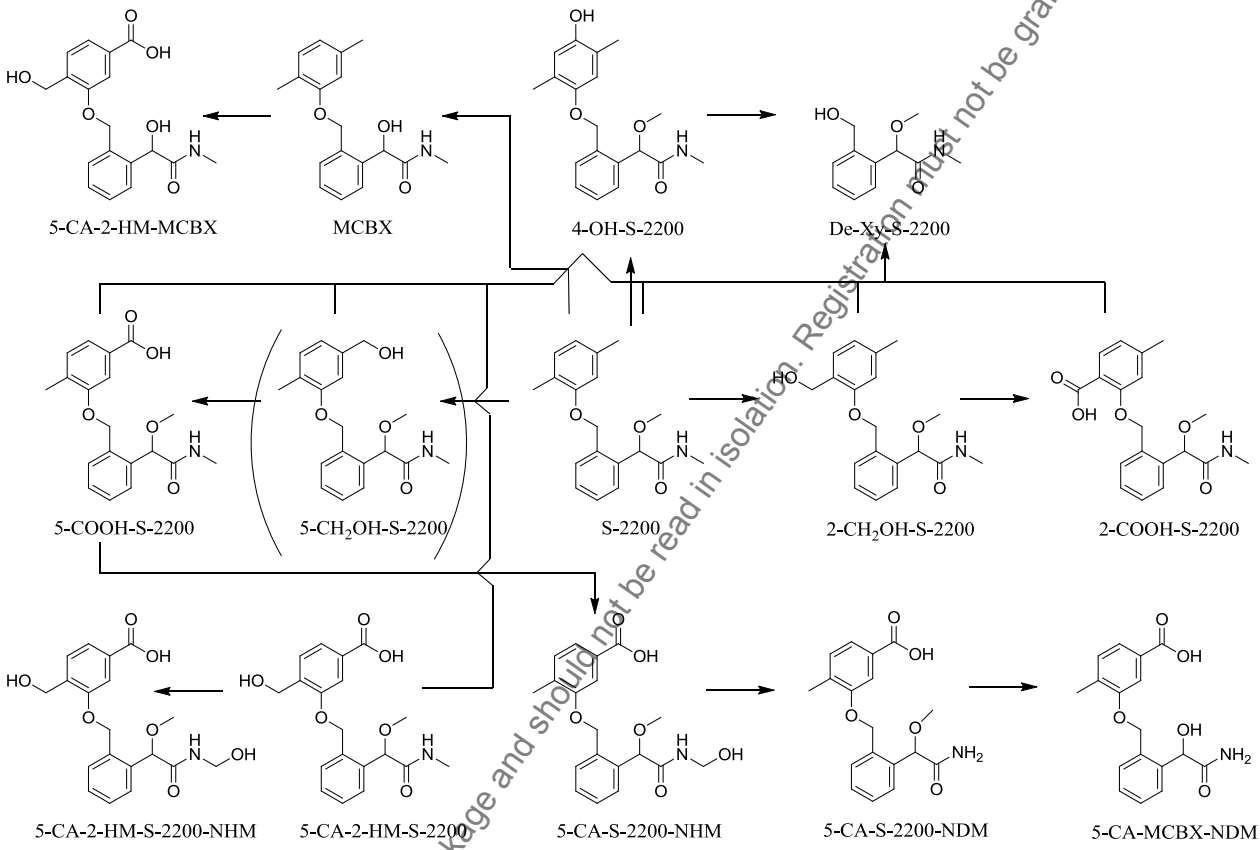
The metabolism of in laying hens proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation and ether hydrolysis.

- ✓ hydroxylation of the phenoxy ring gives 4-OH-S-2200
- ✓ hydroxylation of the methyl groups on the phenoxy ring gives 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 (not isolated in this study)
- ✓ oxidation of the hydroxymethyl groups to the carboxylic acid gives 2-COOH-S-2200, 5-COOH-S-2200 and 5-CA-2-HM-S-2200.
- ✓ hydroxylation on the N-methyl group give 5-CA-S-2200-NHM and 5-CA-2-HM-S-2200-NHM.
- ✓ S-2200 is also subject to hydrolysis of the phenoxy ether link, yielding De-Xy-S-2200.

- ✓ N-demethylation of 5-COOH-S-2200 results in the formation of 5-CA-MCBX-NDM
- ✓ O-demethylation of S-2200 results in the formation of MCBX

The primary metabolites are further metabolised by conjugation, thus S-2200, De-Xy-S-2200, 4-OH-S-2200 and 5-CA-2-HM-S-2200 were present in liver in bound/conjugated form.

Figure B.7.2.1-1: Proposed metabolic pathway of Mandestrobin (S-2200) in poultry



Code Name	Molecular Weight	Chemical Name
S-2200	313.39	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
MCBX	299.36	(RS)-2-hydroxy-N-methyl-2-[α-(2,5- xylyloxy)-o-tolyl]acetamide
5-CA-2-HM-MCBX	345.35	(*RS*)-4-(hydroxymethyl)-3-{2-[1-hydroxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy) benzoic acid
De-Xy-S-2200	209.24	(*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide
4-OH-S-2200	329.39	(RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide
2-CH_2OH-S-2200	329.39	(RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-CH_2OH-S-2200	329.39	(RS)-2-[2-(5-hydroxymethyl-2- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-COOH-S-2200	343.38	(RS)-3-{2-[1-methoxy-1-(N-methylcarbamoyl) methyl]benzyloxy}-4-methylbenzoic acid
5-CA-S-2200-NHM	359.37	(*RS*)-3-{2-[1-(*N*-hydroxymethylcarbamoyl)-1-methoxymethyl]benzyloxy}-4-methylbenzoic acid
5-CA-2-HM-S-2200-NHM	375.37	(*RS*)-4-(hydroxymethyl)-3-{2-[1-(*N*- hydroxymethylcarbamoyl)-1-methoxymethyl] benzyloxy}benzoic acid

Code Name	Molecular Weight	Chemical Name
5-CA-2-HM-S-2200	359.37	(*RS*)-4-(hydroxymethyl)-3-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}benzoic acid
5-CA-S-2200-NDM	329.35	(*RS*)-3-[2-(1-carbamoyl-1-methoxymethyl)benzyloxy]-4-methylbenzoic acid
5-CA-MCBX-NDM	315.32	(*RS*)-3-[2-(1-carbamoyl-1-hydroxymethyl) benzyloxy]-4-methylbenzoic acid

B.7.2.2 Metabolism in lactating ruminant (Section 4, Annex IIA point 6.2.3)

Reference:	**[^{14}C]S-2200 - Absorption, distribution, metabolism and excretion following repeated oral administration to the lactating ruminant**
Author(s), year:	[redacted] (2012b)
Report/ Doc. Number:	[redacted] Report no. 8227546 Sumitomo ref: ROM-0039
Guideline(s):	US EPA OPPTS 860.1300; Nature of the residue – Plants, Livestock EU Guideline 7030/VI/95 - Rev. 3, Metabolism and Distribution in Domestic Animals, 1997. OECD 503, Metabolism in Livestock
GLP:	Yes (certified laboratory)
Deviations:	No.
Validity:	Yes
Comment (RMS):	The study is acceptable

The absorption, distribution, metabolism and excretion of S-2200 was investigated on lactating goats following daily oral administration of [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 for 7 consecutive days at a dose level equivalent to 10 ppm in the diet.
Milk samples were collected each day (in the morning prior to dose administration and in the afternoon) and urine, faeces and cage washes were collected at 24 hour intervals and at necropsy. Approximately 6 to 7 hours after the last dose administration, the animal were killed and samples of liver, kidneys, muscle (flank and loin muscle), fat (omental, renal and subcutaneous fat), blood and plasma were collected for analysis.

Livestock information:

Species/breed	Toggenberg cross/lactating goats *(Capra hircus)*
Number of tested animals	2
Age	ca. 2 years and 4 old
Weight	
at initiation of the study	51 kg and 59 kg
at the end of the study	52 kg and 62 kg
Milk Production	
During acclimatisation	0.844 and 3.897 kg/day
During experimental phase	0.735 and 3.044 kg/day
Housing	Individually in metabolism cages designed for the separate collection of milk and excreta.
Acclimatisation period	approximately 61 days, incl. 3 days in the metabolism cage

Diet	Hay *ad libitum* and 0.75 kg concentrate twice daily
Water	Fresh tap water *ad libitum*
Feed consumption[1)]	
During acclimatisation	1.465 and 3.094 kg dry diet/day
During experimental phase	1.267 and 2.445 kg dry diet/day
Environmental conditions	
Temperature	18-20 C
Humidity	32-80 %
Air changes	approx. 15-20 air changes/hour
Light exposure	16-hour fluorescent light/8-hour dark

[1)]….conversion factor dry : wet Diet= 1.10937
conversion factor dry : wet Hay= 1.107202

Test Material:

Common Name:	S-2200
Name (IUPAC):	(RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers:	S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight:	313.39
CAS No.:	173662-97-0
Purity:	100 %
Log $P_{o/w}$	3.51 (at 25 ± 1°C)
Radiolabel position (specific activity)	[phenoxy-^{14}C] S-2200 (14.0 MBq/mg) [benzyl-^{14}C] S-2200 (14.34 MBq/mg)
Purity	[phenoxy-^{14}C] S-2200: 98.9 % [benzyl-^{14}C] S-2200: 98.7 %
R/S isomer ratio	Formulated [phenoxy-^{14}C] S-2200: 49.54 : 49.6 Formulated [benzyl-^{14}C] S-2200: 49.1 : 48.7

B.7.2.2.1 Material and Methods

The absorption, distribution, metabolism and excretion of [^{14}C]S-2200 were investigated following repeated oral administration of benzyl or phenoxy radiolabelled S-2200 to lactating goats. The test substance was pipetted onto carboxymethylcellulose contained in gelatine capsules (Torpak size 13) to achieve a dose rate equivalent to 10 mg diet, based on the pre-dose dietary intake of dry hay and concentrate. The solvent was allowed to evaporate prior to the capsules being sealed and stored at < - 10°C in the dark. Prior to the preparation of the capsules for dosing, trial capsule preparations for each label were performed to measure the stability of the test substances immediately following preparation and at 4 and 8 days following preparation. The radiochemical purity of the encapsulated S-2200 was also measured prior to the first dose and following the last dose.

Dosing:
Two lactating goats were orally dosed with either [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 once daily following the morning milk collection for a total of 7 consecutive days. The targeted daily dose was 10 ppm in the diet, based on food consumption during the acclimation period. The observed daily dose of [Phenoxy-^{14}C]S-2200 was 16.03 mg/day corresponding to a total dose of 112.2 mg/animal for the duration of the study. The average dose level of [phenoxy-^{14}C]S-2200 was 12.65 mg/kg in the diet. The administered daily dose of [benzyl-^{14}C]S-2200 was 35.05 mg/day corresponding to a total dose of 245.3 mg/animal for the duration of

the study. The average dose level of [benzyl-^{14}C]S-2200 was 14.33 mg/kg in the diet. This dose level represents an exaggerated rate relative to the maximum possible dietary burden to lactating ruminants. The experimental phase was conducted from 20th July 2010 to 26th July 2010. A summary of the dosing regime is given in the table below.

Table B.7.2.2-1: Dosing regime

Label	No. of goats	mean feed consumption per day[1)]	Body weight[2)]	Dose level			
		kg dry weight	kg	mg as/kg feed/day	mg as/day	mg as/animal (total dose)	mg as/kg bw/day
[phenoxy-^{14}C] S-2200	1	1.27	51	12.65	16.03	112.2	0.31
[benzyl-^{14}C] S-2200	1	2.45	59	14.33	35.05	245.3	0.59

1) ….during experimental phase
2) ….at the start of the experimental phase

Sample collection and handling:
The goats were milked in the morning prior to dose administration and in the afternoon. The two milk samples collected each day were kept separate and stored at <-10°C prior to analysis. Urine, faeces and cage washes were collected at 24 hour intervals and at necropsy. After each collection of excreta, cage debris was collected and the cages rinsed with a minimum volume of water. Cage debris was pooled over the duration of the study but was be analysed. Approximately 6 to 7 hours after the last dose administration, the animals were removed from the metabolism cages and killed by anaesthetic overdose and exsanguinated by severance of the major neck blood vessels. The metabolism cage was rinsed with methanol to provide a final cage wash. Samples of liver, kidneys, muscle (flank and loin muscle), fat (omental, renal and subcutaneous fat), blood and plasma were collected for analysis. Milk samples were separated into fat and aqueous fractions by centrifugation on the day of collection, tissues were homogenised on the day of collection and stored at <-10°C.
Aliquots of milk fractions, plasma, urine and cage wash were analysed directly for radioactivity by liquid scintillation counting (LSC). Faeces samples were homogenised and aliquots analysed by combustion. Blood and homogenised tissue samples were solubilised prior to analysis by liquid scintillation counting (LSC).
Tissue samples containing total radioactive residues (TRRs) >0.01 mg/kg were subjected to solvent extraction, protease digestion, acid and base hydrolysis, and the extracts profiled by HPLC. Analysis and identification of residues in the extracts was conducted by radio-HPLC using co-chromatography with authentic reference standards. Selected extracts were also analysed by LC-MS in positive ion mode for metabolite identification.

HPLC Method
Column: Waters Atlantis dC18 (150 x 4.6 mm id, 5 µm particle size)
Flow rate: 1.0 mL/min
UV Detection: 254 nm
Solvent A: 0.05% aqueous formic acid
Solvent B: methanol : acetonitrile (50:50 v/v)
Detection: 500 µL Liquid Cell
Gradient table:

Time (min)	% A	% B
0	90	10
10	85	15

Time (min)	% A	% B
30	50	50
45	45	55
55	30	70
65	30	70
65.1	90	10
75	90	10

Retention time for S-2200: 54.7 min

For LC/MS, a ThermoFisher LTQ Orbitrap hybrid mass spectrometer was coupled to a ThermoFisher Accela HPLC system *via* an API interface. The analytical method used was as above. The eluent was split 1:10 between the mass spectrometer and the fraction collector. Fractions were collected over 12 second intervals into 96 well plates and subjected to LSC by TopCount NXT Microplate Scintillation Counter. Chromatograms were reconstituted from the LSC data. Positive ion electrospray mass spectrometric analysis was carried out with a capillary temperature of 325°C, a capillary voltage of 14 V and a spray voltage of 4kV. The scan range was m/z 100 to 700.

Samples of **milk fat** from day 7 pm (dosed with [phenoxy-^{14}C]S-2200) or (dosed with [benzyl-^{14}C]S-2200), **fat** (omental, perirenal and subcutaneous fat) and **muscle** were pooled by radiolabel and used for the determination of the nature of the residues. A portion of the pooled sample was sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile. The hexane extract was partitioned against acetonitrile. The combined ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were concentrated and analysed by HPLC. The [benzyl-^{14}C]S-2200 labelled **aqueous milk** fraction from day 6 pm was partitioned against hexane. The aqueous residue was freeze-dried and reconstituted in acetonitrile:water (19:1 v/v), methanol, acetonitrile:water (1:1 v/v), ethyl acetate and acetonitrile:water (3:1 v/v). The reconstitute was reduced in volume under nitrogen convection and centrifuged to remove particulate matter 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/kg). **Liver** samples of both radiolabels were sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, ethyl acetate, water, 1M HCl and 1M ammonia solution. The hexane extract was partitioned against acetonitrile. The ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were combined and concentrated for analysis by HPLC. The water, 1M HCl and 1M ammonia solution extracts were combined, freeze dried, reconstituted in acetonitrile:water (19:1 v/v), methanol and acetonitrile:water (1:1 v/v) and reduced in volume under nitrogen and centrifuged prior to analysis by HPLC. The unextracted residue from liver was subjected to protease digestion for 18 hours at 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. The residue remaining after protease digestion was extracted with acetonitrile and then subjected to sequential acid hydrolysis with 10M HCl under reflux followed by base hydrolysis with 10M NaOH under reflux. The acetonitrile extract and acid hydrolysate were analysed by HPLC.

Kidney samples from both radiolabels were sequentially extracted with hexane, ethyl acetate, acetonitrile and 1% formic acid in acetonitrile, water, 1M HCl and 1M ammonia solution. The hexane extract was partitioned against acetonitrile. The ethyl acetate, acetonitrile and 1% formic acid in acetonitrile extracts were combined and concentrated for analysis by HPLC. The water, 1M HCl and 1M ammonia solution extracts were combined, freeze dried, reconstituted in acetonitrile:water (19:1 v/v), methanol and acetonitrile:water (1:1 v/v), reduced in volume under nitrogen and centrifuged prior to analysis by HPLC.

Analysis and identification of residues in the extracts was conducted by radio-HPLC using co-chromatography with authentic reference standards. Selected extracts were also analysed by LC-MS in positive ion mode for metabolite identification.

Storage stability data were not necessary since metabolite profiling was completed within six months after necropsy/sampling.

B.7.2.2.2 Distribution, characterisation and identification of residues

Excretion balance and tissue distribution investigations were performed following repeated oral administrations to 2 lactating goats for 7 consecutive days at a dose level of 10 mg in the diet. Approximately 79 % of the administered radioactivity was recovered from the goat dosed with [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200. The majority of the administered dose was recovered in the excreta (urine and faeces). The TRR in muscle, fat and kidney accounted for <0.1% of the dose, the TRR in liver accounted for 0.2 – 0.3 % of the dose for both labels. The TRR in milk accounted for <0.1% of the dose for both labels. A summary is given in the table below.

Table B.7.2.2-2: Total radioactive residues in milk, tissues and excreta following administration of [^{14}C]S-2200 to lactating goats

Matrix	Total Radioactive Residue			
	[phenoxy-^{14}C]S-2200 12.65 mg/kg diet		[benzyl-^{14}C]S-2200 14.33 mg/kg diet	
	mg/kg	% of dose	mg/kg	% of dose
Liver	0.319	0.225	0.613	0.289
Kidney	0.170	0.022	0.412	0.031
Muscle (flank)	0.012	0.005	0.016	0.003
Muscle (loin)	0.008	0.001	0.014	0.001
Fat (omental)	0.012	0.006	0.028	0.002
Fat (renal)	0.013	0.008	0.034	0.004
Fat (subcutaneous)	0.010	0.001	0.033	<0.001
Blood	0.028	<0.001	0.076	<0.001
Plasma	0.042	<0.001	0.093	<0.001
Total tissues	-	0.27	-	0.33
Milk – fat fraction	0.008-0.033	0.002	0.006-0.035	0.005
Milk – aqueous fraction	0.004-0.010	0.024	0.006-0.018	0.073
Urine	-	35.22	-	39.73
Faeces	-	42.49	-	38.07
Cage wash	-	1.19	-	0.68
Total Recovery	**-**	**79.19**	**-**	**78.89**

For the [phenoxy-^{14}C]S-2200 label radioactive residues in milk fat fraction reached a maximum of 0.033 mg/kg on day 7 *pm* and 0.010 mg/kg on day 6 pm in the aqueous fraction. For the [benzyl-^{14}C]S-2200 label radioactive residues in the milk fat fraction reached a maximum of 0.035 mg/kg on day 5 pm and 0.018 mg/kg in the aqueous fraction on day 3 pm. An overview is given in the following table.

Table B.7.2.2-3: Total radioactive residues in milk over time following administration of [^{14}C]S-2200 to lactating goats

Sampling Time	Total Radioactive Residue in mg/kg							
	Milk – Fat Fraction				Milk – Aqueous Fraction			
	[phenoxy-^{14}C]S-2200 12.65 mg/kg diet		benzyl-^{14}C]S-2200 14.33 mg/kg diet		[phenoxy-^{14}C]S-2200 12.65 mg/kg diet		benzyl-^{14}C]S-2200 14.33 mg/kg diet	
	am	pm	am	pm	am	pm	am	pm
Day 1	NA	0.019	NA	0.025	NA	0.007	NA	0.016
Day 2	0.008	0.028	0.006	0.028	0.004	0.009	0.007	0.015
Day 3	0.008	0.026	0.007	0.031	0.004	0.009	0.006	0.018
Day 4	0.008	0.025	0.009	0.030	0.004	0.009	0.007	0.014
Day 5	0.011	0.025	0.010	0.035	0.004	0.009	0.007	0.015
Day 6	0.009	0.023	0.009	0.031	0.004	0.010	0.007	0.018
Day 7	0.010	0.033	0.008	0.035	0.006	0.009	0.006	0.016
Total % of the dose	0.002		0.005		0.024		0.073	

The dose level used in this study (12.65 and 14.33 mg/kg in the diet, respectively) represents a highly exaggerated rate relative to the maximum possible dietary exposure to ruminants of 0.024 mg/kg diet, however the radioactive residue levels in milk, liver, kidney, muscle and fat were low.

Following administration of **[phenoxy-^{14}C]S-2200** to lactating goats the highest total radioactive residues were found in liver and kidney accounting for 0.32 mg/kg and 0.17 mg/kg S-2200 equivalents, respectively. The total radioactive residues in tissues were low and accounted for 0.008 – 0.012 mg/kg in muscle and 0.010 -0.013 mg/kg in fat, respectively. Total radioactive residues in milk reached a maximum of 0.033 mg/kg in milk fat on day 7 (pm) and 0.010 mg/kg in the aqueous fraction of milk on day 6 (pm). Residues in milk, muscle, kidney and fat were readily extractable with organic and polar solvents (hexane, acetonitrile/water, ethyl actetate, 1% formic acid in acetonitrile) releasing 91.7 % TRR (0.030 mg/kg) in milk fat, 72.3 % TRR (0.007 mg/kg) in muscle, 94.3 % TRR (0.161 mg/kg) in kidney and 87.2 % TRR (0.010 mg/kg) in fat. In liver 70.1% TRR (0.223 mg/kg) were extractable with organic or polar solvents. A TRR of 29.9 % (0.095 mg/kg) remained as post extraction solids, but further 22.7% TRR (0.073 mg/kg) was released by protease hydrolysis and 5.2% TRR (0.017 mg/kg) by strong acid followed by strong base hydrolysis.

Following administration of **[benzyl-^{14}C]S-2200** to lactating goats the highest total radioactive residues were found in liver and kidney accounting for 0.61 mg/kg and 0.41 mg/kg S-2200 equivalents, respectively. The total radioactive residues in tissues were low and accounted for 0.014 – 0.016 mg/kg in muscle and 0.028 - 0.034 mg/kg in fat, respectively.Total radioactive residues in milk reached a maximum of 0.035 mg/kg in milk fat on day 5 (pm) and 0.018 mg/kg in the aqueous fraction of milk on day 3 (pm). Residues in muscle, kidney and fat were readily extractable with organic and polar solvents (hexane, acetonitril, ethyl actetate, 1% formic acid in acetonitrile) releasing 86.8% TRR (0.013 mg/kg) in muscle, 95.2 % TRR (0.392 mg/kg) in kidney and 91.7 % TRR (0.028 mg/kg) in fat. Residues in the milk fat and the milk aqueous fraction were extractable with organic and polar solvents (hexane, acetonitrile/water, methanol, ethyl actetate) and released 100 % TRR (0.035 mg/kg) in milk fat and 96.4 % TRR (0.017 mg/kg) in aqueous fraction of milk. In liver 59.6% TRR (0.364 mg/kg) were extractable with organic or polar solvents. A TRR of 40.4 % (0.248 mg/kg) remained as post extraction solids, but further 26.2% TRR (0.16 mg/kg) was released by protease hydrolysis and 8.4 % TRR (0.052 mg/kg) by strong acid followed by strong base hydrolysis.

The results are summarised in the table below.

Table B.7.2.2-4: Distribution of [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 residues in milk and tissues (expressed as mg/kg S-2200 equivalents and % TRR)

Sample	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		[benzyl-^{14}C]S-2200	
		mg/kg	%TRR	mg/kg	%TRR
Milk fat fraction	Pooled organic fraction	0.026	80.4	0.035	100
	Hexane fraction	0.004	11.3	0.003	9.5
	PES (post extraction solids)	0.003	8.3	-	-
	Total Radioactive Residue (TRR)	**0.033**	**100**	**0.038**	**109.5**
Milk aqueous fraction	Aqueous fraction	Sample not extracted		0.017	96.4
	Hexane fraction			<0.001	3.6
	PES (post extraction solids)			-	-
	Total Radioactive Residue (TRR)	**0.010**	**100**	**0.018**	**100**
Muscle	Pooled organic fraction	0.007	72.0	0.013	86.7
	Hexane fraction	<0.001	0.3	<0.001	0.1
	PES (post extraction solids)	0.003	27.7	0.002	13.2
	Total Radioactive Residue (TRR)	**0.010**	**100**	**0.015**	**100**
Kidney	Pooled organic fraction	0.140	82.2	0.337	81.9
	Hexane fraction	<0.001	<0.1	<0.001	<0.1
	Aqueous extracts	0.021	12.1	0.055	13.3
	PES (post extraction solids)	0.010	5.7	0.020	4.8
	Total Radioactive Residue (TRR)	**0.170**	**100**	**0.412**	**100**
Fat	Pooled organic fraction	0.010	83.8	0.028	89.6
	Hexane fraction	<0.001	3.4	<0.001	2.1
	PES (post extraction solids)	0.002	12.8	0.003	8.3
	Total Radioactive Residue (TRR)	**0.012**	**100**	**0.032**	**100**
Liver	Pooled organic fraction	0.187	58.6	0.289	47.1
	Hexane fraction	<0.001	0.2	<0.001	0.2
	Aqueous extracts	0.036	11.3	0.075	12.3
	PES (post extraction solids)	0.095	29.9	0.248	40.4
	Exhaustive extraction of post-extraction solids				
	Protease digest - Aqueous phase	0.062	19.5	0.132	21.6
	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
	Total Radioactive Residue (TRR)	**0.319**	**100**	**0.613**	**100**

The approximately 2-fold higher tissue residues following dosing with [benzyl-^{14}C]S-2200 compared to [phenoxy-^{14}C]S-2200 (dose level of 0.31 mg/kg bw) could be explained by the approximately 2-fold higher dose level of 0.59 mg/kg bodyweight for the [benzyl-^{14}C]S-2200.

Characterization and identification of metabolites was made HPLC co-chromatography against authentic reference standards. LC/MS was used to confirm the identity of parent S-2200 in milk fat, liver and fat, 2-CH_2OH-S-2200 in liver, 5-COOH-S-2200 in liver and kidney and 4-OH-S-2200 glucuronide in kidney.

Following administration of **[phenoxy-^{14}C]S-2200** to lactating goats the main component of the radioactive residue in milk fat was S-2200, accounting for 0.011 mg/kg (32.7 % TRR). The free metabolites 2-CH_2OH-S-2200, 4-OH-S-2200, 5-CA-MCBX-NDM and 5-CA-S-2200-NDM were found at levels of 5.8 % TRR

(0.002 mg/kg), 6.1 % TRR (0.002 mg/kg), 4.3 % TRR (0.001 mg/kg) and 4.0 % TRR (0.001 mg/kg), respectively. The main component of the extractable residue in liver was 5-COOH-S-2200 (0.062 mg/kg, 19.3 % TRR), S-2200 were present at a level of 1.6 % TRR (0.005 mg/kg). The free metabolites 2-COOH-S-2200, 5-CA-MCBX-NDM and 2-CH_2OH-S-2200 accounted for 1.6 % TRR (0.005 mg/kg), 2.8 % TRR (0.009 mg/kg) and 7.5 % TRR (0.024 mg/kg), respectively. Free 5-CA-2-HM-S-2200 and 5-CH_2OH-S-2200 were found at a level of 2.3 % TRR (0.007 mg/kg) and 2.0 % TRR (0.006 mg/kg), repectively and a number of other minor metabolites were found at levels <0.01 mg/kg. Hydrolysis of the liver post-extraction solids released small amounts of S-2200, 5-CA-2-HM-MCBX, 5-CA-2-HM-S-2200, 2-COOH-S-2200, 2-CH_2OH-S-2200, 5-CA-S-2200-NDM and 5-COOH-S-2200, all individually at levels of < 0.01 mg/kg, indicating that these were present as bound or conjugated residues. Radioactive residues in kidney contained a number minor metabolites (< 0.01 mg/kg), the most significant components of the extractable residue in kidney were 5-COOH-S-2200 (0.043 mg/kg, 25% TRR) and 4-OH-S-2200 glucuronide (0.03 mg/kg, 15% TRR), respectively. S-2200 was present at a level of 2.1 % TRR (0.004 mg/kg). Total radioactive residue levels in muscle were low, S-2200 was present at a level of 23 % TRR (0.002 mg/kg) and the free metabolite 2-CH_2OH-S-2200 was found at 6.0 % TRR (< 0.001 mg/kg). The main component of the radioactive residue in fat was S-2200 accounting for 49.6 % TRR (0.006 mg/kg). The free metabolites 2-COOH-S-2200, 2-CH_2OH-S-2200, 5-CA-S-2200-NDM and 5-COOH-S-2200 were present at low levels (< 0.001 mg/kg). The results are summarised in the table below.

Table B.7.2.2-5: Characterisation and identification of [phenoxy-^{14}C]S-2200 residues in milk and tissues

	Milk fat (Day 7 pm)		Liver		Kidney		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR -Total Radioactive residues	0.033	100	0.319	100	0.170	100	0.010	100	0.012	100
ERR - Extracted Radioactive Residues	**0.030**	**91.7**	**0.224**	**70.1**	**0.162**	**94.3**	**0.008**	**72.3**	**0.011**	**87.2**
S-2200 (free)	0.011	32.7	0.005	1.6	0.004	2.1	0.002	23.0	0.006	49.6
S-2200 (bound/conjugated)	-	-	0.005	1.5						
5-CA-2-HM-MCBX (free)	-	-	0.002	0.7						
5-CA-2-HM-MCBX (bound/conj)	-	-	< 0.001	0.3						
5-CA-2-HM-S-2200-NHM (free)	-	-	0.003	0.9	0.006	3.6	-	-	-	-
5-CA-2-HM-S-2200 (free)	-	-	0.007	2.3	-	-	-	-	-	-
5-CA-2-HM-S-2200 (bound/conjugated)	-	-	0.004	1.1	-	-	-	-	-	-
5-CA-MCBX-NDM (free)	0.001	4.3	0.009	2.8	0.007	4.3	-	-	-	-
2-COOH-S-2200 (free)	-	-	0.005	1.6	0.004	2.2	<0.001	2.0	<0.001	7.0
2-COOH-S-2200 (bound/conjugated)	-	-	0.008	2.6	-	-	-	-	-	-
2-CH_2OH-S-2200 (free)	0.002	5.8	0.024	7.5	0.006	3.5	<0.001	6.0	<0.001	2.6
2-CH_2OH-S-2200 (bound/conjugated)	-	-	0.001	0.3	-	-	-	-	-	-
4-OH-S-2200 (free)	0.002	6.1	0.003	0.8	0.003	1.7	-	-	-	-
5-CH_2OH-S-2200 (free)	-	-	0.006	2.0	0.001	0.6	-	-	-	-
5-CA-S-2200-NHM (bound/conjugated)	-	-	<0.001	0.1	-	-	-	-	-	-
5-CA-S-2200-NDM (free)	0.001	4.0	0.004	1.4	-	-	-	-	<0.001	2.6

	Milk fat (Day 7 pm)		Liver		Kidney		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
5-CA-S-2200-NDM (bound/conjugated)	-	-	< 0.001	0.2	-	-	-	-		-
5-COOH-S-2200 (free)	-	-	0.062	19.3	0.043	25.0	-	-	<0.001	3.5
5-COOH-S-2200 (bound/conjugated)	-	-	0.002	0.8	-	-	-	-	-	-
MCBX (free)	-	-	0.002	0.5	0.002	0.9	-		-	-
4-OH-S-2200 glucuronide	-	-	-	-	0.025	14.9	-	-	-	-
Total identified	**0.017**	**52.9**	**0.154**	**48.2**	**0.100**	**58.8**	**0.003**	**31.0**	**0.008**	**65.2**
URR – Unextracted Radioactive Residues	0.003	8.3	0.095	29.9	0.010	5.7	0.003	27.7	0.002	12.8

Following administration of **[benzyl-^{14}C]S-2200** to lactating goats the main component of the radioactive residue in milk fat was S-2200, accounting for 0.012 mg/kg (35.3 % TRR). The free metabolites 2-CH_2OH-S-2200, De-Xy-S-2200, 5-CA-MCBX-NDM and 5-CA-S-2200-NDM were found at levels of 3.2 % TRR (0.001 mg/kg), 4.9 % TRR (0.002 mg/kg), 2.9 % TRR (0.001 mg/kg) and 2.6 % TRR (0.001 mg/kg), respectively. 4-OH-S-2200 was not detected in the milkfat fraction. Radioactive residues in the aqueous milk fraction contained a number minor metabolites (< 0.001 mg/kg), the main component of the extractable radioactive residue was 5-CA-S-2200-NHM, accounting for 0.003 mg/kg (14.7 % TRR).

The main component of the extractable residue in liver was 5-COOH-S-2200 (0.065 mg/kg, 10.6 % TRR). S-2200 was present at a level of 4.7 % TRR (0.029 mg/kg). The free metabolites De-Xy-S-2200, 2-COOH-S-2200 and 2-CH_2OH-S-2200 accounted for 3.8 % TRR (0.023 mg/kg), 1.2 % TRR (0.007 mg/kg) and 3.6 % TRR (0.022 mg/kg), respectively. Free 5-CA-2-HM-S-2200 and 5-CH_2OH-S-2200 were found at a level of 1.3 % TRR (0.008 mg/kg) and 1.5 % TRR (0.009 mg/kg), repectively and a number of other minor metabolites were found at levels <0.01 mg/kg. Hydrolysis of the liver post-extraction solids from goats dosed with [benzyl-14C]S-2200 released amounts of S-2200 (0.19 mg/kg, 3.0% TRR), De-Xy-S-2200 (0.027 mg/kg, 4.4% TRR), 2-COOH-S-2200 (0.003 mg/kg, 0.5% TRR) and 2-CH2OH-S-2200 (0.017 mg/kg, 2.7% TRR), indicating that these were present as bound or conjugated residues. The identities of S-2200, 2-CH2OH-S-2200 and 5-COOH-S-2200 in liver were confirmed by LC-MS. The most significant component of the extractable residue in kidney were 5-COOH-S-2200 (0.083 mg/kg, 20.2 % TRR) and 4-OH-S-2200 glucuronide (0.055 mg/kg, 13.3 % TRR), respectively. De-Xy-S-2200 (0.019 mg/kg, 4.5% TRR), 5-CA-2-HM-S-2200-NHM (0.02 mg/kg, 5.2% TRR), 2-COOH-S-2200 (0.01 mg/kg, 2.8% TRR) and 2-CH_2OH-S-2200 (0.01 mg/kg, 3.6% TRR) were found, along with S-2200 (0.007 mg/kg, 1.6 % TRR) and 5-CA-2-HM-S-2200 (0.004 mg/kg, 0.9 % TRR).
Total radioactive residue levels in muscle were low, S-2200 was present at a level of 18.2 % TRR (0.003 mg/kg) and the free metabolites 2-CH_2OH-S-2200 and De-Xy-S-2200 were found at 10.1 % TRR (0.002 mg/kg) and 4.7 % TRR (< 0.001 mg/kg), respectively.The main component of the radioactive residue in fat was S-2200 accounting for 22.9 % TRR (0.007 mg/kg). The free metabolites 5-CA-S-2200-NDM, De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and 5-CA-MCBX-NDM were present at levels between 1.9-4.8 % TRR (< 0.001-0.002 mg/kg).The results are summarised in the tables below.

