Document Title

Summary of the residues in or on treated products, food and feed for spiroxamine

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 283/2013

Document MCA

Section 6: Residues in or on treated products, food and feed

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

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Author(s)

[redacted]

ERM
On behalf of Bayer AG
Crop Science Division

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'

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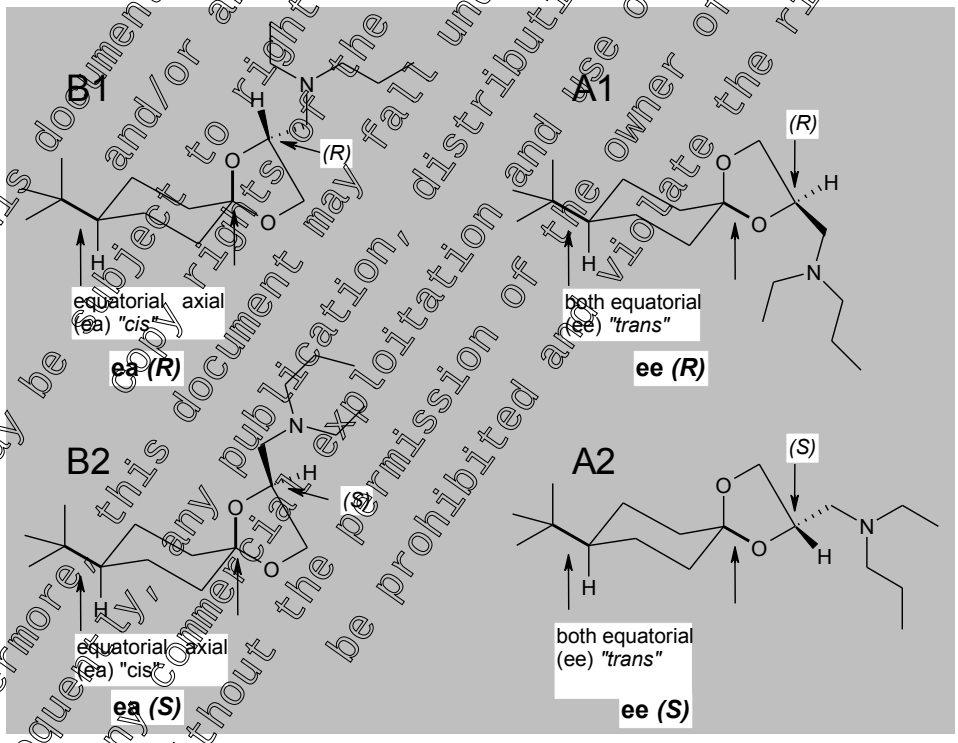
CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

Spiroxamine was included in Annex I to Council Directive 91/414/EEC in 1999 (Directive 1999/73/EC Entry into Force on 1 September 1999). This Supplementary Dossier contains data which were not submitted at the time of the Annex I inclusion and first renewal of spiroxamine under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. However, all studies submitted for the first approval and subsequent first renewal of spiroxamine have also been summarised according to current guidance and included in the dossier. Where studies meet relevant validity criteria, new robust study summaries have been provided in the appropriate dossier section. However, where studies do not meet relevant validity criteria and are not considered acceptable, less detailed summaries may have been provided alongside discussions of study deficiencies. All relied upon study reports are submitted in Document K for this second renewal of approval dossier or in Document K for the previous Annex I inclusion and first renewal submissions.

All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion and first renewal under Council Directive 91/414/EEC are contained in the draft Re-Assessment Report (RAR) 2010 and its revised RAR 2017, and are included in the Baseline Dossier provided by Bayer AG.

Spiroxamine consists of four isomers (two diastereomers each with its corresponding two enantiomers which are in a 1:1 ratio) as shown in the schematic below. The isomer nomenclature presented in some historical documentation may differ with respect to the A/B and corresponding trans/cis notation as a result of a discrepancy in referencing, which is discussed in detail in position paper M-761468-01-1 (see CA 1.7/01). It is recommended that the stereo assignments depicted here, together with the A and B notation should be used exclusively going forward to ensure continuity of information throughout the dossier.



CA 6.1 Storage stability of residues

Data Point:	KCA 6.1/06
Report Author:	[redacted]
Report Year:	2015
Report Title:	Storage stability of spiroxamine in/on grape (fruit) and wheat (grain) for 24 months
Report No:	IF-11/01954154
Document No:	M-525418-01-1
Guideline(s) followed in study:	Chemikaliengesetz, Anhang I zu § 19 a Abs. 1 ChemG in the valid form, OECD document OECD-DOC_ENV/MC/CHEM(98)17, Paris 1998, Storage stability of Residue Samples, EC doc. 7032/VI/95-rev.5, dated 22/7/97
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The study was conducted to evaluate the stability of spiroxamine in frozen storage (≤-18°C) for a period of 24 months in grapes [high acid] and wheat grain, [high starch]. The commodities were spiked with spiroxamine and stored at an average temperature of -18°C or below over a period of 2 years.

The LOQs for spiroxamine in/on grapes and wheat grain were 0.05 mg/kg and 0.01 mg/kg respectively. There was no evidence of any degradation of spiroxamine in any of the sample materials. The results therefore show that spiroxamine was stable for at least 2 years in grapes and wheat grain, with the recovered residue in all sampling dates in all both matrices being above 70%.

The results show that spiroxamine was stable in commodities representative of these matrix types as described in OECD 506, when stored frozen for up to 2 years.

Procedural fortifications were conducted at 0.05 and 1.0 mg/kg for spiroxamine in grapes and 0.01 and 0.2 mg/kg for spiroxamine in wheat grain. Recoveries ranged from 89 to 102%for grapes and 92 to 106% for wheat grain, showing acceptable method performance.

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 88 to 102% with a relative standard deviation in the range of 4.2 to 4.5%.

The final stability data supports all storage intervals for samples of grapes and wheat grain analysed in supervised residue trials (and processing studies), summarised under points CA 6.3 and CA 6.5. Actual details are discussed in the residue study summaries.

I. Materials and methods

The Test Facility obtained samples of untreated control grapes and wheat grain from an organic market in Taunusstein, Germany. All samples were homogenised at the Test Facility and stored at ≤18°C.

Individual 5 g aliquots of the homogenized specimen material were weighed into 20 mL amber glass bottles with plastic caps. Each individual aliquot of grapes and wheat grain were spiked at 1.0 mg/kg for grapes and 0.2 mg/kg for wheat grain with spiroxamine on day 0. The specimens were stored in these containers at an average temperature of ≤-18°C, and were analysed at the nominal storage intervals of 0, 30, 60, 90, 180, 360, 540 and 720 days.

On day 0, 7 spiked specimens (2 at the respective LOQ and 5 at 20 times respective LOQ) and 2 control specimens were analysed for each matrix to confirm the fortification level and performance of the method.

At each subsequent sampling interval, 3 fortified specimens and 3 control specimens were removed from the freezer and allowed to come to room temperature. The stored control specimens were either analysed directly as control specimens or freshly fortified at either 1.0 mg/kg for grapes or 0.20 mg/kg for wheat. The freshly fortified specimens were analysed concurrently with the remaining control specimen and the stored spiked specimens.

Description of analytical procedures

Residues of spiroxamine in grapes (fruit) were analysed using the validated method 01080, report reference M-304677-01-1 (**see Doc MCA Section 4**).

Residues of spiroxamine were extracted with a mixture of acetone/water (2/1, v/v) from 5 g of specimen material using a blender. After rinsing, the extract was filtered into a volumetric flask and made to a known volume with acetone/water (2/1, v/v).

For controls and samples fortified at the LOQ, 200 µL aliquots were transferred into a vial and diluted with 800 µL of methanol/water (1/4, v/v) containing 50 mg/L cysteine hydrochloride. To 200 µL of this solution, 100 µL of the ISTD (spiroxamine-d7) was added and mixed.

For specimens fortified at the 20-times LOQ, a 100 µL aliquot was mixed with 100 µL of ISTD (spiroxamine-d7) and further diluted with 800 µL of control extract in methanol/water (1/4, v/v) containing 50 mg/L cysteine hydrochloride. All samples were determined using LC-MS/MS.

Residues of spiroxamine in wheat (grain) were analysed using the validated method [01013/M001], report reference M-297777-02-1 (**see Doc MCA Section 4**).

After soaking samples in a mixture of 60 mL acetonitrile/water (4/1, v/v) and 4 mL of cysteine hydrochloride for 30 minutes the samples were homogenized for 3 minutes. After rinsing the blender, 2 g of Celite 545 were added and the extracts filtered into volumetric flasks. All extracts were mixed with 0.5 mL of ISTD (spiroxamine-d7) and the solutions were made up to volume. All samples were determined using LC-MS/MS, with dilutions as required.

II. Results and discussion

The mean values of the procedural recovery rates for grapes were in the range of 89-102%, with a relative standard deviation of 4.2%. In wheat grain the mean procedural recovery rates were in the range of 92-106% with a relative standard deviation of 4.5%.

At day 0, average residue levels of spiroxamine were 0.94 mg/kg in grapes (fruit), and 0.20 mg/kg in wheat (grain). In samples analysed after storage for the periods of time described above, storage stability recovered values in the grapes ranged from 92-104%, and in wheat grain from 88-102%. There was no evidence of any significant degradation of spiroxamine in any of the sample materials. At all sampling dates and in all sample materials, the total residues of spiroxamine were above 70% (as shown in Table CA 6.1/06-1 below).

Table CA 6.1/06-1 **Summary of deep-freezer stability data of spiroxamine in grapes or wheat grain after spiking with spiroxamine**

Analyte	Commodity	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	20x LOQ
spiroxamine	Grapes, high acid	0	0.936, 0.922, 0.925, 0.966, 0.929	92, 92, 93, 96, 93 [93]	94	-
		1 [29 days]	0.953, 0.971, 0.967	94, 95, 96 [95]	-	95
		2 [59 days]	0.963, 1.002, 0.985	96, 99, 98 [98]	-	94
		3 [87 days]	0.996, 1.038, 0.983	99, 102, 97 [99]	-	100
		6 [181 days]	0.974, 0.999, 1.004	96, 99, 100 [98]	-	98
		12 [359 days]	0.984, 1.034, 1.025	97, 103, 101 [100]	-	98
		18 [540 days]	1.060, 1.038, 1.061	105, 102, 105 [104]	-	102
		24 [720 days]	0.933, 0.935, 0.929	92, 92, 92 [92]	-	89
	Wheat grain, high starch	0	0.203, 0.201, 0.200, 0.191, 0.196	102, 100, 100, 95, 98 [99]	99	-
		1 [29 days]	0.206, 0.209, 0.182	102, 104, 101 [102]	-	106
		2 [60 days]	0.180, 0.184, 0.182	89, 91, 91 [90]	-	101
		3 [90 days]	0.192, 0.190, 0.195	95, 94, 97 [95]	-	100
		6 [182 days]	0.184, 0.186, 0.186	91, 93, 92 [92]	-	96
		12 [361 days]	0.195, 0.196, 0.197	97, 98, 97 [97]	-	102
		18 [538 days]	0.190, 0.201, 0.191	94, 100, 95 [97]	-	99
		24 [722 days]	0.181, 0.174, 0.174	90, 87, 86 [88]	-	92

III. Conclusions

For grapes [high acid] and wheat grain [high starch], spiroxamine was shown to be stable for the complete storage period of up to 2 years under frozen conditions at ≤-18 °C. These results validate the residue values reported in supervised field trials and processing studies for these matrices with respect to storage stability.

Assessment and conclusion by applicant:

Acceptable study to address the data point.

For grapes (fruit, [high acid]) and wheat (grain, [high starch]) specimen materials, spiroxamine was shown to be stable for the complete storage period of up to 2 years under freezer conditions at -18 °C or below. These results validate the residue values reported in supervised field trials and processing studies with respect to storage stability.

Data Point:	KCA 6.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Determination of storage stability of KWG 4168 in/on barley
Report No:	MR-271/94
Document No:	M-010767-01-1
Guideline(s) followed in study:	No information
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

This study was conducted to determine the freezer storage stability of the total residue of spiroxamine in cereals (barley, [high starch]). Control samples of barley grains were separately fortified with spiroxamine or the metabolite spiroxamine-N-oxide [M03] in acetone. In addition, green plant material and straw were taken from a field residue trial, containing approximately 11 mg/kg and 4.2 mg/kg total spiroxamine residue, respectively. Samples were stored in a freezer ≤-18°C over a period of about 1 year.

The LOQ for total spiroxamine in/on barley (green material, straw and grain) was 0.05 mg/kg. There was no evidence of any degradation of either spiroxamine or spiroxamine N-oxide in any of the sample materials at all sampling intervals, the sample materials contained more than 70% of the original residue value.

The results show that spiroxamine was stable in commodities representative of these matrices as described in OECD 506, when stored frozen for up to 1 year.

Procedural fortifications were conducted at 0.05 and 0.5 mg/kg for spiroxamine in barley (green material, straw and grains). The recovery values were in the range of 77 to 117% (with a mean of 92% and a relative standard deviation of 10.6%). These values reflect a good standard deviation in residue analysis and acceptable method performance.

The mean values of the concurrent recovery rates per sample material and spiking level were in the range of 87-107%, with relative standard deviations in the range of 4.4 to 16.0%.

The final stability data supports all storage intervals for samples of barley grains, straw and green material analysed in supervised residue trials (and processing studies), summarised under points CA 6.3 and CA 6.5. Actual details are discussed in the residue study summaries.

I. Materials and methods

Samples of untreated barley grains, straw and green material were obtained by the Test Facility. Green material and straw were taken from a GLP field residue trial. Part of the frozen laboratory samples of green material and straw were shredded with a cutter in the presence of dry ice. The total residue in incurred samples was 11 mg/kg in green material and 4.2 mg/kg in straw.

Aliquots of green material (50 g) and straw (25 g) of incurred samples were weighed into individual polystyrene boxes.

Aliquots of whole grain (50 g) untreated samples were weighed into individual polystyrene boxes. To avoid contact of the spiking solution with the box, a piece of aluminium foil was used to line the bottom. Samples were fortified with the spiking solution (1 mL) containing 25 µg/mL of spiroxamine or spiroxamine-N-oxide. This fortification resulted in a concentration of 0.3 mg/kg of each compound in the samples. After fortification, the bottles were closed.

For green material and straw, a total of 30 samples were prepared, for grains 60 samples. In addition, 30 untreated samples of each sample material were stored for control and recovery experiments.

The entire sample in the polystyrene box was taken for extraction. Prior to extraction, samples of grains were ground with an Ultra Turrax homogeniser using liquid nitrogen.

The samples were stored in the standard plastic containers in a freezer at ≤-18°C for a period of about one year. Prior to day 0, the method was validated by recovery experiments. Then one control sample and six incurred field samples (green material and straw) or fortified grain samples were analysed at day 0. Samples were analysed at day 0 and after 14-16, 49-51, 97-98, and 377-434 days of storage. At each storage interval after day 0, three treated samples and a control sample were removed from the freezer and allowed to come to room temperature before being extracted and analysed.

Description of analytical procedures

Samples of barley grains, straw and green material were analysed using the validated method 00312, report reference M-018352-02-1 (**see Doc MCA Section 4**).

The residues were extracted from the plant material by refluxing with a mixture of methanol and hydrochloric acid. Through that, the total residue of spiroxamine, i.e. the active ingredient and all metabolites containing the 4-t-butylcyclohexanone moiety, was hydrolysed yielding 4-t-butylcyclohexanone. After filtration, an aliquot of the extract was taken, diluted with water and applied to a reversed phase disposable SPE column. The 4-t-butylcyclohexanone was extracted from the column with dichloromethane. This extract was further purified on a silica gel disposable SPE column. The 4-t-butylcyclohexanone was determined by gas chromatography with a mass selective detector (GC-MSD) in the single ion monitoring mode.

II. Results and discussion

The mean values of the procedural recovery rates per sample material and spiking level were in the range of 87-107%, with relative standard deviations in the range of 4.4 to 16.0%.

At day 0, average residue levels of spiroxamine were 10.7 mg/kg in barley green material and 4.15 mg/kg in barley straw. In the case of barley grains, separate storage of spiroxamine and spiroxamine-N-oxide spiked material was processed, with average residue levels of 0.4 mg/kg and 0.39 mg/kg at day 0 respectively. In samples analysed after storage for the periods of time described above, storage stability values in barley green material ranged from 85-100%; in straw from 93-10%; and in grain 80-88% when spiked with spiroxamine or 72-86% when spiked with KWG 4168 - N-oxide. There was no evidence of any significant degradation of incurred or fortified residue in any of the sample materials. At all sampling dates and in all sample materials, the total recovered residues were above 70% of the expected value (as shown in Table CA 6.1/01-1 below).