Table B.7.2.2-6: Characterisation and identification of [benzyl-^{14}C]S-2200 residues in milk

	Milk - fat fraction Day 5 pm		Milk – aqueous fraction Day 3 pm	
	mg/kg	%TRR	mg/kg	%TRR
TRR -Total Radioactive residues	0.035	100	0.018	100
ERR - Extracted Radioactive Residues	**0.035**	**100**	**0.017**	**96.4**
S-2200	0.012	35.3	<0.001	4.5
De-Xy-S-2200	0.002	4.9	<0.001	2.8

	Milk - fat fraction Day 5 pm		Milk – aqueous fraction Day 3 pm	
	mg/kg	%TRR	mg/kg	%TRR
5-CA-MCBX-NDM	0.001	2.9	<0.001	2.3
2-CH_2OH-S-2200	0.001	3.2	<0.001	2.8
4-OH-S-2200	-	-	<0.001	2.3
5-CH_2OH-S-2200	<0.001	2.6	-	-
5-CA-S-2200-NHM	-	-	0.003	14.7
5-CA-S-2200-NDM	<0.001	2.6	-	-
MCBX	-	-	<0.001	2.8
Total identified	**0.018**	**51.4**	**0.009**	**32.2**
URR – Unextracted Radioactive Residues	-	-		-

Table B.7.2.2-7: Characterisation and identification of [benzyl-^{14}C]S-2200 residues tissues

	Liver		Kidney		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR -Total Radioactive residues	0.613	100	0.412	100	0.015	100	0.032	100
ERR - Extracted Radioactive Residues	**0.365**	**59.6**	**0.392**	**95.2**	**0.013**	**86.8**	**0.029**	**91.7**
S-2200 (free)	0.029	4.7	0.007	1.6	0.003	18.2	0.007	22.9
S-2200 (bound/conjugated)	0.019	3.0	-	-	-	-	-	-
De-Xy-S-2200 (free)	0.023	3.8	0.019	4.5	<0.001	4.7	<0.001	1.9
De-Xy-S-2200 (bound/conjugated)	0.027	4.4	-	-	-	-	-	-
5-CA-2-HM-S-2200-NHM (free)	0.003	0.5	0.021	5.2	-	-	-	-
5-CA-2-HM-S-2200 (free)	0.008	1.3	0.004	0.9	-	-	-	-
5-CA-MCBX-NDM (free)	-	-	-	-	-	-	0.002	4.8
2-COOH-S-2200 (free)	0.007	1.2	0.012	2.8	<0.001	3.3	<0.001	2.2
2-COOH-S-2200 (bound/conjugated)	0.003	0.5	-	-	-	-	-	-
2-CH_2OH-S-2200 (free)	0.022	3.6	0.015	3.6	0.002	10.1	-	-
2-CH_2OH-S-2200 (bound/conjugated)	0.017	2.7	-	-	-	-	-	-
4-OH-S-2200 (free)	0.005	0.8	-	-	-	-	-	-
5-CH_2OH-S-2200 (free)	0.009	1.5	-	-	-	-	-	-
5-CA-S-2200-NHM (free)	-	-	-	-	-	-	-	-
5-CA-S-2200-NDM (free)	0.008	1.3	-	-	-	-	<0.001	1.9
5-COOH-S-2200 (free)	0.065	10.6	0.083	20.2	-	-	0.001	3.8
MCBX (free)	0.004	0.7	0.002	0.6	-	-	-	-
4-OH-S-2200 glucuronide	-	-	0.055	13.3	-	-	-	-
Total identified	**0.248**	**40.4**	**0.217**	**52.7**	**0.005**	**36.5**	**0.012**	**37.5**
URR – Unextracted Radioactive Residues	0.248	40.4	0.020	4.8	0.002	13.2	0.003	8.3

Overall, the most significant residues in tissues from goats dosed with [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 were S-2200, 5-COOH-S-2200 and 4-OH-S-2200 glucuronide.

B.7.2.2.3 Conclusions and metabolic pathway of S-2200 in lactating goats

The absorption, distribution, metabolism and excretion of S-2200 was investigated in 2 lactating goats following daily oral administration of [phenoxy-^{14}C]S-2200 or [benzyl-^{14}C]S-2200 for 7 consecutive days. The average dose level was 12.65 mg/kg and 14.33 mg/kg in the diet, respectively.
Milk samples were collected each day (in the morning prior to dose administration and in the afternoon) and urine, faeces and cage washes were collected at 24 hour intervals and at necropsy. Approximately 6 to 7 hours after the last dose administration, the animal were killed and samples of liver, kidneys, muscle (flank and loin muscle), fat (omental, renal and subcutaneous fat), blood and plasma were collected for analysis. Milk samples were separated into fat and aqueous fractions by centrifugation on the day of collection.
The majority of the administered dose was recovered in the excreta (urine and faeces) with 78 % of the total dose for both labels. The TRR in milk, muscle, fat and kidney accounted for <0.1% of the dose, the TRR in liver accounted for 0.2 – 0.3 % of the dose for both labels.

Total radioactive residues in milk, tissues and excreta following administration of [^{14}C]S-2200 to lactating goats

Matrix	Total Radioactive Residue			
	[Phenoxy-^{14}C]S-2200 12.65 mg/kg diet		[Benzyl-^{14}C]S-2200 14.33 mg/kg diet	
	mg/kg	% of dose	mg/kg	% of dose
Liver	0.319	0.225	0.613	0.289
Kidney	0.170	0.022	0.412	0.031
Muscle	0.028	0.007	0.030	0.004
Fat (omental)	0.035	0.015	0.095	0.006
Milk – fat fraction	0.008-0.033	0.002	0.006-0.035	0.005
Milk – aqueous fraction	0.004-0.010	0.024	0.006-0.018	0.073
Urine	-	35.22	-	39.73
Faeces	-	42.49	-	38.07
Cage wash	-	1.19	-	0.68
Total Recovery	**-**	**79.19**	**-**	**78.89**

For both levels the highest total radioactive residues were found in liver and kidney, 0.319 and 0.613 mg/kg S-2200 equivalents for liver and 0.170 mg/kg and 0.412 mg/kg S-2200 equivalents for kidney. The total radioactive residues found in other tissues were rather low (0.028 up to 0.095 mg/kg). Total radioactive residues in milk fat reached a maximum on day 7 pm (0.033 mg/kg) for the [phenoxy-^{14}C]S-2200 and on day 5 pm (0.035 mg/kg) for [benzyl-^{14}C]S-2200 dosed animals.

[phenoxy-^{14}C]S-2200 treatment

Residues in milk, muscle, kidney and fat were readily extractable with organic and polar solvents releasing 91.7 % TRR (0.030 mg/kg) in milk fat, 72.3 % TRR (0.007 mg/kg) in muscle, 94.3 % TRR (0.161 mg/kg) in kidney and 87.2 % TRR (0.010 mg/kg) in fat.
The main component of the radioactive residue in **milk fat** was S-2200, accounting for 0.011 mg/kg (32.7 % TRR). The free metabolites 2-CH_2OH-S-2200, 4-OH-S-2200, 5-CA-MCBX-NDM and 5-CA-S-2200-NDM were found at levels between 4.0 – 6.1 % TRR (0.001-0.002 mg/kg). In **liver** 70.1% TRR (0.223 mg/kg) was extractable with organic or polar solvents, the main component was 5-COOH-S-2200 (0.062 mg/kg, 19.3 % TRR). S-2200 was present at a level of 1.6 % TRR (0.005 mg/kg) and some other metabolites at levels < 10 % TRR (max. 0.024 mg/kg). Hydrolysis of the post-extraction solids released small amounts released small amounts of S-2200, 5-CA-2-HM-MCBX, 5-CA-2-HM-S-2200, 2-COOH-S-2200, 2-CH_2OH-S-2200, 5-CA-S-2200-NDM and 5-COOH-S-2200, all individually at levels of < 0.01 mg/kg, indicating that these were present as bound or conjugated residues. Radioactive residues in **kidney** contained a number minor metabolites (< 0.01 mg/kg), the most significant components of the extractable residue in kidney were 5-COOH-S-2200 (0.043 mg/kg, 25% TRR) and 4-OH-S-2200 glucuronide (0.025 mg/kg, 15% TRR),

respectively. S-2200 was present at a level of 2.1 % TRR (0.004 mg/kg). Total radioactive residue levels in **muscle** were low, S-2200 was present at a level of 23 % TRR (0.002 mg/kg) and the free metabolite 2-CH_2OH-S-2200 was found at 6.0 % TRR (< 0.001 mg/kg). The main component of the radioactive residue in **fat** was S-2200 accounting for 49.6 % TRR (0.006 mg/kg). All other metabolites were present at low levels (< 0.001 mg/kg).

[benzyl-^{14}C]S-2200 treatment

Residues in muscle, kidney and fat were readily extractable with organic and polar solvents releasing 86.8% TRR (0.013 mg/kg) in muscle, 95.2 % TRR (0.392 mg/kg) in kidney and 91.7 % TRR (0.028 mg/kg) in fat. The extraction of the milk fat and the milk aqueous fraction released residues of 100 % TRR (0.035 mg/kg) in milk fat and 96.4 % TRR (0.017 mg/kg) in aqueous fraction of milk. In liver 59.6% TRR (0.364 mg/kg) were extractable with organic or polar solvents.
The main component of the radioactive residue in **milk fat** was S-2200, accounting for 0.012 mg/kg (35.3 % TRR). The free metabolites 2-CH_2OH-S-2200, De-Xy-S-2200, 5-CA-MCBX-NDM and 5-CA-S-2200-NDM were found at levels between 2.6 – 4.9 % TRR (0.001-0.002 mg/kg). Radioactive residues in the **aqueous milk fraction** contained a number minor metabolites (< 0.001 mg/kg), the main component of the extractable radioactive residue was 5-CA-S-2200-NHM, accounting for 0.003 mg/kg (14.7 % TRR). In **liver** 59.6% TRR (0.364 mg/kg) was extractable with organic or polar solvents, the main component was 5-COOH-S-2200 (0.065 mg/kg, 10.6 % TRR), S-2200 was present at a level of 4.7% TRR (0.029 mg/kg) and some other metabolites at levels < 5 % TRR (max. 0.023 mg/kg). Hydrolysis of the post-extraction solids released small amounts released small amounts S-2200, De-Xy-S-2200, 2-COOH-S-2200 and 2-CH_2OH-S-2200 at levels between 0.5-4.4 % TRR (0.003-0.027 mg/kg) indicating that these were present as bound or conjugated residues. The most significant components of the extractable residue in **kidney** were 5-COOH-S-2200 (0.083 mg/kg, 20.2% TRR) and 4-OH-S-2200 glucuronide (0.055 mg/kg, 13.3% TRR), respectively. S-2200 was present at a level of 1.6 % TRR (0.007 mg/kg). Total radioactive residue levels in **muscle** were low, S-2200 was present at a level of 18.2 % TRR (0.003 mg/kg) and the free metabolites 2-CH_2OH-S-2200 and De-Xy-S-2200 were found at 10.1 % TRR (0.002 mg/kg) and 4.7 % TRR (< 0.001 mg/kg), respectively.The main component of the radioactive residue in **fat** was S-2200 accounting for 22.9 % TRR (0.007 mg/kg). Other metabolites as 5-CA-S-2200-NDM, De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and 5-CA-MCBX-NDM were present at low levels (< 0.001 mg/kg).

Overall residue levels were low in all matrices for both labels. Residues present in a single matrix at levels greater than 0.05 mg/kg were 5-COOH-S-2200 in liver (0.065 mg/kg) and in kidney (0.083 mg/kg) and 4-OH-S-2200 glucuronide in kidney (0.055 mg/kg).

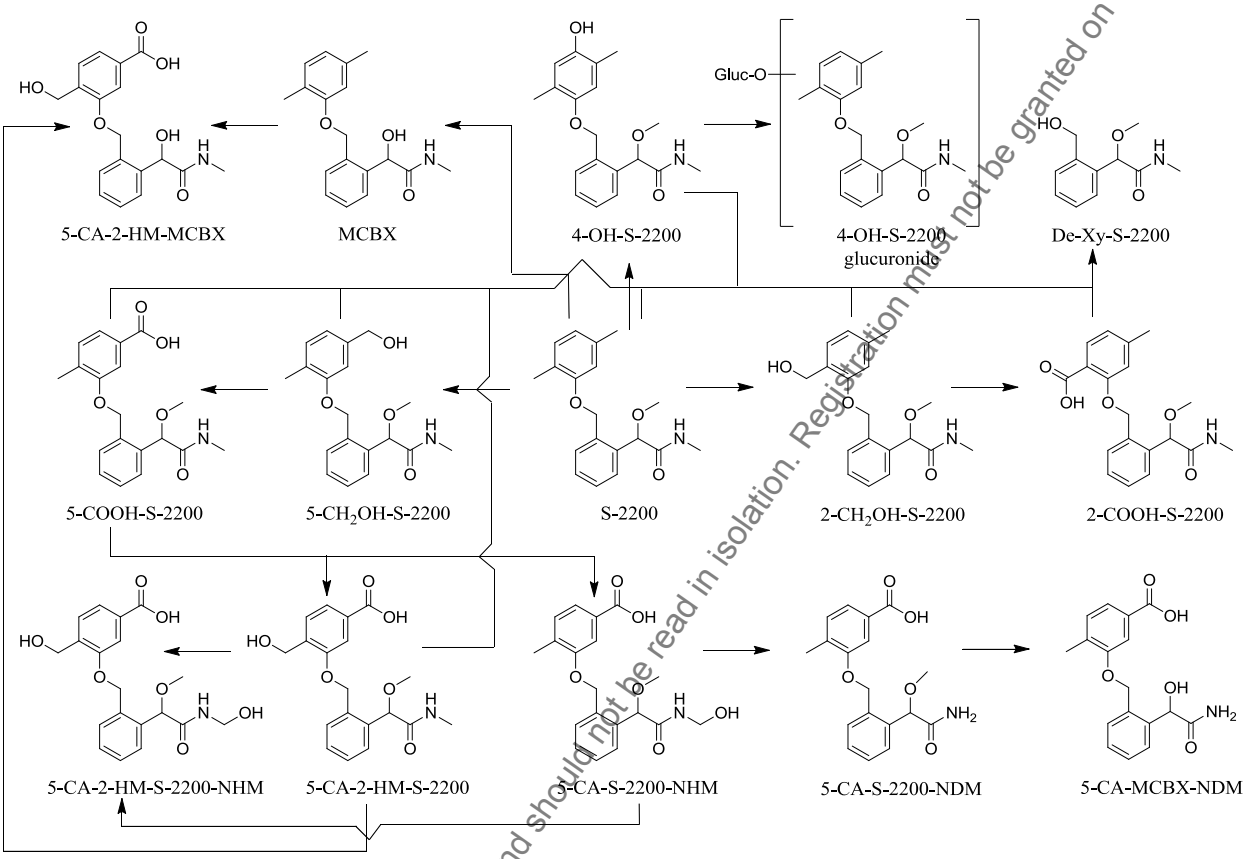
The metabolism of [^{14}C]S-2200 in lactating goats was extensive and proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation, ether hydrolysis and glucuronide conjugation.

- ✓ hydroxylation of the phenoxy ring gives 4-OH-S-2200
- ✓ hydroxylation of the methyl groups on the phenoxy ring gives 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200
- ✓ oxidation of the hydroxymethyl groups to the carboxylic acid gives 2-COOH-S-2200, 5-COOH-S-2200 and 5-CA-2-HM-S-2200.
- ✓ hydroxylation on the N-methyl group give 5-CA-S-2200-NHM and 5-CA-2-HM-S-2200-NHM.
- ✓ S-2200 is also subject to hydrolysis of the phenoxy ether link, yielding De-Xy-S-2200.
- ✓ N-demethylation of 5-COOH-S-2200 results in the formation of 5-CA-MCBX-NDM
- ✓ O-demethylation of S-2200 results in the formation of MCBX

The primary metabolites are further metabolised by conjugation and S-2200, De-Xy-S-2200, 5-CA-2-HM-MCBX, 5-CA-2-HM-S-2200, 2-COOH-S-2200, 2-CH_2OH-S-2200, 5-CA-S-2200-NDM and 5-COOH-S-

2200 were present in liver in bound/conjugated form. 4-OH-S-2200 was found as the glucuronide conjugate in kidney.

Figure B.7.2.2-1: Proposed metabolic pathway of Mandestrobin (S-2200) in lactating goat



Code Name	Molecular Weight	Chemical Name
S-2200	313.39	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
MCBX	299.36	(RS)-2-hydroxy-N-methyl-2-[α-(2,5- xylyloxy)-o-tolyl]acetamide
5-CA-2-HM-MCBX	345.35	(*RS*)-4-(hydroxymethyl)-3-{2-[1-hydroxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy) benzoic acid
4-OH-S-2200	329.39	(RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide
De-Xy-S-2200	209.24	(*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide
2-CH_2OH-S-2200	329.39	(RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
2-COOH-S-2200	343.38	(*RS*)-2-{2-[1-methoxy-1-(*N*-methylcarbamoyl) methyl]benzyloxy}-4-methylbenzoic acid
5-CH_2OH-S-2200	329.39	(RS)-2-[2-(5-hydroxymethyl-2- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-COOH-S-2200	343.38	(RS)-3-{2-[1-methoxy-1-(N-methylcarbamoyl) methyl]benzyloxy}-4-methylbenzoic acid
5-CA-S-2200-NHM	359.37	(*RS*)-3-{2-[1-(*N*-hydroxymethylcarbamoyl)-1-methoxymethyl]benzyloxy}-4-methylbenzoic acid
5-CA-S-2200-NDM	329.35	(*RS*)-3-[2-(1-carbamoyl-1-methoxymethyl)benzyloxy]-4-methylbenzoic acid

Code Name	Molecular Weight	Chemical Name
5-CA-MCBX-NDM	315.32	(*RS*)-3-[2-(1-carbamoyl-1-hydroxymethyl) benzyloxy]-4-methylbenzoic acid
5-CA-2-HM-S-2200	359.37	(*RS*)-4-(hydroxymethyl)-3-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}benzoic acid
5-CA-2-HM-S-2200-NHM	375.37	(*RS*)-4-(hydroxymethyl)-3-{2-[1-(*N*- hydroxymethylcarbamoyl)-1-methoxymethyl] benzyloxy}benzoic acid

B.7.2.3 Metabolism in pigs (Section 4, Annex IIA, point 6.2.4)

A metabolism study on pigs is not required since it was shown that the metabolism of Mandestrobin (S-2200) is similar in rats, poultry and ruminants and no residues above 0.1 mg/kg feed are expected in crops or crop parts that might be fed to livestock.

B.7.2.4 Overall conclusion on the metabolism in livestock

Metabolism studies on laying hens and lactating goats have been investigated following repeated oral administration of [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200. An overview is given in the table below.

Animal tested	Dosing period	Label	Dose level			
			mg as/kg feed per day	mg as/kg bw per day[1)]	mg as/animal per day	mg as/animal (total dose)
Laying hens	14 days	[phenoxy-^{14}C]S-2200	13.3	1.04	1.8	25.2
		[benzyl-^{14}C]S-2200	13.04	0.87	1.8	25.2
Lactating goat	7 days	[phenoxy-^{14}C]S-2200	12.65	0.31	16.03	112.2
		[benzyl-^{14}C]S-2200	14.33	0.59	35.05	245.3

1)..... mean body weight at the begin of dosing for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 label, respectively :
laying hens →1.73 kg and 2.07 kg
lactating goats→ 51 kg and 59 kg

The available metabolism studies on laying hens and lactating goats showed that the metabolic pathways in livestock were similar to that found in the rat. Mandestrobin (S-2200) is extensively metabolised and mainly excreted in the hen (83-98% of the dose) and goat (78 % of the dose). The route of the metabolism of S-2200 has been shown to be similar and proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation and ether hydrolysis. Parent S-2200 was the main component of the residue in eggs, milk fat, muscle (goat) and fat (hen and goat), the main metabolites were 5-COOH-S-2200 (goat kidney and liver), 4-OH-S-2200 (in hen liver and as the glucuronide in goat kidney) and De-Xy-S-2200 (hen liver). The primary metabolites are further metabolised by conjugation, thus S-2200, De-Xy-S-2200, 4-OH-S-2200 and 5-CA-2-HM-S-2200 were present in liver in bound/conjugated form.
Mandestrobin (S-2200) has a log P_{ow} of 3.51and is the main metabolite in milk fat (up to 35 % TRR, 0.012 mg/kg) and in fat (up to 50 % TRR, 0.016 mg/kg in poultry and 50 % TRR, 0.006 mg/kg in goat). However no significant accumulation of residues in tissues, particularly fatty tissues, has been observed.

B.7.3 Residue definitions

B.7.3.1 Residue definition for food of plant origin

The metabolism of Mandestrobin (S-2200) in plants has been studied in oilseed rape (forage and seed), wheat (forage, hay, straw and grain) and lettuce (leaves). These studies showed comparable metabolism in three different crop groups, covering pulses/oilseeds, cereals and leafy crops.

Mandestrobin (S-2200) is extensively metabolised, the main route of metabolism in crops is via a series of hydroxylations and oxidations, and subsequent glycoside conjugation, to yield the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 and their conjugates. Minor metabolic pathways involved demethylation of the methoxy group of the side chain to form MCBX, and cleavage of the ether linkage to form De-Xy-S-2200.

Parent S-2200 was the major residue in lettuce, rapeseed, wheat forage and wheat hay. The major metabolites found at levels >10% TRR were 4-OH-S-2200 (conjugated), 2-CH_2OH-S-2200 (conjugated) and De-Xy-S-2200. A summary of the levels of S-2200 and its main metabolites found in the different crops is presented in the table below.

Table B.7.3.1-1: Occurrence of S-2200 and its main metabolites in the different crops (% TRR and mg/kg)

S-2200 and major metabolites, i.e. > 10% TRR and > 0.05 mg/kg	Crop part	% TRR	mg/kg
S-2200	Rape forage	22.4	0.77
	Rape seed	30.7	0.14
	Wheat forage	59.9	6.25
	Wheat hay	26.2	1.63
	Wheat straw	1.99	0.05
	Wheat grain	nd	nd
	Mature lettuce	91.1	39.3
De-Xy-S-2200	Wheat grain	60.6	0.54
	Wheat straw	11.8	0.29
4-OH-S-2200 conjugate	Rape forage	35.6	1.42
	Rape seed	14.5	0.07
	Wheat hay	13.1	0.81
2-CH_2OH-S-2200 conjugate	Rape forage	12.4	0.43
	Wheat forage	10.6	1.18
	Wheat hay	12.6	1.14

In the confined crop rotation study [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 were applied to bare soil at a rate of 1.6 kg as/ha. The treated soil was aged for a period of 30, 120 and 365 days prior to direct seeding with lettuce, carrots and wheat. The major metabolites identified in following crops were 4-OH-S-2200 (free and conjugated), 2-CH_2OH-S-2200 (free and conjugated) and 5-CH_2OH-S-2200 (free and conjugated). It was demonstrated that Mandestrobin (S-2200) and its metabolites were taken up by rotational crops, extensively metabolized to polar metabolites and incorporated into the constituents of plant. The metabolism in rotational crops was considered to be essentially the same as in primary crops.
In field crop rotation trials conducted on carrots, lettuce, broccoli and barley residues of S-2200, 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 were non-detectable (< LOQ= 0.01 mg/kg) in all following crops at all plant-back intervals (14,120, 365 days).
In field residue trials conducted on oilseed rape, samples were analysed for parent S-2200 and the three major crop metabolites 4-OH-S-2200 (free and conjugated), 2-CH_2OH-S-2200 (free and conjugated) and De-Xy-S-2200. In mature seed samples residues of S-2200 and its metabolites 4-OH-S-2200, 2-CH_2OH-S-2200 and De-Xy-S-2200 were <LOQ (0.01 mg/kg).

Conclusion:
Mandestrobin (S-2200) was the major residue in lettuce, rapeseed, wheat forage and wheat hay. The major

metabolites found at levels >10% TRR were 4-OH-S-2200 (free and conjugated), 2-CH_2OH-S-2200 (free and conjugated) and De-Xy-S-2200. All the free metabolites are also found in the rat, however only 4-OH-S-2200 is a major metabolites in the rat (found at >10% of dose).
Toxicity studies have been performed on the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200 and De-Xy-S-2200, and shown that none of these metabolites are acutely toxic or mutagenic. The toxicity of 4-OH-S-2200 as a major metabolite in the rat is considered to have been adequately covered by the toxicology studies performed on S-2200. The glycoside conjugates of 4-OH-S-2200 and 2-CH_2OH-S-2200 are plant-specific metabolites and are therefore not found in the rat. It was assumed that a cleavage to the free substances takes place after oral ingestion and the toxicological profile is therefore considered compareable with the unconjugated form.

The metabolites detected in the metabolism studies are not of toxicological concern and are covered by the rat metabolism, however in order to cover the whole toxicological burden for the consumer the following residue definitions are proposed:
Residue definition for risk assessment: **sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200).**

Residue definition for monitoring purposes: **mandestrobin (S-2200)**
The conversion factor (CF) from monitoring to risk assessment was based on the the supervised residue trials conducted on oilseed rape is calculated to be 4 (please refer also to B.7.15)

B.7.3.2 Residue definition for food of animal origin

The proposed GAP for Mandestrobin (S-2200) involves application on oilseed rape only. The available metabolism studies on laying hens and lactating goats showed that the metabolic pathways in livestock were similar to that found in the rat. Mandestrobin (S-2200) is extensively metabolised and mainly excreted in the hen and goat. The route of the metabolism of Mandestrobin (S-2200) has been shown to be similar and proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation and ether hydrolysis. S-2200 was the main component of the residue in eggs (51.2 % TRR, 0.06 mg/kg), milk fat (32.7 % TRR, 0.01 mg/kg), in muscle (goat) accounting for 23 % TRR (0.002 mg/kg) and in fat (hen and goat) accounting for 49.6 % TRR (0.006 mg/kg) in the goat and 49.5 % TRR (0.016 mg/kg) in the hen.The main metabolites were 5-COOH-S-2200 (goat kidney and liver), 4-OH-S-2200 (in hen liver and as the glucuronide in goat kidney) and De-Xy-S-2200 (hen liver). The primary metabolites are further metabolised by conjugation, thus S-2200, De-Xy-S-2200, 4-OH-S-2200 and 5-CA-2-HM-S-2200 were present in liver in bound/conjugated form.
With the exception of organs for excretions (liver, kidney), the total radioactive residues were low (0.01-0.05 mg/kg) and individual compounds of degradation do not exceed 0.05 mg/kg. Major compounds in lactating goats are S-2200 (0.03 mg/kg in liver) and the metabolites 5-COOH-S-2200 (0.065 mg/kg in liver and 0.08 mg/kg in kidney) and De-Xy-S-2200 (0.02 mg/kg in liver). Major compounds in hen liver beside S-2200 (< 0.01 mg/kg) are 4-OH-S-2200 (0.04 mg/kg) and De-Xy-S-2200 (0.03 mg/kg).
Mandestrobin (S-2200) has a log P_{ow} of 3.51and is the main metabolite in milk fat (up to 35 % TRR, 0.012 mg/kg) and in fat (up to 50 % TRR, 0.016 mg/kg in poultry and 50 % TRR, 0.006 mg/kg in goat). However no significant accumulation of residues in tissues, particularly fatty tissues, has been observed.

Residues in oilseed rape seed are below the limit of quantification, therefore no determinable residues of S-2200 are expected in products of animal origin, however based on the results provided a residue definition for products of animal origin can be proposed as mandestrobin (S-2200) only for monitoring and risk assessment.

B.7.4 Use pattern

Mandestrobin (S-2200) is a new active substance and is not yet authorised in any Member State in the EU. Mandestrobin (S-2200) is a systemic fungicide and intended to be used against *Sclerotinia* in winter oilseed rape. Details of intended uses (supported by the applicant and for which data are provided) according to Good Agricultural Practice (GAP) are summarized in the table below.

Table B.7.4-1: Good Agricultural Practices (GAPs) proposed for mandestrobin (S-2200)

Crop	N / S	Formulation type, content of as (%)	Application				PHI (d)
			Method latest stage	No of applications	Rate (kg as/ha)	Water (L/ha)	
Winter oilseed rape	N / S	SC, 25	Foliar spraying BBCH 63-67[1]	1	0.2	100-300	-[2]

[1] …BBCH 63: 30% of flowers on main raceme open
BBCH 67: Flowering declining: majority of petals fallen
[2] …the waiting period is covered by the vegetation period which remains between application and harvest.

B.7.5 Identification of the critical GAPs

The proposed critical GAP (Northern and Southern Europe) for Mandestrobin (S-2200) in winter oilseed rape is one application per crop and season at 0.2 kg as/ha at BBCH 63-67 (flowering declining). The pre-harvest interval (PHI) is defined by the application conditions at the BBCH growth stages stated.

Table B.7.5-1: critical GAP for mandestrobin (S-2200)

Crop	N / S	Formulation type, content of as (%)	Application				PHI (d)
			Method latest stage	No of applications	Rate (kg as/ha)	Water (L/ha)	
Winter oilseed rape	N / S	SC, 25	Foliar spraying BBCH 63-67[1]	1	0.2	100-300	-[2]

[1] …BBCH 63: 30% of flowers on main raceme open
BBCH 67: Flowering declining: majority of petals fallen
[2] …the waiting period is covered by the vegetation period which remains between application and harvest.

B.7.6 Residues resulting from supervised trials (Section 4, Annex IIA, point 6.3.1)

A total of twelve residue trials were conducted in winter oilseed rape during 2010 and 2011, eight trials in Northern Europe (N France, Germany, UK) and four trials in Southern Europe (S France).
Crop metabolism studies have shown that in addition to parent S-2200, the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were found at levels >10% TRR. The samples from the oilseed rape residue trials were therefore analysed for parent S-2200 and the metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH_2OH-S-2200.

B.7.6.1 Stability of residues (Section 4, Annex IIA, point 6.1.1)

Studies on the storage stability of S-2200 and the metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH_2OH-S-2200 have been performed on oilseed rape seed (high oil content commodity), lettuce (with high water content commodity), barley grain (dry commodity) and straw. Samples were analysed after storage intervals of 0 days and 1, 3, 6 and 12 months.

Test commodity	Botanical name	Crop part	Analyte	Reference
Oilseed Rape	Brassica napus	seeds	S-2200 R-isomer S-2200 S-isomer	Daneva, E., Taeufer, A. (2011a)
			De-Xy-S-2200 4-OH-S-2200 2-CH_2OH-S-2200	Daneva, E., Taeufer, A. (2012a) Daneva, E., Taeufer, A. (2012b) Daneva, E., Zetzsch, A. (2012a)
Lettuce	Latuca sativa	head	S-2200 R-isomer S-2200 S-isomer	Daneva, E., Taeufer, A. (2011b)
			De-Xy-S-2200 4-OH-S-2200 2-CH_2OH-S-2200	Daneva, E., Taeufer, A. (2012a) Daneva, E., Taeufer, A. (2012b) Daneva, E., Zetzsch, A. (2012a)
Barley	Hordeum vulgare	Grain Straw	S-2200 R-isomer S-2200 S-isomer	Daneva, E., Taeufer, A. (2011b)
			De-Xy-S-2200 4-OH-S-2200 2-CH_2OH-S-2200	Daneva, E., Taeufer, A. (2012a) Daneva, E., Taeufer, A. (2012b) Daneva, E., Zetzsch, A. (2012a)

B.7.6.1.1 Storage stability of S-2200 isomers

Reference:	**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**
Author(s), year:	Daneva, E., Taeufer, A. (2011a)
Report/ Doc. Number:	Eurofins Dr Specht GLP GmbH Report no. SUM-1012 Sumitomo ref: ROR-0007
Guideline(s):	Guidance document SANCO/3029/99 rev. 4 of 11/07/00 EU Commission Working Document 1607/VI/97 rev. 2, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97) US EPA OPPTS 860.1380, Storage Stability Data
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Reference:	**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**
Author(s), year:	Daneva, E., Taeufer, A. (2011b)
Report/ Doc. Number:	Eurofins Agroscience Services Chem GmbH Report no. S10-01949 Sumitomo ref: ROR-0009
Guideline(s):	Guidance document SANCO/3029/99 rev. 4 of 11/07/00 EU Commission Working Document 1607/VI/97 rev. 2, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97) US EPA OPPTS 860.1380, Storage Stability Data
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Homogenised oilseed rape (seed), lettuce, barley grain and barley straw samples were separately fortified with S-2200 *R*-isomer or S-2200 *S*-isomer at 0.1 mg/kg, and deep frozen at ≤–18°C. Samples were analysed for S-2200 *R*-isomer and S-2200 *S*-isomer after storage intervals of 0 days and 1, 3, 6 and 12 months.

Test Material 1:
Common Name: S-2200 *R*-isomer (S-2167, analytical standard)
Name (IUPAC): (*R*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
CAS-Nr.: 394657-24-0
Fortification level: 0.1 mg/kg

Test Material 2:
Common Name: S-2200 *S*-isomer (S-2354, analytical standard)
Name (IUPAC): (*S*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
CAS-Nr.: 1229001-61-9
Fortification level: 0.1 mg/kg

Analytical procedure for S-2200 *R*- and *S*-isomers:
Samples of homogenised **oilseed rape (seeds), barley grain and barley straw** were analysed after extraction with a mixture of cyclohexan/ethyl acetate according to module E9 (ASE) of the DFG method S19. Samples of homogenised **lettuce samples** were analysed after extraction with a mixture of cyclohexan/ethyl acetate according to module E1 of the DFG method S19 as described in B.7.7.1. All specimen extracts were analysed for S-2200 R-isomer and S-2200 S-isomer by chiral LC-MS/MS (liquid chromatography with tandem mass spectrometry) using a ChiralPak-AD-RH column.

At all time points (except on day 0) one untreated control sample, one untreated sample freshly fortified just before analysis at 0.1 mg/kg and two samples (stored at ≤ –18°C) were analysed per analyte.
On day 0, three freshly fortified samples and one untreated control sample per analyte were analysed. The limit of quantitation (LOQ) was 0.005 mg/kg for both S-2200 R-isomer and S-2200 S-isomer.

Findings:
The data indicate that when frozen samples of oilseed rape (seeds), lettuce (head), barley (grain and straw) intended for residue analyses, are stored for up to 12 months at -18°C or below, acceptable storage stability can be expected. The results are summarised in the table below.

Table B.7.6.1.1-1: Storage stability data for S-2200 isomers on oilseed rape (seed), lettuce and barley stored at ≤ -18°C at

Commodity/Analyte	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
OILSEED RAPE (SEED) – fortification level= 0.1 mg/kg						
R-isomer (S-2167)	Individual[1]	91, 89, 83	99, 90	82, 83	92, 91	109, 100
	Mean	88	95	83	92	105
	Procedural	-	103	91	93	110
	% Remaining[2]	100	108	94	105	119
S-isomer (S-2354)	Individual[1]	84, 74, 87	102, 104	99, 96	110, 90	108, 102
	Mean	82	103	98	100	105
	Procedural	-	93	103	94	107
	% Remaining[2]	100	126	120	122	128
LETTUCE – fortification level= 0.1 mg/kg						
R-isomer (S-2167)	Individual[1]	104, 103, 107	93, 84	86, 82	91, 100	97, 97

Commodity/Analyte	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
	Mean	105	89	84	96	97
	Procedural	-	98	84	96	96
	% Remaining[2]	100	85	80	91	93
S-isomer (S-2354)	Individual[1]	80, 85, 86	114, 112	111, 108	109, 98	94, 91
	Mean	84	113	110	104	93
	Procedural	-	119	109	92	89
	% Remaining[2]	100	135	131	124	111
BARLEY (grain) – fortification level= 0.1 mg/kg						
R-isomer (S-2167)	Individual[1]	113, 103, 111	79, 84	89, 84	78, 108	94, 85
	Mean	109	82	87	93	90
	Procedural	-	99	86	102	96
	% Remaining[2]	100	75	79	85	82
S-isomer (S-2354)	Individual[1]	104, 105, 107	91, 96	90, 87	79, 107	89, 88
	Mean	105	94	89	93	89
	Procedural	-	88	89	87	87
	% Remaining[2]	100	89	84	88	84
BARLEY (straw) – fortification level= 0.1 mg/kg						
R-isomer (S-2167)	Individual[1]	110, 109, 98	79, 86	75, 79	106, 95	83, 80
	Mean	106	83	77	101	82
	Procedural	-	87	88	92	88
	% Remaining[2]	100	78	73	95	77
S-isomer (S-2354)	Individual[1]	81, 81, 83	70, 73	77, 74	78, 80	88, 85
	Mean	82	72	76	79	87
	Procedural	-	73	74	79	83
	% Remaining[2]	100	88	92	97	106

[1] Not corrected for freshly fortified procedural recoveries.
[2] Percentage of residue remaining after storage, compared to the initial residue at Day 0.

Conclusion:
Residues of S-2200 R-isomer and S-2200 S-isomer in oilseed rape (seeds), lettuce and barley (grain and straw) are **stable for at least 12 months** when stored at -18°C or below.

B.7.6.1.2 Storage stability of S-2200 metabolites De-Xy-S2200, 4-OH-S-2200, 2-CH_2OH-S-2200

Reference:	**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**
Author(s), year:	Daneva, E., Taeufer, A. (2012a)
Report/ Doc. Number:	Eurofins Agroscience Services Chem GmbH Report no. SUM-1024 Sumitomo ref: ROR-0011
Guideline(s):	Guidance document SANCO/3029/99 rev. 4 of 11/07/00 EU Commission Working Document 1607/VI/97 rev. 2, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97) US EPA OPPTS 860.1380, Storage Stability Data
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Homogenised oilseed rape (seed), lettuce, barley grain and barley straw samples were separately fortified with De-Xy-S-2200 at 0.1 mg/kg, and deep frozen at ≤–18°C. Samples were analysed for De-Xy-S-2200 after storage intervals of 0 days and 1, 3, 6 and 12 months.

Test Material:
Common Name: **De-Xy-S-2200** (analytical standard)
Name (IUPAC): 2-[2-(hydroxymethyl) phenyl]-2-methoxy-N-methylacetamide
CAS-Nr.: not reported
Fortification level: 0.1 mg/kg

Analytical procedure
Samples of homogenised oilseed rape (seeds), lettuce and barley (grain and straw) were analysed after extraction with a mixture of acetone/water followed by clean- up with solid phase extraction using a Bond Elut SI. All specimens were analysed for De-Xy-S-2200 by liquid chromatography with tandem mass spectrometry (LC-MS/MS). At all time points (except on day 0) one untreated control sample, one untreated sample freshly fortified just before analysis at 0.1 mg/kg and two samples (stored at ≤ –18°C) were analysed per analyte. On day 0, three freshly fortified samples and one untreated control sample per analyte were analysed. The limit of quantification (LOQ) for all matrices was 0.01 mg/kg.

Findings:
The data indicate that when frozen samples of oilseed rape (seeds), lettuce (head), barley (grain and straw) intended for residue analyses, are stored for up to 12 months at -18°C or below, acceptable storage stability of metabolite De-Xy-S-2200 can be expected. The results are summarised in the table below

Table B.7.6.1.2-1: Storage stability data for metabolite De-Xy-S-2200 stored at ≤ -18°C at (fortification level= 0.1 mg/kg)

Commodity	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
Lettuce	Individual[1]	80, 70 ,80	81, 81	86, 84	91, 96	89, 90
	Mean	77	81	85	94	90
	Procedural	-	81	78	93	96
	% Remaining[2]	100	106	111	122	117
Oilseed rape (seed)	Individual[1]	92, 87, 88	83, 83	79, 80	92, 79	86, 85

Commodity	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
	Mean	89	83	80	86	86
	Procedural	-	80	82	95	94
	% Remaining[2]	100	93	89	96	96
Barley grain	Individual[1]	92, 80, 97	84, 79	95, 105	91, 89	83, 89
	Mean	90	82	100	90	86
	Procedural	-	81	92	99	88
	% Remaining[2]	100	91	112	100	96
Barley straw	Individual[1]	74, 74, 77	75, 79	75, 78	88, 93	75, 69
	Mean	75	77	77	91	72
	Procedural	-	77	76	98	72
	% Remaining[2]	100	103	102	121	96

[1] Not corrected for freshly fortified procedural recoveries.
[2] Percentage of residue remaining after storage, compared to the initial residue at Day 0.

Conclusion:
Residues of De-Xy-S-2200 in oilseed rape (seeds), lettuce and barley (grain and straw) are **stable for at least 12 months** when stored at -18°C or below.

Reference:	**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**
Author(s), year:	Daneva, E., Taeufer, A. (2012b)
Report/ Doc. Number:	Eurofins Agroscience Services Chem GmbH Report no. SUM-1025 Sumitomo ref: ROR-0012
Guideline(s):	Guidance document SANCO/3029/99 rev. 4 of 11/07/00 EU Commission Working Document 1607/VI/97 rev. 2, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97) US EPA OPPTS 860.1380, Storage Stability Data
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Homogenised oilseed rape (seed), lettuce, barley grain and barley straw samples were separately fortified with 4-OH-S-2200 at 0.1 mg/kg, and deep frozen at ≤–18°C. Samples were analysed for 4-OH-S-2200 after storage intervals of 0 days and 1, 3, 6 and 12 months.

Test Material:
Common Name: **4-OH-S-2200** (analytical standard)
Name (IUPAC): 2-{2-[4-hydroxy-2,5-dimethylphenoxy) methyl] phenyl}-2-methoxy-N-methylacetamide
CAS-Nr.: not reported
Fortification level: 0.1 mg/kg

Analytical procedure
Samples of homogenised oilseed rape (seeds), lettuce and barley (grain and straw) were analysed after extraction with a mixture of acetone/water followed by hydrolysis, purification and further clean- up with solid

phase extraction using a Oasis HLB cartridge. All specimens were analysed for 4-OH-S-2200 by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
At all time points (except on day 0) one untreated control sample, one untreated sample freshly fortified just before analysis at 0.1 mg/kg and two samples (stored at ≤ –18°C) were analysed per analyte. On day 0, three freshly fortified samples and one untreated control sample per analyte were analysed. The limit of quantification (LOQ) for all matrices was 0.01 mg/kg.

Findings:
The data indicate that when frozen samples of oilseed rape (seeds), lettuce (head), barley (grain and straw) intended for residue analyses, are stored for up to 12 months at -18°C or below, acceptable storage stability of metabolite 4-OH-S-2200 can be expected. The results are summarised in the table below.

Table B.7.6.1.2-2: Storage stability data for metabolite 4-OH-S-2200 stored at ≤ -18°C at (fortification level= 0.1 mg/kg)

Commodity	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
Lettuce	Individual[1]	101, 87, 87	94, 99	86, 90	70, 71	68, 73
	Mean	92	97	88	71	71
	Procedural	-	90	83	71	73
	% Remaining[2]	100	105	96	77	77
Oilseed rape (seed)	Individual[1]	75, 86, 97	74, 88	78, 80	72, 76	83, 83
	Mean	86	81	79	74	83
	Procedural	-	71	76	75	88
	% Remaining[2]	100	94	92	86	97
Barley grain	Individual[1]	87, 81, 98	78, 75	90, 91	70, 67	84, 72
	Mean	89	77	91	69	78
	Procedural	-	71	86	71	84
	% Remaining[2]	100	86	102	77	88
Barley straw	Individual[1]	93, 88, 88	74, 78	83, 80	87, 89	81, 86
	Mean	90	76	82	88	84
	Procedural	-	70	81	102	84
	% Remaining[2]	100	85	91	98	93

[1] Not corrected for freshly fortified procedural recoveries.
[2] Percentage of residue remaining after storage, compared to the initial residue at Day 0.

Conclusion:
Residues of 4-OH-S-2200 in oilseed rape (seeds), lettuce and barley (grain and straw) are **stable for at least 12 months** when stored at -18°C or below.

Reference:	**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**
Author(s), year:	Daneva, E., Zetzsch, A. (2012a)
Report/ Doc. Number:	Eurofins Agroscience Services Chem GmbH Report no. SUM-1026 Sumitomo ref: ROR-0013
Guideline(s):	Guidance document SANCO/3029/99 rev. 4 of 11/07/00 EU Commission Working Document 1607/VI/97 rev. 2, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97) US EPA OPPTS 860.1380, Storage Stability Data
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Homogenised oilseed rape (seed), lettuce, barley grain and barley straw samples were separately fortified with 2-CH_2OH-S-2200 at 0.1 mg/kg, and deep frozen at ≤–18°C. Samples were analysed for 2-CH_2OH-S-2200 after storage intervals of 0 days and 1, 3, 6 and 12 months.

Test Material:
Common Name: **2-CH_2OH-S-2200** (analytical standard)
Name (IUPAC): (2-(2-{[2-(hydroxymethyl)-5-methylphenoxy] methyl} phenyl)-2-methoxy-N-methylacetamide
CAS-Nr.: not reported
Fortification level: 0.1 mg/kg

Analytical procedure
Samples of homogenised oilseed rape (seeds), lettuce and barley (grain and straw) were analysed after extraction with a mixture of acetone/water followed by hydrolysis and clean- up with solid phase extraction using a Oasis HLB cartridge. All specimens were analysed for 2-CH_2-OH-S-2200 by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
At all time points (except on day 0) one untreated control sample, one untreated sample freshly fortified just before analysis at 0.1 mg/kg and two samples (stored at ≤ –18°C) were analysed per analyte. On day 0, three freshly fortified samples and one untreated control sample per analyte were analysed. The limit of quantification (LOQ) for all matrices was 0.01 mg/kg.

Findings:
The data indicate that when frozen samples of oilseed rape (seeds), lettuce (head), barley (grain and straw) intended for residue analyses, are stored for up to 12 months at -18°C or below, acceptable storage stability of metabolite 2-CH_2-OH-S-2200 can be expected. The results are summarised in the table below.

Table B.7.6.1.2-3: Storage stability data for metabolite 2-CH_2-OH-S-2200 stored at ≤ -18°C at (fortification level= 0.1 mg/kg)

Commodity	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
Lettuce	Individual[1]	76, 70, 78	81, 83	84, 79	104, 98	91, 87
	Mean	75	82	82	101	89
	Procedural	-	77	72	94	103
	% Remaining[2]	100	110	109	135	119

Commodity	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
Oilseed rape (seed)	Individual[1]	71, 71, 77	71, 91	73, 71	102, 73	80, 81
	Mean	73	81	72	88	81
	Procedural	-	70	87	83	80
	% Remaining[2]	100	111	99	120	110
Barley grain	Individual[1]	82, 84, 88	84, 79	88, 85	100, 86	92, 89
	Mean	85	82	87	93	91
	Procedural	-	86	94	86	98
	% Remaining[2]	100	96	102	110	107
Barley straw	Individual[1]	72, 73, 70	70, 71	74, 81	89, 86	105, 78
	Mean	72	71	78	88	92
	Procedural	-	76	89	93	105
	% Remaining[2]	100	98	108	122	128

[1] Not corrected for freshly fortified procedural recoveries.
[2] Percentage of residue remaining after storage, compared to the initial residue at Day 0.

Conclusion:
Residues of 2-CH_2-OH-S-2200 in oilseed rape (seeds), lettuce and barley (grain and straw) are **stable for at least 12 months** when stored at -18°C or below.

Storage stability data for the rotational crop metabolite 5-CH_2OH-S-2200 have been provided and are summarised below.

Reference:	**Freezer Storage Stability Study of S-2200 Metabolite, 5-CH_2OH-S-2200, in Lettuce (Head) and Barley (Grain and Straw) over 12 Months**
Author(s), year:	Daneva, E., Zetzsch, A. (2012b)
Report/ Doc. Number:	Eurofins Agroscience Services Chem GmbH Report no. SUM-1028 Sumitomo ref: ROR-0014
Guideline(s):	Guidance document SANCO/3029/99 rev. 4 of 11/07/00 EU Commission Working Document 1607/VI/97 rev. 2, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97) US EPA OPPTS 860.1380, Storage Stability Data
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Homogenised lettuce, barley grain and barley straw samples were separately fortified with 5-CH_2OH-S-2200 at 0.1 mg/kg, and deep frozen at ≤–18°C. Samples were analysed for 5-CH_2OH-S-2200 after storage intervals of 0 days and 1, 3, 6 and 12 months.

Test Material:
Common Name: **5-CH_2OH-S-2200** (analytical standard)
Name (IUPAC): 2-(2-{[5-(hydroxymethyl)-5-methylphenoxy] methyl} phenyl)-2-methoxy-N-methylacetamide
CAS-Nr.: not reported
Fortification level: 0.1 mg/kg

Analytical procedure

Samples of homogenised lettuce and barley (grain and straw) were analysed after extraction with a mixture of acetone/water followed by hydrolysisand clean- up by solid phase extraction using an Oasis HLB cartridge. All specimens were analysed for 5-CH_2OH-S-2200 by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
At all time points (except on day 0) one untreated control sample, one untreated sample freshly fortified just before analysis at 0.1 mg/kg and two samples (stored at ≤ –18°C) were analysed per analyte. On day 0, three freshly fortified samples and one untreated control sample per analyte were analysed. The limit of quantification (LOQ) for all matrices was 0.01 mg/kg.

Findings:
The data indicate that when frozen samples of lettuce (head), barley (grain and straw) intended for residue analyses, are stored for up to 12 months at -18°C or below, acceptable storage stability of metabolite 5-CH_2OH-S-2200 can be expected. The results are summarised in the table below

Table B.7.6.1.2-4: Storage stability data for metabolite 5-CH_2OH-S-2200stored at ≤ -18°C at (fortification level= 0.1 mg/kg)

Commodity	Recovery (%)	Interval				
		0 month	1 month	3 month	6 month	12 month
		0 days	31 days	91 days	182 days	364 days
Lettuce	Individual[1]	87, 81, 70	85, 89	79, 82	83, 82	97, 92
	Mean	79	87	81	83	95
	Procedural	-	93	76	82	91
	% Remaining[2]	100	110	101	104	119
Barley grain	Individual[1]	77, 74, 84	77, 72	83, 80	71, 71	71, 72
	Mean	78	75	82	71	72
	Procedural	-	90	79	78	90
	% Remaining[2]	100	95	104	91	91
Barley straw	Individual[1]	77, 82, 83	81, 81	72, 75	72, 73	73, 69
	Mean	81	81	74	73	71
	Procedural	-	80	76	87	77
	% Remaining[2]	100	100	91	90	88

[1] Not corrected for freshly fortified procedural recoveries.
[2] Percentage of residue remaining after storage, compared to the initial residue at Day 0.

Conclusion:
Residues of 5-CH_2OH-S-2200 in lettuce and barley (grain and straw) are stable **for at least 12 months** when stored at -18°C or below.

B.7.6.2 Analytical methods used for the residue trials (Section 4, Annex IIA, point 4.3)

The determination of residues in oilseed rape included the *R*- and *S*-isomers of parent S-2200 and the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated). An overview on the analytical methods provided is given in the table below.

Matrix	Analyte	Method	LOQ	Reference
Oilseed Rape Seeds	S-2200 R-isomer S-2200 S-isomer	LC-MS/MS (DFG-S19)	0.005 mg/kg 0.005 mg/kg	IIA, 4.3/01 Daneva, E. (2010) Report No. ROA-0005
barley (grain and straw), lettuce (head)	S-2200 R-isomer S-2200 S-isomer	LC-MS/MS (DFG-S19)	0.005 mg/kg 0.005 mg/kg	IIA, 4.3/02 Schernikau, N. (2010) Report No. ROA-0007
Oilseed Rape Seeds, barley (grain and straw), lettuce (head)	De-Xy-S-2200	LC-MS/MS	0.01 mg/kg	IIA, 4.3/03 Daneva, E., Breyer, N. & Taeufer, A. (2011a); Report No. ROA-0010
Oilseed Rape Seeds, barley (grain and straw), lettuce (head)	4-OH-S-2200	LC-MS/MS	0.01 mg/kg	IIA, 4.3/04 Daneva, E. & Taeufer, A. (2011a); Report No. ROA-0011
Oilseed Rape Seeds, barley (grain and straw), lettuce	2-CH_2OH-S-2200	LC-MS/MS	0.01 mg/kg	IIA, 4.3/05 Daneva, E., Breyer, N. & Taeufer, A. (2011b); Report No. ROA-0012

Oilseed rape is the only representative crop, therefore the analytical method (IIA, 4.3/02, Schernikau, N., 2010) for the determination of residues of the *R*- and *S*-isomers of S-2200 in barley (grain and straw) and lettuce (head) was not evaluated.

Analytical procedure for S-2200 *R*- and *S*-isomers

Reference:	Adaptation and Validation of Multi-Method DFG S19 for the determination of Residues of S-2200 in Seeds of Oilseed Rape
Author(s), year:	Daneva, E. (June 2010)
Report/ Doc. No.	Sumitomo Chemical Co. Ltd, Report No. ROA-0005, SUM-1011V
Guideline(s):	SANCO/3029/99 rev. 4 of 11/07/00 of the European Commission
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Samples were analysed for S-2200 (R- and S-isomers) using an analytical method based on the extended and revised version of DFG Method S19 (Multi method L 00.00-34 of the Official Collection of Test Methods according to § 64 LFGB).
Seed samples were extracted by accelerated solvent extraction (ASE) according to extraction module E9 of method DFG S19. Homogenised oilseed rape seed samples were mixed with kieselgur, transferred into an extraction cell and extracted with the ASE eluting mixture (ethyl acetate/cyclohexane, 1:1 v/v) under conditions of high temperature and pressure. The resulting extract was filtered, rotary evaporated to a reduced volume and made up to a known volume with ethyl acetate and cyclohexane. An aliquot of the extract was evaporated to dryness and re-dissolved in acetonitrile/water for final determination of S-2200 R-isomer and S-2200 S-isomer by chiral LC-MS/MS using a ChiralPak-AD-RH column.

Whole plant, plant without pods and green pod samples were subjected to extraction with liquid/liquid partition according to extraction module E1 of method DFG S19. Homogenised samples were mixed with water to bring the total water volume to 100 ml. Two volumes of acetone were added and the sample homogenised for 2 minutes. Sodium chloride and ethyl acetate/cyclohexane (1:1 v/v) were added and the sample homogenised again for 1 minute. The phases were allowed to separate and an aliquot of the organic phase filtered through sodium sulphate and evaporated to an aqueous residue. Ethyl acetate was added to the extract and the sample sonicated to ensure complete dissolution. A 1:1 mixture of sodium sulphate/sodium chloride was added to the extract, followed by cyclohexane. After swirling, the salt mixture was allowed to settle. An aliquot of the supernatant was diluted with acetonitrile/water (1:1 v/v) for final determination of S-2200 R-isomer and S-2200 S-isomer by chiral LC-MS/MS.

Recovery Data

Analyte	Matrix	Ions Monitored (m/z)	Mean Recovery at 0.005 mg/kg (%) (n=5)	Mean Recovery at 0.5 mg/kg (%) (n=5)	Overall Mean Recovery (%) (n=10)
S-2200 *R*-isomer	Oilseed rape seed	314→192	79	81	80
S-2200 *R*-isomer	Oilseed rape seed	314→160	82	79	81
S-2200 *S*-isomer	Oilseed rape seed	314→192	81	78	80
S-2200 *S*-isomer	Oilseed rape seed	314→160	80	78	79

Limit of Quantification

The limit of quantitation (LOQ) was **0.005 mg/kg** for both S-2200 R-isomer and S-2200 S-isomer. The limit of detection (LOD) was 0.0015 mg/kg for each isomer.

Analytical procedure for De-Xy-S-2200

Reference:	Validation of an Analytical Method for Determination of S-2200 Metabolite, De-Xy-S-2200, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head)
Author(s), year:	Daneva, E., Breyer, N. & Taeufer, A., (February 2011)
Report/ Doc. No.	Sumitomo Chemical Co. Ltd, Report No. ROA-0010, SUM-1023V
Guideline(s):	SANCO/3029/99 rev. 4 of 11/07/00 of the European Commission
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Samples of oilseed rape (seed), barley (grain and straw) and lettuce were extracted with a mixture of acetone and water (4:1 v/v) followed by a purification stage using a Chem Elut cartridge eluted with dichloromethane. Further clean up of the extract was performed by solid phase extraction using a Mega Bond Elut SI cartridge. De-Xy-S-2200 is eluted with hexane/acetone (1/2, v/v) and evaporated to dryness using a rotary evaporator below 40°C. The sample is reconstituted with acetonitrile/water (1/2, v/v) and the concentrations of De-Xy-S-2200 were determined by the highly specific method of LC/MS-MS with quantification based on two mass transitions (the ion transition m/z 210 → 132 for quantification and m/z 210 → 192 for confirmation). Therefore, an additional confirmatory method was not required.

Recovery Data

Analyte	Matrix	Ions Monitored (m/z)	Mean Recovery at 0.01 mg/kg (%) (n=5)	Mean Recovery at 0.1 mg/kg (%) (n=5)	Overall Mean Recovery (%) (n=10)
De-Xy-S-2200	Lettuce (head)	210→132	86	86	86
De-Xy-S-2200	Lettuce (head)	210→192	87	89	88
De-Xy-S-2200	Oilseed rape seeds	210→132	86	87	86
De-Xy-S-2200	Oilseed rape seeds	210→192	86	86	86
De-Xy-S-2200	Barley grain	210→132	91	88	90
De-Xy-S-2200	Barley grain	210→192	92	89	91
De-Xy-S-2200	Barley straw	210→132	87	88	88
De-Xy-S-2200	Barley straw	210→192	92	89	90

Limit of Quantification

The limit of quantification (LOQ) for De-Xy-S-2200 was **0.01 mg/kg for all matrices**. The limit of detection (LOD), defined as the lowest concentration where a signal of the analyte could be detected, was determined to be 0.003 mg/kg for all matrices.

Analytical procedure for 4-OH-S-2200

Reference:	Validation of an Analytical Method for Determination of S-2200 Metabolites, 4-OH-S-2200 and its Conjugates, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head)
Author(s), year:	Daneva, E. & Taeufer, A., (April 2011)
Report/ Doc. No.	Sumitomo Chemical Co. Ltd, Report No. ROA-0011, SUM-1021V
Guideline(s):	SANCO/3029/99 rev. 4 of 11/07/00 of the European Commission
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Samples of lettuce, oilseed rape (seed) and barley (grain and straw) were extracted with a mixture of acetone and water (4:1 v/v) followed by hydrolysis with sodium hydroxide at pH 11 and then ß-glucosidase in order to release the corresponding aclycone. The hydrolysed extract was then cleaned-up using a Chem Elut cartridge eluted with ethylacetate. The eluate is collected and evaporated to dryness below 40°C using a rotary evaporator. The residues are reconstituted in water, applied to a pre-conditioned Oasis HLB cartridge and eluted with acetonitrile/water (1/1, v/v). The concentrations of 4-OH-S-2200 were determined by the highly specific method of LC/MS-MS with quantification based on two mass transitions (the ion transition m/z 328 → 136 for quantification and m/z 330 → 192 for confirmation). Therefore, an additional confirmatory method was not required.

Recovery Data

Analyte	Matrix	Ions Monitored (m/z)	Mean Recovery at 0.01 mg/kg (%) (n=5)	Mean Recovery at 0.1 mg/kg (%) (n=5)	Overall Mean Recovery (%) (n=10)
4-OH-S-2200	Lettuce (head)	328→136	84	87	86
4-OH-S-2200	Lettuce (head)	330→192	84	85	84
4-OH-S-2200	Oilseed rape seeds	328→136	77	86	81
4-OH-S-2200	Oilseed rape seeds	330→192	74	87	80

Analyte	Matrix	Ions Monitored (m/z)	Mean Recovery at 0.01 mg/kg (%) (n=5)	Mean Recovery at 0.1 mg/kg (%) (n=5)	Overall Mean Recovery (%) (n=10)
4-OH-S-2200	Barley grain	328→136	99	91	95
4-OH-S-2200	Barley grain	330→192	106	90	98
4-OH-S-2200	Barley straw	328→136	101	98	99
4-OH-S-2200	Barley straw	330→192	104	98	101

Limit of Quantification

The limit of quantification (LOQ) for metabolite 4-OH-S-2200 was **0.01 mg/kg for all matrices**. The limit of detection (LODwas determined to be 0.003 mg/kg for all matrices.

Analytical procedure for 2-CH_2OH-S-2200

Reference:	Validation of an Analytical Method for Determination of S-2200 Metabolites, 2-CH_2OH-S-2200 and its Conjugates, in Seeds of Oilseed Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head)
Author(s), year:	Daneva, E.; Breyer, N. & Taeufer, A., (May 2011)
Report/ Doc. No.	Sumitomo Chemical Co. Ltd, Report No. ROA-0012, SUM-1022V
Guideline(s):	SANCO/3029/99 rev. 4 of 11/07/00 of the European Commission
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Samples of lettuce, oilseed rape (seed) and barley (grain and straw) were extracted with a mixture of acetone and water (4:1 v/v) followed by hydrolysis with sodium hydroxide at pH 11 and then ß-glucosidase in order to release the corresponding aclycone. The hydrolysed extract was then cleaned-up using a Chem Elut cartridge eluted with ethylacetate. The eluate is collected and evaporated to dryness below 40°C using a rotary evaporator. The residues are reconstituted in water, applied to a pre-conditioned Oasis HLB cartridge and eluted with acetonitrile/water (1/1, v/v). The concentrations of 2-CH_2OH-S-2200 were determined by the highly specific method of LC/MS-MS with quantification based on two mass transitions (the ion transition m/z 330 → 192 for quantification and m/z 328 → 137 for confirmation). Therefore, an additional confirmatory method was not required.

Recovery Data

Analyte	Matrix	Ions Monitored (m/z)	Mean Recovery at 0.01 mg/kg (%) (n=5)	Mean Recovery at 0.1 mg/kg (%) (n=5)	Overall Mean Recovery (%) (n=10)
2-CH_2OH-S-2200	Lettuce (head)	330→192	90	90	90
2-CH_2OH-S-2200	Lettuce (head)	328→137	89	93	91
2-CH_2OH-S-2200	Oilseed rape seeds	330→192	83	86	85
2-CH_2OH-S-2200	Oilseed rape seeds	328→137	92	89	91
2-CH_2OH-S-2200	Barley grain	330→192	89	80	84
2-CH_2OH-S-2200	Barley grain	328→137	79	78	79
2-CH_2OH-S-2200	Barley straw	330→192	84	78	81
2-CH_2OH-S-2200	Barley straw	328→137	81	79	80

Limit of Quantification
The limit of quantification (LOQ) for metabolite 2-CH_2OH-S-2200 was **0.01 mg/kg for all matrices**. The limit of detection (LOD) was determined to be 0.003 mg/kg for all matrices.

Conclusion
The analytical methods provided were considered to be suitable for the analysis of the two isomers of S-2200 and the 3 metabolites in winter rape seed specimens, barley (grain and straw) and lettuce with satisfactory accuracy and precision.

B.7.6.3 Supervised residue trials on Oilseed Rape (Section 4, Annex IIA, point 6.3.1)

Summary of the critical GAP for the proposed use of Mandestrobin (S-2200) on Winter oilseed rape

North or South of EU	Outdoor/ indoor	Mode of application	Growth stage at last application	Number of applications	Application rate (kg as/ha)	PHI (days)
NEU/SEU	Outdoor	Foliar spray	BBCH 63-67 (flowering)	1	0.20	n.a.

A total of twelve residue trials (6 harvest trials and 6 decline trials) were conducted in winter oilseed rape during 2010 and 2011 in Northern Europe (N France, Germany, UK) and Southern Europe (S France). A summary is given in the table below.

Table B.7.6.3-1: Summary of the Mandestrobin (S-2200) residue trials on oilseed rape

Country	Northern Europe		Southern Europe	
	2010	2011	2010	2011
Northern France	1 decline	1 decline 1 harvest		
Germany	1 decline	1 decline 1 harvest		
UK	1 harvest	1 harvest		
Southern France			1 decline 1 harvest	1 decline 1 harvest
Total number of trials	**3**	**5**	**2**	**2**

The determination of residues in oilseed rape included the *R*- and *S*-isomers of parent S-2200 and the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated).

Crop information:
Crop / crop group: winter rape seed/oilseeds

Test Material:
Common Name: S-2200
Name (IUPAC): (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers: S-isomer (S-2354):R-isomer(S-2167)= 50:50
Formulation type: SC (soluble concentrate)
Nominal concentration: 25 % (w/v)

Reference:	**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**
Author(s), year:	Delmotte, R. (2011)
Report/ Doc. Number:	Staphyt Report no. FLN-10-6267 Sumitomo Chemical Co., Ltd. report no. ROR-0008
Guideline(s):	EU Guideline 7029/VI/95 - Rev. 5, General recommendations for the design, preparation and realization of residue trials. SANCO/3029/99 rev. 4, 11 July 2000 OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

A total of **five supervised residue trials** have been conducted to determine the residue of S-2200 and its metabolites in Raw Agricultural Commodity (RAC) winter oilseed rape (whole plants, whole plants w/o pods, green pods and seed specimens) after **a single application of 0.2 kg S-2200/ha** formulated as 25 % soluble concentrate at crop growth stage BBCH 65 (full flowering: 50 % flowers on main raceme open, older petals falling).
The sites were representative of winter oilseed rape, typical of a producing region in the test countries: Northern France (Champagne-Ardenne), Germany (Baden-Württemberg), United Kingdom (Buckinghamshire) and Southern France (Midi-Pyrénées and Aquitaine). Winter oilseed rape was cultivated according to normal agricultural practices. Application equipment consisted of hand carried boom sprayer or single wheel sprayer with 6 or 12 flat fan nozzles. The actual amount of test item applied ranged from 0.783 to 0.861 L/ha.

Sample collection (decline trials):

- Just after application (within 3 hours) →whole plants whithout roots
- at BBCH 79 → whole plant without roots, whole plants without pods and green pods
- at harvest (71-78 days after application) → rape seeds

In the decline trials, total residues of S-2200 (sum of R- and S-isomer) were 1.5-2.8 mg/kg in whole plant on the day of application and declined to <0.01-0.10 mg/kg in plants without pods and <0.01mg/kg in green pods collected at BBCH 79 (29 days after application) and then to <0.01 mg/kg in mature seed.
Residues of De-Xy-S-2200 and 2-CH_2OH-S-2200 (free and conjugated) were <0.01 mg/kg in all samples at all sampling time points. Residues of 4-OH-S-2200 (free and conjugated) were <0.01 mg/kg in the 0 day whole plant samples, <0.01-0.01 mg/kg in plants without pods and in green pods collected at BBCH 79 (29 days after application), and <0.01 mg/kg in mature seed.

Sample collection (harvest trials):

- at harvest (56-59 days after application) → rape seeds

In mature seed collected 56-59 days after application, residues of S-2200 and of the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were all below the limit of quantification (<0.01 mg/kg).

The ratio of R- and S-isomers of S-2200 remained approximately 50:50 in the oilseed rape samples.

Ongoing quality controls during the analysis were performed with standard reference material as procedural recoveries for each analytical set. For S-2167 (R-isomer of S-2200) and S-2354 (S-isomer of S-2200), fortification levels were 0.005 (LOQ) and 0.05 mg/kg for seeds, green pods, whole plants without pods and whole plants. An additional fortification for whole plants was made at 2.0 mg/kg. Overall mean recovery was 81-96 % for the R-isomer and 88-99 % for the S-isomer. For De-Xy-S-220, sum of free and conjugated forms of 4-OH-S-2200 and sum of free and conjugated forms of 2-CH_2-S-2200, fortifications levels were 0.01 mg/kg (LOQ) and 0.1 mg/kg for seeds, green pods, whole plants without pods and whole plants. Overall mean recovery for the metabolites was between 71 % and 89 %.
The storage stability of S-2200 in specimen extracts in refrigerator was confirmed by procedural recoveries which were analysed in parallel with the field specimens. The maximum storage between sampling and extraction date was 126 days for S-2200 and up to 272 days for the metabolites.