Table CA 6.1/01-1 Summary of deep-freezer stability data of spiroxamine total residue in barley sample materials

Analyte	Commodity	Storage period (months) [actual in days]	Residue level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level or incurred residue) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
spiroxamine	barley green material	0	10.8, 10, 11.1, 10.5, 10.9, 11.1	98, 90, 101, 95, 99, 101 [97]	80	95
		0.5 [16 days]	10.9, 10.2, 10.7	99, 93, 97 [96]	88	93
		1.5 [51 days]	11, 10.9, 11	100, 99, 100 [100]	96	92
		3 [98 days]	11.4, 10.5, 10.4	104, 95, 95 [98]	97	88
		14 [434 days]	10.2, 11, 9.3	93, 100, 85 [93]	102	84
	barley straw	0	3.8, 4.1, 4.2, 4.2, 4.3, 4.3	90, 98, 100, 100, 102, 102 [99]	100	93
		0.5 [14 days]	4.3, 4.3, 3.9	102, 102, 93 [99]	107	87
		1.5 [49 days]	3.8, 3.9, 3.8	90, 93, 90 [91]	106	87
		3 [97 days]	4.1, 4.2, 4.2	98, 100, 100 [99]	107	91
		14 [378 days]	4.2, 4.1, 4.1	100, 98, 98 [99]	114	79
	barley grain [high starch]	0	0.41, 0.4, 0.4, 0.4, 0.38, 0.39	82, 80, 80, 80, 95, 78 [83]	87	82
		0.5 [16 days]	0.44, 0.43, 0.44	88, 86, 88 [87]	77	117
		1.5 [51 days]	0.4, 0.42, 0.4	80, 84, 80 [81]	83	79
		3 [98 days]	0.4, 0.42, 0.44	80, 84, 88 [84]	93	84
		14 [434]	0.4, 0.4, 0.42	80, 80, 84 [81]	98	81
Spiroxamine -N-oxide	barley grain	0	0.39, 0.42, 0.36, 0.37, 0.39, 0.41	78, 84, 72, 74, 78, 82 [78]	-	-
		0.5 [14 days]	0.43, 0.42, 0.43	86, 84, 86 [85]	-	-
		1.5 [49 days]	0.41, 0.39, 0.4	82, 78, 80 [80]	-	-
		3 [97 days]	0.39, 0.38, 0.39	78, 76, 78 [77]	-	-
		14 [377 days]	0.39, 0.36, 0.38	78, 72, 76 [75]	-	-

III. Conclusions

During a storage period of 1 year under frozen ≤-18°C) conditions, the total residue of spiroxamine is stable in barley grains (high starch), straw and green material. Testing was on incurred field samples for green material and straw and for grains used samples fortified with spiroxamine or spiroxamine-N-oxide. Consequently, the results of the storage stability studies validate the residue values reported in supervised field trials with respect to storage stability.

Assessment and conclusion by applicant:

Acceptable study to address the data point.

Stability was demonstrated for spiroxamine and its spiroxamine-N-oxide metabolite in aliquots of barley (high starch) grains, straw and green material under frozen storage at ≤-18°C for 12 months, measured as the total spiroxamine common moiety.

As it was noted by EFSA that the common moiety method used for the analysis of stored samples is not able to confirm stability of spiroxamine residues during frozen storage, a new storage stability study in dry and high acid crops is provided (CA 6.1.05).

Data Point:	KCA 6.1/02
Report Author:	[redacted]
Report Year:	1997
Report Title:	Storage stability of KWG 4168 in/on grapes and processed commodities
Report No:	MR-360/97
Document No:	M-010763-01-1
Guideline(s) followed in study:	US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

This study was conducted to determine the freezer storage stability of the total residue of spiroxamine in grapes (high acid) using control samples of berries, juice and raisins. The commodities were separately spiked with spiroxamine parent compound or with the metabolite spiroxamine-aminodiol [M28] and stored under deep frozen conditions (≤-18°C) over a period of 18 months (529 to 585 days).

The limit of quantification (LOQ) for each of the analytes was 0.05 mg/kg, expressed as spiroxamine equivalents. There was no evidence of any degradation of either of the analytes in any of the sample materials. The results show that spiroxamine is stable in grape and processed commodities with the measured total residue in all sampling dates in all sample materials being above 70%.

The results show that spiroxamine and spiroxamine-aminodiol are stable in commodities representative of grape (high acid) matrix types as described in OECD 506 when stored frozen for up to 18 months.

Procedural fortifications were conducted at 0.05 and 0.5 mg/kg for spiroxamine in grapes and raisins and 0.092 and 0.92 mg/kg for aminodiol in these matrices. In juice, recoveries were conducted at 0.02 and 0.2 mg/kg spiroxamine, and 0.037 and 0.37 mg/kg aminodiol. Recoveries ranged from 70 to 110% (actual 75 to 108%) showing acceptable method performance.

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 84-100%, with relative standard deviations in the range of 2.7 to 14.2%.

The final stability data supports all storage intervals for samples of grapes and processed grape commodities analysed in supervised residue trials (and processing studies), summarised under points CA 6.3 and CA 6.5. Actual details are discussed in the residue study summaries.

I. Materials and methods

Samples of untreated control grapes (berries) plus processed commodities of raisins and grape juice were obtained by the Test Facility. For berries and raisins, the frozen material was homogenised with a cutter and dry ice. Grape juice did not require any further preparation.

Aliquots of grape (berries) and raisins control material (50 g) were weighed into polystyrene boxes. Aluminium foil was used to line the bottom of the boxes. Samples were spiked separately with 1 mL of a spiking solution containing 25 µg/mL of spiroxamine or spiroxamine-aminodiol. This resulted in a concentration of 0.5 mg/kg of spiroxamine and 0.92 mg/kg of spiroxamine-aminodiol (as spiroxamine equivalents) in the samples. After fortification, the boxes were closed.

For juice, aliquots (50 g) of control material were weighed into 100 mL glass bottles. Then the samples were spiked with the spiking solution (1 mL) containing 10 µg/mL of spiroxamine or spiroxamine-

aminodiol. This resulted in a concentration of 0.2 mg/kg of spiroxamine and 0.37 mg/kg of spiroxamine-aminodiol (as spiroxamine equivalents) in the samples. After fortification, the bottles were closed.

In addition, untreated samples of each commodity were weighed and stored for control and recovery testing.

The entire sample in the polystyrene box or the glass bottle was taken for subsequent extraction.

The samples were stored in the standard plastic or glass containers in a freezer at temperatures of ≤-18°C over a period of about 18 months. For day-0 analysis, 4 treated samples of each matrix were selected, as well as one control sample. Samples were then also analysed after 19-21, 82-96, 182-192, 355-369, and 529-585 days of storage. At each of these intervals, 2 treated samples of each matrix were removed from storage and analysed, as well as a control sample. Concurrent recoveries were also performed to check method performance.

Description of analytical procedures

Samples of grapes, raisins and juice were analysed using the validated analytical method 00407, report reference M-019430-01-1 (**see Doc MCA Section 4**).

The residues were extracted from the sample with acetone and water. After filtration an aliquot of the extract was taken, acidified and refluxed. By this procedure, spiroxamine and metabolites with intact N-ethyl-N-propyl-1,2-dihyroxy-3-propanamine- structure are hydrolysed to the common moiety "aminodiol" [M28]. The residues were concentrated to the aqueous remainder. Co-extractives were removed by partitioning with dichloromethane and ethyl acetate. The remaining aqueous layer was cleaned up on a polystyrene-divinylbenzene SPE column. The residues were determined using GC/MS in the single-ion-monitoring mode.

II. Results and discussion

The mean values of the procedural recovery rates per compound, sample material, and spiking level were in the range of 84-100%, with relative standard deviations in the range of 2.7 to 14.2%.

At day 0, average residue levels of spiroxamine were 0.51 mg/kg in grapes (berries), 0.46 mg/kg in raisins, and 0.20 mg/kg in juice. The respective residue levels after fortification of spiroxamine-aminodiol were 0.92, 0.83, and 0.37 mg/kg (expressed in spiroxamine equivalents). In samples analysed after storage for the periods of time described above, storage stability values in the grapes (berries) ranged from 79-102%, in raisins from 70-99% and in juice from 79-105%. There was no evidence of any significant degradation of either of the analytes in any of the sample materials. At all sampling dates and in all sample materials, the total residues of spiroxamine equivalents were above 70% (as shown in Table CA 6.1/02-1 below).

Table CA 6.1/02-1 Summary of deep-frozen stability data of spiroxamine in grape matrices after spiking with either spiroxamine parent compound or KWG 4168 - aminodiol

Analyte	Commodity	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ[2]	10xLOQ[3]
spiroxamine	grape berries (high acid)	0	0.512, 0.520, 0.510, 0.488	102, 104, 102, 98 [102]	106	-
		0.5 [19 days]	0.374, 0.408	75, 82 [79]	-	85
		3 [96 days]	0.390, 0.409	78, 82 [80]	-	88
		6 [192 days]	0.465, 0.467	93, 93 [93]	97, 97	106

Analyte	Commodity	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ[2]	10xLOQ[3]
		12 [369 days]	0.488, 0.466	98, 93 [96]	93, 80	99
		20 [585 days]	0.502, 0.467	100, 93 [97]	93, 111	92
	grape juice	0	0.201, 0.209, 0.202, 0.200	101, 105, 101, 100 [102]	108	-
		1 [21 days]	0.187, 0.183	94, 92 [93]	-	89
		3 [84 days]	0.135, 0.177	68, 89 [79]	-	83
		6 [188 days]	0.188, 0.182	94, 91 [93]	75, 76	94
		12 [355 days]	0.187, 0.191	94, 96 [95]	85, 83	93
		19 [574 days]	0.201, 0.205	101, 103 [102]	107, 96	93
	raisins	0	0.425, 0.454, 0.462, 0.492	85, 91, 93, 98 [92]		93
		1 [19 days]	0.427, 0.476	85, 95 [90]	-	80
		3 [82 days]	0.347, 0.366	69, 73 [71]		95
		6 [182 days]	0.462, 0.474	92, 95 [94]	78, 77	94
		12 [355 days]	0.454, 0.487	91, 97 [94]	88, 88	95
		18 [529 days]	0.469, 0.516	94, 103 [99]	82, 88	83
Spiroxamine -aminodiol	grape berries high acid	0	0.047[1], 0.948, 0.925, 0.879	103, 101, 96 [100]	103	-
		0.5 [19 days]	0.671, 0.784	73, 85 [79]	-	83
		3 [96 days]	0.798, 0.825	87, 90 [88.5]	-	88
		6 [192 days]	0.870, 0.872	95, 95 [95]	95, 103	90
		12 [369 days]	0.874, 0.885	95, 96 [96]	-	-
		20 [585 days]	0.871, 0.927	95, 101 [98]	77, 92	93
	grape juice	0	0.404, 0.382, 0.376, 0.328	109, 103, 102, 89 [101]	106	-
		1 [21 days]	0.313, 0.306	85, 83 [84]	-	88
		3 [84 days]	0.350, 0.348	95, 94 [95]	-	92
		6 [188 days]	0.358, 0.366	97, 99 [98]	-	96
		12 [355 days]	0.344, 0.362	98, 98 [98]	-	-

Analyte	Commodity	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ[2]	10xLOQ[3]
		19 [574 days]	0.387, 0.390	105, 105 [105]	93, 102	100
	raisins	0	0.920, 0.882, 0.807, 0.709	100, 96, 88, 77 [90]	-	100
		1 [19 days]	0.856, 0.844	93, 92 [93]	-	94
		3 [82 days]	0.918, 0.891	100, 97 [99]	-	97
		6 [182 days]	0.686, 0.588	75, 64 [70]	-	98
		12 [355 days]	0.550, 0.905	60, 98 [79]	-	
		18 [529 days]	0.905, 0.858	98, 93 [96]	80, 87	-

1-Outlier, not used for average result
2-LOQ recoveries 0.05 mg/kg for spiroxamine 0.092 mg/kg for spiroxamine-aminodiol (spiroxamine eq)
3-10xLOQ recoveries 0.5 mg/kg for spiroxamine 0.92 mg/kg for spiroxamine-aminodiol (spiroxamine eq)

III. Conclusions

During a storage period of 18 months (529 to 585 days) under frozen (≤-18°C) conditions, the total residues of spiroxamine were stable in grapes (high acid), raisins, and juice from spiking with spiroxamine or spiroxamine-aminodiol. These results validate the residue values reported in supervised field trials and processing studies with respect to storage stability.

Assessment and conclusion by applicant:

Acceptable study to address the data point.

Stability was demonstrated for total spiroxamine and its spiroxamine-aminodiol metabolite in homogenised aliquots of grapes (high acid), raisins, and juice under frozen storage at ≤-18°C for 18 months (529 to 585 days).

As it was noted by EFSA that the common moiety method used for the analysis of stored samples is not able to confirm stability of spiroxamine residues during frozen storage, a new storage stability study in dry and high acid crops is provided (CA 6.1.05).

Data Point:	KCA 6.1/03
Report Author:	[redacted]; [redacted]; [redacted]
Report Year:	1995
Report Title:	Metabolism of KWG 4168 in spring wheat
Report No:	PF4053
Document No:	M-006099-01-1
Guideline(s) followed in study:	US EPA Pesticides Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4, Nature of residues in plants and livestock
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

This study was conducted to determine the freezer storage stability of the total residues of spiroxamine in wheat grain (high starch), parent spiroxamine in wheat forage (high water) and wheat straw (dried commodity); and metabolites spiroxamine-N-oxide (M03), spiroxamine-hydroxy-despropyl (M09) and 3 metabolite groups bearing the intact tert.-butylketone moiety in wheat forage (high water) and straw (dried commodity).

Samples with incurred residues were used from experiment B in the wheat metabolism study (CA 6.2.1/01) and stored for 0-10, 30-32, 60-65, 121-130, 181-200, 364-368 and 517-566 days at <-20°C.

There was no significant decrease in total spiroxamine residues in wheat grain (high starch) or parent spiroxamine in wheat forage (high water) and wheat straw (dry commodity). There was also no significant decrease in metabolites spiroxamine-N-oxide (M03), spiroxamine-hydroxy-despropyl (M09) and 3 metabolite groups bearing the intact tert.-butylketone in wheat forage (high water) or wheat straw (dry commodity).

The results show that spiroxamine (total) was stable in high starch commodities when stored frozen for up to 517 to 566 days. Spiroxamine (parent) and metabolites were stable in high water commodities and dried commodities when stored frozen for up to 517 to 566 days.

I. Materials and methods

Samples of [cyclohexyl-1-^{14}C] spiroxamine treated wheat forage, straw and grain from the wheat metabolism study (CA 6.2.1/01) were analysed at 0-10, 30-32, 60-65, 121-130, 181-200, 364-368 and 517-566 days after storage at <-20°C. For each analysis, two samples were extracted and investigated separately. Pre-packed portions of forage (50 g) and straw (20 g) were homogenised and prepared before storage. Grain (5 g) was taken as needed from one storage container.

The samples were extracted twice with methanol/water and the combined extracts were evaporated to the aqueous residue. The radioactivity was partitioned into dichloromethane and water and analysed by radio-TLC. The radioactivity in the solids was determined by combustion.

II. Results and discussion

The extractions for the storage stability study were conducted at 0-10, 30-32, 60-65, 121-130, 181-200, 364-368 and 517-566 days after storage. The results indicate that parent compound and the following metabolites: spiroxamine-N-oxide (M03), spiroxamine-hydroxy-despropyl (M09) and 3 metabolite groups bearing the intact tert.-butylketone moiety, quantified as one, remained stable under the storage conditions in forage and straw. Quantitative radio-TLC analyses of grain was not possible due to the high matrix content and low amounts of radioactivity. However, a comparable pattern of parent

compound and metabolites was observed and it was concluded that parent compound and metabolites remained stable under the storage conditions.