Table B.7.6.3-2: Overview of European residue data for Mandestrobin (S-2200) and its metabolites in winter oilseed rape conducted in 2010 after application of 1x 0.2 kg/ha

Test site/year	Portion analysed	PHI (days)	Residues (mg/kg)					
			R-isomer	S-isomer	S-2200[1)]	De-Xy-S-2200	CH_2OH-S-2200[2)]	4-OH-S-2200[3)]
Northern Europe								
Trial FLN-10-6267 FR01 **N- France 2010**	Whole plant	0	1.4	1.4	2.8	< 0.01	< 0.01	< 0.01
	Whole plant without pods	29	0.048	0.056	0.10	< 0.01	< 0.01	0.01
	Green pods	29	<0.005	<0.005	< 0.01	< 0.01	< 0.01	< 0.01
	seeds	76	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
Trial FLN-10-6267 GE01 **Germany 2010**	Whole plant	0	0.75	0.75	1.5	< 0.01	< 0.01	< 0.01
	Whole plant without pods	50	<0.005	<0.005	< 0.01	< 0.01	< 0.01	0.01
	Green pods	50	<0.005	<0.005	< 0.01	< 0.01	< 0.01	0.01
	seeds	78	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
Trial FLN-10-6267 UK01 **UK 2010**	seeds	56	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
Southern Europe								
Trial FLN-10-6267 FR02 **S-France 2010**	Whole plant	0	0.77	0.76	1.5	< 0.01	< 0.01	< 0.01
	Whole plant without pods	29	0.014	0.017	0.031	< 0.01	< 0.01	0.01
	Green pods	29	<0.005	<0.005	< 0.01	< 0.01	< 0.01	0.01
	seeds	71	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
Trial FLN-10-6267 FR03 **S-France 2010**	seeds	59	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01

[1)] ….sum of R-isomer and S-isomer
[2)] ….sum of free and conjugated forms of 2-CH_2OH-S-2200
[3)] ….sum of free and conjugated forms of 4-OH-S-2200

Residues of S-2200 or its metabolites were below the LOQ in all control specimens.
The detailed results of the 5 residue trials for S-2200 and its metabolites conducted in 2010 in the northern and the southern Europe on oilseed rape are summarized in Annex Ia.

Reference:	**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**
Author(s), year:	Lebrun, F. (2012)
Report/ Doc. Number:	SGS Institut Fresenius GmbH Report no. IF-11/01898756 Sumitomo Chemical Co., Ltd. report no. ROR-0198
Guideline(s):	EU Guideline 7029/VI/95 - Rev. 5, General recommendations for the design, preparation and realization of residue trials. SANCO/3029/99 rev. 4, 11 July 2000 OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

A total of **seven supervised residue trials** have been conducted to determine the residue of S-2200 and its metabolites in Raw Agricultural Commodity (RAC) Winter Rape (whole plants, whole plants w/o pods, green pods and seed specimens) after a **single application of 0.2 kg S-2200/ha** formulated as 25 % soluble concentrate at crop growth stage BBCH 65 (full flowering: 50 % flowers on main raceme open, older petals falling).
The sites were representative of Winter Rape Seed, typical of a producing region in the test countries: Northern France (Centre and Champagne-Ardenne), Germany (Lower-Saxony), United Kingdom (Central England) and Southern France (Midi-Pyrénées and Aquitaine). Winter Rape Seed were cultivated according to normal agricultural practices. Application equipment consisted of hand carried boom sprayer or single wheel sprayer with 6 or 12 flat fan nozzles. The actual amount of test item applied ranged from 0.794 to 0.842 L/ha.

Sample collection (decline trials):

- Just after application (within 3 hours) →whole plants whithout roots
  whole plants were cut approximately 15cm above soil
- at BBCH 79 → whole plant without roots, whole plants without pods and green pods
  whole plants were harvested by hand and cut 15cm above the soil, then the pods were separated from the plants and retained to form the "green pods" specimens. The remaining plants were retained to form the "plants whithout pods" specimens
- at harvest (60-91 days after application) → rape seeds
  seeds were collected by hand or using a small threshing machine and any waste remaining was removed by sieving

In the decline trials, total residues of S-2200 (sum of R- and S-isomers) were 2.1-3.4 mg/kg in whole plant on the day of application and declined to <0.015-0.36 mg/kg in plants without pods and <0.01-0.029 mg/kg in green pods collected at BBCH 79 (31-40 days after application) and then to <0.01 mg/kg in mature seed. Residues of De-Xy-S-2200 and 2-CH_2OH-S-2200 (free and conjugated) were <0.01 mg/kg in all samples at all sampling time points. Residues of 4-OH-S-2200 (free and conjugated) were <0.01 mg/kg in the 0 day whole plant samples, <0.01-0.03 mg/kg in plants without pods and <0.01-0.02 mg/kg in green pods collected at BBCH 79 (31-40 days after application). Residues were again <0.01 mg/kg in mature seed.

Sample collection (harvest trials):

- at harvest (81-86 days after application) → rape seeds

In mature seed collected 61-86 days after application, residues of S-2200 and of the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were all below the limit of quantification (<0.01 mg/kg).

The ratio of R- and S-isomers of S-2200 remained approximately 50:50 in the oilseed rape samples.
Ongoing quality controls during the analysis were performed with standard reference material as procedural recoveries for each analytical set. For S-2167 (R-isomer of S-2200) and S-2354 (S-isomer of S-2200), fortification levels were 0.005 (LOQ) and 0.05 mg/kg for seeds, green pods, whole plants without pods and whole plants. An additional fortification for whole plants was made at 1.50 mg/kg (R-Isomer) and 2.0 mg/kg (S-Isomer). Overall mean recovery was 83-96 % for the R-isomer and 90-100 % for the S-siomer. For De-Xy-S-220, sum of free and conjugated forms of 4-OH-S-2200 and sum of free and conjugated forms of 2-CH_2-S-2200, fortification levels were 0.01 mg/kg (LOQ) and 0.1 mg/kg for seeds, green pods, whole plants without pods and whole plants. Overall mean recovery for the metabolites was between 75 % and 94 %.
The storage stability of S-2200 in specimen extracts in refrigerator was confirmed by procedural recoveries which were analysed in parallel with the field specimens. The maximum storage between sampling and extraction date was 209 days for S-2200 and up to 226 days for the metabolites.

Table B.7.6.3-3: Overview of European residue data for Mandestrobin (S-2200) and its metabolites in winter oilseed rape conducted in 2011 after application of 1x 0.2 kg/ha

Test site/year	Portion analysed	PHI (days)	Residues (mg/kg)					
			R-isomer	S-isomer	S-2200[1)]	De-Xy-S-2200	2-CH_2OH-S-2200[2)]	4-OH-S-2200[3)]
Northern Europe								
11/01898756-01 **N- France 2011**	seeds	85	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
11/01898756-02 **N- France 2011**	Whole plant	0	0.95	1.1	2.1	< 0.01	< 0.01	< 0.01
	Whole plant without pods	31	0.16	0.20	0.36	< 0.01	< 0.01	< 0.01
	Green pods	31	<0.005	0.005	< 0.01	< 0.01	< 0.01	< 0.01
	seeds	70	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
11/01898756-05 **Germany 2011**	seeds	83	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
11/01898756-06 **Germany 2011**	Whole plant	0	0.95	1.1	2.2	< 0.01	< 0.01	< 0.01
	Whole plant without pods	40	0.006	0.009	0.015	< 0.01	< 0.01	< 0.01
	Green pods	40	<0.005	<0.005	< 0.01	< 0.01	< 0.01	< 0.01
	seeds	91	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
11/01898756-07 **UK 2011**	seeds	86	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
Southern Europe								
11/01898756-03 **S-France 2011**	seeds	61	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01
11/01898756	Whole plant	0	1.5	1.9	3.4	< 0.01	< 0.01	< 0.01

Test site/year	Portion analysed	PHI (days)	Residues (mg/kg)					
			R-isomer	S-isomer	**S-2200[1)]**	De-Xy-S-2200	2-CH_2OH-S-2200[2)]	4-OH-S-2200[3)]
-04 **S-France 2011**	Whole plant without pods	32	0.043	0.058	0.10	< 0.01	< 0.01	0.03
	Green pods	32	0.012	0.017	0.029	< 0.01	< 0.01	0.02
	seeds	60	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01

1)….sum of R-isomer and S-isomer
2)….sum of free and conjugated forms of 2-CH_2OH-S-2200
3)….sum of free and conjugated forms of 4-OH-S-2200

Residues of S-2200 or its metabolites were below the LOQ in all control speciments.
The detailed results of the 7 residue trials for S-2200 and its metabolites conducted in 2011 in the northern and the southern Europe on oilseed rape are summarized in Annex Ib.

Overall conclusion

A total of 12 supervised residue trials conducted in 2010 and 2011 have been provided to support the use of S-2200 on oilseed rape seed in Northern and Southern Europe. S-2200 was applied once at 200 g/ha to oilseed rape at growth stage BBCH 65. In mature seed collected 56-91 days after application, residues of S-2200 and residues of the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were all below the limit of quantification (<0.01 mg/kg).
The ratio of R- and S-isomers of S-2200 remained approximately 50:50 in the oilseed rape samples.

Table B.7.6.3-4: Residue data for Mandestrobin (S-2200) for monitoring and risk assessment

Commodity	Trial results relevant to critical GAP (mg/kg)		STMR mg/kg	HR mg/kg	MRL proposal	CF[1)]
	Monitoring mandestrobin (S-2200)	**Riskassessment** sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200)				
Oilseed rape (EU north)	8x < 0.01	8x < 0.04	< 0.01	< 0.01	0.01*	4
Oilseed rape (EU South)	4x < 0.01	4x < 0.04	< 0.01	< 0.01	0.01*	4

1)….MG (S-2200)= 313.39; MG (De-Xy)= 209; MG (4-OH-S-2200, 2-CH_2OH-S-2200) = 329
f (S-2200/De-Xy) = 1.4995; f (S-2200/4-OH)= 0.9526; f (S-2200/2-CH_2OH)= 0.9526 kg

According to the SANCO document 7525/VI/95 rev.9 (March 2011) oilseed rape is a major crop in Northern Europe (NEU) and southern Europe (SEU) and therefore results from 8 supervised trials each are required to support the applied use on it. When at least two residue trials confirm a “no residue” situation further trials are normally not necessary. Thus residue data from the residue trials provided (8x NEU and 4x SEU) carried out within two growing seasons are considered sufficient and acceptable.

All oilseed rape samples collected in the residue trials were stored frozen and analysed within a maximum of 9 months after sampling and covered by the storage stabililty studies provided and described in chapter B.7.6.1.

B.7.7 Effects of industrial processing and/or household preparation (Section 4, Annex IIA, point 6.5.1)

B.7.7.1 Effects on the nature of the residue (Section 4, Annex IIA, point 6.5.1)

Hydrolysis is the parameter that is most likely to affect the nature of the residue during food processing operations. In this study the hydrolysis of [phenoxy-^{14}C]S-2200 was investigated in sterile buffered aqueous solution under a range of hydrolysis conditions simulating processes such as pasteurisation, baking/brewing/boiling and sterilisation.

Reference:	**[^{14}C]S-2200: Nature of the Residue (High Temperature Hydrolysis) Study**
Author(s), year:	Dixon K., Gilbert J. (2011)
Report/ Doc. Number:	Covance Laboratories Ltd Report no. 8239214 Sumitomo ref: ROM-0027
Guideline(s):	EU Document 7035/VI/95 rev.5 (22/7/97), Appendix E Processing Studies OECD Test Guideline 507: Nature of the Pesticide Residues in Processed Commodities – High temperature Hydrolysis (16 October 2007).
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Test Material:
Common Name: S-2200
Name (IUPAC): (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers: S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight: 313.39
CAS No.: 173662-97-0
Radiolabel position: [Phenoxy-^{14}C] S-2200

Material and Methods:
The hydrolysis of [Phenoxy-^{14}C]S-2200 was investigated by incubation in sterile buffered aqueous solution under the conditions given in the table below:

Temperature (°C)	Time (min)	pH	Process represented
90	20	4	Pasteurisation
100	60	5	Baking/brewing/boiling
120	20	6	Sterilisation

[Phenoxy-^{14}C]S-2200 application solutions were prepared in acetonitrile. The application solutions were injected into incubation vessels containing sterile aqueous buffer solution to give a final concentration of ca. 1.0 µg a.s./ml and a final acetonitrile concentration < 1% v/v. The samples were incubated at 90°C (pH 4) for 20 minutes, 100°C (pH 5) for 60 minutes or 120°C (pH 6) for 20 minutes.
For each set of conditions, the total radioactivity present was determined by LSC at time zero and at the end of the incubation period after cooling. Radioactivity in the samples was analysed by reverse phase HPLC and selected samples were also analysed by normal phase 2D-TLC to confirm the identity of S-2200. Chiral HPLC was employed to determine the ratio of R- and S-isomers of S-2200.

Results:

Mean recovery of applied radioactivity as S-2200 was 94.2 to 100.4% at each sampling interval. There were no differences in distribution between incubation conditions. One minor degradation product was observed at a maximum of 1.5% of the applied radioactivity.

Process	Test conditions	Recovery of the applied Radioactivity in %			
		Before incubation (time zero)		After incubation	
		S-2200	Unknown	S-2200	Unknown
Pasteurisation	pH 4, 90°C, 20 minutes	95.5	0.8	94.2	0.8
Baking/brewing/boiling	pH 5, 100°C, 60 minutes	98.3	1.5	99.8	0.7
Sterilisation	pH 6, 120°C, 20 minutes	100.4	1.1	99.6	1.3

There was no noticeable change in isomer ratio occurring during the incubation period. Levels of [^{14}C]S-2167 and [^{14}C]S-2354 remained at 46 to 48% (pH 4), at 49 to 45 % (PH 5) and at 47 to 47 % (pH 6), respectively, after the incubation period.

Conclusion:
The nature of the residue during processing showed that Mandestrobin (S-2200) was stable under conditions representing pasteurisation and baking/brewing/boiling and sterilisation. No degradation would be expected during food processing.

B.7.7.2 Distribution of the residue in peel / pulp (Section 4, Annex IIA, point 6.5.2)

This distribution of the residue in peel/pulp is not relevant to oilseed rape and not required.

B.7.7.3 Residue levels – balance studies (Section 4, Annex IIA, point 6.5.3)

Residues of Mandestrobin (S-2200) and its metabolites in oilseed rape seed were all <0.01 mg/kg and therefore processing studies are not required.

B.7.7.4 Residue levels – follow-up studies (Section 4, Annex IIA, point 6.5.4)

Residues of Mandestrobin (S-2200) and its metabolites in oilseed rape seed were all <0.01 mg/kg and therefore processing studies are not required.

B.7.8 Livestock feeding studies (Section 4, Annex IIA, point 6.4)

The proposed GAP of Mandestrobin (S-2200) involves application of the active substance to winter oilseed rape. Mandestrobin is applied to oilseed rape after the growth stage at which oilseed rape forage may be harvested to be fed to livestock, therefore only the residues in oilseed rape seed have been considered for calculation of livestock dietary burdens. Residues of Mandestrobin (S-2200) and metabolites in oilseed rape seed were <LOQ (0.01 mg/kg) in all trials. However as oilpresscakes might be fed to livestock it was assumed that in case of processing of rape seed to oil the residues levels of Mandestrobin (S-2200) in rape seed are expected to be in the press cake (worst case). With respect to an allocated yield of 40% oil and 60% press cake a theoretical transfer factor for Mandestrobin (S-2200) from rape seed (STMR < 0.01 mg/kg) to cake was calculated as 1.7.
The dietary burden calculation was performed according to the EFSA Pesticide Livestock Calculator as a worst case assumption based on the median residue levels (STMR) obtained from the supervised residue trials considering a conversion factor (CF) of 4 and a processing factor (PF) of 1.7.

Input values for the dietary burden calculation

Group		Crop/commodity	Median dietary burden		Maximum dietary burden	
			Input value (mg/kg)	comment	Input value (mg/kg)	comment
Risk assessment residue definition: *sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH2OH-S-2200 conjugate, expressed as mandestrobin (S-2200).*						
VI	Oilseed (meal, cake)	Rapeseed	0.068	STMR-p x CF	Not applicable	

Results of the dietary burden calculation

	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Median dietary burden (mg/kg DM)	Trigger exceeded?
Risk assessment residue definition: *sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH2OH-S-2200 conjugate, expressed as mandestrobin (S-2200).*					
Dairy ruminants	Not applicable	0.00086	Rapeseed cake	0.024	no
Meat ruminants	Not applicable	0.00102	Rapeseed cake	0.024	no
Poultry	Not applicable	0.00050	Rapeseed cake	0.008	no
Pigs	Not applicable	0.00063	Rapeseed cake	0.016	no

The calculated livestock dietary burden was below the the trigger value of 0.1 mg/kg DM for all livestock species.
The available metabolism studies on laying hens and lactating goats showed that Mandestrobin (S-2200) is extensively metabolised and mainly excreted. No significant accumulation of residues (above 0.01 mg/kg) in tissues, particularly fatty tissues (log P_{ow} of 3.51) has been observed. Therefore livestock feeding studies are considered not necessary.

B.7.9 Residues in succeeding crops (Section 4, Annex IIA, point 6.6)

B.7.9.1 Metabolism and distribution studies on representative crops (Section 4, Annex IIA, point 6.6.2)

Reference:	**Confined rotational crop study with [^{14}C]S-2200**
Author(s), year:	Panthani A., Connor S., Malekani K. (2011)
Report/ Doc. Number:	Smithers Viscient Report no. 13048.6630 Sumitomo ref: ROM-0032
Guideline(s):	US EPA OPPTS 860.1850 Confined Accumulation in Rotational Crops OECD 502 - Guideline for the Testing of Chemicals - Metabolism in Rotational Crops
GLP:	Yes (certified laboratory)
Deviations:	No
Validity:	Yes
Comment (RMS):	The study is acceptable

Test Site information:
Test site: Madera, California
Testing environment: outdoor test plots

Soil characteristics:

pH	7.5
CEC (meq/100g)	10.1
OM (%)	1.4
Sand (%)	74
Silt (%)	14
Clay (%)	12
Soil classification	sandy loam

Crop information:

Crop / crop group: Wheat/Cereals
Lettuce/leafy vegetables
Carrot/root vegetables

Variety: Blanca Royale (Wheat)
Salad Bowl (Lettuce)
Danvers Half Long 126 (Carrot)

Botanical name: Triticum aestivum
Lactuca sativa
Daucus carota

Crop parts processed: Wheat: Forage, hay, straw and grain
Lettuce: Immature and mature leaves
Carrot: Mature tops and roots

Test Material:

Common Name: S-2200
Name (IUPAC): (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide
Rate of isomers: S-isomer (S-2354):R-isomer(S-2167)= 50:50
Molecular Weight: 313.39
CAS No.: 173662-97-0
Purity: 100%
*Log $P_{o/w}$= 3.51 (*at 25 ± 1°C)
Radiolabel position, specific activity: [Phenoxy-^{14}C] S-2200 (120 mCi/mmol)
[Benzyl-^{14}C] S-2200 (123 mCi/mmol

Material and Methods

The confined rotational crop study was conducted to quantify the total ^{14}C levels in lettuce (immature and mature), wheat (forage, hay, straw, grain) and carrot (root, foliage), representating the crop groups leafy vegetable (lettuce), root (carrot) and small grain (wheat).The study was carried out under outdoor growing conditions using two radiolabeled forms of [^{14}C]S-2200, [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 as a 25 % SC formulation.

Above ground wooden boxes (plots) were filled with soil (surface area of 0.83 m^2/ plot) and a soil column depth of ca. 30 cm. Each plot was equipped with a means of draining excess water from the bottom of the container to prevent root rot. A total of 12 plots (4 treated with [phenoxy-^{14}C]S-2200, 4 treated with [benzyl-^{14}C]S-2200, and 4 untreated) were used for the study. Plastic sheeting approximately 2.1 m high was erected all around each of the plots to block wind.The treated plots each received one application of [^{14}C]S-2200 at a rate of 1.6 kg as/ha manually by means of trigger-pulled pump sprayer. The plots remained fallow and the treated soil was aged for a period of 30, 120 and 365 days prior to direct seeding with lettuce, carrots and wheat. At each planting time, wheat was planted in one plot, lettuce and carrots were planted in separate sections of another plot, i.e. the lettuce and the carrot crops shared 50% of the space in each box. Plots were maintained under open field conditions and the crops grown and harvested according to normal agricultural practices.

The application rates were verified by LSC of the formulated test substances. The purity of the radiolabelled test substances was confirmed by HPLC before and after application.

Sampling

Soil samples were taken collected at the time of application, planting (30, 120, 365 days after treatment) and at the time of final crop harvest. Wheat forage (immature) was collected when plants were at the 6-8 inches stage to stem elongation (jointing) stage. Wheat hay was collected when plants were at the early flower (boot) to soft dough stage of grain development and air dried to a moisture content of approximately 28-38%. Mature wheat was separated into wheat grain and straw. The wheat chaff was not retained for analysis. Immature (50% mature size) and mature lettuce heads were harvested by cutting above ground level. Carrots were harvested at maturity and were separated into tops (foliage) and roots. The carrot roots were gently washed to remove adhering soil. Samples were stored frozen between sampling and analysis.

Extraction and analysis

Samples were homogenised in the presence of dry ice. The total radioactive residues (TRR) in plant samples were determined initially by combustion analysis and then by extraction followed by combustion of the debris. Extracts were analysed by liquid scintillation counting (LSC).
Homogenised plant samples were extracted 2-3 times by high speed homogenisation with acetone/water (80:20; v/v) and up to 3 times with acetone/water/hydrochloric acid (80:20:1; v/v/v). Each sample was centrifuged to separate the extracts from the post-extraction solids (PES). The radioactivity in the extracts was determined by liquid scintillation counting (LSC). The post-extraction solids (PES) were allowed to dry and then analysed by combustion analysis. The extractable fractions were combined, concentrated and profiled by radio-HPLC. The regions or peaks of interest were isolated from selected crop samples by reverse-phase HPLC. The metabolites were characterised by HPLC and TLC using co-chromatography with reference standards. The R/S isomer ratio of the S-2200 residues was determined by chiral HPLC.

Distribution of residues

The total radioactive residue (TRR) in each sample was determined by combustion of the homogenised sample, and by liquid scintillation counting (LSC) of extracts and combusted residues. No radioactivity was detected in the control crops.

[phenoxy-^{14}C]S-2200

The TRR concentrations in **wheat** forage, hay and straw samples were higher in the 30 DAT samples (2.74 mg/kg, 1.56 mg/kg and 1.32 mg/kg, respectively) compared to the 120 DAT samples (0.14 mg/kg, 0.29 mg/kg and 0.34 mg/kg, respectively) and the 365 DAT samples (0.10 mg/kg, 0.35 mg/kg and 0.31 mg/kg, respectively). The TRR levels in wheat grain were 0.04 mg/kg (30 and 120 DAT) and < 0.01 mg/kg for the 365 DAT sample. The TRR levels in immature **lettuce** decreased from 0.32 mg/kg (30DAT) to 0.07 mg/kg at 365 DAT. The TRR levels in mature lettuce were lower compared to immature lettuce samples for all plant back intervals (0.08 mg/kg at 30 DAT and 0.02 mg/kg at 365 DAT). TRR levels were higher in **carrot** foliage (0.11 mg/kg for 30 DAT, 0.05 mg/kg for 120 DAT and 0.03 mg/kg for 365 DAT) compared to the roots (0.05 mg/kg, 0.03 mg/kg and < 0.01 mg/kg, respectively).

[benzyl-^{14}C]S-2200

The TRR concentrations in **wheat** forage, hay and straw samples were higher in the 30 DAT samples (2.54 mg/kg, 4.54 mg/kg and 0.82 mg/kg, respectively) compared to the 120 DAT samples (0.31 mg/kg, 0.72 mg/kg and 0.59 mg/kg, respectively) and the 365 DAT samples (0.26 mg/kg, 0.74 mg/kg and 0.38 mg/kg, respectively). The TRR levels in wheat grain were 0.12 mg/kg (30 DAT), 0.20 mg/kg (120 DAT) and < 0.01 mg/kg for the 365 DAT sample. The TRR levels in immature **lettuce** decreased from 0.33 mg/kg (30 DAT) to 0.07 mg/kg at 365 DAT. The TRR levels in mature lettuce were rather similar compared to immature lettuce samples for all plant back intervals (0.22 mg/kg at 30 DAT and 0.02 mg/kg at 365 DAT). TRR levels in **carrot** foliage and carrot roots were rather similar with 0.07 mg/kg and 0.04 mg/kg (30 DAT), 0.08 mg/kg and 0.04 mg/kg (120 DAT) and 0.03 and < 0.01 mg/kg (365 DAT).
The results are summarised in the table below.

B.7.9.1-1: Total radioactive residues in confined rotational crops grown in aged soil treated with [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200

Rotational crop sample		Total Radioactive Residue (mg/kg S-2200 equivalents)[1)]					
		[Phenoxy-^{14}C]S-2200			[Benzyl-^{14}C]S-2200		
		30 DAT	120 DAT	365 DAT	30 DAT	120 DAT	365 DAT
Wheat	Forage	2.73	0.14	0.10	2.54	0.31	0.26
	Hay	1.56	0.29	0.35	4.54	0.72	0.74
	Straw	1.32	0.34	0.31	0.82	0.59	0.38
	Grain	0.04	0.04	0.003[2)]	0.12	0.20	0.005[2)]
Lettuce	Immature	0.33	0.03	0.07	0.32	0.08	0.07
	Mature	0.08	0.02	0.02	0.22	0.05	0.02
Carrot	Mature roots	0.05	0.03	<0.01	0.04	0.04	<0.01
	Mature foliage	0.11	0.05	0.03	0.07	0.08	0.03

DAT: Days after treatment
[1)]…TRR determined by extraction followed by combustion of the debris
[2)]….Sample not extracted. TRR determined by combustion analysis.

In wheat, the extractable residues amounted to 75-84% TRR in the 30-365 DAT forage samples, 59-80% TRR in 30-365 DAT hay and 47-76% TRR in 30-365 DAT straw. In wheat grain grown in [phenoxy-^{14}C]S-2200 treated soil after 30-120 DAT, only 10-11% TRR was extractable, whereas 68-70% TRR was extractable from wheat grain grown in [benzyl-^{14}C]S-2200 treated soil. The 365 DAT wheat grain samples contained TRRs of <0.01 mg/kg and were not extracted. Most of the TRR was extractable from the lettuce (67-94% TRR) and carrot samples (77-93% TRR) at all plant back intervals. The results are summarised in the table below.

Table B.7.9.1-2: Distribution of radioactivity in rotational crops grown in aged soil treated with [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200

	[Phenoxy-^{14}C]S-2200						[Benzyl-^{14}C]S-2200					
	30 DAT		120 DAT		365 DAT		30 DAT		120 DAT		365 DAT	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Wheat forage												
extractable	2.205	80.87	0.106	75.40	0.073	75.20	2.083	82.14	0.258	84.29	0.219	84.13
PES	0.521	19.13	0.035	24.60	0.024	24.80	0.453	17.86	0.048	15.71	0.041	15.87
Total	**2.726**	**100**	**0.141**	**100**	**0.097**	**100**	**2.536**	**100**	**0.306**	**100**	**0.260**	**100**
Wheat hay												
extractable	1.056	67.79	0.211	73.98	0.205	58.55	3.433	75.70	0.578	79.84	0.503	68.28
PES	0.502	32.21	0.074	26.02	0.146	41.45	1.102	24.30	0.146	20.16	0.234	31.72
Total	**1.558**	**100**	**0.285**	**100**	**0.351**	**100**	**4.535**	**100**	**0.724**	**100**	**0.737**	**100**
Wheat straw												
extractable	0.622	46.99	0.248	72.58	0.154	50.17	0.477	58.33	0.451	75.97	0.228	59.72
PES	0.702	53.01	0.093	27.42	0.153	49.83	0.341	41.67	0.143	24.03	0.154	40.28
Total	**1.324**	**100**	**0.341**	**100**	**0.307**	**100**	**0.818**	**100**	**0.594**	**100**	**0.382**	**100**
Wheat grain												
extractable	0.004	11.29	0.004	10.40	na	na	0.082	67.58	0.141	70.47	na	na
PES	0.032	88.71	0.032	89.60	na	na	0.039	32.42	0.059	29.53	na	na
Total	**0.036**	**100**	**0.036**	**100**	**0.003**	**100**	**0.121**	**100**	**0.200**	**100**	**0.005**	**100**
Immature lettuce												
extractable	0.315	94.37	0.024	71.46	0.053	80.57	0.297	94.03	0.065	85.66	0.061	82.25

	[Phenoxy-^{14}C]S-2200						[Benzyl-^{14}C]S-2200					
	30 DAT		120 DAT		365 DAT		30 DAT		120 DAT		365 DAT	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
PES	0.019	5.63	0.010	28.54	0.013	19.43	0.019	5.97	0.011	14.53	0.013	17.75
Total	**0.334**	**100**	**0.034**	**100**	**0.066**	**100**	**0.316**	**100**	**0.076**	**100**	**0.074**	**100**
Mature lettuce												
extractable	0.075	90.03	0.015	67.19	0.015	78.87	0.202	93.12	0.039	82.29	0.016	74.55
PES	0.008	9.97	0.007	32.81	0.004	21.13	0.015	6.88	0.009	17.71	0.006	25.45
Total	**0.083**	**100**	**0.022**	**100**	**0.019**	**100**	**0.217**	**100**	**0.048**	**100**	**0.022**	**100**
Carrot roots												
extractable	0.046	90.70	0.025	87.65	0.006	77.24	0.037	93.09	0.037	93.15	0.008	84.50
PES	0.005	9.30	0.003	12.35	0.002	22.76	0.003	6.91	0.003	6.85	0.001	15.50
Total	**0.051**	**100**	**0.028**	**100**	**0.008**	**100**	**0.040**	**100**	**0.040**	**100**	**0.009**	**100**
Carrot foliage												
extractable	0.105	93.16	0.046	89.46	0.025	81.68	0.066	89.29	0.069	89.76	0.022	80.75
PES	0.008	6.84	0.005	10.54	0.006	18.32	0.008	10.71	0.008	10.24	0.005	19.25
Total	**0.113**	**100**	**0.051**	**100**	**0.031**	**100**	**0.074**	**100**	**0.077**	**100**	**0.027**	**100**

Post-extraction solids (PES) of wheat samples (30 DAT) of both labels were sequentially hydrolyzed with enzyme, acid and base.

Forage and hay samples were further characterised by sequential enzyme hydrolysis with Driselase followed by weak acid hydrolysis (0.1M HCl, 40°C, overnight), strong acid hydrolysis (6M HCl, 80°C, 4 hours), weak base hydrolysis (0.1M NaOH, 40°C, overnight) and strong base hydrolysis (6M NaOH, 80°C, 4 hours).

Wheat straw samples were further characterised by weak acid hydrolysis (1M HCl, 40°C, overnight) followed by strong acid hydrolysis (6M HCl, 80°C, 4 hours), weak base hydrolysis (1M NaOH, 40°C, overnight) and strong base hydrolysis (6M NaOH, 80°C, 4 hours).

Wheat grain samples were further characterised by sequential enzyme hydrolysis with amylase followed by protease, weak acid hydrolysis (1M HCl, 40°C, overnight) and weak base hydrolysis (1M NaOH, 40°C, overnight).

The results are summarized in the table below.

Table B.7.9.1-3: Distribution of radioactivity in the PES of wheat samples (30 DAT)

Crop	Fraction	Total Radioactive Residue					
		[phenoxy-^{14}C]S-2200			[benzyl-^{14}C]S-2200		
		% of PES[1)]	mg/kg	% TRR[2)]	% of PES[1)]	mg/kg	% TRR[2)]
Wheat forage	Enzyme hydroysis	44.18	0.230	8.45	42.16	0.191	7.53
	Weak Acid Hydrolysis (0.1M HCl)	12.04	0.063	2.30	15.05	0.068	2.69
	Strong Acid Hydrolysis (6M HCl)	2.02	0.011	0.39	13.03	0.059	2.33
	Weak Basic Hydrolysis (0.1M NaOH)	20.21	0.105	3.87	15.38	0.070	2.75
	Strong Basic Hydrolysis (6M NaOH)	18.46	0.096	3.53	13.00	0.059	2.32
	Bound Residue	3.09	0.016	0.59	1.38	0.006	0.25
	Total	**100**	**0.521**	**19.13**	**100**	**0.453**	**17.86**
Wheat hay	Enzyme hydroysis	13.27	0.067	4.27	23.21	0.256	5.64
	Weak Acid Hydrolysis (0.1M HCl)	7.48	0.038	2.41	10.84	0.119	2.63
	Strong Acid Hydrolysis (6M HCl)	2.56	0.013	0.83	15.02	0.165	3.65
	Weak Basic Hydrolysis (0.1M NaOH)	8.61	0.043	2.77	12.90	0.142	3.14
	Strong Basic Hydrolysis (6M NaOH)	63.51	0.319	20.45	27.29	0.301	6.63
	Bound Residue	4.57	0.023	1.47	10.73	0.118	2.61
	Total	**100**	**0.502**	**32.21**	**100**	**1.102**	**24.30**

Wheat straw	Weak Acid Hydrolysis (1M HCl)	7.51	0.053	3.98	10.82	0.037	4.51
	Strong Acid Hydrolysis (6M HCl)	4.26	0.030	2.26	6.71	0.023	2.80
	Weak Basic Hydrolysis (1M NaOH)	41.54	0.292	22.02	35.84	0.122	14.93
	Strong Basic Hydrolysis (6M NaOH)	29.55	0.207	15.66	32.61	0.111	13.59
	Bound Residue	17.14	0.120	9.08	14.01	0.048	5.84
	Total	**100**	**0.702**	**53.01**	**100**	**0.341**	**41.67**
Wheat grain	Enzyme hydrolysis[3)]	7.55	0.002	6.70	22.25	0.009	7.21
	Enzyme hydrolysis[4)]	5.77	0.002	5.12	11.57	0.005	3.75
	Weak Acid Hydrolysis (1M HCl)	3.43	0.001	3.05	4.47	0.002	1.45
	Weak Basic Hydrolysis (1M NaOH)	36.95	0.012	32.78	31.73	0.012	10.29
	Bound Residue	46.30	0.015	41.07	29.98	0.012	9.72
	Total	**100**	**0.032**	**88.71**	**100**	**0.039**	**32.42**

[1)]……. % of PES = (Radioactivity released (dpm)) /(total dpm available) * 100
[2)].…….% TRR = (% of PES) * (% PES TRR) / 100
[3)]……Amylase enzyme hydrolysis
[4)]……Protease enzyme hydrolysis

Small amounts of radioactivity were released by each hydrolysis step, however for hay, straw and grain, the greatest proportion of the bound radioactivity was released by 1M and/or 6M base hydrolysis**.**

Characterisation and identification of residues

The extractable fractions were combined, concentrated and profiled by radio-HPLC. The regions or peaks of interest were isolated from selected crop samples by reverse-phase HPLC. S-2200 and metabolites were isolated and identified by HPLC co-chromatography with reference standards. The identity of metabolites was confirmed by 1D TLC. The R/S isomer ratio of the S-2200 residues was determined by chiral HPLC.
For the confirmation of the S-2200 R/S isomer ratio the ^{14}C-S-2200 peak was isolated from benzyl-[^{14}C]S-2200 treated mature lettuce (30 DAT) and [phenoxy-^{14}C]S-2200 treated wheat forage (30 DAT) using HPLC Method 1 (please refer to chapter B.7.1.1) and was analysed by chiral HPLC to determine the isomer ratio. The R:S isomer ratios were found to be 41:59 in lettuce and 42:58 in wheat forage. The R:S isomer ratio in both radiolabelled test substances was 50.3:49.7.
The identity of [^{14}C]S-2200 was confirmed by HPLC and TLC chromatographic data.The [^{14}C]S-2200 isolates from the extractables of [benzyl-^{14}C]S-2200 treated mature lettuce and [phenoxy-^{14}C]S-2200 treated wheat forage were also analyzed by 1D-TLC, co-spotting the isolates with authentic S-2200 standard. It was demonstrated that [^{14}C]S-2200 isolate and S-2200 reference standard coeluted using both chromatographic techniques. The identity of metabolites was also confirmed by1D-TLC and co-spotting with authentic reference standards.
Extraction efficiency of S-2200 was not conducted using control matrices from this study since S-2200 was shown to be quantitatively extracted from lettuce and wheat matrices based on the extraction efficiency experiments conducted during the lettuce and wheat metabolism study.
Freezer storage stability of S-2200 and metabolites was conducted in lettuce and wheat matrices. Representative [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 treated lettuce and wheat matrices were extracted and analyzed up to approximately 6 and 9 months of freezer storage, respectively The metabolite profiles from the two analyses (the initial and final analyses) were very similar indicating that [^{14}C]S-2200 metabolites were stable in the lettuce and wheat matrices under freezer storage conditions. The experiments clearly demonstrated that S-2200 and metabolites are stable during freezer storage. Carrot samples were all analyzed within 3 months of sample collection.

[Phenoxy-^{14}C]S-2200 treated **Wheat (PBI 30 days)**

Un-metabolised S-2200 was not detected in any of the [phenoxy-^{14}C]S-2200 treated wheat samples at the 30 days plant back interval.
In **forage** regions of interest were detected at 14, 15, 16 and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (8 components), the largest accounted for 0.049 mg/kg (1.81 % TRR). The 15-minute region contained one

component (0.027 mg/kg, 0.99% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region showed five components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.245 mg/kg, 8.98% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.043 mg/kg, 1.58% TRR), 2-CH_2OH-S-2200-glycoside (0.386 mg/kg, 14.17% TRR), 4-OH-S-2200-malonyl glycoside (0.394 mg/kg, 14.46% TRR) and one unknown component accounting for 0.008 mg/kg (0.28% TRR). The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.140 mg/kg, 5.13% TRR).

In **hay** the known S-2200 metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 were detected at levels of 0.158 mg/kg (10.15 % TRR), 0.018 mg/kg (1.18 % TRR) and 0.140 mg/kg (8.97 % TRR), respectively. Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (6 components), the largest accounted for 0.034 mg/kg (2.20 % TRR). The 15-minute region contained one component (0.222 mg/kg, 14.26% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region contained one component (0.158 mg/kg, 10.15 % TRR) which was identified as 5-CH_2OH-S-2200-glycoside 1 by co-chromatography, 1D-TLC and LC-MS/MS.

In **straw** the metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 were detected at levels of 0.035 mg/kg (2.64 % TRR), 0.029 mg/kg (2.23 % TRR) and 0.067 mg/kg (5.07 % TRR), respectively. Regions of interest were detected at 14 and 16 minutes (HPLC method 1). The analysis of the 14 minute region using HPLC method 4 showed one component at 0.036 mg/kg (2.73 % TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region contained one component (0.046 mg/kg, 3.44 % TRR) which was identified as 5-CH_2OH-S-2200-glycoside.

In **grain** ^{14}C peaks were < 0.002 mg/kg in the extractable fraction. The results for the [phenoxy-^{14}C]S-2200 treated wheat samples at 30 days plant back interval are summarised in the table below.

Table B.7.9.1-4: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 treated wheat samples planted at 30 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200							
	30 DAT Forage		30 DAT Hay		30 DAT Straw		30 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**2.726**	**100**	**1.558**	**100**	**1.324**	**100**	**0.036**	**100**
S-2200	ND	ND	ND	ND	ND	ND	<LOD	0.43
4-OH-S-2200-glycoside (15min)	0.027	0.99	0.222	14.26	0.036	2.73	ND	ND
5-CH_2OH-S-2200-glycoside 1 (16min)	0.245	8.98	0.158	10.15	0.046	3.44	ND	ND
5-CH_2OH-S-2200-glycoside 2 (16min)	0.043	1.58	ND	ND	ND	ND	ND	ND
2-CH_2OH-S-2200-glycoside (16min)	0.386	14.17	ND	ND	ND	ND	ND	ND
4-OH-S-2200-malonyl glycoside (16min)	0.394	14.46	ND	ND	ND	ND	ND	ND
5-CH_2OH-S-2200-glycoside 3 (19min)	0.140	5.13	ND	ND	ND	ND	ND	ND
2-CH_2OH-S-2200	ND	ND	0.158	10.15	0.035	2.64	ND	ND
5-CH_2OH-S-2200	ND	ND	0.018	1.18	0.029	2.23	ND	ND
4-OH-S-2200	0.012	0.43	ND	ND	ND	ND	ND	ND
5-COOH-S-2200	0.012	0.43	0.140	8.97	0.067	5.07	ND	ND

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200							
	30 DAT Forage		30 DAT Hay		30 DAT Straw		30 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Unknown (2 min)	ND	ND	0.016	1.03	0.018	1.40	0.002	5.12
Unknown (8 min)	0.002	0.06	0.005	0.29	0.037	2.83	<LOD	0.47
Unknown (9 min)	0.008	0.30	ND	ND	0.007	0.54	ND	ND
Unknown (9.5 min)	<LOD	0.02	ND	ND	0.006	0.47	ND	ND
Unknown (10 min)	0.050	1.84	ND	ND	ND	ND	ND	ND
Unknown (11 min)	0.010	0.37	0.082	5.29	0.016	1.24	0.001	1.49
Unknown (14 min region)	0.254[1)]	9.31[1)]	0.106[2)]	6.75[2)]	0.020	1.42	0.001	1.79
Unknown (16min)	0.008	0.28	ND	ND	ND	ND	ND	ND
Unknown (20 min)	0.023	0.83	ND	ND	ND	ND	ND	ND
Unknown (22 min)	0.009	0.34	ND	ND	ND	ND	ND	ND
Others	0.472	17.31	0.030	1.91	0.305	22.99	<LOD	1.10
ERR-extracted radioactive residues	**2.205**	**80.9**	**1.056**	**67.8**	**0.622**	**47.0**	**0.004**	**11.3**
URR – Unextracted Radioactive Residues	**0.521**	**19.1**	**0.502**	**32.2**	**0.702**	**53.0**	**0.032**	**88.7**

[1)] the 14-minute region consists of multiple minor components (8 components), the largest accounted for 0.049 mg/kg (1.81 % TRR).
[2)] the 14-minute region consists of multiple minor components (6 components), the largest accounted for 0.034 mg/kg (2.20 % TRR)

[Benzyl-^{14}C]S-2200 treated **Wheat (PBI 30 days)**

Un-metabolised S-2200 was not detected in the [benzyl-^{14}C]S-2200 treated wheat samples at the 30 days plant back interval, except in forage at an amount of 0.045 mg/kg (1.77 %TRR).

In **forage** no other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 16 and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (4 components), the largest accounted for 0.115 mg/kg (4.55 % TRR). The 16-minute region showed 4 components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.019 mg/kg, 0.76% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.522 mg/kg, 20.6% TRR), 4-OH-S-2200-malonyl glycoside (0.602 mg/kg, 23.75% TRR). The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.147 mg/kg, 5.82% TRR).

In **hay** the known S-2200 metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 were detected at levels of 0.202 mg/kg (4.46 % TRR), 0.056 mg/kg (1.24 % TRR) and 0.060 mg/kg (1.32 % TRR), respectively. Regions of interest were detected at 14, 15, 16 and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (6 components), the largest accounted for 0.235 mg/kg (5.18 % TRR). The 15-minute region contained one component (0.456mg/kg, 10.07% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region showed 4 components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.174 mg/kg, 3.83% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.072 mg/kg, 1.60% TRR), 2-CH_2OH-S-2200-glycoside (0.038 mg/kg, 0.85% TRR) and 4-OH-S-2200- malonyl glycoside (0.072 mg/kg, 1.58% TRR). The 19 minute region showed one component which was identified as 5-CH_2OH-S-2200-glycoside 3 (0.068 mg/kg, 1.50% TRR).

In **straw** the metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 were detected at levels of 0.006 mg/kg (0.69 % TRR), 0.011 mg/kg (1.41 % TRR) and 0.014 mg/kg (1.68 % TRR), respectively. Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). The analysis of the 14 minute region using HPLC method 4 showed one component at 0.014 mg/kg (1.76 % TRR). The 15-minute region contained one component (0.067mg/kg, 8.14 % TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region contained one component (0.016 mg/kg,

1.91 % TRR) which was identified as 5-CH_2OH-S-2200-glycoside 1.
In **grain** no S-2200 or any known metabolites of S-2200 were detected. The results for the [benzyl-^{14}C]S-2200 treated wheat samples at 30 days plant back interval are summarised in the table below.

Table B.7.9.1-5: Identification and characterisation of residues in [benzyl-^{14}C]S-2200 treated wheat samples planted at 30 DAT

Metabolite/Residue fraction	[benzyl-^{14}C]S-2200							
	30 DAT Forage		30 DAT Hay		30 DAT Straw		30 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**2.536**	**100**	**4.535**	**100**	**0.818**	**100**	**0.121**	**100**
S-2200	0.045	1.77	ND	ND	ND	ND	ND	ND
4-OH-S-2200-glycoside (15min)	ND	ND	0.456	10.07	0.067	8.14	ND	ND
5-CH_2OH-S-2200-glycoside 1 (16min)	0.019	0.76	0.174	3.83	0.016	1.91	ND	ND
5-CH_2OH-S-2200-glycoside 2 (16min)	0.522	20.60	0.072	1.60	ND	ND	ND	ND
2-CH_2OH-S-2200-glycoside (16min)	ND	ND	0.038	0.85	ND	ND	ND	ND
4-OH-S-2200-malonyl glycoside (16min)	0.602	23.75	0.072	1.58	ND	ND	ND	ND
5-CH_2OH-S-2200-glycoside 3 (19min)	0.147	5.82	0.068	1.50	ND	ND	ND	ND
2-CH_2OH-S-2200	ND	ND	0.202	4.46	0.006	0.69	ND	ND
5-CH_2OH-S-2200	ND	ND	0.056	1.24	0.011	1.41	ND	ND
4-OH-S-2200	ND	ND	ND	ND	ND	ND	ND	ND
5-COOH-S-2200	ND	ND	0.060	1.32	0.014	1.68	ND	ND
Unknown (2 min)	0.234	9.22	ND	ND	ND	ND	ND	ND
Unknown (8 min)	ND	ND	0.037	0.83	ND	ND	ND	ND
Unknown (9 min)	ND	ND	0.105	2.32	0.009	1.14	ND	ND
Unknown (9.5 min)	0.039	1.52	0.244[c]	5.38	0.034[c]	4.11[c]	0.013	10.78
Unknown (10 min)	0.026	1.01	0.479	10.57[c]	0.047	5.72	0.010	8.17
Unknown (10.5 min)	0.125	4.93	ND	ND	ND	ND	ND	ND
Unknown (11 min)	0.077	3.03	0.413[c]	9.11[c]	0.072[c]	8.77	0.027	22.31
Unknown (14 min region)	0.221[1)]	8.72[1)]	0.653[2)]	14.4[2)]	0.014	1.76	0.032	26.32
Unknown (16min)	ND	ND	ND	ND	ND	ND	ND	ND
Unknown (20 min)	ND	ND	ND	ND	ND	ND	ND	ND
Unknown (22 min)	ND	ND	ND	ND	ND	ND	ND	ND
Others	0.026	1.01	0.302	6.65	0.188	23.00	<LOD	0.01
ERR-extracted radioactive residues	**2.083**	**82.14**	**3.433**	**75.70**	**0.477**	**58.33**	**0.082**	**67.58**
URR – Unextracted Radioactive Residues	**0.453**	**17.86**	**1.102**	**24.30**	**0.341**	**41.67**	**0.039**	**32.42**

[1)] the 14-minute region consists of multiple minor components (4 components), the largest accounted for 0.115 mg/kg (4.55 % TRR).
[2)] the 14-minute region consists of multiple minor components (6 components), the largest accounted for 0.235 mg/kg (5.18 % TRR)

[Phenoxy-^{14}C]S-2200 treated **Wheat (PBI 120 days)**

In **forage** S-2200 and metabolite 2-CH_2OH-S-2200 were present at levels of 0.001 mg/kg (0.89 %TRR) and 0.008 mg/kg (5.90 %TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest at 14, 15, 16 and 19 minutes (HPLC method 1) were detected. Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (7 components), the largest accounted for 0.006 mg/kg (4.34 % TRR). The 15-minute region contained one component (0.016 mg/kg, 11.69% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region showed four components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.011 mg/kg, 8.07% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.001 mg/kg, 1.01% TRR), 2-CH_2OH-S-2200-glycoside (0.014 mg/kg, 9.87% TRR) and 4-OH-S-2200- malonyl glycoside (0.008 mg/kg, 5.88% TRR). The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.003 mg/kg, 2.48% TRR).

In **hay** unmetabolized S-2200 was not detected, the S-2200 metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200, 4-OH-S-2200 and 5-COOH-S-2200 were present at levels of 0.003-0.014 mg/kg (1-5 % TRR). Regions of interest were detected at 14, 15, 16 and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (3 components), the largest accounted for 0.053 mg/kg (18.61 % TRR). The 15-minute region contained one component (0.027 mg/kg, 9.40% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into three components which were identified as 5-CH_2OH-S-2200-glycoside (0.023 mg/kg, 7.97 %TRR), 5-CH_2OH-S-2200-glycoside 2 (0.006 mg/kg, 2.18% TRR) and 4-OH-S-2200-malonyl glycoside (0.015 mg/kg, 5.17% TRR). The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.010 mg/kg, 3.42% TRR).

In **straw** unmetabolized S-2200 was detected at a level of 0.004 mg/kg (1.15 %TRR). The metabolites 2-CH_2OHS-2200, MCBX, 5-CH_2OH-S-2200, 4-OH-S-2200 and 5-COOH-S-2200 were detected at levels between 0.007-0.016 mg/kg (1.97-4.70 %TRR). Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (3 components), the largest accounted for 0.026 mg/kg (7.73 % TRR). The 15-minute region contained one component (0.017 mg/kg, 4.94% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into two components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.008 mg/kg, 2.29 %TRR) and 2-CH_2OH-S-2200-glycoside (0.016 mg/kg, 4.81% TRR).

In **grain** the extractable radioactivity was not analyzed by HPLC due to the low level of radioactivity present (< 0.01 mg/kg). The results for the [phenoxy-^{14}C]S-2200 treated wheat samples at 120 days plant back interval are summarised in the table below.

Table B.7.9.1-6: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 treated wheat samples planted at 120 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200							
	120 DAT Forage		120DAT Hay		120 DAT Straw		120 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.141**	**100**	**0.285**	**100**	**0.341**	**100**	**0.036**	**100**
S-2200	0.001	0.89	ND	ND	0.004	1.15	Not profiled	
4-OH-S-2200-glycoside (15min)	0.016	11.69	0.027	9.40	0.017	4.94		
5-CH_2OH-S-2200-glycoside 1 (16min)	0.011	8.07	0.023	7.97	0.008	2.29		
5-CH_2OH-S-2200-glycoside 2 (16min)	0.001	1.01	0.006	2.18	ND	ND		
2-CH_2OH-S-2200-glycoside (16min)	0.014	9.87	ND	ND	0.016	4.81		
4-OH-S-2200-malonyl	0.008	5.88	0.015	5.17	ND	ND		

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200							
	120 DAT Forage		120DAT Hay		120 DAT Straw		120 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
glycoside (16min)								
5-CH_2OH-S-2200-glycoside 3 (19min)	0.003	2.48	0.010	3.42	ND	ND		
2-CH_2OH-S-2200	0.008	5.90	0.014	4.94	0.007	1.97		
MCBX	ND	ND	ND	ND	0.015	4.54		
5-CH_2OH-S-2200	ND	ND	0.005	1.88	0.016	4.70		
4-OH-S-2200	ND	ND	0.005	1.76	0.009	2.72		
5-COOH-S-2200	ND	ND	0.003	1.08	0.013	3.83		
Unknown (2 min)	ND	ND	0.010	3.60	ND	ND		
Unknown (8 min)	ND	ND	ND	ND	0.013	3.87		
Unknown (9 min)	ND	ND	0.003	0.98	0.013	3.67		
Unknown (9.5 min)	ND	ND	ND	ND	ND	ND		
Unknown (10 min)	0.001	0.53	ND	ND	ND	ND		
Unknown (11 min)	0.001	0.69	ND	ND	0.008	2.34		
Unknown (14 min region)	0.021[1)]	14.35[1)]	0.088[2)]	30.79[2)]	0.059[3)]	17.53[3)]		
Unknown (20 min)	ND	ND	ND	ND	ND	ND		
Unknown (22 min)	ND	ND	ND	ND	ND	ND		
Others	0.020[d]	14.03[d]	0.002	0.81	0.049[d]	14.24[d]		
ERR-extracted radioactive residues	**0.106**	**75.40**	**0.211**	**73.98**	**0.248**	**72.60**	**0.004**	**10.40**
URR – Unextracted Radioactive Residues	**0.035**	**24.60**	**0.074**	**26.02**	**0.093**	**27.40**	**0.032**	**89.60**

[1)] the 14-minute region consists of multiple minor components (7 components), the largest accounted for 0.006 mg/kg (4.34 % TRR).
[2)] the 14-minute region consists of multiple minor components (3 components), the largest accounted for 0.053 mg/kg (18.61 % TRR) and was further characterized with 30DAT samples
[3)] the 14-minute region consists of multiple minor components (3 components), the largest accounted for 0.026 mg/kg (7.73 % TRR).

[Benzyl-^{14}C]S-2200 treated **Wheat (PBI 120 days)**

Un-metabolised S-2200 was not detected in the [benzyl-^{14}C]S-2200 treated wheat samples at the 120 days plant back interval, except in straw at an amount of 0.009 mg/kg (1.49 %TRR).

In **forage** the S-2200 metabolites 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-COOH-S-2200 were detected at levels of ≤ 0.01 mg/kg. Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (7 components), the largest accounted for 0.031 mg/kg (10.27 % TRR). The 15-minute region contained one component (0.009mg/kg, 2.80% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into three components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.009 mg/kg, 3.08 %TRR), 2-CH_2OH-S-2200-glycoside (0.010 mg/kg, 3.29% TRR) and 4-OH-S-2200-malonyl glycoside (0.011 mg/kg, 3.57% TRR). The 19 minute region showed one component which was identified as 5-CH_2OH-S-2200-glycoside 3 (0.008 mg/kg, 2.48% TRR).

In **hay** the S-2200 metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 were detected at levels of ≤ 0.01 mg/kg. Regions of interest were detected at 14, 15, 16 and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (7 components), the largest accounted for 0.101 mg/kg (13.97 % TRR). The 15-minute region contained one component (0.048mg/kg, 6.68% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into three components

which were identified as 5-CH_2OH-S-2200-glycoside 1, 5-CH_2OH-S-2200-glycoside 2 and 2-CH_2OH-S-2200-glycoside with levels between < 0.005 – 0.02 mg/kg (< 0.5-2.8 %TRR)
In **straw** the metabolites 2-CH_2OHS-2200, MCBX, 5-CH_2OH-S-2200, 4-OH-S-2200 and 5-COOH-S-2200 were detected at levels between 0.011-0.030 mg/kg (1.9 - 5.1 %TRR). Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (3 components), the largest accounted for 0.056 mg/kg (9.41 % TRR). The 15-minute region contained one component (0.030 mg/kg, 4.98% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into two components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.013 mg/kg, 2.25 %TRR) and 2-CH_2OH-S-2200-glycoside (0.033 mg/kg, 5.47% TRR).
In **grain** no S-2200 or any known metabolites of S-2200 were detected. Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (6 components), the largest accounted for 0.044 mg/kg (22.0 % TRR). The results for the [benzyl-^{14}C]S-2200 treated wheat samples at 120 days plant back interval are summarised in the table below.

Table B.7.9.1-7: Identification and characterisation of residues in [benzyl-^{14}C]S-2200 treated wheat samples planted at 120 DAT

Metabolite/Residue fraction	[benzyl-^{14}C]S-2200							
	120 DAT Forage		120DAT Hay		120 DAT Straw		120 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.306**	**100**	**0.724**	**100**	**0.594**	**100**	**0.200**	**100**
S-2200	ND	ND	ND	ND	0.009	1.49	ND	ND
4-OH-S-2200-glycoside (15min)	0.009	2.80	0.048	6.68	0.030	4.98	0.008	3.84
5-CH_2OH-S-2200-glycoside 1 (16min)	0.009	3.08	0.020	2.79	0.013	2.25	ND	ND
5-CH_2OH-S-2200-glycoside 2 (16min)	ND	ND	0.001	0.08	ND	ND	ND	ND
2-CH_2OH-S-2200-glycoside (16min)	0.010	3.29	0.002	0.31	0.033	5.47	ND	ND
4-OH-S-2200-malonyl glycoside (16min)	0.011	3.57	ND	ND	ND	ND	ND	ND
5-CH_2OH-S-2200-glycoside 3 (19min)	0.008	2.48	ND	ND	ND	ND	ND	ND
2-CH_2OH-S-2200	0.010	3.19	0.003	0.42	0.011	1.88	ND	ND
MCBX	ND	ND	ND	ND	0.028	4.76	ND	ND
5-CH_2OH-S-2200	0.004	1.31	ND	ND	0.030	5.08	ND	ND
4-OH-S-2200	ND	ND	0.002	0.28	0.016	2.71	ND	ND
5-COOH-S-2200	0.002	0.56	0.004	0.59	0.024	3.98	ND	ND
Unknown (2 min)	ND	ND	ND	ND	ND	ND	ND	ND
Unknown (8 min)	ND	ND	0.018	2.42	0.020	3.42	ND	ND
Unknown (9 min)	0.008	2.72	0.058	7.98	0.028	4.73	0.003	1.53
Unknown (9.5 min)	0.025	8.15	0.018	2.43	ND	ND	0.003	1.60
Unknown (10 min)	0.009	2.93	0.052	7.15	ND	ND	0.007	3.45
Unknown (11 min)	0.019	6.36	0.063	8.71	0.018	3.04	0.003	1.66
Unknown (14 min region)	0.083[1)]	27.07[1)]	0.134[2)]	18.48[2)]	0.117[3)]	19.76[3)]	0.110[4)]	55.33[4)]
Unknown (16min)	ND	ND	ND	ND	ND	ND	ND	ND
Unknown (20 min)	ND	ND	ND	ND	ND	ND	ND	ND
Unknown (22 min)	0.002	0.56	ND	ND	ND	ND	ND	ND
Others	0.050	16.22	0.155	21.52	0.074	12.41	0.007	3.06

Metabolite/Residue fraction	[benzyl-^{14}C]S-2200							
	120 DAT Forage		120DAT Hay		120 DAT Straw		120 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
ERR-extracted radioactive residues	**0.258**	**84.29**	**0.578**	**79.84**	**0.451**	**75.97**	**0.141**	**70.47**
URR – Unextracted Radioactive Residues	**0.048**	**15.71**	**0.146**	**20.16**	**0.143**	**24.03**	**0.059**	**29.53**

1) the 14-minute region consists of multiple minor components (7 components), the largest accounted for 0.031 mg/kg (10.27 % TRR) and was further characterized with 30DAT samples
2) the 14-minute region consists of multiple minor components (7 components), the largest accounted for 0.101 mg/kg (13.97 % TRR) and was further characterized with 30DAT samples
3) the 14-minute region consists of multiple minor components (3 components), the largest accounted for 0.056 mg/kg (9.41 % TRR) and was further characterized with 30DAT samples
4) the 14-minute region consists of multiple minor components (6 components), the largest accounted for 0.044 mg/kg (22.0 % TRR) and was further characterized with 30DAT samples

[Phenoxy-^{14}C]S-2200 treated **Wheat (PBI 365 days)**

In **forage** unmetabolized S-2200 was not detected, the S-2200 metabolites 2-CH_2OH-S-2200, MCBX and 4-OH-S-2200 were present at levels of < 0.01 mg/kg (1-6 % TRR). In **hay** unmetabolized S-2200 was detected at 0.002 mg/kg (0.85 % TRR). The S-2200 metabolites 2-CH_2OH-S-2200, MCBX, 5-CH_2OH-S-2200 and 5-COOH-S-2200 were present at levels of ≤ 0.01 mg/kg (1.7-6.6 % TRR). In both crops, forage and hay, a number of minor ^{14}C peaks were present at low concentrations (< 0.01 mg/kg). In **straw** unmetabolized S-2200 was detected at a level of < LOD (0.003 mg/kg). The metabolites 2-CH_2OH-S-2200, MCBX and 5-COOH-S-2200 were detected at a level of < 0.01 mg/kg (up to 4.6 %TRR). Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (4 components), the largest accounted for 0.006 mg/kg (4.52 % TRR). The 15-minute region contained one component (0.006 mg/kg, 4.35% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into three components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.006 mg/kg, 4.30 %TRR), 5-CH_2OH-S-2200-glycoside 2 (0.003 mg/kg, 2.65 %TRR) and 4-OH-S-2200-malonyl glycoside (0.002 mg/kg, 1.66% TRR). In **grain** the extractable radioactivity was not analyzed by HPLC due to the low level of radioactivity present (< 0.01 mg/kg). The results for the [phenoxy-^{14}C]S-2200 treated wheat samples at 120 days plant back interval are summarised in the table below.

Table B.7.9.1-8: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 treated wheat samples planted at 365 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200							
	365 DAT Forage		365DAT Hay		365 DAT Straw		365 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.097**	**100**	**0.351**	**100**	**0.307**	**100**	**0.003**	**100**
S-2200	ND	ND	0.002	0.85	<LOD	0.24	Not extracted for profiling	
4-OH-S-2200-glycoside (15min)	0.005	5.49	0.007	3.40	0.006	4.35		
5-CH_2OH-S-2200-glycoside 1 (16min)	0.005	4.96	0.009	4.40	0.006	4.30		
5-CH_2OH-S-2200-glycoside 2 (16min)	0.004	4.27	ND	ND	0.003	2.65		
2-CH_2OH-S-2200-glycoside (16min)	ND	ND	0.002	1.07	ND	ND		
4-OH-S-2200-malonyl glycoside (16min)	0.003	3.41	0.003	1.22	0.002	1.66		
5-CH_2OH-S-2200-glycoside 3 (19min)	0.002	1.99	0.005	2.45	<LOD	0.27		

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200							
	365 DAT Forage		365DAT Hay		365 DAT Straw		365 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
2-CH_2OH-S-2200	0.006	6.20	0.012	6.60	0.006	4.67		
MCBX	0.001	1.26	0.004	1.73	0.003	2.56		
5-CH_2OH-S-2200	ND	ND	0.004	1.77	ND	ND		
4-OH-S-2200	0.002	2.53	ND	ND	ND	ND		
5-COOH-S-2200	0.002	1.85	0.007	3.23	0.004	3.36		
Unknown (2 min)	0.003	2.82	0.004	2.10	0.002	1.25		
Unknown (8 min)	ND	ND	ND	ND	ND	ND		
Unknown (9 min)	0.003	2.83	0.001	0.45	0.001	0.77		
Unknown (9.5 min)	0.001	0.95	0.001	0.65	0.003	2.37		
Unknown (10 min)	ND	ND	0.003	1.55	0.001	0.89		
Unknown (11 min)	0.004	4.04	0.008	3.69	0.002	1.52		
Unknown (14 min region)	0.018[1]	18.69[1]	0.032[2]	15.72[2]	0.019[3]	14.59[3]		
Unknown (20 min)	0.002	2.00	ND	ND	ND	ND		
Unknown (22 min)	ND	ND	ND	ND	ND	ND		
Others	0.012[a]	11.92[a]	0.101	7.67	0.071	4.72		
ERR-extracted radioactive residues	**0.073**	**75.20**	**0.205**	**58.55**	**0.154**	**50.17**		
URR – Unextracted Radioactive Residues	**0.024**	**24.80**	**0.146**	**41.45**	**0.153**	**49.83**		

LOD= 0.0015 mg/kg (S-2200) and 0.003 mg/kg (metabolites)

[1)] the 14-minute region consists of multiple minor components

[2)] the 14-minute region consists of multiple minor components (4 components), the largest accounted for 0.015 mg/kg (7.13 % TRR)

[3)] the 14-minute region consists of multiple minor components (4 components), the largest accounted for 0.006 mg/kg (4.52 % TRR).

[Benzyl-^{14}C]S-2200 treated **Wheat (PBI 365 days)**

In **forage** unmetabolized S-2200 was not detected, the S-2200 metabolite 2-CH_2OH-S-2200was present at 0.003 mg/kg (1.14 % TRR). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. In **hay** unmetabolized S-2200 was detected at 0.002 mg/kg (0.37 % TRR). The S-2200 metabolites 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-COOH-S-2200 were present at levels of < 0.01 mg/kg (0.6-2.5 % TRR). Moreover a number of minor ^{14}C peaks were present at very low concentrations (< 0.01 mg/kg). In **straw** unmetabolized S-2200 was detected at a level of < 0.005 mg/kg (2.17 %TRR). The metabolites 2-CH_2OH-S-2200, MCBX, 5-CH_2OHS-2200 and 5-COOH-S-2200 were detected at a level of < 0.01 mg/kg. An unknown region at 11-minutes accounting for 0.025 mg/kg (10.91% TRR) consisted of multiple minor components. Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of two minor components, the largest accounted for 0.025 mg/kg (10.99 % TRR). The 15-minute region contained one component (0.020 mg/kg, 8.81% TRR) which was identified as 4-OH-S-2200-glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into four components which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.006 mg/kg, 2.60 %TRR), 5-CH_2OH-S-2200-glycoside 2 (0.004 mg/kg, 1.71 %TRR), 2-CH_2OH-S-2200-glycoside (0.003 mg/kg, 1.40 %TRR) and 4-OH-S-2200-malonyl glycoside (0.004 mg/kg, 1.58% TRR).

In **grain** the extractable radioactivity was not analyzed by HPLC due to the low level of radioactivity present (< 0.01 mg/kg). The results for the [benzyl-^{14}C]S-2200 treated wheat samples at 365 days plant back interval are summarised in the table below.

Table B.7.9.1-9: Identification and characterisation of residues in [benzyl-^{14}C]S-2200 treated wheat samples planted at 365 DAT

Metabolite/Residue fraction	[benzyl-^{14}C]S-2200							
	365 DAT Forage		365 DAT Hay		365 DAT Straw		365 DAT Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.260**	**100**	**0.737**	**100**	**0.382**	**100**	**0.005**	**100**
S-2200	ND	ND	0.002	0.37	0.005	2.17	Not extracted for profiling	
4-OH-S-2200-glycoside (15min)	0.009	3.55	0.003	0.62	0.020	8.81		
5-CH_2OH-S-2200-glycoside 1 (16min)	0.008	3.04	0.007	1.39	0.006	2.60		
5-CH_2OH-S-2200-glycoside 2 (16min)	ND	ND	0.007	1.41	0.004	1.71		
2-CH_2OH-S-2200-glycoside (16min)	0.008	3.09	ND	ND	0.003	1.40		
4-OH-S-2200-malonyl glycoside (16min)	0.006	2.31	ND	ND	0.004	1.58		
5-CH_2OH-S-2200-glycoside 3 (19min)	0.004	1.67	ND	ND	0.002	0.90		
2-CH_2OH-S-2200	0.003	1.14	0.013	2.51	0.003	1.41		
MCBX	ND	ND	ND	ND	0.003	1.49		
5-CH_2OH-S-2200	ND	ND	0.007	1.35	0.006	2.50		
4-OH-S-2200	ND	ND	0.005	1.07	ND	ND		
5-COOH-S-2200	ND	ND	0.003	0.55	0.005	2.31		
Unknown (2 min)	ND	ND	ND	ND	0.002	1.09		
Unknown (8 min)	0.003	1.09	0.001	0.29	ND	ND		
Unknown (9 min)	0.003	1.09	0.002	0.43	0.002	0.75		
Unknown (9.5 min)	0.027[a]	10.47[a]	0.047	9.37	0.005	1.98		
Unknown (10 min)	0.025	9.77	0.006	1.12	ND	ND		
Unknown (11 min)	0.003	1.23	0.018	3.66	0.025	10.91		
Unknown (14 min region)	0.092[1)]	36.81[1)]	0.065[2)]	12.99[2)]	0.034[3)]	14.78[3)]		
Unknown (16min)	ND	ND	ND	ND	ND	ND		
Unknown (20 min)	0.005	1.91	ND	ND	ND	ND		
Unknown (22 min)	ND	ND	ND	ND	ND	ND		
Others	0.018[a]	6.98[a]	0.317[a]	31.15[a]	0.093	0.63		
ERR-extracted radioactive residues	**0.219**	**84.13**	**0.503**	**68.28**	**0.228**	**59.72**		
URR – Unextracted Radioactive Residues	**0.041**	**15.87**	**0.234**	**31.72**	**0.154**	**40.28**		

[1)] the 14-minute region consists of multiple minor components (8 components), the largest accounted for 0.023 mg/kg (8.99 % TRR)
[2)] the 14-minute region consists of multiple minor components (7 components), the largest accounted for 0.019 mg/kg (3.78 % TRR)
[3)] the 14-minute region consists of multiple minor components (2 components), the largest accounted for 0.025 mg/kg (10.99 % TRR)

[Phenoxy-^{14}C]S-2200 treated **Lettuce (PBI 30 days)**

In **immature lettuce** un-metabolised S-2200 was detected at level of 0.003 mg/kg (1.03% TRR). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 15, 16, and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components (4 components), the largest accounted for < 0.01 mg/kg (1.94 % TRR). The 15-minute region contained one component

(0.007 mg/kg, 2.08% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into four peaks which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.003 mg/kg, 0.81% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.041 mg/kg, 12.34% TRR), 2-CH_2OH-S-2200-glycoside (0.018 mg/kg, 5.30% TRR) and 4-OH-S-2200- malonyl glycoside (0.108 mg/kg, 32.32% TRR). The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.043 mg/kg, 12.82% TRR). There were two unknown peaks detected at 20 minutes (0.024 mg/kg, 7.12% TRR) and 22 minutes (0.006 mg/kg, 1.76% TRR).
In **mature lettuce** un-metabolised S-2200 and MCBX were detected at a level of 0.013 mg/kg (13.25% TRR) and 0.002 mg/kg (2.58% TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 16, and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region comprised one component accounting for 0.002 mg/kg (2.49 % TRR). The 16-minute region was separated into three peaks which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.002 mg/kg, 1.91% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.006 mg/kg, 7.72% TRR) and 4-OH-S-2200- malonyl glycoside (0.019 mg/kg, 22.93% TRR) by co-chromatography, 1D-TLC and LC-MS/MS. The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.018 mg/kg, 22.07% TRR).