Table CA 6.1/03-1 Summary of deep-freezer stability data of spiroxamine total residue in wheat sample materials

Days	Residue compound[1]	Residue recovery					
		Wheat forage [High water]		Wheat straw [Dried commodity]		Wheat grain [High starch]	
		% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
1-10	Parent	n/a	n/a	26.80	7.77	-	-
	Metabolites[2]	n/a	n/a	15.10	4.38	-	
	Total residues	-	-	-	-	35.10	0.018
30-32	Parent	53.15	3.98	24.30	6.73	-	-
	Metabolites[2]	7.45	0.55	22.45	6.22	-	
	Total residues	-	-	-	-	20.40	0.009
60-65	Parent	53.15	3.90	36.35	10.40	-	-
	Metabolites[2]	10.45	0.77	12.45	3.57	-	
	Total residues	-	-	-	-	22.95	0.011
121-130	Parent	50.10	3.93	25.3	7.27	-	-
	Metabolites[2]	10.60	0.83	22.10	6.35	-	-
	Total residues	-	-	-	-	31.25	0.016
181-200	Parent	55.10	3.79	25.85	7.48	-	-
	Metabolites[2]	8.50	0.58	21.00	6.07	-	-
	Total residues	-	-	-	-	123.50	0.051
364-368	Parent	51.20	3.12	20.90	5.85	-	-
	Metabolites[2]	7.80	0.48	21.35	5.95	-	-
	Total residues	-	-	-	-	32.75	0.016
517-566	Parent	48.95	4.21	23.85	6.73	-	-
	Metabolites[2]	8.40	0.73	25.85	7.30	-	-
	Total residues	-	-	-	-	31.00	0.015

1 – analysis of dichloromethane phase, mean of two analyses
2 - covering spiroxamine-N-oxide (M03), spiroxamine -hydroxy-despropyl (M09) and 3 metabolite groups bearing the intact tert.-butylketone moiety

III. Conclusions

The total residues of spiroxamine are stable in wheat grains (high starch) under frozen conditions (<-20°C) for 517 to 566 days. Parent spiroxamine and metabolites are also stable in wheat forage (high water) and wheat straw (dry commodity) under frozen conditions (<-20°C) for 517 to 566 days.

Assessment and conclusion by applicant:

Acceptable study to address the data point.

Stability was demonstrated for total spiroxamine in wheat grain, parent spiroxamine and metabolites in wheat forage and wheat straw under frozen storage at <-20°C for 517 to 566 days.

Data Point:	KCA 6.1/04
Report Author:	[redacted]
Report Year:	2004
Report Title:	Determination of the storage stability of KWG 4168 and aminodiol residues in fortified analytical samples of hops and banana during frozen storage
Report No:	MR-178/03
Document No:	M-060190-01-1
Guideline(s) followed in study:	US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A deep-freezer storage stability study was conducted to determine the freezer storage stability of the total residue of spiroxamine in hops (green cones, beer, spent hops) and banana (fruit, [high water]). The commodities were separately spiked with spiroxamine or with the metabolite spiroxamine-aminodiol and stored frozen at ≤-18°C over a period of 24 months. The samples spiked with spiroxamine were analysed for both parent compound and as total spiroxamine (determined as aminodiol and expressed as spiroxamine equivalents). The samples spiked with spiroxamine-aminodiol were analysed for total spiroxamine (determined as aminodiol and expressed as spiroxamine equivalents).

The LOQ for each of the analytes was 0.05 mg/kg for all matrices. There was no evidence of any degradation of either of the analytes in any of the sample materials. The results show that spiroxamine was stable in hops (green cones, beer, spent hops) and banana (fruit).

After a maximum storage period of 'day X' + 21 months, corresponding to about 2 years (713 to 723 days) at ≤-18°C, spiroxamine parent compound and as total spiroxamine (determined as aminodiol and expressed as spiroxamine equivalents) were shown to be stable. Both analytes were also stable in beer and spent hops after a maximum storage period of day X + 2 months, corresponding to about 5 to 7 months (147 to198 days). 'Day X' referred to the number of storage days between sample fortification and date of first extraction. It varied between 75 and 106 days. This was because the method was not fully validated on the day of sample spiking and the sample analysis was done later, once an appropriately validated method was available.

The results show that spiroxamine was stable in commodities representative of these matrix types as described in OECD 506, when stored frozen for up to 24 months.

Procedural fortifications were conducted at a level of 0.05 and 0.5 mg/kg for spiroxamine in all matrices. Recoveries ranged from 71 to 115% showing acceptable method performance.

The mean concurrent recovery rates per compound and spiking level were in the range of 84-115% for banana (fruit), 82-106% for green cones, 96-107% for beer and 88-115% for spent hops.

The final stability data supports storage intervals for samples of bananas or hops commodities up to the intervals investigated, however this dossier does not consider these crops but the data can be evaluated to support the overall stability of spiroxamine in crops.

I. Materials and methods

The bulk control material used for fortification was either from previous residue studies, a German hops processing factory or a grocery (in the case of beer). For the preparation of homogeneous laboratory samples (solid samples), each sample material was macerated in the presence of dry ice in a rotating cutter bowl at the Laboratory (PVTL). Parts of the frozen laboratory samples were then transferred into polystyrene boxes and stored at ≤-18°C.

Aliquots of the sample materials (5 g for all matrices except 2 g aliquots for spent hops) were weighed into brown-glass bottles with plastic caps. In addition to the bottles required for analysis, 12 reserve samples were prepared for each sample material (6 control samples and 6 spiked samples).

Samples were extracted at the nominal storage intervals of 'day X' and then at nominal 2, 5, 18 and 21 months thereafter. 'Day X' corresponded to the number of storage days between sample spiking (fortification) and date of first extraction (first sample interval) and 'day X' varied between 75 and 106 days. Since the method was not yet validated on the day of sample spiking, the sample analysis was done later when the appropriate validated method was available. The total nominal storage period for beer and spent hops was 'day X' + 2 months (147 to 198 days) and 'day X' + 21 months (713 to 723 days) for banana fruit and green hop cones.

The aliquots specified as "spiked sample" were fortified at either 0.5 mg/kg of spiroxamine or KWG 4168 - aminodiol. After evaporation of the solvent (approximately 15 minutes), the glass bottles were sealed. The beer sample bottles were sealed immediately. The samples were then stored in a deep-freezer at -18°C or below.

On 'day X' (first sample analysis interval), five stored spiked samples were analysed along with one control sample for each sample matrix. At each further sampling date, for each sample material three spiked samples and the required number of control samples were removed from the freezer and allowed to come to room temperature. The stored control samples were either analysed directly as a control sample or as freshly fortified for recoveries. The two freshly fortified samples (concurrent recoveries) were then extracted, cleaned up and analysed concurrently with the remaining control sample and the stored spiked samples.

Description of analytical procedures

Samples of banana (fruit) and hops (green cones, beer and spent hops) were either spiked with spiroxamine parent compound and analysed for parent and aminodiol, or spiked with KWG 4168 - aminodiol and analysed for aminodiol. Spiroxamine was determined with two separate method approaches, covering the two different residue definitions then used in the USA and in the EU.

Total spiroxamine (common moiety method)

Samples for total spiroxamine as a common moiety (aminodiol moiety) were analysed using the validated analytical method 00407/E002, report reference M-019430-01-1 **(see Doc MCA Section 4).**

This method was applied for samples fortified with spiroxamine-aminodiol.

Residues were extracted from the sample material with acetone and water. After filtration, an aliquot of the extract was taken, acidified and refluxed. By this, spiroxamine and any metabolites / degradates with intact N-ethyl-N-propyl-1,2-dihydroxy-3-aminopropane-structure were hydrolysed to the common moiety, aminodiol. The residues were concentrated to the aqueous remainder. Co-extractives were removed by shaking with dichloromethane and ethyl acetate. The aqueous layer was cleaned up on a polystyrene divinylbenzene SPE column and the residues were determined after silylation with GC/MS in the single-ion-monitoring mode. Samples were analysed as aminodiol and expressed as total spiroxamine equivalents.

Spiroxamine

Samples for spiroxamine were analysed using the validated analytical method 00721, report reference M-058392-01-1 **(see Doc MCA Section 4).**

Residues of spiroxamine were extracted from the homogenised samples with an acetone/water mixture by high-speed blending. After filtration, the acetone/water-phase was diluted for measurement by LC-MS/MS. The analyte was chromatographed by reversed-phase HPLC on a C18 column using an isocratic acetonitrile/water eluent containing acetic acid. A triple-stage mass spectrometer with an electrospray interface operated in the positive ion mode under multiple reaction monitoring (MRM) conditions was coupled to the outlet of the HPLC column to obtain highly sensitive and selective detection (RP-LC-ESI_MS/MS).

II. Results and discussion

The mean concurrent recovery rates per compound and spiking level were in the range of 84-115% for banana (fruit), 82-106% for green cones, 96-107% for beer and 88-115% for spent hops.

Both repeatability and successful spiking were shown for all matrices on 'day X' (varying between 75 and 106 days after fortification) with mean recoveries between 89 and 102% and relative standard deviations (RSD) between 0.44 and 9.5% related to sets of 5 individual treated samples. This was true for samples spiked with spiroxamine and determined both for spiroxamine parent compound and as the total spiroxamine (determined as aminodiol and expressed as spiroxamine equivalents).

After a maximum storage period of 'day X' + 21 months, corresponding to about 2 years (actual 713 and 723 days) at -18°C and below recoveries of spiroxamine (both analysed as aminodiol and as parent in the respective sample sets), the mean recoveries in banana fruit and hops, green cones were between 84 and 100%. Concerning the aminodiol spiked samples 83 and 90% were recovered in banana, fruit and hops, green cones after about 2 years of storage.

Regarding the sample materials beer and spent hops, the mean recoveries of spiroxamine were between 101 and 109% after a storage period between 5 and 7 months. In the aminodiol spiked samples 105 and 104% were found. Both analytes are stable in the sample materials for the respective storage periods investigated. The mean values at all sampling dates and in all sample materials, the total residues of spiroxamine were above 70% (as shown in Table CA 6.1/04-1 to Table CA 6.1/04-4 below).

Table CA 6.1/04-1 Summary of deep-freezer stability data of spiroxamine in banana (fruit) after spiking with either spiroxamine (as common moiety or parent) or aminodiol

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
banana fruit high water	spiroxamine as aminodiol (common moiety)	2 (day X) [75 days]	0.495, 0.46, 0.48, 0.49, 0.49	99, 92, 95, 97, 97 [96]	-	84, 96
		4 [131 days]	0.52, 0.46, 0.47	104, 91, 94 [96]	80, 96 91	92, 98
		7 [208 days]	0.54, 0.51, 0.55	107, 101, 110 [106]	-	108, 111
		21 [621 days]	0.48, 0.47, 0.44	96, 94, 88 [93]	61[1], 109, 103	84, 89
		24 [719 days]	0.49, 0.47, 0.48	98, 93, 96 [96]	-	97, 102

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
	KWG 4168 - aminodiol as aminodiol (common moiety)	2 (day X) [75 days]	0.52, 0.44, 0.45, 0.44, 0.45	91, 88, 89, 88, 89 [89]	-	90, 97
		4 [131 days]	0.46, 0.495, 0.42	92, 99, 83 [91]	-	95, 96
		7 [208 days]	0.55, 0.54, 0.51	109, 108, 102 [106]	-	104, 102
		21 [621 days]	0.46, 0.55, 0.49	92, 109, 98 [100]	-	99, 97
		24 [719 days]	0.46, 0.35, 0.45	91, 69, 89 [83]	-	92, 93
	spiroxamine as parent	3 (day X) [104 days]	0.45, 0.46, 0.46, 0.47, 0.45	89, 92, 91, 93, 89 [91]	90, 91, 94	89, 88
		5 [140 days]	0.495, 0.5, 0.49	99, 100, 97 [99]	-	100, 98
		7 [222 days]	0.5, 0.44, 0.47	100, 88, 94 [94]	92, 96, 98	100, 99
		21 [629 days]	0.44, 0.45, 0.42	88, 90, 83 [87]	-	92, 92
		24 [722 days]	0.46, 0.40, 0.42	91, 79, 83 [84]	-	93, 74
		5 [147 days]	0.53, 0.53, 0.48	106, 106, 96 [103]	-	98, 93
	spiroxamine as parent	3 (day X) [106 days]	0.48, 0.48, 0.49, 0.47, 0.48	96, 95, 98, 93, 95 [95]	114, 115, 214[2]	98, 94
		4 [128 days]	0.48, 0.47, 0.46	95, 93, 92 [93]	-	98, 98
		5 [149 days]	0.5, 0.495, 0.64	100, 99, 127 [109]	-	93, 93

1-invalid value, because derivatisation was incomplete
2-invalid value, because of erroneous sample work-up

Table CA 6.1/04-2 Summary of deep-freezer stability data of spiroxamine in hops (green cones) after spiking with either spiroxamine (as common moiety or parent) or aminodiol

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
hops (green cones)	spiroxamine as aminodiol (common moiety)	3 (day X) [84 days]	0.49, 0.55, 0.47, 0.51, 0.53	98, 109, 94, 102, 106 [102]	-	98, 92
		5 [139 days]	0.51, 0.53, 0.39	101, 105, 78 [95]	105, 97, 92	81, 85
		7 [216 days]	0.51, 0.54, 0.53	102, 107, 106 [105]	-	89, 111

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
		21 [615 days]	0.47, 0.34, 0.46	94, 67, 91 [84]	100, 35[1], 89	71, 92
		24 [713 days]	0.48, 0.48, 0.47	96, 95, 93 [95]	-	84, 96
	KWG 4168 - aminodiol as aminodiol (common moiety)	3 (day X) [84 days]	0.52, 0.54, 0.51, 0.47, 0.47	104, 107, 101, 94, 95 [100]	-	97, 102
		5 [139 days]	0.44, 0.52, 0.52	87, 104, 104 [98]	-	95, 94
		7 [216 days]	0.51, 0.495, 0.52	108, 99, 103 [103]	-	95, 96
		21 [615 days]	0.4, 0.26, 0.42	80, 51, 84 [72]	-	93, 93
		24 [713 days]	0.44, 0.46, 0.46	87, 91, 91 [90]	-	91, 93
	spiroxamine as parent	4 (day X) [105 days]	0.46, 0.46, 0.46, 0.455, 0.43	92, 92, 92, 91, 86 [91]	100, 98, 105	89, 91
		5 [141 days]	0.49, 0.49, 0.54	97, 97, 107 [100]	-	97, 96
		7 [223 days]	0.495, 0.495, 0.505	99, 99, 101 [100]	-	102, 102
		21 [630 days]	0.47, 0.47, 0.47	93, 93, 93 [93]	84, 95, 93	100, 99
		24 [723 days]	0.49, 0.51, 0.5	97, 102, 100 [100]	-	104, 107

1- invalid value because of erroneous sample work-up

Table CA 6.1/04-3 Summary of deep-freezer stability data of spiroxamine in hops (beer) after spiking with either spiroxamine (as common moiety or parent) or aminodiol

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
hops (beer)	spiroxamine as aminodiol (common moiety)	3 (day X) [98 days]	0.53, 0.43, 0.47, 0.53, 0.47	106, 85, 93, 106, 93 [97]	-	53[1], 51[1]
		4 [126 days]	0.56, 0.57, 0.54	112, 113, 107 [111]	-	106, 107
		5 [155 days]	0.125[1], 0.13[1], 0.36	25[1], 26[1], 71 [-]	-	34[2], 112[2]
		7[3] [198 days]	0.55, 0.545, 0.53	110, 109, 105 [108]	-	99, 112
	KWG 4168 - aminodiol as aminodiol (common moiety)	3 (day X) [98 days]	0.49, 0.52, 0.51, 0.5, 0.495	98, 104, 102, 100, 99 [101]	-	96, 96
		4 [126 days]	0.49, 0.53, 0.56	98, 105, 111 [105]	-	99, 100

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
		5 [155 days]	0.54, 0.51, 0.53	107, 101, 106 [105]	-	103, 105
	spiroxamine as parent	3 (day X) [101 days]	0.5, 0.51, 0.51, 0.51, 0.51	100, 101, 101, 101, 101 [101]	102, 106, 106 [105]	103, 93
		4 [128 days]	0.49, 0.495, 0.5	98, 97, 100 [98]	-	97, 99
		5 [149 days]	0.49, 0.51, 0.52	98, 101, 103 [101]		99, 98

1:- inappropriate spiking; the fortified amount was only half the projected amount
2:- invalid recoveries, because of extraction error, whole sample set was repeated
3:- repetition of sample extraction

Table CA 6.1/04-4 Summary of deep-freezer stability data of spiroxamine in hops (spent hops) after spiking with either spiroxamine (as common moiety or parent) or aminodiol

Commodity	Analyte	Storage period (months) [actual in days]	Residue Level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)	
					LOQ	10xLOQ
hops (spent hops)	spiroxamine as aminodiol (common moiety)	3 (day X) [91 days]	0.49, 0.48, 0.45, 0.47, 0.47	98, 96, 89, 94, 93 [94]	-	95, 81
		4 [119 days]	0.48, 0.44, 0.495	96, 88, 99 [94]	-	89, 94
		5 [147 days]	0.47, 0.59, 0.54	79, 117, 107 [101]	-	94, 112
	KWG 4168 - aminodiol as aminodiol (common moiety)	3 (day X) [91 days]	0.46, 0.46, 0.45, 0.43, 0.42	92, 92, 90, 86, 84 [89]	-	89, 91
		4 [119 days]	0.48, 0.47, 0.47	96, 94, 94 [95]	-	96, 100
		5 [147 days]	0.53, 0.53, 0.48	106, 106, 96 [103]	-	98, 93
	spiroxamine as parent	3 (day X) [106 days]	0.48, 0.48, 0.49, 0.47, 0.48	96, 95, 98, 93, 95 [95]	114, 115, 214[1] [115]	98, 94
		4 [128 days]	0.48, 0.47, 0.46	95, 93 92 [93]	-	98, 98
		5 [149 days]	0.5, 0.495, 0.64	100, 99, 127 [109]	-	93, 93

1- invalid value, because of erroneous sample work-up

III. Conclusions

After a maximum storage period of approximately 24 months (713 to 723 days) at ≤-18°C, total residues of spiroxamine were shown to be stable in banana fruit (high water) and green hop cones. Both analytes were also stable in beer and spent hops after a maximum storage period of 5 to 7 months respectively (147 to198 days).