[Benzyl-^{14}C]S-2200 treated **Lettuce (PBI 30 days)**
In **immature lettuce** un-metabolised S-2200 and the metabolites MCBX and 5-COOH-S-2200 were detected at level of 0.021 mg/kg (6.52% TRR), 0.004 mg/kg (1.24% TRR) and 0.002 mg/kg (0.50% TRR), respectively. Regions of interest were detected at 14, 15, 16, and 19 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components, the largest accounted for 0.008 mg/kg (2.57 % TRR). The 15-minute region contained one component (0.004 mg/kg, 1.32% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into four peaks which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.001 mg/kg, 0.41% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.024 mg/kg, 7.50% TRR), 2-CH_2OH-S-2200-glycoside (0.008 mg/kg, 2.51% TRR) and 4-OH-S-2200- malonyl glycoside (0.08 mg/kg, 25.43% TRR). The 19 minute region showed one component identified as 5-CH_2OH-S-2200-glycoside 3 (0.026 mg/kg, 8.36% TRR).
In **mature lettuce** un-metabolised S-2200 and the metabolite MCBX were detected at level of 0.014 mg/kg (6.50% TRR) and 0.005 mg/kg (2.15% TRR), respectively. Regions of interest were detected at 14, 15, 16 and 19 (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components < 0.01 mg/kg (< 1 %TRR). The 15-minute region contained one component (0.006 mg/kg, 2.80% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into three peaks which were identified as 5-CH_2OH-S-2200-glycoside 2 (0.014 mg/kg, 6.22% TRR), 2-CH_2OH-S-2200-glycoside (0.004 mg/kg, 1.69% TRR) and 4-OH-S-2200- malonyl glycoside (0.054 mg/kg, 24.76% TRR. The 19 minute region showed one component which was identified as 5-CH_2OH-S-2200-glycoside 3 (0.032 mg/kg, 14.63% TRR).
The results for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 treated lettuce samples at 30 days plant back interval are summarised in the table below.

Table B.7.9.1-10: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 and in [benzyl-^{14}C]S-2200 treated lettuce samples planted at 30 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	30 DAT Immature Lettuce		30 DAT Mature Lettuce		30 DAT Immature Lettuce		30 DAT Mature Lettuce	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.334**	**100**	**0.083**	**100**	**0.316**	**100**	**0.217**	**100**
S-2200	0.003	1.03	0.013	13.25	0.021	6.52	0.014	6.50
4-OH-S-2200-glycoside (15min)	0.007	2.08	<LOD	0.32	0.004	1.32	0.006	2.80
5-CH_2OH-S-2200-glycoside 1 (16min)	0.003	0.81	0.002	1.91	0.001	0.41	ND	ND
5-CH_2OH-S-2200-glycoside 2 (16min)	0.041	12.34	0.006	7.72	0.024	7.50	0.014	6.22
2-CH_2OH-S-2200-glycoside (16min)	0.018	5.30	<LOD	0.32	0.008	2.51	0.004	1.69
4-OH-S-2200-malonyl glycoside (16min)	0.108	32.31	0.019	22.93	0.080	25.43	0.054	24.76
5-CH_2OH-S-2200-glycoside 3 (19min)	0.043	12.82	0.018	22.07	0.026	8.36	0.032	14.63
2-CH_2OH-S-2200	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	0.002	2.58	0.004	1.24	0.005	2.15
5-COOH-S-2200	ND	ND	ND	ND	0.002	0.50	ND	ND
Unknown (2 min)	0.004	1.26	0.003	3.27	0.003	0.99	0.001	0.68
Unknown (8 min)	ND	ND	ND	ND	0.004	1.11	0.001	0.68
Unknown (9 min)	ND	ND	ND	ND	0.006	2.06	0.003	1.44
Unknown (9.5 min)	ND	ND	ND	ND	0.014	4.54	0.010	4.72
Unknown (10 min)	ND	ND	ND	ND	0.005	1.58	0.003	1.47
Unknown (11 min)	ND	ND	ND	ND	0.012[c]	3.87[c]	0.010[e]	4.79[e]
Unknown (14 min region)	0.017[1)]	5.13[1)]	0.002[2]	2.49[2)]	0.027[d]	8.90[d]	0.006[4]	3.01[4]
Unknown (20 min)	0.024	7.12	0.003	3.82	0.008	2.46	0.011	4.89
Unknown (22 min)	0.006	1.76	ND	ND	0.004	1.16	ND	ND
Others	0.041	12.39	0.006	7.19	0.043[g]	13.57	0.028[g]	12.69
ERR-extracted radioactive residues	**0.315**	**94.37**	**0.075**	**90.03**	**0.297**	**94.03**	**0.202**	**93.12**
URR – Unextracted Radioactive Residues	**0.019**	**5.63**	**0.008**	**9.97**	**0.019**	**5.97**	**0.015**	**6.88**

[1)] the 14-minute region consists of multiple minor components, the largest accounted for 0.006 mg/kg (1.94 % TRR).
[2)] the 14-minute region comprised 1 component at 0.002 mg/kg (2.49 % TRR)
[3)] the 14-minute region consists of multiple minor components, the largest accounted for 0.008 mg/kg (2.57 % TRR).
[4)] the 14-minute region consists of multiple minor components, the largest accounted for 0.002 mg/kg (1.12 % TRR).

[Phenoxy-^{14}C]S-2200 treated **Lettuce (PBI 120 days)**

In **immature lettuce** un-metabolised S-2200 and metabolites were present at low levels (< 0.001 mg/kg). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 15, 16, and 19 minutes (HPLC method 1), but the concentrations of individual ^{14}C residues were < 0.005 mg/kg.

In **mature lettuce** un-metabolised S-2200 was detected at a level of 0.002 mg/kg (8.36% TRR), other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present at low levels (< 0.005 mg/kg). Regions of interest were detected at 14, 15, and 16 minutes (HPLC method 1). The

individual components of the specific regions were separated by HPLC analysis (Method 4) and showing multiple components at low levels (≤ 0.005 mg/kg).

[Benzyl-^{14}C]S-2200 treated **Lettuce (PBI 120 days)**

In **immature lettuce** un-metabolised S-2200 and metabolites were not detected. Regions of interest were detected at 14, 15, and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple minor components, the largest accounted for 0.015 mg/kg (19.65 %TRR). The individual components of the other specific regions showed that no individual component exceeded the 0.01 mg/kg level.

In **mature lettuce** un-metabolised S-2200 was detected at a level of 0.004 mg/kg (9.36 %TRR). Regions of interest were detected at 14, 15, and 16 minutes (HPLC method 1). The individual components of the 14 and 16 minutes regions were separated by HPLC analysis (Method 4) showing multiple components at low levels (< 0.005 mg/kg). The 15-minute region contained one component (0.005 mg/kg, 9.92% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS.

The results for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 treated lettuce samples at 120 days plant back interval are summarised in the table below.

Table B.7.9.1-11: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 and in [benzyl-^{14}C]S-2200 treated lettuce samples planted at 120 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	120 DAT Immature Lettuce		120 DAT Mature Lettuce		120 DAT Immature Lettuce		120 DAT Mature Lettuce	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.034**	**100**	**0.022**	**100**	**0.076**	**100**	**0.048**	**100**
S-2200	<LOD	1.34	0.002	8.36	ND	ND	0.004	9.36
4-OH-S-2200-glycoside (15min)	<LOD	0.31	0.001	2.71	0.006	8.15	0.005	9.92
5-CH_2OH-S-2200-glycoside 1 (16min)	0.001	4.33	<LOD	1.71	0.002	2.65	0.002	4.97
5-CH_2OH-S-2200-glycoside 2 (16min)	<LOD	0.22	ND	ND	ND	ND	<LOD	0.17
2-CH_2OH-S-2200-glycoside (16min)	<LOD	0.22	ND	ND	ND	ND	<LOD	0.73
4-OH-S-2200-malonyl glycoside (16min)	0.002	6.95	ND	ND	0.008	10.40	ND	ND
5-CH_2OH-S-2200-glycoside 3 (19min)	0.001	3.02	ND	ND	0.002	2.47	ND	ND
2-CH_2OH-S-2200	ND	ND	0.001	3.36	ND	ND	0.001	1.67
MCBX	ND	ND	<LOD	1.55	ND	ND	0.001	2.90
5-CH_2OH-S-2200	ND	ND	ND	ND	ND	ND	<LOD	0.98
4-OH-S-2200	ND	ND	ND	ND	ND	ND	0.001	2.53
5-COOH-S-2200	ND	ND	<LOD	1.46	ND	ND	<LOD	0.97
Unknown (7 min)	0.003	10.41	0.004	18.38	0.008	11.08	0.001	2.90
Unknown (8 min)	ND	ND	ND	ND	ND	ND	0.001	1.05
Unknown (9 min)	<LOD	0.34	<LOD	2.15	ND	ND	0.003	6.02
Unknown (9.5 min)	<LOD	0.37	ND	ND	ND	ND	0.001	1.97
Unknown (10 min)	ND	ND	ND	ND	ND	ND	0.001	1.76
Unknown (11 min)	ND	ND	ND	ND	0.004	5.68	ND	ND

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	120 DAT Immature Lettuce		120 DAT Mature Lettuce		120 DAT Immature Lettuce		120 DAT Mature Lettuce	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Unknown (14 min region)	0.003[1]	12.27[1]	0.002[2]	10.70[2]	0.030[3]	37.67[3]	0.005[4]	11.80[4]
Unknown (20 min)	0.001	1.56	ND	ND	ND	ND	ND	ND
Unknown (22 min)	0.001	4.45	ND	ND	ND	ND	0.001	2.80
Others	0.009	25.43	0.004	16.61	0.005	7.57	0.009	19.80
ERR-extracted radioactive residues	**0.024**	**71.46**	**0.015**	**67.19**	**0.065**	**85.66**	**0.039**	**82.29**
URR – Unextracted Radioactive Residues	**0.010**	**28.54**	**0.007**	**32.81**	**0.011**	**14.34**	**0.009**	**17.71**

[1] the 14-minute region consists of multiple minor components.
[2] the 14-minute region comprised 1 component at 0.002 mg/kg (10.70 % TRR)
[3] the 14-minute region consists of multiple minor components, the largest accounted for 0.015 mg/kg (19.65 % TRR), further characterised with wheat samples
[4] the 14-minute region consists of multiple minor components, the largest accounted for 0.003 mg/kg (6.82 % TRR).

[Phenoxy-^{14}C]S-2200 treated **Lettuce (PBI 365 days)**

In **immature lettuce** un-metabolised S-2200 and metabolites MCBX and 4-OH-S-2200 were detetcted at levels of 0.013 mg/kg (19.54 % TRR), 0.002 mg/kg (3.35 % TRR) and 0.002 mg/kg (2.77 % TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 15, 16, and 19 minutes (HPLC method 1). The 16-minute region was separated into two peaks which were identified as 5-CH_2OH-S-2200-glycoside 2 (0.012 mg/kg, 18.50% TRR) and 4-OH-S-2200- malonyl glycoside (0.008 mg/kg, 12.60% TRR) by co-chromatography, 1D-TLC and LC-MS/MS. The individual components of the other specific regions were separated by HPLC analysis (Method 4) showing multiple components at low levels (< 0.005 mg/kg).
In **mature lettuce** un-metabolised S-2200 was detected only at a low level (< 0.001 mg/kg). Detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were not present or its level not greater than 0.005 mg/kg.

[Benzyl-^{14}C]S-2200 treated **Lettuce (PBI 365 days)**

In **immature lettuce** un-metabolised S-2200 and metabolite 4-OH-S-2200 were detetcted at levels of 0.009 mg/kg (11.63 % TRR) and 0.001 mg/kg (0.70 % TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 15, 16, and 19 minutes (HPLC method 1). The individual components of the other specific regions were separated by HPLC analysis (Method 4) showing multiple components at low levels (≤ 0.005 mg/kg) except one component (0.013 mg/kg, 17.3% TRR) which was identified as 4-OH-S-2200-malonyl glycoside by co-chromatography, 1D-TLC and LC-MS/MS.
In **mature lettuce** un-metabolised S-2200 was not detected. No other single ^{14}C component was present at a level greater than 0.002 mg/kg.
The results for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 treated lettuce samples at 365 days plant back interval are summarised in the table below.

Table B.7.9.1-12: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 and in [benzyl-^{14}C]S-2200 treated lettuce samples planted at 365 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	365 DAT Immature Lettuce		365 DAT Mature Lettuce		365 DAT Immature Lettuce		365 DAT Mature Lettuce	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.066**	**100**	**0.019**	**100**	**0.074**	**100**	**0.022**	**100**
S-2200	0.013	19.54	<LOD	0.49	0.009	11.63	ND	ND
4-OH-S-2200-glycoside (15min)	ND	ND	0.001	3.31	0.002	2.43	<LOD	0.57
5-CH_2OH-S-2200-glycoside 1 (16min)	ND	ND	<LOD	1.96	0.001	1.41	<LOD	0.89
5-CH_2OH-S-2200-glycoside 2 (16min)	0.012	18.50	0.003	14.94	0.002	2.56	<LOD	2.05
2-CH_2OH-S-2200-glycoside (16min)	ND	ND	ND	ND	ND	ND	<LOD	1.17
4-OH-S-2200-malonyl glycoside (16min)	0.008	12.60	0.002	8.21	0.013	17.26	0.001	6.78
5-CH_2OH-S-2200-glycoside 3 (19min)	0.001	1.97	0.001	6.90	0.002	3.13	0.001	4.23
2-CH_2OH-S-2200	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	0.002	3.35	<LOD	2.43	ND	ND	ND	ND
5-CH_2OH-S-2200	ND	ND	ND	ND	ND	ND	ND	ND
4-OH-S-2200	0.002	2.77	ND	ND	0.001	0.70	ND	ND
5-COOH-S-2200	ND	ND	ND	ND	ND	ND	<LOD	0.95
Unknown (2 min)	0.001	1.62	0.002	9.57	0.004	5.24	0.004	19.16
Unknown (8 min)	ND	ND	ND	ND	0.003	3.90	<LOD	1.05
Unknown (9 min)	ND	ND	<LOD	0.49	0.001	1.27	0.001	6.39
Unknown (9.5 min)	<LOD	0.11	ND	ND	0.005	7.35	ND	ND
Unknown (10 min)	ND	ND	ND	ND	0.001	1.15	ND	ND
Unknown (11 min)	ND	ND	<LOD	1.03	<LOD	0.64	0.001	4.00
Unknown (14 min region)	0.006[1]	8.32[1]	0.004[2]	14.94[2]	0.009[3]	13.03[3]	0.004[4]	18.72[4]
Unknown (20 min)	0.004	5.78	ND	ND	0.006	8.44	ND	ND
Unknown (22 min)	ND	ND	0.002	10.77	ND	ND	0.001	5.94
Others	0.004	6.01	0.001	3.83	0.002	2.11	0.001	2.63
ERR-extracted radioactive residues	**0.053**	**80.57**	**0.015**	**78.87**	**0.061**	**82.25**	**0.016**	**74.55**
URR – Unextracted Radioactive Residues	**0.013**	**19.43**	**0.004**	**21.13**	**0.013**	**17.75**	**0.006**	**25.45**

[1] the 14-minute region consists of multiple minor components, the largest accounted for 0.002 mg/kg (3.01 % TRR).
[2] the 14-minute region consists of multiple minor components, the largest accounted for 0.001 mg/kg (4.14 % TRR).
[3] the 14-minute region consists of multiple minor components, the largest accounted for 0.005 mg/kg (6.90 % TRR
[4] the 14-minute region consists of multiple minor components, the largest accounted for 0.002 mg/kg (7.06 % TRR).

[Phenoxy-^{14}C]S-2200 treated **Carrots (PBI 30 days)**

In **carrot roots** un-metabolised S-2200 was detected at level of 0.040 mg/kg (78.47% TRR). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present except for one unknown polar ^{14}C peak eluting at 2 minutes (HPLC method 1) with a concentration of 0.006 mg/kg (12.24% TRR).

In **carrot foliage** un-metabolised S-2200 was detected at a level of 0.001 mg/kg (1.21% TRR). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14, 15, and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple components, the largest accounted for 0.027 mg/kg (23.6 % TRR). The 15-minute region contained one component (0.002 mg/kg, 1.43% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into three peaks which were identified as 5-CH_2OH-S-2200-glycoside 1 (0.008 mg/kg, 7.21% TRR), 5-CH_2OH-S-2200-glycoside 2 (0.001 mg/kg, 0.93% TRR) and 4-OH-S-2200- malonyl glycoside (0.003 mg/kg, 2.38% TRR). One unknown polar ^{14}C region eluting at 2 minutes (HPLC method 1) at a level of 0.014 mg/kg (12.62 % TRR) did not correspond to any known metabolite of S-2200.

[Benzyl-^{14}C]S-2200 treated **Carrot (PBI 30 days)**

In **carrot roots** un-metabolised S-2200 was detected at level of 0.018 mg/kg (46.77% TRR). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present except for except for one unknown polar ^{14}C region eluting at 2 minutes (HPLC method 1) with a concentration of 0.018 mg/kg (46.32% TRR).

In **carrot foliage** un-metabolised S-2200 was detected at a level of 0.002 mg/kg (3.21% TRR). No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple components, the largest accounted for 0.008 mg/kg (11.4 % TRR). The 16-minute region consisted of one single peak which was identified as 5-CH_2OH-S-2200-glycoside 1 (0.002 mg/kg, 2.23% TRR), by co-chromatography, 1D-TLC and LC-MS/MS. One unknown polar ^{14}C region eluting at 2 minutes (HPLC method 1) at a level of 0.010 mg/kg (13.32 % TRR) did not correspond to any known metabolite of S-2200.

The results for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 treated carrot samples at 30 days plant back interval are summarised in the table below.

Table B.7.9.1-13: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 and in [benzyl-^{14}C]S-2200 treated carrot samples planted at 30 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	30 DAT root		30 DAT foliage		30 DAT root		30 DAT foliage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.051**	**100**	**0.112**	**100**	**0.039**	**100**	**0.074**	**100**
S-2200	0.040	78.47	0.001	1.21	0.018	46.77	0.002	3.21
4-OH-S-2200-glycoside (15min)	ND	ND	0.002	1.43	ND	ND	ND	ND
5-CH_2OH-S-2200-glycoside 1 (16min)	ND	ND	0.008	7.21	ND	ND	0.002	2.23
5-CH_2OH-S-2200-glycoside 2 (16min)	ND	ND	0.001	0.93	ND	ND	ND	ND
4-OH-S-2200-malonyl glycoside (16min)	ND	ND	0.003	2.38	ND	ND	ND	ND
2-CH_2OH-S-2200	ND	ND	ND	ND	ND	ND	ND	ND
MCBX	ND	ND	ND	ND	ND	ND	ND	ND
5-COOH-S-2200	ND	ND	ND	ND	ND	ND	ND	ND

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	30 DAT root		30 DAT foliage		30 DAT root		30 DAT foliage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Unknown (2 min)	0.006[1]	12.24[1]	0.014[1]	12.62[1]	0.018[1]	46.32[1]	0.010[1]	13.32[1]
Unknown (8 min)	ND	ND	0.003	2.29	ND	ND	0.007	9.79
Unknown (9 min)	ND	ND	0.004	3.42	ND	ND	0.003	4.28
Unknown (9.5 min)	ND	ND	ND	ND	ND	ND	0.004	6.08
Unknown (10 min)	ND	ND	0.004	3.20	ND	ND	ND	ND
Unknown (11 min)	ND	ND	0.006	5.43	ND	ND	0.006	7.63
Unknown (14 min region)	ND	ND	0.058[2]	51.15[2]	ND	ND	0.031[3]	42.73[3]
Others	ND	ND	0.001	1.89	ND	ND	ND	ND
ERR-extracted radioactive residues	**0.046**	**90.70**	**0.105**	**93.16**	**0.037**	**93.09**	**0.066**	**89.29**
URR – Unextracted Radioactive Residues	**0.005**	**9.30**	**0.008**	**6.84**	**0.003**	**6.91**	**0.008**	**10.71**

[1)] consists of multiple minor components
[2)] the 14-minute region consists of multiple minor components, the largest accounted for 0.0276 mg/kg (23.6 % TRR).
[3)] the 14-minute region consists of multiple minor components, the largest accounted for 0.008 mg/kg (11.4 % TRR).

[Phenoxy-^{14}C]S-2200 treated **Carrots (PBI 120 days)**

In **carrot roots** un-metabolised S-2200 was detected at level of 0.015 mg/kg (53.40% TRR). The metabolites 2-CH_2OH-S-2200, MCBX and 5-COOH-S-2200 were detected at levels of 0.004 mg/kg (14.35 % TRR), < 0.001 mg/kg (1.72 % TRR) and 0.003 mg/kg (9.4 % TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present.
In **carrot foliage** un-metabolised S-2200 was detected at level of 0.003 mg/kg (4.87% TRR). The metabolites 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-COOH-S-2200 were detected at levels of 0.002 mg/kg (3.10 % TRR), 0.001 mg/kg (1.36 % TRR) and 0.001 mg/kg (1.33 % TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple components, the largest accounted for 0.007 mg/kg (12.85 % TRR). The 16-minute region was separated into two peaks which were identified as 5-CH_2OH-S-2200-glycoside 2 (0.003 mg/kg, 6.18% TRR) and 4-OH-S-2200- malonyl glycoside (0.001 mg/kg, 2.21% TRR) by co-chromatography, 1D-TLC and LC-MS/MS. A number of minor ^{14}C peaks which were grouped together and designated as others accounted for 0.009 mg/kg (17.47% TRR).

[Benzyl-^{14}C]S-2200 treated **Carrot (PBI 120 days)**

In **carrot roots** un-metabolised S-2200 was detected at level of 0.024 mg/kg (60.75% TRR). The metabolites 2-CH_2OH-S-2200 and 5-COOH-S-2200 were detected at levels of 0.001 mg/kg (2.97 % TRR) and 0.001 mg/kg (1.56 % TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present.
In **carrot foliage** un-metabolised S-2200 and metabolite 5-CH_2OH-S-2200 were detected at a level of 0.010 mg/kg (12.42% TRR) and 0.001 mg/kg (1.54% TRR), respectively. No other detectable amounts of ^{14}C residues that corresponded to known metabolites of S-2200 were present. Regions of interest were detected at 14 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple components, the largest accounted for 0.007 mg/kg (8.9 % TRR). The 16-minute region was separated into three peaks which were identified as 5-CH_2OH-S-2200-glycoside 2 (0.002

mg/kg, 2.03% TRR), 2-CH_2OH-S-2200-glycoside (0.001 mg/kg, 1.50% TRR) and 4-OH-S-2200- malonyl glycoside (0.002 mg/kg, 2.56% TRR) by co-chromatography, 1D-TLC and LC-MS/MS. A number of minor ^{14}C peaks which were grouped together and designated as others accounted for 0.011 mg/kg (13.7% TRR). The results for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 treated carrot samples at 120 days plant back interval are summarised in the table below.

Table B.7.9.1-14: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 and in [benzyl-^{14}C]S-2200 treated carrot samples planted at 120 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	120 DAT root		120 DAT foliage		120 DAT root		120 DAT foliage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.028**	**100**	**0.051**	**100**	**0.040**	**100**	**0.077**	**100**
S-2200	0.015	53.40	0.003	4.87	0.024	60.75	0.010	12.42
5-CH_2OH-S-2200-glycoside 1 (16min)	ND	ND	ND	ND	ND	ND	ND	ND
5-CH_2OH-S-2200-glycoside 2 (16min)	ND	ND	0.003	6.18	ND	ND	0.002	2.03
2-CH_2OH-S-2200-glycoside (16min)	ND	ND	ND	ND	ND	ND	0.001	1.50
4-OH-S-2200-malonyl glycoside (16min)	ND	ND	0.001	2.21	ND	ND	0.002	2.56
5-CH_2OH-S-2200-glycoside 3 (19min)	ND	ND	0.001	1.19	ND	ND	ND	ND
2-CH_2OH-S-2200	0.004	14.35	0.002	3.10	0.001	2.97	ND	ND
MCBX	<LOD	1.72	ND	ND	ND	ND	ND	ND
5-CH_2OH-S-2200	ND	ND	ND	ND	ND	ND	0.001	1.54
4-OH-S-2200	ND	ND	0.001	1.36	ND	ND	ND	ND
5-COOH-S-2200	0.003	9.40	0.001	1.33	0.001	1.56	ND	ND
Unknown (2 min)	ND	ND	ND	ND	ND	ND	0.001	1.66
Unknown (8 min)	ND	ND	<LOD	0.47	ND	ND	0.001	0.96
Unknown (9 min)	ND	ND	ND	ND	ND	ND	0.002	2.87
Unknown (9.5 min)	ND	ND	0.001	1.91	ND	ND	0.004	4.78
Unknown (10 min)	ND	ND	0.001	1.41	ND	ND	0.007	9.31
Unknown (11 min)	ND	ND	0.001	1.76	0.004	11.22	0.003	3.76
Unknown (14 min region)	0.001	1.88	0.021[1]	39.82[1]	0.005[2]	11.46[2]	0.024[3]	30.96[3]
Unknown (22 min)	ND	ND	0.002	3.53	ND	ND	0.001	0.96
Others	0.002	5.58	0.009	17.47	0.002	5.18	0.011[4]	13.66[4]
ERR-extracted radioactive residues	**0.025**	**87.65**	**0.046**	**89.46**	**0.037**	**93.15**	**0.069**	**89.76**
URR – Unextracted Radioactive Residues	**0.003**	**12.35**	**0.005**	**10.54**	**0.003**	**6.85**	**0.008**	**10.24**

[1] the 14-minute region consists of multiple minor components, the largest accounted for 0.007 mg/kg (12.85 % TRR).
[2] the 14-minute region consists of multiple minor components, the largest accounted for 0.003 mg/kg (6.40 % TRR).
[3] the 14-minute region consists of multiple minor components, the largest accounted for 0.007 mg/kg (8.9 % TRR).
[4] consists of multiple minor components

[Phenoxy-^{14}C]S-2200 treated **Carrots (PBI 365 days)**

Carrot roots were not further analysed due to the low total radioactive residue (TRR) concentration of 0.008 mg/kg.

In **carrot foliage** un-metabolised S-2200 was not detected. The known metabolites 2-CH_2OH-S-2200, MCBX and 5-COOH-S-2200 were present at levels < 0.001 mg/kg (1-2 %TRR). Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple components, the largest accounted for 0.007 mg/kg (24.17 % TRR). The 15-minute region contained one component (0.001 mg/kg, 4.7% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into four peaks at levels between 0.001-0.002 mg/kg which were identified as 5-CH_2OH-S-2200-glycoside 1 (2.25% TRR), 5-CH_2OH-S-2200-glycoside 2 (5.8% TRR), 2-CH_2OH-S-2200-glycoside (2.16% TRR) and 4-OH-S-2200- malonyl glycoside (3.8% TRR). A number of minor ^{14}C peaks which were grouped together and designated as others accounted for 0.003 mg/kg (10.2% TRR).

[Benzyl-^{14}C]S-2200 treated **Carrot (PBI 365 days)**

Carrot roots were not further analysed due to the low total radioactive residue (TRR) concentration of 0.009 mg/kg.

In **carrot foliage** un-metabolised S-2200 was detected at a level of < 0.001 mg/kg (1.74 %TRR). The known metabolites 2-CH_2OH-S-2200 and 5-COOH-S-2200 were present at levels < 0.001 mg/kg (1 %TRR). Regions of interest were detected at 14, 15 and 16 minutes (HPLC method 1). Analysis of the 14-minute region using HPLC method 4 showed that the region consists of multiple components, the largest accounted for 0.004 mg/kg (15.52 % TRR). The 15-minute region contained one component (0.001 mg/kg, 4.2% TRR) which was identified as 4-OH-S-2200- glycoside by co-chromatography, 1D-TLC and LC-MS/MS. The 16-minute region was separated into four peaks at levels between 0.001-0.002 mg/kg which were identified as 5-CH_2OH-S-2200-glycoside 1 (2.7% TRR), 5-CH_2OH-S-2200-glycoside 2 (6.6% TRR), 2-CH_2OH-S-2200-glycoside (2.2% TRR) and 4-OH-S-2200- malonyl glycoside (4.7% TRR).

The results for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 treated carrot samples at 365 days plant back interval are summarised in the table below.

Table B.7.9.1-15: Identification and characterisation of residues in [phenoxy-^{14}C]S-2200 and in [benzyl-^{14}C]S-2200 treated carrot samples planted at 365 DAT

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	365 DAT root		365 DAT foliage		365 DAT root		365 DAT foliage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR-total radioactive residues	**0.008**	**100**	**0.031**	**100**	**0.009**	**100**	**0.027**	**100**
S-2200	Not profiled		ND	ND	Not profiled		<LOD	1.74
4-OH-S-2200-glycoside (15min)			0.001	4.69			0.001	4.18
5-CH_2-OH-S-2200-glycoside 1 (16min)			0.001	2.25			0.001	2.67
5-CH_2-OH-S-2200-glycoside 2 (16min)			0.002	5.81			0.002	6.56
2-CH_2-OH-S-2200-glycoside (16min)			0.001	2.16			0.001	2.19
4-OH-S-2200-malonyl glycoside (16min)			0.001	3.82			0.001	4.71
5-CH_2-OH-S-2200-glycoside 3 (19min)			0.001	1.80			<LOD	1.80

Metabolite/Residue fraction	[Phenoxy-^{14}C]S-2200				[benzyl-^{14}C]S-2200			
	365 DAT root		365 DAT foliage		365 DAT root		365 DAT foliage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
2-CH_2OH-S-2200			0.001	2.06			<LOD	0.70
MCBX			<LOD	1.37			ND	ND
5-CH2OH-S-2200			ND	ND			ND	ND
5-COOH-S-2200			<LOD	1.14			<LOD	1.27
Unknown (2 min)			0.002	5.11			0.001	3.88
Unknown (8 min)			<LOD	0.43			<LOD	1.45
Unknown (9 min)			ND	ND			<LOD	1.71
Unknown (9.5 min)			ND	ND			ND	ND
Unknown (10 min)			ND	ND			ND	ND
Unknown (11 min)			0.001	3.65			0.001	5.10
Unknown (14 min region)			0.011[1]	36.27[1]			0.009[2]	34.03[2]
Unknown (20 min)			<LOD	0.46			<LOD	0.65
Unknown (22 min)			<LOD	0.47			<LOD	0.63
Others			0.003	10.19			0.002	7.47
ERR-extracted radioactive residues	**0.006**	**77.24**	**0.025**	**81.68**	**0.008**	**84.50**	**0.022**	**80.75**
URR – Unextracted Radioactive Residues	**0.002**	**22.76**	**0.006**	**18.32**	**0.001**	**15.50**	**0.005**	**19.25**

[1)] the 14-minute region consists of multiple minor components, the largest accounted for 0.007 mg/kg (24.17 % TRR).
[2)] the 14-minute region consists of multiple minor components, the largest accounted for 0.004 mg/kg (15.52 % TRR).

Conclusion

A confined accumulation study on rotational crops was conducted to determine the total [^{14}C] residues in lettuce, wheat and carrots and to identify and characterize the nature of the major [^{14}C] residues. [Phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200 was applied to bare soil at a rate of 1.6 kg as/ha. The treated soil was aged for a period of 30, 120 and 365 days prior to direct seeding with lettuce, carrots and wheat. The crops were grown to maturity.

Table B.7.9.1-16: Total radioactive residues in confined rotational crops grown in aged soil treated with [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200

Rotational crop sample		Total Radioactive Residue (mg/kg S-2200 equivalents)[1)]					
		[Phenoxy-^{14}C]S-2200			[Benzyl-^{14}C]S-2200		
		30 DAT	120 DAT	365 DAT	30 DAT	120 DAT	365 DAT
Wheat	Forage	2.73	0.14	0.10	2.54	0.31	0.26
	Hay	1.56	0.29	0.35	4.54	0.72	0.74
	Straw	1.32	0.34	0.31	0.82	0.59	0.38
	Grain	0.04	0.04	0.003[2)]	0.12	0.20	0.005[2)]
Lettuce	Immature	0.33	0.03	0.07	0.32	0.08	0.07
	Mature	0.08	0.02	0.02	0.22	0.05	0.02
Carrot	Mature roots	0.05	0.03	<0.01	0.04	0.04	<0.01
	Mature foliage	0.11	0.05	0.03	0.07	0.08	0.03

DAT: Days after treatment
[1)]…TRR determined by extraction followed by combustion of the debris

[2)]….Sample not extracted. TRR determined by combustion analysis.

In all crops Mandestrobin (S-2200) and the plant metabolites 2-CH_2OH-S-2200, 5-CH_2OH-S-2200, 5-COOH-S-2200, 4-OH-S-2200 and MCBX were found. Mandestrobin (S-2200) was the main component of the residue in 30 and 120 DAT carrot root. Conjugated residues were found in all crops, the most significant metabolites were 4-OH-S-2200 (as glycoside and malonyl-glycoside conjugates), 2-CH_2OH-S-2200 (free and as a glycoside conjugate) and 5-CH_2OH-S-2200 (as glycoside conjugates).

Table B.7.9.1-17: Occurrence of S-2200 and its main metabolites (i.e. > 10% TRR and > 0.05 mg/kg) in the rotational crops (% TRR and mg/kg)

S-2200 and major metabolites	Crop part	PBI	% TRR	mg/kg
S-2200	Carrot root	30 DAT	78.5	0.04
	Carrot root	120 DAT	60.8	0.02
4-OH-S-2200 glycoside	Wheat hay	30 DAT	10.1	0.46
4-OH-S-2200-malonyl glycoside	Wheat forage	30 DAT	23.8	0.60
	Lettuce immature	30 DAT	32.3	0.11
	Lettuce mature	30 DAT	24.8	0.05
2-CH_2OH-S-2200 glycoside	Wheat forage	30 DAT	14.2	0.39
2-CH_2OH-S-2200	Wheat hay	30 DAT	10.2	0.16
5-CH_2OH-S-2200 glycoside	Wheat forage	30 DAT	20.1	0.52

The dose level used in this study (1.6 kg as/ha) represents a highly exaggerated rate relative to the intended application rate of 0.2 kg as/ha for oilseed rape. The results of the study demonstrated that S-2200 and metabolites were taken up by rotational crops, extensively metabolized to polar metabolites and incorporated into the constituents of plant. The metabolism in rotational crops was considered to be essentially the same as in primary crops.