Assessment and conclusion by applicant:

Acceptable study to address the data point.

Residues of spiroxamine and total spiroxamine were shown to be stable in banana fruit (high water) and green hop cones remain stable for at least 24 months when samples are stored under deep frozen conditions (≤-18 °C). Both analytes were also stable in beer and spent hops after a maximum storage period of five to seven months respectively (147 to 198 days) when samples are stored under deep frozen conditions (≤-18 °C).

Data Point:	KCA 6.1/05
Report Author:	[redacted]
Report Year:	1996
Report Title:	KWG 4168 - Cattle feeding study
Report No:	MR-91/96
Document No:	M-006159-02-1
Guideline(s) followed in study:	U.S. EPA FIFRA Guideline, Subdivision O, No. 171-4(b)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The study was conducted to obtain data on the storage stability of spiroxamine-acid (M06) in milk and tissues from bovine origin under deep frozen conditions (≤-18°C) in the dark over a storage period up to 228 days, in support of a cattle feeding study.

The results show that spiroxamine-acid is stable in commodities representative of milk, kidney and muscle (meat) from bovine origin when stored frozen for up to 228 days. In fat, average residues at the 228 day storage interval are 64%, suggesting some degradation has occurred, Procedural fortifications were conducted at 0.5 mg/kg for spiroxamine-acid, with recoveries generally ranging from 70 to 110% (actual 80 to 103%) showing acceptable method performance.

The stability data supports all storage intervals for milk, kidney and muscle samples analysed in a cattle livestock feeding study and summarised under point CA 6.4.2. In fat, uncorrected residue of spiroxamine-acid metabolite was just below 70%. In liver, residues of spiroxamine-acid had declined to less than 50% of the initial level after 222 days. However, interpolation of fat and liver residues would imply that spiroxamine-acid is stable in these matrices for at least 100 days frozen storage. Frozen stability has been demonstrated in muscle (representative of bovine meat) and other edible offal, i.e. kidney, so it is considered that adequate storage stability is achieved.

I. Materials and methods

The purpose of this study was to quantify the residues of spiroxamine-acid (M06) in the milk and tissues of lactating dairy cows following oral administration of spiroxamine for a minimum period of 28 days. To support the data, an assessment of stability was also performed as part of the study and this is summarised here.

Control material was bought from a grocery store. Fat and muscle samples were homogenised with a mincing machine. For liver and kidney, whole portions of the tissues were taken.

Aliquots of each of the five matrices (whole milk, liver, kidney, muscle and fat) were weighed into containers and fortified at 0.50 mg/kg (25 or 50 x LOQ) with spiroxamine-acid in acetonitrile using a syringe from a stock solution. .

The containers were then stored in a freezer at approximately -18°C until removal for analysis. The samples were stored alongside samples from the feeding study. At day 0 (day of fortification): 1 spiked samples, 1 control, and 1 recovery sample were analysed. Then depending on the matrix, further samples were analysed after approximately 4 weeks and up to 228 days after fortification. At these later storage intervals, 2 spiked samples, 1 control and 1 recovery sample were analysed.

Samples of tissues and milk were analysed for spiroxamine-acid using the validated analytical method 00355, report reference M-019051-02-1 (**see Doc MCA Section 4**).

Milk and muscle

Spiroxamine-acid metabolite was extracted twice from the samples using methanol (milk) or acetonitrile and an acetonitrile/water mix (muscle). After centrifugation, the combined extracts were concentrated to the aqueous remainder. Acetonitrile and water were used to make up to a known volume (25 or 50 mL). Prior to analysis by HPLC, extracts were filtered. Quantitation was performed by LC-MS/MS in the multiple reaction mode.

The limit of quantification was 0.01 mg/kg in milk and 0.02 mg/kg in tissues.

Fat, liver and kidney

Spiroxamine-acid metabolite was extracted twice from the samples using acetonitrile and an acetonitrile/methanol mix (liver and kidney). The same metabolite was extracted from fat twice with a methanol/acetonitrile/cyclohexane mixture (cyclohexane layer discarded). After centrifugation, the combined extracts were concentrated to the aqueous remainder. An XAD-4 column was used to purify the samples. Acetonitrile and water were used to make up to a known volume (25 or 50 mL). Prior to analysis by HPLC, extracts were filtered. Quantitation was performed by LC-MS/MS in the multiple reaction mode.

Untreated and fortified samples were analysed in single assay. No residues above 30% of the limit of quantification were found in any of the untreated samples.

The limit of quantification for all compounds in all matrices was 0.01 mg/kg.

II. Results and discussion

Procedural recoveries run concurrently with fortified test samples at levels of 0.5 mg/kg gave recoveries ranging from 70 to 110% spiroxamine-acid over the tested storage interval.

A summary of the storage stability results for spiroxamine-acid in bovine commodities is presented in Table CA 6.1/05-1 according to the format proposed in OECD 506.

For kidney, milk and muscle, the average amount of spiroxamine-acid recovered was at least 70% at any testing interval, which demonstrates acceptable storage stability for spiroxamine-acid. In fat, uncorrected recoveries were just below 70% (average 64%) and in liver, average recoveries at the 222 day storage interval indicated that over 30% of the fortified residue could not be recovered after frozen storage.

In the study report and previous evaluation of the data, residue levels were corrected for procedural recoveries. This is no longer considered acceptable and has not been applied in this study summary. As demonstrated below, the uncorrected value for fat was just below 70% (average of 64% from 2 values) with a procedural recovery of 88%. A crude interpolation of the results would imply that residues of spiroxamine-acid are stable in fat for approximately 200 days. This would therefore satisfactorily cover

the storage periods for fat seen in the cattle feeding study described in CA 6.4.2. Additionally, fat is an intrinsic component of meat, and frozen storage stability in muscle was fully acceptable with little or no degradation over time seen in this tissue. It can therefore be concluded that stability of spiroxamine-acid in ruminant meat is stable under the conditions of storage.

Significant degradation on frozen storage over time was noted in liver tissue with recoveries of less than 50% of the original spiked residue found after 222 days. In the study report and subsequent evaluations, an explanation was provided detailing the differences in storage between whole ruminant liver in the feeding study and the macerated liver tissues used for frozen storage stability (FSS) evaluation. When evaluating FSS, it was not possible to store whole tissue, therefore untreated liver was cut into cubes of approximately 12.5g in weight which were then spiked with a solution of spiroxamine-acid at ambient temperature. Cutting the parenchyma in liver tissue is known to release the highly active hepatic enzymes and this is thought to explain the degradation of the spiroxamine-acid moiety in liver tissue as a result of increased enzyme activity. It would be anticipated that this degradation is far slower in the liver tissue stored as part of the cattle feeding study. A crude extrapolation of the results would imply that residues of spiroxamine-acid are stable in liver for at least 100 days. Additionally, residues in other edible tissue such as kidney were deemed to be stable under the applied conditions of frozen storage.

Table CA 6.1/05-1 Analytical results for tissue and whole milk samples fortified with spiroxamine-acid

Commodity	Analyte	Storage period (days)	Residue level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)
Fat	Spiroxamine-acid	0	0.458, 0.436, 0.463, 0.453	92, 87, 93, 91 [91]	94
		28	0.553, 0.486	111, 97 [104]	101
		228	0.333, 0.301	67, 60 [64]	88
Kidney	Spiroxamine-acid	0	0.316, 0.388, 0.400, 0.394	63, 78, 80, 79 [75]	80
		28	0.283, 0.446	57, 89 [73]	92
		224	0.450, 0.423	90, 85 [88]	100
Liver	Spiroxamine-acid	0	0.459, 0.460, 0.516, 0.430	92, 92, 103, 86 [93]	95
		28	0.464, 0.416	93, 83 [88]	102
		222	0.261, 0.140	52, 28 [40]	81
Whole milk	Spiroxamine-acid	0	0.533, 0.498, 0.539, 0.459	107, 100, 108, 92 [102]	95
		32	0.519, 0.514	104, 103 [104]	101
		192	0.535, 0.562	107, 112 [110]	100
Muscle	Spiroxamine-acid	0	0.513, 0.490, 0.526, 0.576	103, 98, 105, 115 [105]	103
		32	0.490, 0.500	98, 100 [99]	102
		175	0.575, 0.573	115, 115 [115]	94

III. Conclusions

Acceptable stability was demonstrated for spiroxamine-acid in aliquots of whole milk, kidney and muscle under frozen storage at ≤-18°C for up to 228 days (approximately 7.5 months).

In fat, uncorrected residue of spiroxamine-acid metabolite was just below 70% (average of 64% from 2 values) with a procedural recovery of 88%. A crude interpolation of the results would imply that residues of spiroxamine-acid are stable in fat for approximately 200 days. Additionally, fat is an intrinsic component of meat, and frozen storage stability in muscle was fully acceptable with little or no degradation over time seen in this tissue. It can therefore be concluded that residues of spiroxamine-acid in ruminant meat are stable under the conditions of storage.

After 222 days frozen, cubed portions of spiked liver showed degradation of spiroxamine-acid to less than 50% of the initial recovery. This was thought to be due in large part to the tissue being roughly macerated releasing degradative liver enzymes. Livers in the cattle feeding study were stored whole, and similar degradation would not be expected. However, a crude extrapolation of the results would imply that residues of spiroxamine-acid are stable in macerated liver for approximately 100 days. In other edible offal, i.e. kidney, residues of spiroxamine-acid are shown to be stable over the duration of the study.

The stability data supports all storage intervals for these bovine matrices analysed in the cattle feeding study summarised under point CA 6.4.2.

Assessment and conclusion by applicant:

Acceptable study to address the data point.

Stability was demonstrated for spiroxamine-acid, CM06, the major metabolite in animal matrices following repeated dosing of spiroxamine) in aliquots of whole milk, kidney and muscle under frozen storage at ≤-18°C for up to 228 days.

In fat, uncorrected residue of spiroxamine-acid metabolite was just below 70%. In liver, residues of spiroxamine-acid had declined to less than 50% of the initial level after 222 days. However, interpolation of fat and liver residues would imply that spiroxamine-acid is stable in these matrices for at least 100 days frozen storage. Frozen stability has been demonstrated in muscle (representative of bovine meat) and other edible offal, i e. kidney, so it is considered that adequate storage stability is demonstrated.

Data Point:	KCA 6.1/07
Report Author:	[redacted]
Report Year:	2021
Report Title:	Enantiomer specific residue analytical method and short term storage stability of spiroxamine as sum of its enantiomers A1, A2, B1 and B2 in/on honey, pollen and nectar by HPLC-MS/MS
Report No:	M-763118-01-1
Document No:	M-763118-01-1
Guideline(s) followed in study:	Storage stability component of report in accordance with OECD 506
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The study validated the analytical method 01480/M001 to determine the residues of spiroxamine in/on honey, pollen and nectar as a sum of its four enantiomers: A1, A2, B1 and B2 by HPLC–MS/MS detection. In addition, a short-term storage stability of spiroxamine (as individual enantiomers) was performed in matrices honey and pollen at ≤-18°C (target) in the dark over a storage period up to 6 months. This summary describes the storage stability component. Details of the method validation are described in MCA 4.

The samples were spiked with spiroxamine and analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers.

The LOQ, per enantiomer, based on the composition of the reference material, was established at 0.0027 mg/kg for enantiomers A1 and A2 and at 0.0023 mg/kg for B1 and B2. The LOQ based on the composition of the reference material (sum of four enantiomers) was established at 0.01 mg/kg.

After deep-freeze storage (≤-18°C) for a period of about 6 months, the mean recovery rates from the stored samples were 77% for A1, 84% for A2, 89% for B1 and 92% for B2 in honey and 83% for A1, 89% for A2, 80% for B1 and 72% for B2 in pollen.

Procedural fortifications were conducted at levels of 0.10 mg/kg for spiroxamine (as sum of the four enantiomers) in both matrices. The mean concurrent recovery rates per for each individual isomer at the 0.1 mg/kg spiking level were in the range of 78-107% for pollen and 92-110% for honey.

Altogether, the study results demonstrate that the residues of the analytes are stable in honey and pollen for at least 6 months under deep-freezer storage conditions (≤-18°C).

I. Materials and methods

Honey (organic) was obtained from a local supermarket and pollen was obtained from control plants (Phacelia/sunflower).

After weighing, control samples were deep-frozen at ≤-18°C until their preparation.

Aliquots of homogenised matrix material were transferred into a plastic tube with screw cap and fortified with spiroxamine (as the sum of the four enantiomers) at 0.10 mg/kg (i.e. 10 x LOQ) using a solution in acetonitrile. The sample tubes were sealed immediately. Day 0 samples were analysed immediately. The remaining samples were stored in a deep-freezer at -18°C or below. Samples were analysed after nominal storage intervals of 0, 30, 90 and 180 days.

On day 0 (first sample analysis interval), four spiked samples were analysed along with one control sample for each sample matrix. At each further sampling date, two fortified samples per matrix and the required number of control samples were removed from the freezer and allowed to come to room temperature. Subsequently, one blank and two recovery samples per matrix were prepared to determine the concurrent recoveries (fortification levels were at the same concentration as the spiked storage samples). These samples were extracted and analysed concurrently with the remaining (unfortified) control sample and the spiked storage samples.

Description of analytical procedures

Honey

Samples (1.0 g) were diluted with a methanol/water mixture (3/1, v/v). After centrifugation, the raw extract was filtered and an aliquot was analysed by high performance liquid chromatography, chromatographed under chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size).

Pollen

Water (1 mL) was added to each of the samples (0.200 g). 3 mL methanol was then added after 10 mins and the samples were shaken before being centrifuged. An aliquot (3 mL) was transferred to another tube and diluted with a methanol/water mixture (3/1, v/v). After centrifugation, the raw extract was filtered, an aliquot was analysed by high performance liquid chromatography, chromatographed under chiral reverse phase column chromatography as detailed above.

Spiroxamine parent enantiomers

Residue analysis of samples of honey and pollen for the determination of the four enantiomers of spiroxamine was conducted using the validated analytical method no. 01480/M001, report reference M-763118-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

The quantification was carried out by internal standardization using the stable d7 ISTD of spiroxamine, which is also separated into its four enantiomers.

II. Results and discussion

The mean concurrent recovery rates for each individual isomer and spiking level were in the range of 78-107% for pollen and 92-110% for honey.

At day 0, average residues in pollen for A1 and A2 enantiomers were between 0.02295 – 0.02781 mg/kg, and for B1 and B2 enantiomers, between 0.0184 – 0.0204 mg/kg, equivalent to 80 – 103% of the spiked material. At day 0, average residues in honey for A1 and A2 enantiomers were between 0.02619 – 0.02754 mg/kg, and for B1 and B2 enantiomers, between 0.02277 – 0.02484 mg/kg, equivalent to 85 to 103% of the spiked material.