The major metabolism pathway included

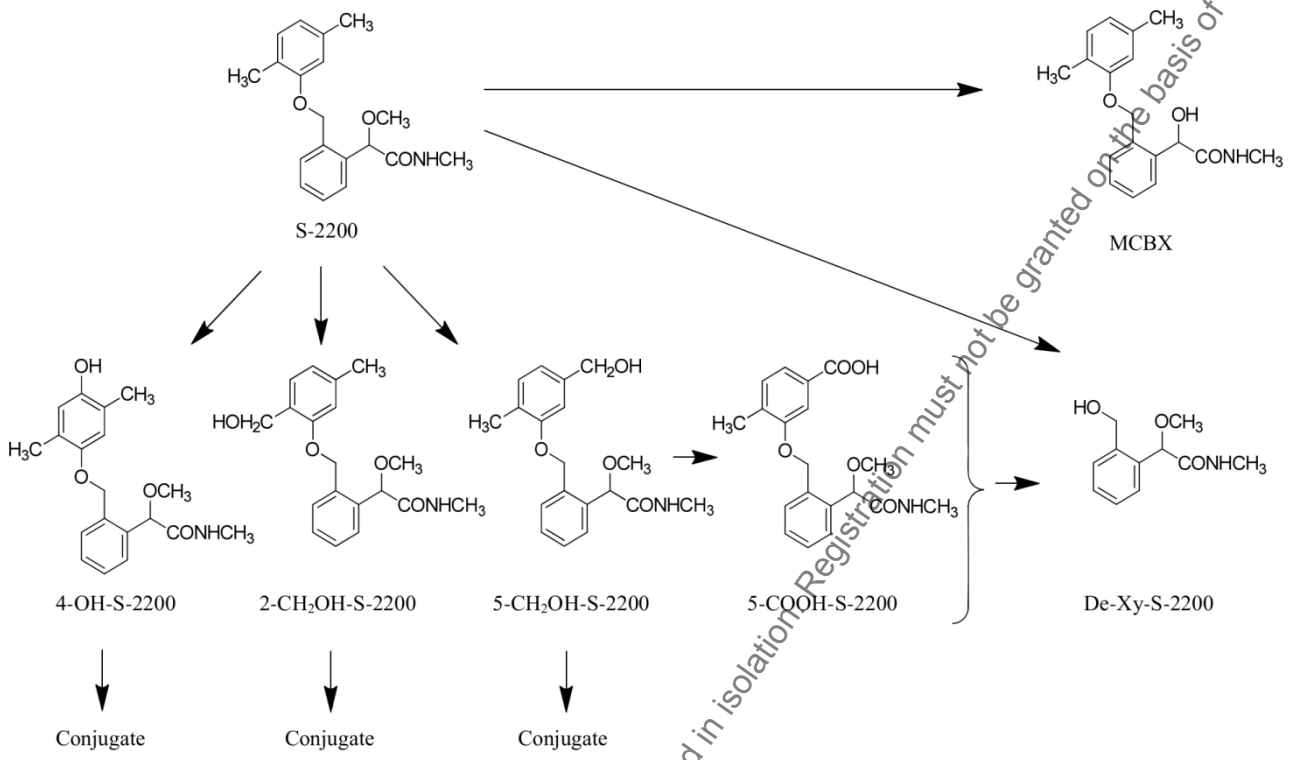
- ✓ hydroxylation of the dimethylphenoxy ring to form 4-OH-S-2200 and subsequent formation of glycoside and malonyl glycoside conjugate
- ✓ oxidation of the methyl group attached to the 2- and the 5-positions of the dimethylphenoxy ring to form 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and the corresponding glycoside conjugate

Minor metabolic pathways included

- ✓ the demethylation of the methoxy group of the side chain to form MCBX and
- ✓ cleavage of the ether link to form De-Xy-S-2200 and
- ✓ further oxidation of 5-CH_2OH-S-2200 to form 5-COOH-S-2200

Further metabolism occurred to form other minor metabolites and polar products.

Figure B.7.9.1-1: Proposed metabolic pathway of Mandestrobin (S-2200) in rotational crops



Code Name	Molecular Weight	Chemical Name
S-2200	313.39	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
MCBX	299.36	(RS)-2-hydroxy-N-methyl-2-[α-(2,5- xylyloxy)-o-tolyl]acetamide
4-OH-S-2200	329.39	(RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide
2-CH_2OH-S-2200	329.39	(RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-CH_2OH-S-2200	329.39	(RS)-2-[2-(5-hydroxymethyl-2- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide
5-COOH-S-2200	343.38	(RS)-3-{2-[1-methoxy-1-(N-methylcarbamoyl) methyl]benzyloxy}-4-methylbenzoic acid

B.7.9.2 Field trials on representative crops (Section 4, Annex IIA, point 6.6.3)

A study was performed in Northern and Southern Europe (France and Spain) following a single application of S-2200 as a 25 SC formulation to winter rapeseed in order to determine residues in succeeding crops of carrots (roots and leaves), lettuce (heads), broccoli (inflorescences) and barley (grain and straw).

Reference:	**Rotational field-crops residue study after application of S-2200 25 SC (25 % w/v) to winter rapeseed**
Author(s), year:	Roussel, Ch.H. (2012)
Report/ Doc. Number:	Staphyt Report no. FLN-10-6268 Sumitomo ref: ROR-0202
Guideline(s):	OECD Guideline 504 EU Document 7029/VI/95 rev.5 (22/7/1997) Appendix B SANCO/825/00 rev.7, 17 March 2004 SANCO/3029/99 rev.4, 11 July 2000 Guidance Document on Pesticide Residue Analytical methods ENV/JM/MONO(2007)17
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Test commodity

Trial-1→ FR01 (Picardie, Northern France)
Primary Crop → Winter oilseed rape (Adriana)
Following Crops → Lettuce (Appia)
Carrot (Nantaise, Touchon)
Broccoli (Marathon F1)
Barley (Sebastian, Cervoise)

Trial-2→ SP01 (Alava, Spain)
Primary Crop → Winter oilseed rape (Corail)
Following Crops → Lettuce (Romana, Batabia)
Carrot (Nantesa
Broccoli (Italica, Clipper)
Barley (Quik, Mane)

Crop parts processed *Oilseed rape* → whole plants
Lettuce → *heads*
Carrot → roots and leaves
broccoli → inflorescences
Barley → grain and straw

Soil physiochemical properties

FR01 (Northern France)	pH (in water)	7.5
	CEC (meq/100g)	10.5
	Organic matter (%)	1.7
	Sand (%)	40
	Silt (%)	45
	Clay (%)	15
	Soil classification (USDA)	Loam
SP01 (Spain)	pH (in water)	7.6
	CEC (meq/100g)	6.8
	Organic matter (%)	2.2
	Sand (%)	78
	Silt (%)	10
	Clay (%)	12
	Soil classification	Sandy

	(USDA)	loam

Test Material
Formulation → S-2200 25 SC
Formulation type → Soluble concentrate
Purity → 23.82 % w/v (250.9 g/L)
CAS → 173662-97-0
Expiry date → October 2012
Fortification levels → S-2200 R-isomer and S-2200 S-isomer: 0.005, 0.05 and 1.0 mg/kg
4-OH-S-2200, 2-CH2OH-S-2200 and 5-CH2OH-S-2200: 0.01 and 0.1 mg/kg

Material and Methods

Two supervised field trials were conducted in Northern France (trial FR01) and Spain (trial SP01). Each trial consisted of two plots, one untreated (U) and one treated plot (T). Each plot was divided into three subplots for the plant back intervals (PBI) of 14,120 and 365 days. Each PBI subplot was then divided into 4 individual subplots for the rotational crops carrots, lettuce, broccoli and barley at each plant back interval. S-2200 was applied once as a 25SC formulation on the preceding crop (winter rapeseed) at growth stage BBCH 65 (full flowering, 50% flowers on main raceme open, older petals falling) at a rate of 200 g a.s./ha and spray volume 300 L/ha. 14 days after application, the oilseed rape crop was mechanically destroyed (crushed) and incorporated into the soil. After destruction of the preceding winter oilseed rape crop, each plot was divided into 12 sub-plots. Succeeding crops (carrot, lettuce, broccoli and barley) were sown or transplanted into the plots at nominal plant-back intervals of 14, 120 and 365 days after treatment (DAT). Crops were maintained according to normal agricultural practices, and irrigated as required.

Sampling

Samples of oilseed rape whole plant preceding crop were collected within 4 hours after application and analysed for the R- and S-isomers of S-2200.
The succeeding crops were harvested at their commercial harvest dates and the following commodities collected: carrot roots and leaves, lettuce heads, broccoli inflorescences and barley grain and straw.
Rotational crop samples were analysed for S-2200 (R- and S-isomers) and the metabolites 4-OH-S-2200 (free and conjugated), 2-CH_2OH-S-2200 (free and conjugated) and 5-CH_2OH-S-2200 (free and conjugated).

The quantities of barley grain harvested from trial FR01 (14 day plant back interval) were low as the growth conditions did not allow normal development of the crop. The analytical method was therefore adapted for grain to allow the use of smaller sample sizes.

Analytical procedure for S-2200 *R*- and *S*-isomers (see also B.7.6.2)

Samples were analysed for S-2200 (R- and S-isomers) using an analytical method based on the extended and revised version of DFG Method S19 (Multi method L 00.00-34 of the Official Collection of Test Methods according to § 64 LFGB). The method consisted of extraction with a mixture of acetone and water 2/1, v/v) according to module E1 (for winter rapeseed, lettuce, carrot leaves and roots and broccoli) and ethyl acetate / cyclohexane (1/1, v/v) according to module E9 (for barley grains and straw) of modular DFG S19 method followed by chiral LC-MS/MS detection of S-2200 (R- and Sisomers).
The limit of quantitation (LOQ) for both S-2200 R-isomer and S-2200 S-isomer was 0.005 mg/kg. The limit of detection (LOD) was 0.0015 mg/kg for each isomer.
Recovery values for the R-isomer (S-2167) and the S-isomer (S-2354) of S-2200 are given in the table below.

Table B.7.9.2-1: Recoveries of S-2200 *R*-isomer and S-2200 *S*-isomer for the rotational crops

Matrix	Fortification level	Analytical Recovery					
		S-2200 *R*-isomer (S-2167)			S-2200 *S*-isomer (S-2354)		
		Individual (%)	Mean (%)	RDS (%)	Individual (%)	Mean (%)	RDS (%)
Winter oilseed rape whole plant	0.005	82	87	5.4	85	87	3.7
	0.05	89			91		
	1.0	91			86		
Lettuce heads	0.005	86, 93, 89	89	3.4	91, 88, 95	94	6.8
	0.05	85, 88, 91			93, 105		
Carrot roots	0.005	92, 92, 95, 96	93	2.5	78, 92, 73	82	12
	0.05	91, 96, 91			83, 94, 72		
Carrot leaves	0.005	95, 96, 82	93	6.4	84, 77, 71	77	6.6
	0.05	99, 91,95			80, 71, 77		
Broccoli	0.005	92, 83	84	6.1	85, 93, 85	91	7.7
	0.05	78, 82, 85			91, 102		
Barley grain	0.005	91, 75, 76	81	9.0	86, 80, 79	83	11
	0.05	76, 86			98, 72, 81		
Barley grain*	0.005	88, 91, 91	90	1.9	97, 95, 110	101	8.1
	0.05	72, 89, 90	84	12	83, 96, 97	92	8.5
Barley straw	0.005	75, 72	80	17	81, 94	83	7.5
	0.05	82, 70, 103			79, 79, 83		

*Analysis performed using reduced sample size of 1g

Metabolites to be analyzed were selected based on the results of the confined rotational crop study, in which 2-CH_2OH-S-2200 (conj), 2- CH_2OH-S-2200 (free), 4-OH-S-2200 (conj) and 5-CH_2OH-S-2200 (conj), were found greater than 10%TRR. The analytical procedure for the metabolites 4-OH-S-2200 and 2-CH_2OH-S-2200 has already been described in chapter B.7.6.2.

Analytical procedure for 5-CH_2OH-S-2200

Reference:	Validation of an Analytical Method for Determination of S-2200 Metabolites, 5-CH_2OH-S-2200 and its Conjugates, in Barley (Grain and Straw) and Lettuce (Head)
Author(s), year:	Daneva, E. & Taeufer, A., (2011b)
Report/ Doc. No.	Sumitomo Chemical Co. Ltd, Report No. ROA-0013, SUM-1027V
Guideline(s):	SANCO/3029/99 rev. 4 of 11/07/00 of the European Commission
GLP:	Yes (certified laboratory)
Deviations:	No deviations.
Validity:	Yes
Comment (RMS):	The study is acceptable

Samples of lettuce and barley (grain and straw) were extracted twice with a mixture of acetone and water (4/1, v/v). The pH was adjusted to pH 11 with 0.1M sodium hydroxide, and left for 1 hour at room temperature. The pH was then adjusted to pH 7 by the addition of 0.1M hydrochloric acid, 10 mM sodium acetate-acetic acid buffer solution (pH 5) and ß-glucosidase were added. The hydrolysed extract was then cleaned-up using a Chem Elut cartridge followed by solid phase extraction using an Oasis HLB cartridge. The final eluate in acetonitrile/water (1:1 v/v) was diluted with acetonitrile/water (1:1 v/v) for determination of

5-CH_2OH-S-2200 by liquid chromatography with tandem mass spectrometry (LC-MS/MS) based on two mass transitions (the ion transition m/z 328 → 137 for quantification and m/z 330 → 192 for confirmation). The limit of quantification (LOQ) for 5-CH_2OH-S-2200 was 0.01 mg/kg for all matrices. The limit of detection (LOD), defined as the lowest concentration where a signal of the analyte could be detected, was determined to be 0.003 mg/kg for all matrices.

Recovery values for all individual metabolites 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 are given in the table below.

Table B.7.9.2-2: Recoveries of 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200

Matrix	Fortification level	Analytical Recovery								
		4-OH-S-2200			2-CH_2OH-S-2200			5-CH_2OH-S-2200		
		Individual (%)	Mean (%)	RDS (%)	Individual (%)	Mean (%)	RDS (%)	Individual (%)	Mean (%)	RDS (%)
Lettuce heads	0.01	106	88	-	102, 83	94	9.5	90, 87	89	2.5
	0.1	70			100, 90			92, 88		
Carrot roots	0.01	87, 88, 95, 110	96	12	92, 97	93	8.1	91, 100, 81, 94	87	8.7
	0.1	80, 110, 99			89, 89, 106, 85			80, 84, 84, 79		
Carrot leaves	0.01	74, 74, 74, 85	78	6.2	79, 98, 100	92	13	93, 75, 93, 74	79	11
	0.1	82, 80			78, 106, 90			74, 75, 71, 79		
Broccoli	0.01	70, 73	77	13	72	85	-	71, 83	80	9.9
	0.1	91, 73			98			86		
Barley grain	0.01	92, 72, 92	86	9.5	93, 101, 94, 87	90	7.9	85, 95, 76	80	11
	0.1	91, 80, 88			94, 88, 78, 84			79, 76, 71		
Barley grain*	0.01	88, 112, 101	100	12	80, 84, 75	80	5.7	76, 83, 81	80	4.5
	0.1	75, 71, 70	72	3.7	103, 81, 86	90	13	85, 82, 82	83	2.1
Barley straw	0.01	87, 71, 103	85	14	90, 76	81	12	80	79	9.6
	0.1	86, 77			88, 70			86, 71		

*Analysis performed using reduced sample size of 1g

Results

Residues of S-2200 (sum of isomers) in preceding oilseed rape were 1.9-2.6 mg/kg. No detectable residues of the R- or S-isomers of S-2200 or the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 were found in any of the succeeding crop samples. No residues of S-2200 were found in the control samples. The results are summarised in the tables below.

Table B.7.9.2-3: Residues of S-2200 and metabolites in field rotational crops grown in N-France

Plant back interval	Crop		Trial FR01 (Northern France): Residue levels (mg/kg)					
		DALA*	S-2200 *R*-isomer	S-2200 *S*-isomer	Total S-2200	4-OH-S-2200 (free + conj)	2-CH_2OH-S-2200 (free + conj)	5-CH_2OH-S-2200 (free + conj)
Primary crop	Oilseed rape whole plant	0	1.4	1.2	2.6			
14 days	Lettuce	74	ND	ND	ND	ND	ND	ND
	Carrot roots Carrot leaves	144	ND	ND	ND	ND	ND	ND
	Broccoli	144	ND	ND	ND	ND	ND	ND
	Barley grain Barley straw	151	ND	ND	ND	ND	ND	ND
120 days	Lettuce	194	ND	ND	ND	ND	ND	ND
	Carrot roots Carrot leaves	382	ND	ND	ND	ND	ND	ND
	Broccoli	167	ND	ND	ND	ND	ND	ND
	Barley grain Barley straw	416	ND	ND	ND	ND	ND	ND
365days	Lettuce	441	ND	ND	ND	ND	ND	ND
	Carrot roots Carrot leaves	508	ND	ND	ND	ND	ND	ND
	Broccoli	441	ND	ND	ND	ND	ND	ND
	Barley grain Barley straw	480	ND	ND	ND	ND	ND	ND

*DALA = Days after last application of S-2200 25 SC to the primary crop (winter oilseed rape).
ND = not detectable.
LOD = 0.0015 mg/kg for S-2200 *R*-isomer and S-2200 *S*-isomer.
LOD = 0.003 mg/kg for 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200.

Table B.7.9.2-4: Residues of S-2200 and metabolites in field rotational crops grown in Spain

Plant back interval	Crop		Trial FR01 (Northern France): Residue levels (mg/kg)					
		DALA*	S-2200 *R*-isomer	S-2200 *S*-isomer	Total S-2200	4-OH-S-2200 (free + conj)	2-CH_2OH-S-2200 (free + conj)	5-CH_2OH-S-2200 (free + conj)
Primary crop	Oilseed rape whole plant	0	0.97	0.94	1.9			
14 days	Lettuce	58	ND	ND	ND	ND	ND	ND
	Carrot roots Carrot leaves	106	ND	ND	ND	ND	ND	ND
	Broccoli	103	ND	ND	ND	ND	ND	ND
	Barley grain Barley straw	106	ND	ND	ND	ND	ND	ND
120 days	Lettuce	173	ND	ND	ND	ND	ND	ND
	Carrot roots Carrot leaves	326	ND	ND	ND	ND	ND	ND
	Broccoli	240	ND	ND	ND	ND	ND	ND
	Barley grain Barley straw	296	ND	ND	ND	ND	ND	ND
365days	Lettuce	414	ND	ND	ND	ND	ND	ND

Plant back interval	Crop	DALA*	Trial FR01 (Northern France): Residue levels (mg/kg)					
			S-2200 *R*-isomer	S-2200 *S*-isomer	Total S-2200	4-OH-S-2200 (free + conj)	2-CH_2OH-S-2200 (free + conj)	5-CH_2OH-S-2200 (free + conj)
	Carrot roots Carrot leaves	454	ND	ND	ND	ND	ND	ND
	Broccoli	452	ND	ND	ND	ND	ND	ND
	Barley grain Barley straw	456	ND	ND	ND	ND	ND	ND

*DALA = Days after last application of S-2200 25 SC to the primary crop (winter oilseed rape).
ND = not detectable.
LOD = 0.0015 mg/kg for S-2200 *R*-isomer and S-2200 *S*-isomer.
LOD = 0.003 mg/kg for 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200.

The storage stability of both S-2200 isomers and metabolites in specimen extracts in the refrigerator was confirmed by procedural recoveries which were analysed in parallel with the field specimens.

Conclusion
Residues of S-2200 in the preceding crop oilseed rape whole plant were 2.6 mg/kg (N-France) and 1.9 mg/kg (Spain), respectively on the day of application. No detectable residues of the R- or S-isomers of S-2200 or the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 were found in any of the succeeding crop samples planted 14, 120 or 365 days after application to winter oilseed rape.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post harvest uses

Based on the intended use and the residue data provided for oilseed rape the definition of a waiting period is considered not necessary. The latest application of mandestrobin (S-2200) was at growth stage BBCH 63-67 (flowering), the proposed pre harvest interval (PHI) for rape seed is covered by the vegetation period remaining between last application and harvest.

B.7.11 Withholding period for animal feedingstuffs

Residues in rape seed at harvest were less than the LOQ (0.01 mg/kg). Based on the intended use and the residue data provided the definition of a waiting period is considered not necessary.

B.7.12 Waiting period between last treatment and handling treated products

Refer to section B.6, Toxicology.

B.7.13 Waiting period between last application and sowing or planting succeeding crops

Studies on residues in succeeding crops show that the setting of a waiting period between the last application and sowing or planting of succeeding crops is not necessary.

B.7.14 Community MRL and MRLs in EU Member States

Mandestrobin (S-2200) is a new active substance in the EU. Therefore no EU MRLs currently exist.
The application for a MRL according to Regulation 396/2005 was submitted at the same time as for the approval of the active substance. The intended use is identical for both the approval of the actie substance and the application for MRL setting. Following the agreed procedure, the draft assessment report (DAR) provided is the basis for MRL setting assessment (please refer also to point B.7.20).

B.7.15 Proposed EU MRLs and justification for the acceptability of those MRLs

Mandestrobin (S-2200) is a systemic fungicide and intended to be used against *Sclerotinia* in Winter Oilseed Rape. Details of intended uses (supported by the applicant and for which data are provided) according to Good Agricultural Practice (GAP) are summarized in the table below.

Table B.7.15-1: Good Agricultural Practices (GAPs) for mandestrobin (S-2200) in oilseed rape

Crop	N / S	Formulation type, content of as (%)	Application				PHI (d)
			Method latest stage	No of applications	Rate (kg as/ha)	Water (L/ha)	
Winter oilseed rape	N / S	SC, 25	Foliar spraying BBCH 63-67[1]	1	0.2	100-300	-[2]

[1] …BBCH 63: 30% of flowers on main raceme open
BBCH 67: Flowering declining: majority of petals fallen
[2] …the waiting period is covered by the vegetation period which remains between application and harvest

A total of 12 supervised residue trials (6 harvest trials and 6 decline trials) were conducted in winter oilseed rape during 2010 and 2011 in Northern Europe (N France, Germany, UK) and Southern Europe (S France). Mandestrobin (S-2200) was applied once at 200 g/ha to oilseed rape at growth stage BBCH 65.
Crop metabolism studies have shown that in addition to parent S-2200, the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were found at levels >10% TRR. Thus the determination of residues in oilseed rape included the *R*- and *S*-isomers of parent S-2200 and the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated).
In mature seed collected 56-91 days after application, residues of S-2200 and residues of the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were all below the limit of quantification (<0.01 mg/kg).
The ratio of R- and S-isomers of S-2200 remained approximately 50:50 in the oilseed rape samples.

Table B.7.15-2: Residue data for Mandestrobin (S-2200) for monitoring

Commodity	Trial results relevant to critical GAP (mg/kg)	No. of trials	STMR (mg/kg)	HR (mg/kg)	Proposed MRL	
					EU	OECD
Rape seed (EU north)	< 0.01 (8x)	8	< 0.01	< 0.01	0.01*	0.01*
Rape seed (EU south)	< 0.01 (4x)	4	< 0.01	< 0.01	0.01*	0.01*

Table B.7.15-3: Residue data for Mandestrobin (S-2200) for riskassessment

Commodity		mg/kg	No trials	f[2)]	mg as-equiv./kg	CF[4)] median
Rape seed (EU north)	**S-2200[1)]**	< 0.01 (8x)	8		< 0.01	4.0
	De-Xy-S-2200	< 0.01 (8x)	8	1.4995	< 0.015 (8x)	
	4-OH-S-2200	< 0.01 (8x)	8	0.9526	< 0.0095 (8x)	
	2-CH_2OH-S-2200	< 0.01 (8x)	8	0.9526	< 0.0095 (8x)	
	S-2200 + metabolites				**< 0.04**	
Rape seed (EU south)	**S-2200[1)]**	< 0.01 (4x)	4		< 0.01	4.0
	De-Xy-S-2200	< 0.01 (4x)	4	1.4995	< 0.015 (4x)	
	4-OH-S-2200	< 0.01 (4x)	4	0.9526	< 0.0095 (4x)	
	2-CH_2OH-S-2200	< 0.01 (4x)	4	0.9526	< 0.0095 (4x)	
	S-2200 + metabolites				**< 0.04**	

[1)] sum of R-isomer and S-isomer

[2] f= S-2200/metabolite →S-2200/De-Xy-S-2200= 313.39/209, S-2200/4-OH-S-2200= 313.39/329), S-2200/2-CH_2OH-S-2200= 313.39/329
[3] metabolites expressed as mg as equiv./kg →mg/kg*f
[4] Conversion factor (CF) for monitoring→ riskassessment = S-2200+ metabolites/S-2200

Based on the available residue data a MRL of 0.01* mg/kg for Mandestrobin (S-2200) in winter oilseed rape is proposed.

B.7.16 Proposed EU import tolerances and justification for the acceptability of those residues

Mandestrobin (S-2200) is a new active substance, no import tolerances are proposed and a listing of MRLs established in exporting countries is not needed at the moment.

B.7.17 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

Mandestrobin (S-2200) has not been scheduled for evaluation by the JMPR (Joint Meeting on Pesticide Residues). Data or information has not yet been provided to JMPR.

B.7.18 Estimates of potential and actual dietary exposure through diet and other means

The dietary risk was identified due to residues resulting from the use of Mandestrobin (S-2200) according to the uses in oilseed rape. According to the residue definition for risk assessment the exposure assessment is performed for the sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200). The toxicological reference values are summarised in the following table:

Table B.7.18-1: Toxicological reference values

End Point	Value	Comment
ADI	0.19 mg/kg bw/d	52 week dog study, SF 100
ARfD	-	Not necessary

Table B.7.18-2: Summary of residue data according to the Residue definition

Commodity	Trial results relevant to critical GAP (mg/kg)		STMR mg/kg	HR mg/kg	MRL proposal	CF[1]
	Monitoring mandestrobin (S-2200)	Riskassessment sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200)				
Oilseed rape (EU north)	8x < 0.01	8x < 0.04	< 0.01	< 0.01	0.01*	4
Oilseed rape (EU South)	4x < 0.01	4x < 0.04	< 0.01	< 0.01	0.01*	4

[1] CF= conversion factor
MW (S-2200)= 313.39; MW (De-Xy)= 209; MW (4-OH-S-2200, 2-CH_2OH-S-2200) = 329
f (S-2200/De-Xy) = 1.4995; f (S-2200/4-OH)= 0.9526; f (S-2200/2-CH_2OH)= 0.9526 kg

Table B.7.18-3: Input values for the consumer risk assessment

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition for risk assessment: sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200)				
Oilseed rape	0.04	proposed MRL x CF[1)] according to residue definition for riskassessment	No ARfD set	No acute riskassessment performed

[1)]….CF= conversion factor= 4

B.7.18.1 Estimation of chronic exposure through diet

The chronic risk assessment for Mandestrobin (S-2200) was carried out by means of the EFSA chronic acute RA model rev.2 taking into account the proposed MRL for oilseed rape resulted from the supervised residue trials.

Table B.7.18.1-1: Theoretical Maximum Daily Intake (TMDI) of Mandestrobin residues presented as % exhaustion of the Acceptable Daily Intake

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities
0,013	WHO cluster diet E	0,013	Rape seed
0,007	WHO Cluster diet F	0,007	Rape seed
0,003	WHO regional European diet	0,003	Rape seed
0,001	WHO Cluster diet B	0,001	Rape seed

The estimated Theoretical Maximum Daily Intakes (TMDI) for Mandestrobin (S-2200) is below the ADI (< 0.1 %) for all consumer groups. Thus no chronic consumer risk could be identified.

B.7.18.2 Estimation of the acute exposure through diet

No ARfD has been proposed by the RMS. No residues above the limit of quantification are to be expected and therefore no acute exposure assessment has been performed.

B.7.19 Summary and evaluation of residue behaviour

Mandestrobin (S-2200) is a new active substance and a fungicide used for the control of white mould (*Sclerotinia sclerotiorum*). The envisaged use is oilseed rape only. **Metabolism, distribution and expression of residues in plants** have been investigated on rapeseed (oilseeds), wheat (cereal crop) and lettuce (leafy crop) using two radiolabeled forms of [^{14}C]S-2200, [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200, as a 25% SC formulation. An overview on the application details is given in the table below.

Crop	Plant parts analysed	Category*	Application rate [g as/ha]	x GAP**	Comment RMS
Oilseed rape	Forage, seed	Pulses & oilseeds	2x 400 1x 400	2x	Valid
Wheat	Forage, hay, straw, grain	Cereals	1x 300	1.5x	Valid

Crop	Plant parts analysed	Category*	Application rate [g as/ha]	x GAP**	Comment RMS
Lettuce	Immature lettuce Mature lettuce	Leafy crops	2x 800	4x	Valid

* according Document 7028/VI/95 rev.3 (22.7.97), Appendix A, Metabolism, distribution and expression of residues in plants

** proposed GAP for oilseed rape: 1x 200 g as/ha

The results are compareable for both radiolables. The distribution of [^{14}C] residues in the different crops is presented in the tables below.

B.7.19-1: Oilseed rape (forage and seed)

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		**[benzyl-^{14}C]S-2200**	
		mg/kg	%TRR	mg/kg	%TRR
Forage 2 x 400 g/ha PHI 14 days	Surface wash	1.47	36.7	1.18	34.16
	Sum of extracts [1)]	2.19	54.9	2.02	58.32
	PES (post extraction solids)	0.34	8.4	0.26	7.53
	Total Radioactive Residue (TRR)	**3.99**	**100**	**3.44**	**100**
	Total identified	2.9	72.8	2.25	65.0
	Total characterised	0.75	18.8	0.95	27.5
Seed 2 x 400 g/ha PHI 40 days	Surface wash	not performed			
	Sum of extracts	0.40	85.4	0.64	99.9
	PES (post extraction solids)	0.07	14.6	<0.01	0.1
	Total Radioactive Residue (TRR)	**0.47**	**100**	**0.64**	**100**
	Total identified	0.27	58.1	0.32	46.2
	Total characterised	0.14	27.2	0.33	53.8
Seed 1 x 400 g/ha PHI 54 days	Surface wash	not performed			
	Sum of extracts	0.04	81.45	0.10	90.74
	PES (post extraction solids)	<0.01	18.55	0.01	9.26
	Total Radioactive Residue (TRR)	**0.05**	**100**	**0.11**	**100**
	Total identified	<0.01	20.3	-	-
	Total characterised	0.02	61.2	0.10	90.7

[1)].....excluding surface wash

Table B.7.19-2: Wheat (forage, hay, straw and grain)

	Fraction	**Total Radioactive Residue**			
		[phenoxy-^{14}C]S-2200		**[benzyl-^{14}C]S-2200**	
		mg/kg	**%TRR**	**mg/kg**	**%TRR**
Wheat forage 1 x 300 g/ha PHI 7 days	Surface wash	4.57	41.0	3.54	33.9
	Sum of extracts [1)]	5.93	53.2	6.33	60.6
	PES (post extraction solids)	0.64	5.8	0.57	5.5
	Total Radioactive Residue (TRR)	**11.14**	**100**	**10.44**	**100**
	Total identified	7.96	71.4	8.48	81.3
	Total characterised	2.55	22.8	1.39	13.2
Wheat hay 1 x 300 g/ha PHI 14 days	Surface wash	1.45	23.3	1.73	19.1
	Sum of extracts [1)]	4.09	65.8	6.58	72.6
	PES (post extraction solids)	0.68	10.9	0.73	8.1
	Total Radioactive Residue (TRR)	**6.21**	**100**	**9.04**	**100**
	Total identified	3.66	58.9	4.61	50.9
	Total characterised	1.89	30.2	3.71	40.9

Wheat straw 1 x 300 g/ha PHI 104 days	Surface wash	0.07	3.7	0.07	2.8
	Sum of extracts [1)]	1.09	58.7	1.61	64.7
	PES (post extraction solids)	0.70	37.6	0.81	32.5
	Total Radioactive Residue (TRR)	**1.85**	**100**	**2.49**	**100**
	Total identified	0.33	17.7	0.73	29.6
	Total characterised	0.83	44.7	0.96	37.9
Wheat grain 1 x 300 g/ha PHI 104 days	Surface wash	Not performed		Not performed	
	Sum of extracts [1)]	<0.01	67.0	0.07	72.7
	PES (post extraction solids)	<0.01	33.0	0.02	27.3
	Total Radioactive Residue (TRR)	**0.01**	**100**	**0.09**	**100**
	Total identified	n.a.	n.a.	0.06	66.4
	Total characterised	<0.01	66.97	0.01	9.0

[1)].....excluding surface wash [2)].....including surface wash n.a. =not applicable

Table B.7.19-3: Lettuce (immature and mature)

	Fraction	Total Radioactive Residue			
		[phenoxy-^{14}C]S-2200		**[benzyl-^{14}C]S-2200**	
		mg/kg	%TRR	mg/kg	%TRR
Immature Lettuce 2 x 800 g/ha PHI 5 days	Surface wash	31.03	88.4	24.5	87.8
	Sum of extracts [1)]	4.01	11.4	3.3	12.0
	PES (post extraction solids)	0.07	0.2	0.06	0.2
	Total Radioactive Residue (TRR)	**35.11**	**100**	**27.94**	**100**
	Total identified	34.46	97.1	27.05	96.8
	Total characterised	0.59	1.65	0.84	2.2
Mature Lettuce 2 x 800 g/ha PHI 5 days	Surface wash	35.32	81.9	32.64	78.5
	Sum of extracts [1)]	7.33	17.0	8.50	20.4
	PES (post extraction solids)	0.49	1.1	0.44	1.1
	Total Radioactive Residue (TRR)	**43.14**	**100**	**41.59**	**100**
	Total identified	41.5	96.2	39.72	95.5
	Total characterised	1.17	2.7	1.43	3.4

[1)].....excluding surface wash

Mandestrobin (S-2200) is extensively metabolised in crops. The route of the metabolism of Mandestrobin (S-2200) has been shown to be similar in all three crop groups. The main route of metabolism in crops is via hydroxylations and oxidations, and subsequent glycoside conjugation, to yield the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200, 5-CH_2OH-S-2200 and 5-COOH-S-2200 and their conjugates. Minor metabolic pathways involved demethylation of the methoxy group of the side chain to form MCBX, and cleavage of the ether linkage to form De-Xy-S-2200.