In samples analysed after storage for the periods of time described above, storage stability recovered values in pollen ranged from 72-102% and in honey from 77-108%. There was no evidence of any significant degradation of spiroxamine in any of the sample materials.

The mean values at all sampling dates and in all sample materials, the total residues of spiroxamine were above 70% (as shown in Table CA 6.1/07-1 to Table CA 6.1/07-2 below).

Table CA 6.1/07-1 Summary of deep freezer storage stability in pollen for the determination of spiroxamine enantiomers

Commodity	Analyte	Storage period (days)	Residue level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)
Pollen	A1 enantiomer	0	0.0230, 0.0228	85, 84 [85]	85
		29	0.0272, 0.0272	101, 101 [101]	106
		90	0.0201, 0.0208	74, 77 [76]	84
		187	0.0204, 0.0242	76, 90 [83]	101
Pollen	A2 enantiomer	0	0.0253, 0.0299	94, 111 [103]	99
		29	0.0252, 0.0243	93, 90 [92]	102
		90	0.0205, 0.0197	76, 73 [75]	83
		187	0.0242, 0.0266	79, 99 [89]	103
Pollen	B1 enantiomer	0	0.0195, 0.0173	85, 75 [80]	101
		29	0.0204, 0.0199	89, 87 [88]	103
		90	0.0168, 0.0174	73, 76 [75]	83
		187	0.0195, 0.0172	85, 75 [80]	83
Pollen	B2 enantiomer	0	0.0214, 0.0189	99, 82 [88]	92
		29	0.0237, 0.0232	103, 101 [102]	107
		90	0.0172, 0.0177	75, 77 [76]	78
		187	0.0161, 0.0170	70, 74 [72]	79

Table CA 6.1/07-2 Summary of deep freezer storage stability in honey for the determination of spiroxamine enantiomers

Commodity	Analyte	Storage period (days)	Residue level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)
Honey	A1 enantiomer	0	0.0256, 0.0294	95, 109 [102]	97
		31	0.0242, 0.0230	90, 85 [88]	104
		92	0.0238, 0.0296	88, 110 [99]	108
		189	0.0193, 0.0224	71, 83 [77]	96
Honey	A2 enantiomer	0	0.0238, 0.0286	88, 106 [97]	99
		31	0.0242, 0.0220	90, 82 [86]	97
		92	0.0262, 0.0298	97, 110 [94]	106
		189	0.0202, 0.0248	75, 92 [84]	101
Honey	B1 enantiomer	0	0.0224, 0.0232	97, 101 [99]	92
		31	0.0202, 0.0218	88, 95 [92]	107
		92	0.0226, 0.0236	98, 103 [101]	110
		189	0.0192, 0.0216	93, 94 [89]	99
Honey	B2 enantiomer	0	0.0244, 0.0250	106, 109 [108]	98
		31	0.0202, 0.0212	88, 92 [90]	106
		92	0.0220, 0.0240	96, 104 [100]	107
		189	0.0210, 0.0214	91, 93 [92]	104

III. Conclusions

After a maximum storage period of approximately 6 months (187 to 189 days) at ≤-18°C, residues of individual enantiomers of spiroxamine were shown to be stable in pollen and honey.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted.

Residues of individual enantiomers of spiroxamine were shown to be stable in pollen and honey for at least 6 months when samples are stored under deep frozen conditions (≤-18 °C).

Data Point:	KCA 6.1/08
Report Author:	[redacted]
Report Year:	2021
Report Title:	Analytical method for the determination of the total residue of spiroxamine and short term storage stability in honey by HPLC-MS/MS
Report No:	S20-01487
Document No:	M-763119-01-1
Guideline(s) followed in study:	Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 OECD 506, 2007, OECD Guideline for the Testing of Chemicals - Stability of Pesticide Residues in Stored Commodities SANTE/11956/2016 rev.9
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The study validated the analytical method 00312/M004 to determine the total residues of spiroxamine in/on honey as 4-*t*-butylcyclohexanone (4-TBCH) by LC-MS/MS detection. In addition, a short-term storage stability of spiroxamine (recovered as 4-TBCH) was performed in honey at ≤-18°C in the dark over a storage period up to 6 months. This summary describes the storage stability component. Details of the method validation are described in MCA 4.

The samples were spiked with spiroxamine and analysed for spiroxamine as 4-TBCH.

The LOQ, was established at 0.01 mg/kg for total spiroxamine.

After deep-freeze storage (≤-18°C) for a period of about 6 months, the mean recovery rates from the stored samples were 71% for spiroxamine, measured as 4-TBCH, in honey.

Procedural fortifications were conducted at levels of 0.10 mg/kg for spiroxamine (as 4-TBCH) in honey. Mean recoveries ranged from 72 to 80% showing acceptable method performance.

Overall, the study results demonstrate that residues of spiroxamine are stable in honey for at least 6 months under deep-freeze storage conditions (≤-18°C) and can be recovered by a common moiety methods as 4-TBCH.

I. Materials and methods

Honey (organic) was obtained from a local supermarket.

After weighing, control samples were deep-frozen at≤-18°C until their preparation.

Aliquots of homogenised matrix material were transferred into a plastic tube with screw cap and fortified with spiroxamine at 0.10 mg/kg (i.e. 10 x LOQ) using a solution in acetonitrile. The sample tubes were sealed immediately. Day 0 samples were analysed immediately. The remaining samples were stored in a deep-freezer at -18°C or below. Samples were analysed after nominal storage intervals of 0, 30, 90 and 180 days.

On day 0 (first sample analysis interval), four spiked samples were analysed along with one control sample for each sample matrix representing two samples for stability and two procedural recoveries. At each further sampling date, two fortified samples per matrix and the required number of control samples were removed from the freezer and allowed to come to room temperature. Subsequently, one blank and two recovery samples per matrix were prepared to determine the concurrent recoveries (fortification levels were at the same concentration as the spiked storage samples). These samples were extracted and analysed concurrently with the remaining (unfortified) control sample and the spiked storage samples.

Description of analytical procedures

Analysis of spiroxamine residues in honey was performed according to validated common moiety method 00312/M004, report reference M-763149-01-1 **(see Doc MCA Section 4)**

Samples (approximately 1.0 g) were diluted/dissolved with a methanol/water mixture (3/1, v/v) and hydrolysed with hydrochloric acid under reflux conditions. After extraction with cyclohexane and concentration, the extracts were derivatised with 2,4-dinitrophenylhydrazine solution in sulphuric acid/methanol (1/4, v/v) to the corresponding hydrazone. The mixture was basified and filtered and an aliquot was analysed by high performance liquid chromatography, chromatographed under reversed-phase column chromatography and detected by Tandem Mass Spectrometry with electrospray ionisation. Residues of derivatised 4-TBCH were quantified using solvent standards with an isotopic stable-labelled internal standard.

II. Results and discussion

The mean concurrent recovery rates for spiroxamine (as 4-TBCH) spiking level were in the range of 72-80% for honey.

At day 0, average residues in honey for spiroxamine were 0.0782 and 0.0790 mg/kg, equivalent to 78 and 79% of the spiked level.

In samples analysed after storage for 181 days (6 months), mean storage stability recovered values in honey were 71%. There was no evidence of significant degradation of spiroxamine in honey.

For all sampling dates in honey, the mean total recovered residues of spiroxamine were above 70% as shown in Table CA 6.1/08-1.

Table CA 6.1/08-1 Summary of deep freezer storage stability in honey for the determination of total spiroxamine as 4-TBCH

Commodity	Analyte	Storage period (days)	Residue level in freezer storage stability sample (mg/kg)	Residue level in freezer storage stability sample (% of nominal spiking level) [mean]	Procedural recovery for freshly spiked control sample (%)
Honey	Spiroxamine measured as 4-TBCH	0	0.0782, 0.0790	78, 79 [79]	74, 79
		29	0.0777, 0.0796	78, 80 [79]	67, 77
		91	0.0775, 0.0856	77, 86 [82]	73, 86
		181	0.072, 0.0703	72, 70 [71]	75, 78

III. Conclusions

After a maximum storage period of approximately 6 months (181 days) at ≤-18 °C, residues of spiroxamine recovered as 4-TBCH were shown to be stable in honey.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted.

Residues of spiroxamine, recovered as 4-TBCH, were shown to be stable in honey for at least 6 months when samples are stored under deep frozen conditions (≤-18 °C).

The following new study is currently on-going and an interim report will be submitted at an agreed submission top-up time-point:

Dossier node	Draft title	Study ID	Planned submission
KCA 6.1	Storage stability of residues of spiroxamine enantiomers in/on grapes and cereal grain under deep frozen conditions	S20-04326	Interim report Not before May 2021

Overall conclusions storage stability

It can be concluded that spiroxamine is stable for up to 722 days (approximately 24 months) in grapes and wheat grain, representative of the high acid and high starch crops covered in this Renewal of Approval dossier, respectively. The samples in the residues trials were stored for up to 420 – 500 days in grapes (parent spiroxamine enantiomers and total spiroxamine via aminodiol respectively), 566 - 656 days in barley (parent spiroxamine enantiomers and total spiroxamine via aminodiol) and 654 - 678 days in wheat (parent spiroxamine enantiomers and total spiroxamine via aminodiol) and are therefore within the storage conditions reported.

Therefore, for the representative uses of spiroxamine in grapes, wheat and barley discussed in this renewal of Approval dossier, the frozen storage stability in each of these crops is covered by the study (not previously submitted), summarised under CA 6.1.06, where adequate stability of spiroxamine was demonstrated.

Spiroxamine as parent spiroxamine or as the aminodiol common moiety was also demonstrated to be stable in hops (green cones) and bananas for 24 months. Stability in the processed fractions of spent hops and beer was also demonstrated for between 5 – 7 months.

Additionally, a number of studies are presented as supporting information, where it was acknowledged by EFSA that the common moiety method used for the analysis of stored samples is not able to confirm stability of parent spiroxamine residues during frozen storage. However, these studies also demonstrate the frozen storage stability of total spiroxamine by either common moiety approach, for at least 18 months in grapes or as spiroxamine plus metabolites (covering spiroxamine N-oxide, (M03) spiroxamine-hydroxy-despropyl (M09) and three metabolite groups bearing the t-butylketone moiety).

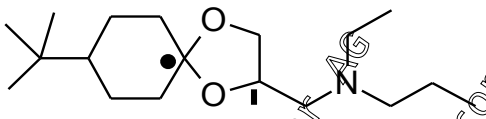
Stability was demonstrated for spiroxamine-acid, (M06) the major metabolite in animal matrices following repeated dosing of spiroxamine, in aliquots of whole milk, kidney (representing other edible offal) and muscle under frozen storage at ≤-18°C for up to 228 days. The stability data therefore supports the storage intervals for samples of poultry and bovine matrices analysed in the hen and cattle feeding studies summarised under point CA 6.4.

Residues of spiroxamine in pollen and honey remain stable for at least 6 months (187 - 189 days) when samples are stored under deep frozen conditions (≤-18°C).

CA 6.2 Metabolism, distribution and expression of residues

Metabolism studies were conducted using [^{14}C]-radiolabelled spiroxamine with two radiolabelled forms used as shown below in Figure CA 6.2-1.

Figure CA 6.2-1 Label positions for [^{14}C]-radiolabelled spiroxamine



●: [Cyclohexyl-1-^{14}C]-spiroxamine
■: [1,3-dioxolane-4-^{14}C]-spiroxamine

Studies on the metabolism and distribution of spiroxamine applied as foliar spray on spring/winter wheat, grapes and banana (covering two crops groups: grasses/cereals and fruit crops) were submitted and reviewed for the first inclusion/first renewal of spiroxamine according to EU directive 91/414/EEC.

The metabolism pathway of spiroxamine in plants has been assessed in the studies presented in Annex Point CA 6.2.1 and in Annex Point CA 6.6.1. These are listed in Table CA 6.2-1.

The metabolism pathway of spiroxamine in livestock has been assessed in the studies presented in Annex Point CA 6.2.2 and in Annex Point CA 6.5.3. These are listed in Table CA 6.2-2.

Table CA 6.2-1 [^{14}C]-radiolabelled spiroxamine test items used in plant metabolism studies

Reference	Study Title	spiroxamine radiolabels tested
Primary plant metabolism		
CA 6.2.1/01 M-006099-01-1	Metabolism of KWG 4168 in spring wheat	[Cyclohexyl-1-^{14}C]-spiroxamine
CA 6.2.1/02 M-006112-01-1	Metabolism of KWG 4168 in winter wheat	[1,3-dioxolane-4-^{14}C]-spiroxamine
CA 6.2.1/03 M-006107-01-1	Metabolism of [Cyclohexyl-1-^{14}C]KWG 4168 in Grapes	[Cyclohexyl-1-^{14}C]-spiroxamine
CA 6.2.1/04 M-006104-01-1	Metabolism of [1,3-dioxolane-4-^{14}C] KWG 4168 in grapes	[1,3-dioxolane-4-^{14}C]-spiroxamine
CA 6.2.1/05 M-026783-01-1	Metabolism of [^{14}C]-KWG 4168 in banana (in-life phase)	[Cyclohexyl-1-^{14}C]-spiroxamine [1,3-dioxolane-4-^{14}C]-spiroxamine
CA 6.2.1/06 M-030423-01-1	Metabolism of [Cyclohexyl-1-^{14}C]KWG4168 in banana (analytical part)	[Cyclohexyl-1-^{14}C]-spiroxamine
CA 6.2.1/07 M-088457-01-1	Metabolism of [1,3-dioxolane-4-^{14}C]KWG4168 in banana (analytical part)	[1,3-dioxolane-4-^{14}C]-spiroxamine
Rotational crop metabolism		
CA 6.6.1/01 M-006096-01-1	[Cyclohexyl-1-^{14}C]KWG 4168 Residues in following crops	[Cyclohexyl-1-^{14}C]-spiroxamine

Reference	Study Title	spiroxamine radiolabels tested
CA 6.6.1/02 M-303904-01-1	Metabolism of [1,3-dioxolane-4-^{14}C]KWG 4168 in confined rotational crops- first part: first rotation	[1,3-dioxolane-4-^{14}C]-spiroxamine
CA 6.6.1/03 M-344278-01-1	Metabolism of [1,3-dioxolane-4-^{14}C]KWG 4168 in confined rotational crops- second part: 2nd and 3rdst rotation	[1,3-dioxolane-4-^{14}C]-spiroxamine

Table CA 6.2-2 [^{14}C]-radiolabelled spiroxamine test items used in livestock metabolism studies

Reference	Study Title	spiroxamine radiolabels tested
CA 6.2.2/01 M-006038-01-1	[Cyclohexyl-1-^{14}C]KWG 4168: Absorption, distribution, excretion and metabolism in laying hens	[Cyclohexyl-1-^{14}C]-spiroxamine
CA 6.2.3/01 M-006039-01-1	[Cyclohexyl-1-^{14}C]KWG 4168: Absorption, distribution, excretion and metabolism in a lactating goat	[Cyclohexyl-1-^{14}C]-spiroxamine
CA 6.2.5/01 M-006169-01-1	[Cyclohexyl-1-^{14}C] KWG 4168: Metabolism in the Edible Parts of Bluegill Sunfish	[Cyclohexyl-1-^{14}C]-spiroxamine

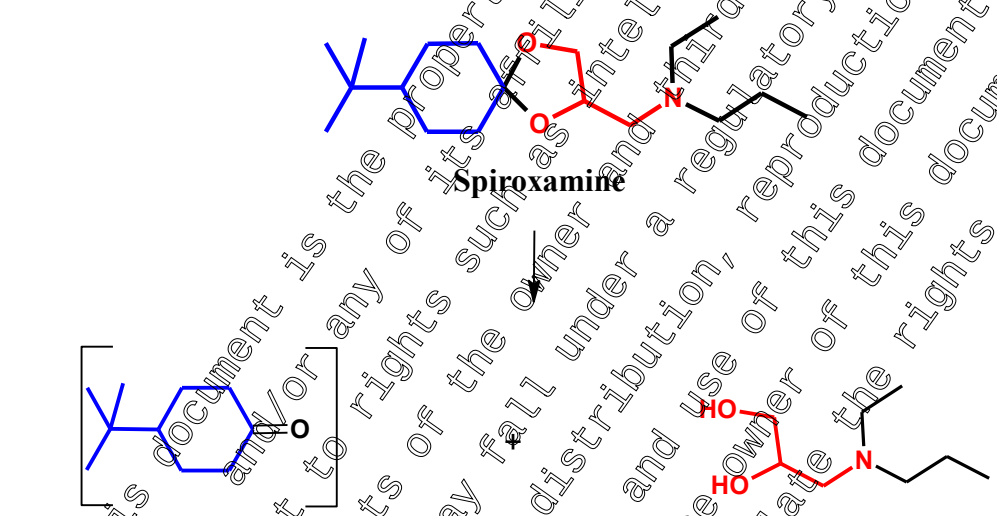
CA 6.2.1 Metabolism, distribution and expression of residues in plants

Background to plant metabolism

As shown below in Figure CA 6.2.1-1, the metabolism pathway of spiroxamine in plants and in particular for fruit crops involves a first step which results in the formation of Group B and Group C metabolites by hydrolytic cleavage of the spiroketal structure of spiroxamine resulting in the formation of the metabolites spiroxamine-ketone (M15) and spiroxamine-aminodiol (M28), both of which are found in animals and plants.

Following the formation of metabolites M15 and M28, a series of metabolic pathway steps in fruit crops can occur, resulting in formation of additional Group B and Group C metabolites. These steps are described in the updated study summaries presented in this section. Metabolites retaining the intact spiroxamine moiety structure are referred to as Group A metabolites. All metabolites with details of codes, structures, grouping etc are shown in document N3 of this submission.

Figure CA 6.2.1-01 Hydrolytic cleavage of spiroxamine in plants



Data Point:	KCA 6.2.1/01
Report Author:	[redacted]; [redacted]; [redacted]
Report Year:	1995
Report Title:	Metabolism of KWG 4168 in spring wheat
Report No:	PF4053
Document No:	M-006099-01-1
Guideline(s) followed in study:	US EPA Pesticides Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4, Nature of residues in plants and livestock
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

[Cyclohexyl-1-^{14}C]-labelled spiroxamine (KWG 4168), formulated as a 500 EC formulation, was applied twice to spring wheat plants in a controlled vegetation area, at growth stages BBCH 30 (stem elongation) and BBCH 51 (ear emergence). The total amount applied was equivalent to 831 g a.s./ha (equivalent to 1.1N maximum seasonal rate). Forage was sampled immediately after the second application (day 0) and at day 14, with straw and grain sampled at harvest (day 61).

The total radioactive residue (TRR) in forage accounted for 7.93 mg/kg parent compound equivalents at day 0. The major residue found in forage (day 0) was parent spiroxamine (76%TRR, 6.03 mg/kg). Other metabolites identified were spiroxamine-N-oxide (M03) (8.0%TRR, 0.63 mg/kg), spiroxamine-N-formyl-desethyl (M04) (2.1%TRR, 0.17 mg/kg,), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (3.2%TRR, 0.25 mg/kg), spiroxamine-despropyl (M02) (4.3%TRR, 0.34 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (0.2%TRR, 0.02 mg/kg). Identified hydrolysis products included conjugates of diol and hydroxyl-despropyl (0.2-2.0% TRR, 0.02-0.16 mg/kg).

At day 14, the total radioactive residue (TRR) in forage accounted for 10.67 mg/kg parent compound equivalents. The majority of the residue in forage (day 14) was parent spiroxamine (53.8%TRR, 5.74 mg/kg). Other metabolites identified were spiroxamine-N-oxide (M03) (9.0%TRR, 0.96 mg/kg), spiroxamine-N-formyl-desethyl (M04) (5.3%TRR, 0.56 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (5.1%TRR, 0.54 mg/kg), spiroxamine-despropyl (M02) (4.6%TRR, 0.49 mg/kg), spiroxamine-hydroxy-N-oxide glucoside (M20) (0.7%TRR, 0.08 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (2.0%TRR, 0.21 mg/kg). Identified hydrolysis products included conjugates of diol, conjugates of hydroxyl-ketone and hydroxyl-despropyl (1.0-1.6% TRR, 0.11-0.17 mg/kg).

At harvest (day 61), the TRR in straw accounted for 34.92 mg/kg parent equivalents. Parent spiroxamine and metabolite spiroxamine-N-oxide (M03) were the major components of the residue in straw, accounting for 25.1% and 22% of the TRR (8.76 mg/kg and 7.68 mg/kg), respectively. Other metabolites identified were spiroxamine-N-formyl-desethyl (M04) (7.5%TRR, 2.62 mg/kg), spiroxamine-hydroxyl (M05) (2.4%TRR, 0.84 mg/kg), spiroxamine-desethyl (M01) (2.0%TRR, 0.70 mg/kg), spiroxamine-despropyl (M02) (3.2%TRR, 1.12 mg/kg), spiroxamine-hydroxy-despropyl(M09) (0.3%TRR, 0.1 mg/kg) and spiroxamine-hydroxy-N-oxide glucoside (M20) (2.0%TRR, 0.70 mg/kg). Identified hydrolysis products included conjugates of diol and conjugates of hydroxyl-ketone (1.3-1.8% TRR, 0.46-0.63 mg/kg). Identified hydrolysis products from exhaustive extraction included tert.-butylketone, diol (M14) and spiroxamine-hydroxy-ketone (M16) (0.2-5.5%TRR, 0.07-1.93 mg/kg).

The TRR in grain was very low (0.07 mg/kg). Parent spiroxamine and spiroxamine-N-oxide (M03) were the only significant residues detected in grain, accounting for 14.3% (0.010 mg/kg) of the TRR

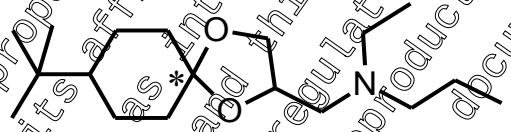
and 17.8% (0.012 mg/kg) of the TRR. Metabolites spiroxamine-N-formyl-desethyl (M04), spiroxamine-hydroxyl (M05), spiroxamine-desethyl (M01), and spiroxamine-despropyl (M02) were detected at very low levels (<10% of the TRR, <0.01 mg/kg). Identified hydrolysis products from exhaustive extraction included tert.-butylketone, diol (M14), spiroxamine-hydroxy-ketone (M16) and tert.-butylcyclohexanol (2.4-7.6%TRR, 0.001-0.005 mg/kg, M13).

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the spiroxamine-N-oxide, M03) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl, M05). The metabolites with an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide glucoside (M20) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)). Some metabolites were formed by desalkylation (spiroxamine-desethyl, M01, despropyl- spiroxamine, M02) which were partly metabolised by formylation (spiroxamine-N-formyl-desethyl, M04). Only small amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

I. Materials and methods

A. Test material

[Cyclohexyl-1-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (MBq/mg):	0.972 (26.3 µCi/mg) a.s. formulated as 500 EC (container A) 0.960 (25.9 µCi/mg) a.s. formulated as 500 EC (container B)
Lot/Batch No.:	Not stated
Purity:	Radiochemical purity > 99%
Storage condition:	Not stated
CAS No.:	118134-30-8

B. Study design

Test system

Soil

Table CA 6.2.1.01-1 Soil classification and physico-chemical properties

Soil Type DIN 19682	pH (KCl)	OM %	Sand %	Silt %	Clay %	Moisture holding capacity mL / 100g)	CEC meq / 100 g
Loamy silt	6.63	1.14	17.1	71.8	11.1	Not reported	15

The study was conducted in the vegetation area of the Institute for Metabolism Research Monheim, Bayer AG. Spring wheat, variety Kadett, was sown in a container at a density of 550 seeds/m^2. Two containers were used, one for the metabolism study (experiment A) and one for the storage stability investigations and validation of residue method (experiment B). Treatment with other plant protection products was conducted as required according to Good Agricultural Practice.

Test design

The dates when the experimental work was conducted are not explicitly stated in the report, however, storage stability analysis was conducted for up to 566 days, so it is assumed that this covers the experimental phase from sampling to analysis. The study started in May 1991 and from report

information, the final date for experimental work is March 1993. The in-life phase was conducted in the Institute for Metabolism Research, Bayer AG, Monheim Germany. The analytical phase and final reporting was conducted at the Institute for Metabolism Research and Residue Analysis, Bayer AG, Monheim, Germany.

Experimental conditions

[Cyclohexyl-1-^{14}C]-spiroxamine, formulated as a 500 EC formulation, was applied twice as a spray application to spring wheat plants at BBCH 30 (stem elongation) and BBCH 51 (ear emergence) at a total rate of 831 g a.s./ha.

A use on cereals is included in the representative GAP for spiroxamine. The rate employed in this study (total seasonal rate of 831 g a.s./ha) is equivalent to 1.1N the maximum seasonal use proposed for spiroxamine (750 g a.s./ha). This study is considered representative and appropriate for the objectives of establishing the nature and magnitude of spiroxamine in cereal crops.

Preparation of application solutions/applications

Experiment A: 80 mg of the 500 EC formulation was applied at the first application and 87 mg of the formulation was applied at the second application. In total 167 mg of the formulation was applied which corresponded to 83.1 mg active ingredient or 80.78 MBq (2.183 mCi) ^{14}C-radioactivity equivalent to a field rate of 831 g a.s./ha. Each application was conducted using 40 mL of spray solution. A small amount of the spray solution was taken before and after the applications and stability measured.

Experiment B: 83.9 mg of the 500 EC formulation was applied at the first application and 78 mg of the formulation at the second application. In total 161.9 mg of formulation was applied which corresponded to 80.6 mg active ingredient or 77.38 MBq (2.091 mCi) ^{14}C-radioactivity. Each application was conducted using 40 mL of spray solution. The use rate (806 g a.s./ha) was comparable to the use rate of experiment A.

Sampling

Experiment A: The first forage sample (day 0) was taken immediately after the second application (June 6, 1991) from approximately 10% of the treated area. The second forage sample (day 14) was taken 2 weeks later (June 20, 1991) from approximately 30% of the treated area. The plant material was homogenised in liquid nitrogen and stored in different aliquots (50.0 g - 200.0 g) at -20°C or below.

The spring wheat from the remaining treated area (approximately 60%) was harvested on day 61 (August 6, 1991) at maturity. The plants were separated into straw, grain and glumes. The glumes and the straw were combined and homogenised in liquid nitrogen. Homogenised straw was stored in aliquots (60 g). The homogenised straw and the unground grain were stored at -20°C or below.

Experiment B: One forage sample was taken 14 days after the second application (June 20, 1991) from 1/3 of the treated area. The plant material was homogenised in liquid N_2 and stored in 18 x 50.0 g portions plus 414 g separately at -20°C or below.

The spring wheat from the remaining treated area (2/3) was harvested on day 61 (August 6, 1991) at maturity. The plants were separated into straw, grain and glumes. The glumes and the straw were combined and homogenised in liquid N_2 and stored in 18 x 20.0 g portions plus 204 g separately at -20°C or below. The whole grain and the homogenised straw were stored at -20°C or below.

Sample preparation and extraction

Forage

An aliquot (50.0 g) of the homogenised forage from day 0 and day 14 was successively macerated with methanol (2 x 300 mL) and methanol/water 1:1 (v/v). The suspension was filtered by suction, yielding the methanol/water extract (combined filtrates) and the solids (non-extractable residue). The methanol/water extract was evaporated to the aqueous remainder using a rotary evaporator. The aqueous

remainder was extracted with dichloromethane (3 x 150 mL) resulting in the organic phase and aqueous phase. Aliquots from extracts, phases and solids were taken for radioactivity measurement.

Straw

First extraction: An aliquot (60.0 g) of the homogenised straw from day 61 was successively macerated with methanol (2 x 450 mL) and methanol/water 1:1 (v/v). The suspension was filtered by suction yielding the methanol/water extract and solid residue 1. The methanol/water extract was evaporated to an aqueous remainder using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3 x 300 mL) resulting in organic phase 1 and aqueous phase 1. An aliquot (3.5 g) of solid residue 1 was further extracted with methanol/1N HCl 1:1 (v/v) under reflux for 1 hour. The suspension was filtered by suction, the filter cake washed using methanol, and the methanol/1N HCl extract and the solids (non-extractable residue) were obtained. Aliquots from all relevant extracts, phases, solid residue 1 and solids were taken for radioactivity measurement.

Second extraction: In order to have enough solid residue 1 available for the investigation of the hydrolysis products of organic phase 2 and aqueous phase 2, a second extraction of straw was conducted under the same conditions as described for the first extraction and the exhaustive extraction of solid residue 1 was done using the following quantities: An aliquot of solid residue 1 was extracted with methanol/1N HCl 1:1 (v/v) under reflux for 1 hour. The suspension was filtered by suction, the filter cake washed using methanol, and the methanol/1N HCl extract and the solids (non-extractable residue) were obtained. An aliquot (100 mL) of the methanol/1N HCl extract was neutralised using a $NaHCO_3$-solution, evaporated to the aqueous residue and extracted with dichloromethane resulting in organic phase 2 and aqueous phase 2.

Grain

An aliquot (20.0 g) of grain from day 61 was homogenised in liquid nitrogen and macerated with methanol/water 4:1 (v/v, 2 x 300 mL). The suspension was filtered by suction yielding the methanol/water extract and solid residue 1. The methanol/water extract was evaporated to an aqueous remainder using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3 x 100 mL) resulting in organic phase 1 and aqueous phase 1.

An aliquot (5.0 g) of solid residue 1 was further extracted by a repeated enzymatic hydrolysis procedure using diastase: 5.0 g solid residue 1 were combined with diastase and 55 mL citrate/NaOH-buffer (pH 6, Titrisol) containing 0.02% sodium azide (NaN_3) and incubated for 72 hours at room temperature. The suspension was filtered by suction and the dissolved amount of solid residue 1 was determined by weighing of the remaining solids. The undissolved residue was repeatedly treated with diastase (50 mg) and buffer solution (50 mL) and shaken or stirred with a magnetic stirrer at room temperature. The procedure was stopped (seven extractions in total) when the final residue (solids, non-extractable residue) had decreased to 0.67 g, which was completely combusted. The aqueous enzyme extracts (filtrates) were combined and extracted with ethyl acetate resulting in organic phase 2 and aqueous phase 2. Aliquots from all relevant extracts, phases or solids were taken for radioactivity measurement.

Hydrolysis of metabolites from the organic phase 1 of straw

Metabolite numbers are designated in the methods section as per the study report.

Acidic hydrolysis of metabolites: metabolites purified by TLC, were dissolved in methanol and 1N HCl, and heated for 1 hour at 90°C. After cooling, the reaction mixture was neutralised with solid $NaHCO_3$ and investigated by radio-TLC.

Acidic hydrolysis of metabolite group 11 and metabolite group 14: the metabolite group, purified by TLC, was dissolved in methanol and 1N HCl, and heated for 1 hour at 90°C. After cooling, methanol was added and the reaction mixture was analysed without neutralisation by radio-TLC.

Alkaline hydrolysis of metabolites: metabolites purified by TLC, were dissolved in methanol and 1N NaOH, and heated for 16 hours at 100°C. After cooling, the methanol was removed using a stream of

nitrogen, the residue neutralised with 1N HCl and extracted with dichloromethane. The combined dichloromethane phases were investigated by radio-TLC. Metabolites, purified by HPLC and TLC, were hydrolysed with 1N NaOH for 2 hours at 100°C. After cooling, the reaction mixture was analysed without neutralisation by radio-TLC.

Acidic hydrolysis of [cyclohexyl-1-^{14}C]spiroxamine: Approximately 38.5 kBq of [cyclohexyl-1-^{14}C]spiroxamine, dissolved in acetonitrile (100 µL), was combined with methanol (100 µL) and 1N HCl (100 µL) and heated (5 mL) for 30 minutes at 80°C. After cooling, the reaction mixture was neutralised with a saturated Na_2CO_3 solution, water was added up to a volume of 1 mL and extracted with dichloromethane (3 x 2 mL). The combined dichloromethane phases were investigated by radio-TLC. The [cyclohexyl-1-^{14}C]tert.-butylketone obtained in high purity was co-chromatographed by TLC (SS-II and dichloromethane) with spiroxamine-ketone (M15) and used as a standard solution for different chromatographic comparisons.