S-2200 was a major component of the residue in all crops. The *R*/*S* ratio of [^{14}C]S-2200 remained approximately 1:1 indicating no *R*/*S* isomerization in all tested crops. The major metabolites found at levels >10% TRR were 4-OH-S-2200 (conjugated), 2-CH_2OH-S-2200 (conjugated) and De-Xy-S-2200. An overview is given in the table below.

Table B.7.19-4: Occurrence of S-2200 and its main metabolites in the primary crops (> 10% TRR and > 0.05 mg/kg)

	Crop part	% TRR	mg/kg
S-2200	Rape forage	22.4	0.77
	Rape seed	30.7	0.14
	Wheat forage	59.9	6.25
	Wheat hay	26.2	1.63
	Wheat straw	1.99	0.05
	Wheat grain	nd	nd
	Mature lettuce	91.1	39.3
De-Xy-S-2200	Wheat grain	60.6	0.54
	Wheat straw	11.8	0.29
4-OH-S-2200 conjugate	Rape forage	35.6	1.42
	Rape seed	14.5	0.07
	Wheat hay	13.1	0.81
2-CH_2OH-S-2200 conjugate	Rape forage	12.4	0.43
	Wheat forage	10.6	1.18
	Wheat hay	12.6	1.14

The metabolites detected in the metabolism studies are not of toxicological concern and are covered by the rat metabolism, however in order to cover the whole toxicological burden for the consumer the following residue definitions are proposed: **Residue definition for monitoring purposes:** mandestrobin (S-2200), **Residue definition for risk assessment:** sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200).

A metabolism study each on poultry and on a lactating goat was designed to assess the **excretion, distribution and metabolism of mandestrobin (S-2200) in livestock**. The studies on two groups of ten laying hens laying hens and two lactating goats have been investigated following repeated oral administration of [phenoxy-^{14}C]S-2200 and [benzyl-^{14}C]S-2200. A summary of the dosing regime is given in the table below.

Animal tested	Dosing period	Label	Dose level			
			mg as/kg feed per day	mg as/kg bw per day[1)]	mg as/animal per day	mg as/animal (total dose)
Laying hens	14 days	[phenoxy-^{14}C]S-2200	13.24	1.04	1.8	25.2
		[benzyl-^{14}C]S-2200	13.04	0.87	1.8	25.2
Lactating goats	7 days	[phenoxy-^{14}C]S-2200	12.65	0.31	16.03	112.2
		[benzyl-^{14}C]S-2200	14.33	0.59	35.05	245.3

1)..... mean body weight at the begin of dosing for the [phenoxy-^{14}C]S-2200 and the [benzyl-^{14}C]S-2200 label, respectively :
laying hens → 1.73 kg and 2.07 kg
lactating goats → 51 kg and 59 kg

The available metabolism studies on laying hens and lactating goats showed that the metabolic pathways in livestock were similar to that found in the rat. Mandestrobin (S-2200) is extensively metabolised and mainly excreted in the hen (83-98% of the dose) and goat. The route of the metabolism of S-2200 has been shown to be similar and proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation and ether hydrolysis. Parent S-2200 was the main component of the residue in eggs, milk fat, muscle (goat) and fat (hen and goat), the main metabolites were 5-COOH-S-2200 (goat kidney and liver), 4-OH-S-2200 (in hen liver and as the glucuronide in goat kidney) and De-Xy-S-2200 (hen liver). The primary metabolites are

further metabolised by conjugation, thus S-2200, De-Xy-S-2200, 4-OH-S-2200 and 5-CA-2-HM-S-2200 were present in liver in bound/conjugated form. No significant accumulation of residues in tissues, particularly fatty tissues, has been observed.
With the exception of organs for excretions (liver, kidney), the total radioactive residues were low (0.01-0.05 mg/kg) and individual compounds of degradation do not exceed 0.05 mg/kg. Residues in oilseed rape seed are below the limit of quantification, therefore no determinable residues of S-2200 are expected in products of animal origin, however based on the results provided **a residue definition for products of animal origin** can be proposed as mandestrobin (S-2200) for monitoring and risk assessment.

The **proposed critical GAP** (Northern and Southern Europe) for Mandestrobin (S-2200) in winter oilseed rape is one application per crop and season at 0.2 kg as/ha at BBCH 63-67 (flowering declining). The pre-harvest interval (PHI) is defined by the application conditions at the BBCH growth stages stated.

Crop	N / S	Formulation type, content of as (%)	Application				PHI (d)
			Method latest stage	No of applications	Rate (kg as/ha)	Water (L/ha)	
Winter oilseed rape	N / S	SC, 25	Foliar spraying BBCH 63-67[1]	1	0.2	100-300	-[2]

[1] …BBCH 63: 30% of flowers on main raceme open
BBCH 67: Flowering declining: majority of petals fallen
[2] …the waiting period is covered by the vegetation period which remains between application and harvest.

A total of 12 supervised residue trials conducted in 2010 and 2011 have been provided to support the use of Mandestrobin (S-2200) on oilseed rape seed in Northern and Southern Europe. Mandestrobin (S-2200) was applied once at 0.2 kg/ha to oilseed rape at growth stage BBCH 65. In mature seed collected 56-91 days after application, residues of S-2200 and residues of the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were all below the limit of quantification (<0.01 mg/kg). The ratio of R- and S-isomers of S-2200 remained approximately 50:50 in the oilseed rape samples.

Table B.7.19-5: Residue data for Mandestrobin (S-2200) for monitoring and risk assessment

Commodity	Trial results relevant to critical GAP (mg/kg)		STMR mg/kg	HR mg/kg	MRL proposal	CF[1)]
	Monitoring mandestrobin (S-2200)	Riskassessment sum of mandestrobin (S-2200), De-Xy-S-2200, 4-OH-S-2200 conjugate, 2-CH_2OH-S-2200 conjugate, expressed as mandestrobin (S-2200)				
Oilseed rape (EU north)	8x < 0.01	8x < 0.04	< 0.01	< 0.01	0.01*	4
Oilseed rape (EU South)	4x < 0.01	4x < 0.04	< 0.01	< 0.01	0.01*	4

1) ….MW (S-2200)= 313.39; MW (De-Xy)= 209; MW (4-OH-S-2200, 2-CH_2OH-S-2200) = 329
f (S-2200/De-Xy) = 1.4995; f (S-2200/4-OH)= 0.9526; f (S-2200/2-CH_2OH)= 0.9526 kg

According to Article 6(2) of Regulation (EC) 396/2005 Austria as the RMS and Evaluating Member State (EMS) received an application form from Sumitomo Chemical Agro Europe S.A.S. for setting a maximum residue level (MRL) for S-2200 (Mandestrobin) in oilseed rape. Following the agreed procedure, the assessment for MRL setting is prepared within the draft assessment report (DAR). The residue data on oilseed rape (8x NEU and 4x SEU) carried out within two growing seasons are considered sufficient to propose **a MRL of 0.01* mg/kg for oilseed rape for Mandestrobin (S-2200)**

Studies on the **storage stability** of Mandestrobin (S-2200) and the metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH_2OH-S-2200 have been performed on oilseed rape seed (high oil content commodity), lettuce (with high water content commodity), barley grain (dry commodity) and straw. Storage stability of the rotational crop metabolite 5-CH_2OH-S-2200 was also studied in lettuce and barley grain and straw. Storge stability was demonstrated to be at least 12 month for Mandestrobin (S-2200) and all metabolites tested.

Studies to investigate the **effects of industrial processing and/or household preparation** are not required since residues of Mandestrobin (S-2200) and its metabolites in oilseed rape seed were all <0.01 mg/kg. Studies on the nature of the residue during processing showed that Mandestrobin (S-2200) was stable under conditions representing pasteurisation and baking/brewing/boiling and sterilisation.

No **feeding studies** have been submitted and are considered not necessary. Residues of Mandestrobin (S-2200) and metabolites in oilseed rape seed were <LOQ (0.01 mg/kg) in all trials. The calculated livestock dietary burden was below the the trigger value of 0.1 mg/kg DM for all livestock species and the available metabolism studies on laying hens and lactating goats showed that Mandestrobin (S-2200) is extensively metabolised and mainly excreted.

The **metabolism and distribution** of Mandestrobin S-2200 has been studied in confined **rotational crops** (wheat, lettuce, carrots) at 30, 120 and 365 day plant-back intervals. There was moderate uptake of S-2200 residues from soil into rotational crops the metabolism in rotational crops was essentially the same as in primary crops. In a field study on representative crops (carrots, lettuce, broccoli and barley) no detectable residues S-2200 or the metabolites 4-OH-S-2200, 2-CH_2OH-S-2200 and 5-CH_2OH-S-2200 were found in any of the succeeding crop samples at any plant back interval after application of 0.2 kg as/ha on the preceding crop winter rapeseed.

The **chronic dietary exposure** of Mandestrobin (S-2200) residues was evaluated by calculation of the Theoretical Maximum Daily Intake (TMDI) from the proposed MRLs and carried out by means of the EFSA chronic acute RA model rev.2. No determinable residues of Mandestrobin (S-2200) are to be expected in products of animal origin, which therefore have not been included in the dietary risk assessment. The calculated TMDIs were compared to the ADI value of 0.19 mg/kg bw/day as proposed by the RMS indicating that the chronic intake of residues of Mandestrobin (S-2200) with regard to the representative use will be below ADI (< 0.1%) for all consumer groups. **No acute dietary exposure assessment** was done as no ARfD was set by the RMS.

B.7.20 Assessment according to Article 10 of the Regulation (EC) No 396/2005

According to Article 6(2) of Regulation (EC) 396/2005 Austria as the RMS and Evaluating Member State (EMS) received an application form from Sumitomo Chemical Agro Europe S.A.S. for setting a maximum residue level (MRL) for S-2200 (Mandestrobin) in oilseed rape. Following the agreed procedure, the assessment for MRL setting is prepared within the draft assessment report (DAR).

A total of **12 supervised residue trials** conducted in 2010 and 2011 have been provided to support the use of S-2200 on oilseed rape seed in Northern and Southern Europe. S-2200 was applied once at 0.2 kg as/ha to oilseed rape at growth stage BBCH 65. In mature seed collected 56-91 days after application, residues of S-2200 and residues of the metabolites De-Xy-S-2200, 4-OH-S-2200 (free and conjugated) and 2-CH_2OH-S-2200 (free and conjugated) were all below the limit of quantification (<0.01 mg/kg).

MRL setting was done considering SANCO 10634/2010 Rev 0 (“Classes to be used for the setting of EU pesticide Maximum Residue Levels”) and according to the EFSA PROFile (2.3) MRL calculator.

Table B.7.20-1: Residue data for Mandestrobin (S-2200) for monitoring

	EU-north (n= 8)	EU-South (n= 4)
Individual data points	8x < 0.01*mg/kg	4x < 0.01*mg/kg
Mean residue	0.01 mg/kg	0.01 mg/kg
Highest residue (HR)	0.01 mg/kg	0.01 mg/kg
STMR	0.01 mg/kg	0.01 mg/kg
Method I: Rmax	0.01 mg/kg	0.01 mg/kg
Method II : Rber	0.02 mg/kg	0.02 mg/kg
MRL proposal (OECD)	0.01 mg/kg	0.01 mg/kg
MRL proposal	**0.01 mg/kg**	**0.01 mg/kg**

*.... indicates LOQ

The residue data on oilseed rape (8x NEU and 4x SEU) carried out over two growing seasons are considered sufficient and acceptable. Thus a MRL of 0.01* mg/kg for oilseed rape for S-2200 (Mandestrobin) is proposed.

B.7.21 References relied on

Annex Point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or unpublished	Data Protection claimed Y/N	Owner
IIA, 6.1.1 / 01	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 seeds of Oilseed Rape Eurofins Dr Specht GLP GmbH report no. SUM-1012 Sumitomo Chemical Co., Ltd. ROR-0007 GLP, unpublished	Y	SUM
IIA, 6.1.1 / 02	Daneva, E. & Taeufer, A.	2011b	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 report no. S10-01949 Sumitomo Chemical Co., Ltd. ROR-0009 GLP, unpublished	Y	SUM
IIA, 6.1.1 / 03	Daneva, E. & Taeufer, A.	2012a	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 report no. SUM-1024 Sumitomo Chemical Co., Ltd. ROR-0011 GLP, unpublished	Y	SUM
IIA, 6.1.1 / 04	Daneva, E. & Taeufer, A.	2012b	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 report no. SUM-1025 Sumitomo Chemical Co., Ltd. ROR-0012 GLP, unpublished	Y	SUM
IIA, 6.1.1 / 05	Daneva, E. & Zetzsch, A.	2012a	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 report no. SUM-1026 Sumitomo Chemical Co., Ltd. ROR-0013 GLP, unpublished	Y	SUM

Annex Point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or unpublished	Data Protection claimed Y/N	Owner
IIA, 6.1.1 / 06	Daneva, E. & Zetzsch, A.	2012b	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 report no. SUM-1028 Sumitomo Chemical Co., Ltd. ROR-0014 GLP, unpublished	Y	SUM
IIA, 6.2.1 / 01	Panthani, A. & Connor, S.	2011	Metabolism of [^{14}C]S-2200 in Rapeseed Plants Smithers Viscient Report No: 13048.6618 Sumitomo Chemical Co., Ltd. ROM-0026 GLP, unpublished	Y	SUM
IIA, 6.2.1 / 02	Panthani, A. & Lentz, N R.	2010a	Metabolism of [^{14}C]S-2200 in Wheat Springborn Smithers Laboratories Report No: 13048.6619 Sumitomo Chemical Co., Ltd. ROM-0009 GLP, unpublished	Y	SUM
IIA, 6.2.1 / 03	Panthani, A. & Lentz, N R.	2010b	Metabolism of [^{14}C]S-2200 in Lettuce Plants Springborn Smithers Laboratories Report No: 13048.6631 Sumitomo Chemical Co., Ltd. ROM-0008 GLP, unpublished	Y	SUM
IIA, 6.2.2 / 01	[redacted]	2012a	Amended Final Report 1 and 2: [^{14}C]S-2200 - Absorption, distribution, metabolism and excretion following repeated oral administration to the laying hen [redacted] Sumitomo Chemical Co., Ltd. ROM-0040 GLP, unpublished	Y	SUM
IIA, 6.2.3 / 01	[redacted]	2012b	Amended Final Report 1: [^{14}C]S-2200 - Absorption, distribution, metabolism and excretion following repeated oral administration to the lactating ruminant [redacted] Sumitomo Chemical Co., Ltd. ROM-0039 GLP, unpublished	Y	SUM
IIA, 6.3.1 / 01	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 study no. FLN-10-6267 Sumitomo Chemical Co., Ltd. ROR-0008 GLP, unpublished	Y	SUM

Annex Point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or unpublished	Data Protection claimed Y/N	Owner
IIA, 6.3.1 / 02	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 study no. IF-11/01898756 Sumitomo Chemical Co., Ltd. ROR-0198 GLP, unpublished	Y	SUM
IIA, 6.5.1/ 01	Dixon, K. & Gilbert, J.	2011	[^{14}C]S-2200: Nature of the Residue (High Temperature Hydrolysis) Study Covance Laboratories Ltd Report No.: 8239214 Sumitomo Chemical Co., Ltd. ROM-0027 GLP, unpublished	Y	SUM
IIA, 6.6.2 / 01	Panthani, A., Connor, S. & Malekani, K.	2011	Confined Rotational Crop Study with [^{14}C]S-2200 Smithers Viscient Report No.: 13048.6630 Sumitomo Chemical Co., Ltd. ROM-0032 GLP, unpublished	Y	SUM
IIA, 6.6.3 / 01	Roussel, Ch.H.	2012	Rotational Field-Crops Residue Study after Application of S-2200 25 SC (25 % w/v) to Winter Rapeseed Staphyt Report No.: FLN-10-6268 Sumitomo Chemical Co., Ltd. ROR-0202 GLP, unpublished	Y	SUM
IIA, 4.3/01	Daneva, E.	2010	Adaptation and Validation of Multi-Method DFG S 19 for the Determination of Residues of S-2200 in Seeds of Oilseed Rape Eurofins Dr Specht GLP GmbH, Report no. SUM-1011V Sumitomo Chemical Co., Ltd. ROA-0005 GLP, Unpublished	Y	SUM
IIA, 4.3/03	Daneva, E., Breyer, N. & Taeufer, A.	2011a	Validation of an analytical method for 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, Report no. S10-02910 Sumitomo Chemical Co., Ltd. ROA-0010 GLP, Unpublished	Y	SUM
IIA, 4.3/04	Daneva, E., & Taeufer, A.	2011a	Validation of an analytical method for 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, Report no. S10-02908 Sumitomo Chemical Co., Ltd. ROA-0011 GLP, Unpublished	Y	SUM

Annex Point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or unpublished	Data Protection claimed Y/N	Owner
IIA, 4.3/05	Daneva, E., Breyer, N. & Taeufer, A.	2011b	Validation of an analytical method for determination of S-2200 metabolites, 2-CH2OH-S-2200 and its conjugates, in seeds of oilseed rape, barley (grain and straw) and lettuce (head) Eurofins Dr Specht GLP GmbH, Report no. S10-02909 Sumitomo Chemical Co., Ltd. ROA-0012 GLP, Unpublished	Y	SUM
IIA, 4.3/06	Daneva, E., Taeufer, A.	2011b	Validation of an analytical method for determination of S-2200 metabolites, 5-CH2OH-S-2200 and its conjugates, in barley (grain and straw) and lettuce (head) Eurofins Dr Specht GLP GmbH, Report no. S10-03385 Sumitomo Chemical Co., Ltd. ROA-0013 GLP, Unpublished	Y	SUM

B.7.22 Annex Ia: Supervised residue trials on OILSEED RAPE conducted in 2010

B.7.22.1 Residues of S-2200 (R- and S-isomer)

Active Substance:	S-2200	**Commercial Product:**	S-2200 25SC
Crop:	Winter Oilseed Rape	**Producer:**	Sumitomo Chemical Co., Ltd., Tokyo, Japan
Responsible for reporting:	STAPHYT – F-37110 Dame Marie les Bois		
Country:	**France, Germany, UK (EU North)** **France (EU South)**	**Indoor/glasshouse/outdoor:**	Outdoor
Content of as:	25% w/v	**Other a.s. in formulation:**	None
Formulation:	SC	**Residue calculated as:**	**S-2200 R and S-isomers**

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				S-2200 R-isomer (S-2167)	S-2200 S-isomer (S-2354)	**S-2200 Sum of isomers**		
FLN-10-6267 51490 Epoye, Champagne-Ardennes Northern France Decline Curve FR01	Winter oilseed rape Safran	1. 27.08.2009 2. 30.04.2010 – 10.05.2010 3. 20.07.2010	Foliar application	0.20	200	0.10	05.05.2010	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	1.4 0.048 <0.005 <0.005	1.4 0.056 <0.005 <0.005	2.8 0.10 <0.01 <u><0.01</u>	0 29 29 <u>76</u>	All untreated controls < LOQ Method validation report SUM-1011V & SUM-1016V Max. storage interval from sampling to analysis: 119 days
FLN-10-6267 74673 Mulfingen/ Railhof, Baden-Württemberg, Germany Decline Curve GE01	Winter oilseed rape Visby	1. 19.08.2009 2. 28.04.2010 – 26.05.2010 3. 20.07.2010	Foliar application	0.20	300	0.067	03.05.2010	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	0.75 <0.005 <0.005 <0.005	0.75 <0.005 <0.005 <0.005	1.5 <0.01 <0.01 <u><0.01</u>	0 50 50 <u>78</u>	All untreated controls < LOQ Method validation report SUM-1011V & SUM-1016V Max. storage interval from sampling to analysis: 121 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				S-2200 R-isomer (S-2167)	S-2200 S-isomer (S-2354)	**S-2200 Sum of isomers**		
FLN-10-6267 Moulsoe, Buckinghamshire, MK16 0HR, United Kingdom At Harvest Trial UK01	Winter oilseed rape DK Cabernet	1. 28.08.2009 2. mid May - mid June 2010 3. 30.07.2010	Foliar application	0.20	200	0.100	04.06.2010	BBCH 65	Seeds	<0.005	<0.005	<u><0.01</u>	<u>56</u>	All untreated controls < LOQ Method validation report SUM-1011V & SUM-1016V Max. storage interval from sampling to analysis: 31 days
FLN-10-6267 31290 Montgaillard Lauragais, Midi-Pyrénées, **Southern France** Decline Curve FR02	Winter oilseed rape Cokeliko	1. 02.09.2009 2. 10.04.2010 – 10.05.2010 3. 08.07.2010	Foliar application	0.20	300	0.067	28.04.2010	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	0.77 0.014 <0.005 <0.005	0.76 0.017 <0.005 <0.005	1.5 0.031 <0.01 <u><0.01</u>	0 29 29 <u>71</u>	All untreated controls < LOQ Method validation report SUM-1011V & SUM-1016V Max. storage interval from sampling to analysis: 126 days
FLN-10-6267 33210 St Pardon de Conques, Aquitaine, **Southern France** At Harvest Trial FR03	Winter oilseed rape Standol	1. 06.09.2009 2. end April 2010 3. 24.06.2010	Foliar application	0.20	300	0.067	26.04.2010	BBCH 65	Seeds	<0.005	<0.005	<u><0.01</u>	<u>59</u>	All untreated controls < LOQ Method validation report SUM-1011V & SUM-1016V Max. storage interval from sampling to analysis: 67 days

B.7.22.2 Residues of De-Xy-S-2200, Sum of free and conjugated 4-OH-S-2200, Sum of free and conjugated 2-CH_2OH-S-2200

Active Substance:	S-2200	**Commercial Product:**	S-2200 25SC
Crop:	Winter Oilseed Rape	**Producer:**	Sumitomo Chemical Co., Ltd., Tokyo, Japan
Responsible for reporting:	STAPHYT – F-37110 Dame Marie les Bois		
Country:	**France, Germany, UK (EU North)** **France (EU South)**	**Indoor/glasshouse/outdoor:**	Outdoor
Content of as:	25% w/v	**Other a.s. in formulation:**	None
Formulation:	SC	**Residue calculated as:**	**De-Xy-S-2200** **Sum of free and conjugated 4-OH-S-2200** **Sum of free and conjugated 2-CH_2OH-S-2200**

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				De-Xy-S-2200	4-OH-S-2200	2-CH_2OH-S-2200		
FLN-10-6267 51490 Epoye, Champagne-Ardennes Northern France Decline Curve FR01	Winter oilseed rape Safran	1. 27.08.2009 2. 30.04.2010 – 10.05.2010 3. 20.07.2010	Foliar application	0.20	200	0.100	05.05.2010	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	<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 29 29 76	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V Max. storage interval: 218 days 4-OH-S-2200: Method validation report SUM-1021V Max. storage interval: 246 days 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 218 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				De-Xy-S-2200	4-OH-S-2200	2-CH_2OH-S-2200		
FLN-10-6267 74673 Mulfingen/ Railhof, Baden-Württemberg, Germany Decline Curve GE01	Winter oilseed rape Visby	1. 19.08.2009 2. 28.04.2010 – 26.05.2010 3. 20.07.2010	Foliar application	0.20	300	0.067	03.05.2010	BBCH 65	Whole plants	<0.01	<0.01	<0.01	0	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V Max. storage interval: 220 days 4-OH-S-2200: Method validation report SUM-1021V Max. storage interval: 252 days 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 220 days
									Whole plants w/o pods	<0.01	0.01	<0.01	50	
									Green pods	<0.01	<0.01	<0.01	50	
									Seeds	<0.01	<0.01	<0.01	78	
FLN-10-6267 Moulsoe, Buckinghamshire, MK16 0HR, United Kingdom At Harvest Trial UK01	Winter oilseed rape DK Cabernet	1. 28.08.2009 2. mid May - mid June 2010 3. 30.07.2010	Foliar application	0.20	200	0.100	04.06.2010	BBCH 65	Seeds	<0.01	<0.01	<0.01	56	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V Max. storage interval: 161 days 4-OH-S-2200: Method validation report SUM-1021V Max. storage interval: 236 days 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 177 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				De-Xy-S-2200	4-OH-S-2200	2-CH_2OH-S-2200		
FLN-10-6267 31290 Montgaillard Lauragais, Midi-Pyrénées, **Southern France** Decline Curve FR02	Winter oilseed rape Cokeliko	1. 02.09.2009 2. 10.04.2010 – 10.05.2010 3. 08.07.2010	Foliar application	0.20	300	0.067	28.04.2010	BBCH 65	Whole plants	<0.01	<0.01	<0.01	0	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V Max. storage interval: 225 days 4-OH-S-2200: Method validation report SUM-1021V Max. storage interval: 258 days 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 225 days
									Whole plants w/o pods	<0.01	0.01	<0.01	29	
									Green pods	<0.01	0.01	<0.01	29	
									Seeds	<0.01	<0.01	<0.01	<u>71</u>	
FLN-10-6267 33210 St Pardon de Conques, Aquitaine, **Southern France** At Harvest Trial FR03	Winter oilseed rape Standol	1. 06.09.2009 2. end April 2010 3. 24.06.2010	Foliar application	0.20	300	0.067	26.04.2010	BBCH 65	Seeds	<0.01	<0.01	<0.01	<u>59</u>	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V Max. storage interval: 197 days 4-OH-S-2200: Method validation report SUM-1021V Max. storage interval: 272 days 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 213 days

B.7.23 Annex Ib: Supervised residue trials on OILSEED RAPE conducted in 2011

B.7.23.1 Residues of S-2200 (R- and S-isomer)

Active Substance:	S-2200	**Commercial Product:**	S-2200 25SC
Crop:	Winter Oilseed Rape	**Producer:**	Sumitomo Chemical Co., Ltd., Tokyo, Japan
Responsible for reporting:	SGS INSTITUTE FRESENIUS GmbH – 7 rue de la Minée, F-49620 La Pommeraye - FRANCE		
Country:	**France, Germany, UK (EU North)** **France (EU South)**	**Indoor/glasshouse/outdoor:**	Outdoor
Content of as:	25% w/v	**Other a.s. in formulation:**	None
Formulation:	SC	**Residue calculated as:**	**S-2200 R and S-isomers**

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				S-2200 R-isomer (S-2167)	S-2200 S-isomer (S-2354)	**S-2200 Sum of isomers**		
IF-11/01898756 37370 Saint Paterne Racan, Centre, N France At Harvest Trial -01	Winter oilseed rape Zeruca	1. 17.09.2010 2. 02.04.2011 – 27.04.2011 3. 05.07.2011	Foliar application	0.20	200	0.100	13.04.2011	BBCH 65	Seeds	<0.005	<0.005	<0.01	85	All untreated controls < LOQ Method validation report SUM-1011V Max. storage interval from sampling to analysis: 114 days
IF-11/01898756 51220 Brimont, Champagne-Ardennes, N France Decline Curve - 02	Winter oilseed rape Ovasion	1. 21.08.2010 2. 07.04.2011 – 12.04.2011 3. 01.07.2011	Foliar application	0.20	200	0.100	18.04.2011	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	0.95 0.16 <0.005 <0.005	1.1 0.20 <0.005 <0.005	2.1 0.36 <0.01 <0.01	0 31 31 70	All untreated controls < LOQ Method validation report SUM-1011V Max. storage interval from sampling to analysis: 193 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				S-2200 R-isomer (S-2167)	S-2200 S-isomer (S-2354)	**S-2200 Sum of isomers**		
IF-11/01898756 21220 Seevetal, Lower-Saxony, Germany At Harvest Trial -05	Winter oilseed rape NK Petrol	1. 26.08.2010 2. 14.04.2011 – 12.05.2011 3. 27.07.2011	Foliar application	0.20	200	0.1	29.04.2011	BBCH 65	Seeds	<0.005	<0.005	<0.01	83	All untreated controls < LOQ Method validation report SUM-1011V Max. storage interval from sampling to analysis: 83 days
IF-11/01898756 26169 Altenoythe, Lower-Saxony, Germany Decline Curve - 06	Winter oilseed rape Visby	1. 25.08.2010 2. 14.04.2011 – 12.05.2011 3. 27.07.2011	Foliar application	0.20	200	0.1	28.04.2011	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	0.95 0.006 <0.005 <0.005	1.2 0.009 <0.005 <0.005	2.2 0.015 <0.01 <0.01	0 40 40 91	All untreated controls < LOQ Method validation report SUM-1011V & SUM-1016V Max. storage interval from sampling to analysis: 209 days
IF-11/01898756 CV35 944 Wellesbourne, Central England, UK At Harvest Trial -07	Winter oilseed rape DK Cabernet	1. 05.09.2010 2. 04.04.2011 – 20.05.2011 3. 18.07.2011	Foliar application	0.20	200	0.1	19.04.2011	BBCH 65	Seeds	<0.005	<0.005	<0.01	86	All untreated controls < LOQ Method validation report SUM-1011V Max. storage interval from sampling to analysis: 90 days
IF-11/01898756 32700 Pergain Taillac, Midi-Pyrénées, **S France** At Harvest Trial -03	Winter oilseed rape Mercury	1. 06.09.2010 2. 27.03.2011 – 06.05.2011 3. 06.06.2011	Foliar application	0.20	250	0.08	06.04.2011	BBCH 65	Seeds	<0.005	<0.005	<0.01	61	All untreated controls < LOQ Method validation report SUM-1011V Max. storage interval from sampling to analysis: 145 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				S-2200 R-isomer (S-2167)	S-2200 S-isomer (S-2354)	**S-2200 Sum of isomers**		
IF-11/01898756 47400 Gontaud de Nogaret, Aquitaine, **S France** Decline Curve - 04	Winter oilseed rape Hybri Star	1. 15.09.2010 2. 24.03.2011 – 25.04.2011 3. 10.06.2011	Foliar application	0.20	250	0.08	07.04.2011	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	1.5 0.043 0.012 <0.005	1.9 0.058 0.017 <0.005	3.4 0.10 0.029 <0.01	0 32 32 60	All untreated controls < LOQ Method validation report SUM-1011V Max. storage interval from sampling to analysis: 204 days

B.7.23.2 Residues of De-Xy-S-2200, Sum of free and conjugated 4-OH-S-2200, Sum of free and conjugated 2-CH_2OH-S-2200

Active Substance:	S-2200	**Commercial Product:**	S-2200 25SC
Crop:	Winter Oilseed Rape	**Producer:**	Sumitomo Chemical Co., Ltd., Tokyo, Japan
Responsible for reporting:	SGS INSTITUTE FRESENIUS GmbH – 7 rue de la Minée, F-49620 La Pommeraye - FRANCE		
Country:	**France, Germany, UK (EU North)** **France (EU South)**	**Indoor/glasshouse/outdoor:**	Outdoor
Content of as:	25% w/v	**Other a.s. in formulation:**	None
Formulation:	SC	**Residue calculated as:**	**De-Xy-S-2200** **Sum of free and conjugated 4-OH-S-2200** **Sum of free and conjugated 2-CH_2OH-S-2200**

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				**De-Xy-S-2200**	**4-OH-S-2200**	**2-CH_2OH-S-2200**		
IF-11/01898756 37370 Saint Paterne Racan, Centre, N France At Harvest Trial -01	Winter oilseed rape Zeruca	1. 17.09.2010 2. 02.04.2011 – 27.04.2011 3. 05.07.2011	Foliar application	0.20	200	0.100	13.04.2011	BBCH 65	Seeds	<0.01	<0.01	<0.01	85	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 148 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				**De-Xy-S-2200**	**4-OH-S-2200**	**2-CH_2OH-S-2200**		
IF-11/01898756 51220 Brimont, Champagne-Ardennes, N France Decline Curve -02	Winter oilseed rape Ovasion	1. 21.08.2010 2. 07.04.2011 – 12.04.2011 3. 01.07.2011	Foliar application	0.20	200	0.100	18.04.2011	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	<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 31 31 70	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 197 days
IF-11/01898756 21220 Seevetal, Lower-Saxony, Germany At Harvest Trial -05	Winter oilseed rape NK Petrol	1. 26.08.2010 2. 14.04.2011 – 12.05.2011 3. 27.07.2011	Foliar application	0.20	200	0.1	29.04.2011	BBCH 65	Seeds	<0.01	<0.01	<0.01	83	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 126 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				**De-Xy-S-2200**	**4-OH-S-2200**	**2-CH_2OH-S-2200**		
IF-11/01898756 26169 Altenoythe, Lower-Saxony, Germany Decline Curve -06	Winter oilseed rape Visby	1. 25.08.2010 2. 14.04.2011 – 12.05.2011 3. 27.07.2011	Foliar application	0.20	200	0.1	28.04.2011	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	<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 40 40 91	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 187 days
IF-11/01898756 CV35 944 Wellesbourne, Central England, UK At Harvest Trial -07	Winter oilseed rape DK Cabernet	1. 05.09.2010 2. 04.04.2011 – 20.05.2011 3. 18.07.2011	Foliar application	0.20	200	0.1	19.04.2011	BBCH 65	Seeds	<0.01	<0.01	<0.01	86	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 133 days

1	2	3	4	5			6	7	8	9			10	11
Report No.	Commodity Variety	Date of 1-Planting 2-Flowering 3- Harvest	Method of treatment	Application rate per treatment			Date of treatment	Crop growth stage at treatment BBCH	Portion analysed	Residue (mg/kg)			PHI (d)	Remarks
				kg as/ha	Water (l/ha)	kg as/hl				**De-Xy-S-2200**	**4-OH-S-2200**	**2-CH_2OH-S-2200**		
IF-11/01898756 32700 Pergain Taillac, Midi-Pyrénées, **S France** At Harvest Trial -03	Winter oilseed rape Mercury	1. 06.09.2010 2. 27.03.2011 – 06.05.2011 3. 06.06.2011	Foliar application	0.20	250	0.08	06.04.2011	BBCH 65	Seeds	<0.01	<0.01	<0.01	61	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 179 days
IF-11/01898756 47400 Gontaud de Nogaret, Aquitaine, **S France** Decline Curve -04	Winter oilseed rape Hybri Star	1. 15.09.2010 2. 24.03.2011 – 25.04.2011 3. 10.06.2011	Foliar application	0.20	250	0.08	07.04.2011	BBCH 65	Whole plants Whole plants w/o pods Green pods Seeds	<0.01 <0.01 <0.01 <0.01	<0.01 0.03 0.02 <0.01	<0.01 <0.01 <0.01 <0.01	0 32 32 60	All untreated controls < LOQ De-Xy-S-2200: Method validation report SUM-1023V 4-OH-S-2200: Method validation report SUM-1021V 2-CH_2OH-S-2200: Method validation report SUM-1022V Max. storage interval: 226 days