Purification of aqueous phase 1 of straw

XAD-4 column

A glass column (50 cm length, 3 cm diameter) was filled with XAD-4 polystyrene resin (50 g, Serva). The resin was washed with methanol and water. An aliquot of the aqueous phase 1 of straw was transferred onto the column. The column was washed with water. The wash solutions contained negligible amounts of radioactivity and were discarded. The absorbed residue was eluted using methanol, the methanol eluate concentrated and the radioactivity measured. No losses were observed. The solution was used for the quantitation of metabolites by TLC.

Similar XAD-4 columns were also used for the purification of aliquots of aqueous phase 1 for the isolation of metabolites. The metabolite fraction was eluted using methanol, then concentrated and redissolved in water for further purification using a carboxylic acid cartridge.

Carboxylic acid cartridge

For further purification, a carboxylic acid cartridge (3 mL, Baker) was used. The cartridge was pre-washed with methanol and water. An aliquot of the metabolite fraction dissolved in water was added to the cartridge. The cartridge was successively eluted using the following solvents:

1. 5 mL water
2. 5 mL methanol
3. 5 mL methanol:25% ammonia 1:1 (v/v)

Each fraction was evaporated to dryness and the residue redissolved in methanol. The first and second eluate was used for the isolation of metabolites. The third eluate was not further investigated.

Hydrolysis and derivatisation of metabolites from the aqueous phase 1 of straw

Hydrolysis with cellulase

A cellulase stock solution was obtained using 50 mg Cellulase (Serva) dissolved in 5 mL buffer (0.05M Sodium acetate, pH 5 containing 0.2 g/l NaN_3). An aliquot of the solution containing metabolites 17 and 18 (diol conjugate), purified by HPLC and TLC, was dried under a stream of nitrogen. The residue was dissolved in 500 µL cellulase stock solution and stirred for 24 hours at 37°C. The buffer solution was extracted with ethyl acetate. The combined ethyl acetate phases were concentrated with a stream of nitrogen and investigated by radio-TLC. Metabolites spiroxamine-hydroxy-N-oxide glucoside and spiroxamine-hydroxy-ketone conjugate were similarly hydrolysed with cellulase.

Methylation

To a solution of spiroxamine-hydroxy-N-oxide malonyl glucoside (9 mL methanol and 1 mL diethyl ether), purified by HPLC and TLC, a diazomethane/diethyl ether mixture was added over a period of

30 minutes. After 1 hour the solution was evaporated to dryness. The residue was dissolved in small amounts of methanol and the solution investigated by radio-TLC.

Reduction

Spiroxamine-hydroxy-N-oxide glucoside was stirred in methanol and triphenylphosphine (10 mg) at 50°C for 16 hours. The reaction mixture was concentrated and evaluated by radio-TLC analysis.

Acidic hydrolysis of the purified aqueous phase 1 of straw

A concentrated aliquot of the methanol eluate, obtained by XAD-4 purification of aqueous phase 1 of straw, was hydrolysed with 1N HCl in a Reacti-Vial for 1 hour at 100°C. After cooling, the reaction mixture was extracted with ethyl acetate. The combined ethyl acetate phases were investigated by radio-TLC.

Hydrolysis of spiroxamine-hydroxyl and spiroxamine-desethyl of aqueous phase 1 from grain

Acidic hydrolysis of metabolites

Metabolites, purified from the organic phase 1 of grain by TLC, were dissolved methanol and 1N HCl, and heated for 1 hour at 90°C. After cooling, the reaction mixture was analysed without neutralisation by radio-TLC.

Acidic hydrolysis of aqueous phase 1

Aqueous phase 1 (30 mL, obtained from a second extraction of grain under identical conditions to have more radioactivity available) in 1N HCl (*ca.* 3.3 mL 36% HCl added), was heated in a glass bulb for 1 hour under reflux. After cooling, the reaction mixture was extracted with dichloromethane. The combined dichloromethane phases were concentrated using a stream of nitrogen and investigated by radio-TLC.

Radiochemical analysis

Radioactivity determination

The ^{14}C-radioactivity in liquid samples was determined in liquid-scintillation counters: PW4700 (Philips/Raytest), LKB 1219 Rackbeta (LKB-Wallac), LS 6000LL (Beckman Instruments) or LS 6500 (Beckman Instruments) with Instant-Scint-Gel (Packard), Quicksafe A (Zinsser Analytic) or Quickszint 401 (Zinsser Analytic).

Solid samples were combusted in an Oxidiser 306 (Packard), Harvey OX 300 (Zinsser Analytic) or OX 500 (Zinsser Analytic). The CO_2 produced by combustion was absorbed in a scintillation-cocktail [8 mL Carbosorb + 10 mL Permafluor V (Packard) or Oxysolve C-400 (Zinsser Analytic)] and the radioactivity measured by LSC.

Thin layer chromatography (TLC)

The radioactive solutions were investigated by TLC using silica gel plates (silica gel 60 F_{254}, 0.25 mm thickness, 20 cm x 20 cm, Merck) and the following solvent systems (SS):

I: dichloromethane:ethyl acetate 9:2 (v/v)
II: acetonitrile:water:25% :ammonia 320:18:2 (v/v)
III: dichloromethane:methanol:2-butanone 6:2:1 (v/v)
IV: acetonitrile:water:25% ammonia 80:18:2 (v/v)
V: chloroform:methanol:25% ammonia 65:28:8 (v/v)

The radioactive compounds were detected and evaluated quantitatively using a Linear Analyser (Rita 3200 or IM 3016, Raytest) or a Bio-Imaging Analyser (BAS 2000, Fuji). The reference compounds used in co-chromatography were visualised by UV-light (254 nm) or in an iodine chamber.

High performance liquid chromatography (HPLC)

For chromatographic comparison or for purification of metabolites by HPLC a HP 1090 Liquid Chromatograph (Hewlett Packard) with DAD-Detector (wave length 220 nm) and a radioactivity flow through monitor Ramona-5 (Raytest) with a solid scintillator glass cell were used.

Method 1:

RP-8 column (Lichrosorb 5 µm, 250 x 4 mm, Merck) without pre-column. Elution was conducted with the following gradient and a flow rate of 1 mL/min:

Eluent A: 1000 mL water + 5 mL triethylamine + 1.5 mL phosphoric acid
Eluent B: acetonitrile

Method 2:

RP-18 column (Lichrosorb 7 µm, 250 x 4 mm, Merck) with a RP-18 pre-column (25 x 4 mm, Merck). Elution was conducted with the following gradient and a flow rate of 1 mL/min.

Eluent A: water
eluent B: methanol

Other methods:

For the purification and isolation of metabolites from aqueous phase 1 of straw different columns (RP-8, RP-18 or ODS-30) and different elution gradients (water/triethylamine/phosphoric acid, water/acetonitrile or water/methanol) were used.

Mass spectroscopy (MS)

GC/MS-spectra were recorded with a mass selective detector HP 5970 (Hewlett Packard) and a GC 5880A (Hewlett Packard). The capillary column used was a 12.5 m Ultra2 (Hewlett Packard) or a 15 m SE54 (CS Chromatography Service). The oven temperature program was as follows: 60°C isothermal for 1 minute, heated at 10 °/min up to 280°C and isothermal at 280°C for 20 minutes. The injector was operated in the splitless mode at 280°C.

Direct chemical ionisation (DCI) spectra were recorded on a Finnigan 8230 instrument. The ion source temperature was 200°C, the electron energy 70 eV, the emission current 0.2 mA. Ammonia was used as reagent gas.

Electrospray (ESI) and fast atom bombardment (FAB) mass spectra were recorded on a TSQ 700 (Finnigan), laboratory Dr. Wesener (Bayer AG, ZF-D2A/SE, Leverkusen). Glycerol was used as FAB-matrix, xenon as FAB-gas.

^{1}H-NMR-spectroscopy

The 1H-NMR-spectra were recorded on a Bruker AC 300 spectrometer, the samples were dissolved in CD_3OD (Merck) for analysis.

Derivatisation

Silylation was performed with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) containing 5% trimethylchlorosilane at 70°C for ten minutes. Injection into the GC was directly from the reaction mixture.

II. Results and discussion

Total radioactive residue (TRR)

The TRR in forage (day 0) accounted for 7.93 mg/kg parent compound equivalents as determined by summation of the radioactivity in the dichloromethane phase, aqueous phase and in the solids. The radioactivity in forage was readily extracted using methanol/water and partitioned almost completely into the dichloromethane phase (97.6% of the TRR, 7.74 mg/kg). Only 1.6% (0.12 mg/kg) of the TRR

was found in the aqueous phase and 0.8% (0.07 mg/kg) in the solids. The radioactivity detected in the combined methanol/water extracts was recovered quantitatively (>99%) in the organic and aqueous phases.

The TRR in forage (day 14) accounted for 10.67 mg/kg parent compound equivalents. The radioactivity in forage was readily extracted (95.4% of the TRR) using methanol/water and the majority was partitioned into the dichloromethane phase (86.8% of the TRR, 9.26 mg/kg). Smaller amounts (8.6% of the TRR, 0.92 mg/kg) were present in the aqueous phase and in the solids (4.6%, 0.49 mg/kg). The radioactivity detected in the combined methanol/water extracts was also recovered nearly quantitatively (>98%) in the organic and aqueous phases.

The TRR in straw accounted for 34.92 mg/kg parent compound equivalents as determined by combustion. The predominant part of the radioactivity was extracted with methanol and methanol/water and partitioned into organic phase 1 (73.2% of the TRR, 25.55 mg/kg). Significantly smaller amounts were found in aqueous phase 1 (11.9% of the TRR, 4.17 mg/kg) and in solid residue 1 (14.9% of the TRR, 5.20 mg/kg) which was further extracted. Following methanol/1 N HCl extraction 7.0% of the TRR (2.45 mg/kg) was partitioned into organic phase 2 and 2.2% (0.75 mg/kg) into aqueous phase 2. The radioactivity remaining in the solids (non-extractable residue) was equivalent to 5.7% (2.00 mg/kg) of the TRR. No significant losses of radioactivity were observed during evaporation and partitioning. The second extraction of straw (was conducted under the same conditions as the first one to reproduce larger amounts of solid residue 1 needed for the quantitative investigation of the hydrolysis products in organic phase 2 and aqueous phase 2.

The TRR in grain was very low and accounted for 0.070 mg/kg parent compound equivalents as determined by combustion. Intensive efforts were made to solubilise the metabolites as quantitatively as possible before acidic hydrolysis was used. Therefore, the extraction procedure commenced conventionally with methanol/water and the remainder (17.5 g solid residue 1 resulting from 20.0 g grain) was hydrolysed enzymatically with diastase to solubilise the starch fraction. The radioactivity in the dichloromethane phase, obtained following evaporation of methanol, accounted for 36.6% (0.026 mg/kg) of the TRR. Comparable amounts (28.5% of the TRR, 0.020 mg/kg) partitioned into aqueous phase 1 and 34.9% (0.024 mg/kg) of the TRR remained in solid residue 1. The combined enzyme hydrolysis solutions obtained from solid residue 1 were extracted with ethyl acetate. Finally, 13.9% (0.010 mg/kg) of the TRR partitioned into the ethyl acetate phase and 11.0% (0.008 mg/kg) into aqueous phase 2 and 10.0% (0.007 mg/kg) of the TRR remained in the solids (non-extractable residue). No significant losses of radioactivity were observed during evaporation and partitioning. The quantitation of radioactivity in fractions was based on the combustion of grain (total), solid residue 1 (subtotal) and solids.

TRR and distribution of radioactivity in wheat are given in Table CA 6.2.1/01-2.

Table CA 6.2.1/01-2 Distribution of radioactive residues in wheat following two spray applications with [cyclohexyl-1-^{14}C]-spiroxamine

Radioactive residues in wheat	Forage (0 DALA)		Forage (14 DALA)		Straw (61 DALA)		Grain (61 DALA)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR[1]	7.93	100	10.67	100	34.92	100	0.070	100.0
Organic phase 1 (dichloromethane)	7.74	97.6	9.26	86.8	25.55	73.2	0.026	36.6
Aqueous phase 1	0.12	1.6	0.92	8.6	4.17	11.9	0.020	28.5
Organic phase 2 (dichloromethane)	-	-	-	-	2.45	7.0	-	
Organic phase 2 (ethyl acetate)	-	-	-	-	-	-	0.010	13.9
Aqueous phase 2	-	-	-	-	0.75	2.2	0.008	11.0
Post extraction solids (PES)	0.07	0.8	0.49	4.6	2.00	5.7	0.007	10.0

1 - Total radioactive residues given in mg parent substance equivalents per kg, determined by summation of all fractions after extraction for forage and by combustion for straw and grain.

Characterisation

Available synthesised compounds for characterisation were parent test substance (spiroxamine (KWG 4168)) and the following potential metabolites:

KWG 4557 (spiroxamine-desethyl) (M01)

KWG 4669 (spiroxamine-despropyl) (M02)

WAK6301/1 (spiroxamine-N-oxide) (M03)

WAK 6782 (spiroxamine-N-formyl-desethyl) (M04)

BNF 5567B (acetyl-spiroxamine-desethyl)

BNF 5567A (acetyl-spiroxamine-despropyl)

WAK 6072 (spiroxamine-amine)

WAK 5868 (spiroxamine-hydroxyl) (M05)

WAK 6084/1(spiroxamine-hydroxy-desethyl) (M41)

WAK 6079/1 (spiroxamine–hydroxy-despropyl) (M09)

WAK 5708 (spiroxamine-acid) (M06)

WAK 5756B (spiroxamine-desethyl acid) (M11)

BNF 5534A (spiroxamine-despropyl acid) (M12)

WAK 5428 (spiroxamine-ketone) (M15)

BNF 5544A (spiroxamine-hydroxy-ketone) (M16)

BNF 5550A (spiroxamine-cyclohexanol) (M13)

WAK6482-4 (spiroxamine-diol) (M14)

WAK 6131-2-7 (spiroxamine-ketone acid) (M17)

Each metabolite was identified by co-chromatography with reference standards.

Residues in [^{14}C]spiroxamine treated wheat

Metabolites forage (day 0)

Organo-extractable residue

The dichloromethane phase of forage (day 0) contained 97.6% (7.74 mg/kg) of the TRR and unchanged parent compound accounted for 75.5% (5.99 mg/kg). The main metabolite spiroxamine-N-oxide (M03) accounted for 8.0%TRR (0.63 mg/kg). Further metabolites were identified as spiroxamine-N-formyl-desethyl (M04) (2.1%TRR, 0.17 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (3.2%TRR, 0.25 mg/kg) and spiroxamine-despropyl (M02) (4.3%TRR, 0.34 mg/kg). Metabolite 1 (1.5%TRR, 0.12 mg/kg) was characterised as being based on tert-butylketone. Spiroxamine-hydroxy-despropyl (M09) and unknown metabolite 9 (based on tert.-butylketone) co-chromatographed in solvent system IV and the sum of both was 2.0%TRR (0.16 mg/kg). Due to the relatively low amounts, their individual amount was not determined in forage but only in straw, where spiroxamine-hydroxy-despropyl (M09) and metabolite 9 were isolated, then hydrolysed and finally quantified as spiroxamine-hydroxy-ketone and tert.-butylketone. The metabolite group 11 (1.0%TRR or 0.08 mg/kg) was not further investigated in forage. Group 11 metabolites, as named in the report, were isolated from the dichloromethane phase and hydrolysed under acidic conditions. They were based on the tert-butylketone and hydroxyketone and contained at least 6 components.

Aqueous soluble residue

Only very small amounts of radioactivity (1.6%, 0.12 mg/kg of the TRR) were present in the aqueous phase. Traces of parent spiroxamine accounted for 0.5%TRR (0.04 mg/kg). Metabolites diol conjugate (0.2%TRR, 0.02 mg/kg) and glucoside conjugate (tentatively assigned as a spiroxamine-hydroxy-N-oxide malonyl glucoside) of the hydroxy-N-oxide (M21) (0.2%TRR, 0.02 mg/kg) were identified. Each of the minor polar metabolites 21-23 were quantified as < 0.3%TRR (< 0.02 mg/kg).

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/01-3. The identified components of the terminal residue in forage, are summarised in Table CA 6.2.1/01-6.

The sum of identified and characterised metabolites was 97.5% (7.73 mg/kg) of the TRR in forage (day 0) and only 0.8% (0.07 mg/kg) remained in the solids.

Metabolites forage (day 14)

Organo-extractable residue

The dichloromethane phase of forage (day 14) contained 86.8% (9.26 mg/kg) of the TRR, and unchanged parent compound accounted for 53.3%TRR (5.69 mg/kg). The main metabolite spiroxamine-N-oxide (M03) accounted for 9.0%TRR (0.96 mg/kg). Further metabolites were identified as spiroxamine-N-formyl-desethyl (M04) (5.3%TRR, 0.56 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (5.1%TRR, 0.54 mg/kg) and spiroxamine-despropyl (M02) (4.6%TRR, 0.49 mg/kg). Metabolite 1 (5.8%TRR, 0.62 mg/kg), was characterised as being based on tert.-butylketone. Spiroxamine-hydroxy-despropyl (M09) and metabolite 9 (based on tert.-butylketone) co-chromatographed in solvent system IV and the sum of both was 1.6% TRR (0.17 mg/kg). Due to the relatively low amounts, their individual amount was not determined in forage. Metabolite group 11 (1.2%TRR or 0.13 mg/kg) and metabolite group 14 (0.5%TRR or 0.05 mg/kg) were not further investigated in forage. Group 14 metabolites, as named in the report, were isolated from the dichloromethane phase and hydrolysed under acidic conditions. They were based on the tert-butylketone and hydroxyketone and contained approximately 4 separate components. A further 0.4% TRR (0.04 mg/kg) remained at the TLC-origin.

Aqueous soluble residue

Only 8.6% (0.92 mg/kg) of the TRR was present in the aqueous phase. Traces of parent spiroxamine accounted for 0.5%TRR (0.05 mg/kg). Two glucoside conjugates were identified as spiroxamine-hydroxy-N-oxide glucoside (M20) (0.7%TRR, 0.08 mg/kg) and the spiroxamine-hydroxy-N-oxide malonyl glucoside conjugate of the hydroxy-N-oxide (M21) (2.0%TRR, 0.21 mg/kg). Spiroxamine-hydroxy-ketone conjugate (1.1% RR, 0.12 mg/kg) and diol conjugate (1.0% TRR, 0.11 mg/kg) were also identified. The minor metabolites or metabolite groups 11, 14, 21, 22 and 23 (each quantified as <1.3%TRR, <0.14 mg/kg) were not further investigated.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/01-3. The identified components of the terminal residue in forage, are summarised in Table CA 6.2.1/01-6.

The sum of identified and characterised metabolites was 90.0% (9.60 mg/kg) of the TRR in forage (day 14). At least 5 minor metabolites (nos. 11, 14, 21, 22, 23) ranging from 0.5-1.6%TRR (0.05-0.17 mg/kg) and radioactivity at the TLC-origin (0.4%TRR, 0.04 mg/kg) remained unidentified and 4.6%TRR (0.49 mg/kg) were detected in the solids.

Metabolites straw (day 61)

Organo-extractable residue

Organic phase 1 (dichloromethane) contained 73.2% (25.55 mg/kg) of the TRR, and unchanged parent compound accounted for 25.1%TRR (8.76 mg/kg). The main metabolite spiroxamine-N-oxide (M03) accounted for 22.0%TRR (7.68 mg/kg). Further metabolites were identified as spiroxamine-N-formyl-desethyl (M04) (7.5%TRR, 2.62 mg/kg), spiroxamine-hydroxyl (M05) (2.4%TRR, 0.84 mg/kg), spiroxamine-desethyl (M01) (2.0%TRR, 0.70 mg/kg), spiroxamine-despropyl (M02) (3.2%TRR, 1.12 mg/kg) and spiroxamine-hydroxy-despropyl (M09) (0.3%TRR, 0.11 mg/kg). Metabolite 1 (5.8%TRR, 2.02 mg/kg) and metabolite 9 (0.9%TRR, 0.31 mg/kg) were characterised as being based on tert.-butylketone. Metabolite groups 11 and 14 were hydrolysed and finally characterised as being based on tert.-butylketone (0.8%TRR, 0.28 mg/kg, M15), spiroxamine-hydroxy ketone (0.7%TRR, 0.24 mg/kg) and unknown hydrolysis products (0.7%TRR, 0.24 mg/kg). Finally, 1.1%TRR (0.38 mg/kg) remained at the TLC origin and another 0.7%TRR (0.24 mg/kg) was obtained as radioactivity at the TLC-origin following hydrolysis of metabolites spiroxamine-hydroxyl (M05), spiroxamine-desethyl (M01), spiroxamine-hydroxy-despropyl(M09), metabolite 9 and of metabolite groups 11 and 14.

Organic phase 2 (dichloromethane) contained 7.0% (2.45 mg/kg) of the TRR. As this phase was obtained from the exhaustive extraction of solid residue 1 under acidic conditions, only hydrolysis products of metabolites were present. The main component was identified as tert.-butylketone. Three hydrolysis products were identified as tert.-butylketone (5.5%TRR, 1.93 mg/kg, M15), spiroxamine-hydroxy ketone (1.0%TRR, 0.35 mg/kg) and spiroxamine-diol (0.2%TRR, 0.07 mg/kg, M13). Minor amounts (0.3%TRR, 0.10 mg/kg) were due to an unknown hydrolysis product.

Aqueous soluble residue

Aqueous phase 1 of straw contained 11.9% (4.17 mg/kg) of the TRR and contained at least 13 metabolites or metabolite groups in concentrations of 0.5 - 2.3% (0.18 - 0.81 mg/kg) of the TRR. In order to reduce the large number of metabolites to common moieties, acidic and enzymatic hydrolyses of aqueous phase 1 of straw were additionally performed.

Following HCl hydrolysis of the aqueous phase 1 of straw, the major portion (93.8%) of the radioactivity partitioned into ethyl acetate representing 11.2% of the TRR in straw. The main hydrolysis products in the ethyl acetate phase were spiroxamine-hydroxy-ketone (8.9% of the TRR) and spiroxamine-diol (1.3% of the TRR). Enzymatic hydrolysis experiments of the XAD-4 purified aqueous phase 1 confirmed the results obtained from the acidic hydrolysis experiments.

Aqueous phase 2 of straw (2.2%, 0.75 mg/kg) consisted of polar compounds near the TLC-origin. One unknown component accounted for 1.1% (0.37 mg/kg) of the TRR. Another 1.1%TRR (0.38 mg/kg) remained at the TLC-origin.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/01-4. The identified components of the terminal residue in forage are summarised in Table CA 6.2.1/01-6.

The sum of identified and characterised metabolites was 86.5% (30.20 mg/kg) of the TRR. At least 5 minor metabolites or metabolite groups accounted for 4.8%TRR (1.69 mg/kg) in total, a further 2.9%TRR (1.00 mg/kg) remained at the TLC-origin and 5.7% (2.00 mg/kg) was detected in the solids.

Metabolites in grain (Day 61)

Organo-extractable residue

Organic phase 1 (dichloromethane) contained very low amounts of radioactivity and the metabolites were identified by comparison with the metabolite pattern from the dichloromethane phase of straw. The dichloromethane phase of grain contained 36.6% (0.026 mg/kg) of the TRR, and unchanged parent compound accounted for 14.3% (0.010 mg/kg). The main metabolite spiroxamine-N-oxide (M03) accounted for 8.6%TRR (0.006 mg/kg). Further metabolites were identified as spiroxamine-N-formyl-desethyl (M04) (4.5%TRR, 0.003 mg/kg), spiroxamine-hydroxyl (M05) (1.6%TRR, 0.001 mg/kg), spiroxamine-desethyl (M01) (0.5%TRR, <0.001 mg/kg) and spiroxamine-despropyl (M02) (3.0%TRR, 0.002 mg/kg). The characterised metabolite 1, which was based on tert.-butylketone, accounted for 1.5%TRR (0.001 mg/kg) and 1.0%TRR (0.001 mg/kg) remained at the TLC-origin. The quantitation of spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) was conducted as described for the dichloromethane phase of straw following acidic hydrolysis. As a result, tert.-butylketone, spiroxamine-hydroxy ketone and two unknown hydrolysis products near the TLC origin were detected. Metabolites 5.1 and 5.2 accounted for 0.7%TRR (<0.001 mg/kg) and 0.9%TRR (<0.001 mg/kg), respectively.

Organic phase 2 (ethyl acetate) of grain consisted of metabolites, which were dissolved following enzymatic treatment of solid residue 1 using diastase and were partitioned into ethyl acetate. Organic phase 2 contained 13.9% (0.010 mg/kg) of the TRR. A small amount of tert.-butylketone (1.7%TRR, 0.001 mg/kg) was observed which probably was obtained as a by-product under the special extraction conditions. Spiroxamine-N-formyl-desethyl (M04) (2.4%TRR, 0.002 mg/kg) and spiroxamine-N-oxide (M03) (9.2%TRR, 0.006 mg/kg) were identified and 0.6%TRR (<0.001 mg/kg) remained at the TLC-origin.

Organic phase 3 (dichloromethane) of grain contained 18.5% (0.013 mg/kg) of the TRR. The hydrolysis products were identified as tert.-butylketone (2.9%TRR, 0.002 mg/kg), tert.-butylcyclohexanol (2.4%TRR, 0.002 mg/kg, M15), spiroxamine-hydroxy ketone (7.6%TRR, 0.005 mg/kg), spiroxamine-diol (2.6%TRR, 0.002 mg/kg, M13), hydrolysis product 1.5 (0.6%TRR, 0.001mg/kg), hydrolysis product 1.6 (1.1%TRR, <0.001 mg/kg), hydrolysis product 1.7 (0.5%TRR, 0.001 mg/kg) and TLC-origin (0.8%TRR, 0.001 mg/kg). The hydrolysis products 1.5 - 1.7 were detected near the origin.

Aqueous soluble residue

Aqueous phase 1 of grain contained 38.5% (0.020 mg/kg) of the TRR. However, this phase could not be chromatographed by TLC due to the high matrix content and could not be purified without losses. Therefore, aqueous phase 1 was completely hydrolysed using 1N HCl and the cleavage products were distributed into organic phase 3 and aqueous phase 3.

The aqueous phases 2 and 3 contained 11.0% (0.008 mg/kg) and 10.0% (0.007 mg/kg) of the TRR, respectively and could not be further investigated. Aqueous phase 2 represented polar metabolites which were characterised as being dissolved following enzymatic hydrolysis using diastase and being unextracted by ethyl acetate. Similarly, aqueous phase 3 represented polar metabolites which were

originally dissolved in aqueous phase 1 and were based on unknown hydrolysis products which were not extracted by dichloromethane.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/01-5. The identified components of the terminal residue in forage, are summarised in Table CA 6.2.1/01-6.

The sum of identified and characterised metabolites was 87.6% (0.061 mg/kg) of the TRR and 10.0% (0.007 mg/kg) was detected in the solids.

Table CA 6.2.1/01-3 Summary of extractable radioactive residue fractions in wheat forage following two spray applications with [cyclohexyl-1-^{14}C]-spiroxamine

	Forage (0 DACA)		Forage (14 DALA)	
	mg/kg	%TRR	mg/kg	%TRR
Organic (dichloromethane) phase [1]	**7.74**	**92.5**	**9.26**	**86.8**
Spiroxamine	5.99	75.5	5.69	53.3
Metabolite 1 (based on tert.-butylketone)	0.12	1.5	0.62	5.8
Spiroxamine-N-formyl-desethyl (M04)	0.17	2.1	0.56	5.3
Spiroxamine-hydroxyl (M05) and Spiroxamine-desethyl (M01)	0.25	3.2	0.54	5.1
Spiroxamine-despropyl (M02)	0.34	4.3	0.49	4.6
Spiroxamine-hydroxy-despropyl(M09) plus metabolite 9 (based on tert.-butylketone)	0.16	2.0	0.17	1.6
Spiroxamine-N-oxide (M03)	0.63	8.0	0.96	9.0
Metabolite group 11	0.08	1.0	0.13	1.2
Metabolite group 14	-	-	0.05	0.5
Polar material (TLC-origin)			0.04	0.4
Aqueous phase [1]	**0.12**	**1.6**	**0.92**	**8.6**
Spiroxamine, parent	0.04	0.5	0.05	0.5
Conjugate of M14 (diol conjugate)	0.02	0.2	0.11	1.0
Spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)[2]	0.02	0.2	0.21	2.0
Metabolite group 14	-	-	0.04	0.4
Metabolite group 14		-	0.03	0.3
Metabolite 21 (unknown)	0.02	0.2	0.05	0.5
Metabolite 22 (unknown)	0.02	0.2	0.09	0.8
Metabolite group 23 (unknown)	0.02	0.3	0.14	1.3
Spiroxamine-hydroxy-N-oxide glucoside (M20)	-	-	0.08	0.7
Conjugate of M16 (spiroxamine-hydroxy-ketone conjugate)	-	-	0.12	1.1
Non-extractable, bound residue	0.07	0.8	0.49	4.6
Total residue	**7.93**	**100.0**	**10.67**	**100.0**

1 – designation of unknown metabolites follows designation in the study report
2 – glucoside conjugate (probably a spiroxamine-hydroxy-N-oxide malonyl glucoside) of the hydroxy-n-oxide

Table CA 6.2.1/01-4 Summary of extractable radioactive residue fractions in wheat straw following two spray applications with [cyclohexyl-1-^{14}C]-spiroxamine

	Straw (61 DALA)	
	mg/kg	%TRR
Organic (dichloromethane) phase 1[1]	**25.55**	**73.2**
Spiroxamine, parent	8.76	25.1
Metabolite 1 (based on tert.-butylketone)	2.02	5.8
Spiroxamine-N-formyl-desethyl (M04)	2.62	7.5
Spiroxamine-hydroxyl (M05)	0.84	2.4
Spiroxamine-desethyl (M01)	0.70	2.0
Spiroxamine-despropyl (M02)	1.12	3.2
Spiroxamine-hydroxy-despropyl(M09)	0.11	0.3
Metabolite 9 (based on tert.-butylketone)	0.31	0.9
Spiroxamine-N-oxide (M03)	7.68	22.0
tert.-butylketone following hydrolysis of the metabolite groups 11 and 14	0.28	0.8
spiroxamine-hydroxy ketone following hydrolysis of the metabolite groups 11 and 14	0.24	0.7
unknown hydrolysis products obtained from metabolite groups 11 and 14	0.24	0.7
Polar material (TLC-origin)	0.38	1.1
Polar material (TLC-origin following hydrolysis of spiroxamine-hydroxyl, spiroxamine-desethyl, spiroxamine-hydroxyl - despropyl, metabolite 9 and of metabolite groups 11 and 14)	0.24	0.7
Aqueous phase 1[1]	**4.17**	**11.9**
Metabolite group 11	0.21	0.6
Spiroxamine-hydroxy-N-oxide glucoside (M20)	0.70	2.0
Metabolite group 14	0.18	0.5
Conjugate of M16 (spiroxamine-hydroxy-ketone conjugate)	0.63	1.8
Conjugate of M14 (diol conjugate)	0.46	1.3
Spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)[2]	0.67	1.9
Metabolite 21 (unknown)	0.21	0.6
Metabolite 22 (unknown)	0.28	0.8
Metabolite group 23 (unknown)	0.81	2.3
Organic (dichloromethane) phase 2	**2.45**	**7.0**
tert.-butylketone (M15)	1.93	5.5
Unknown hydrolysis product	0.10	0.3
Spiroxamine-hydroxy-ketone (M16)	0.35	1.0
Diol (M14)	0.07	0.2
Aqueous phase 2[1]	**0.75**	**2.2**
Unknown hydrolysis product	0.37	1.1
Polar material (TLC origin)	0.38	1.1
Non-extractable bound residue	2.0	5.7
Total residue	34.92	100.0

1 – designation of unknown metabolites follows designation in the study report
2 – glucoside conjugate (probably a spiroxamine-hydroxy-N-oxide malonyl glucoside) of the hydroxy-n-oxide

Table CA 6.2.1/01-5 Summary of extractable radioactive residue fractions in wheat grain following two spray applications with [cyclohexyl-1-^{14}C]-spiroxamine

	Grain (61 DALA)	
	mg/kg	%TRR
Organic (dichloromethane) phase 1[1]	**0.026**	**36.6**
Spiroxamine, parent	0.010	14.3
Metabolite 1 (based on tert.-butylketone)	0.001	1.5
Spiroxamine-N-formyl-desethyl (M04)	0.003	4.5
Spiroxamine-hydroxyl (M05)	0.001	1.6
Spiroxamine-desethyl (M01)	<0.001	0.5
Metabolite 5.1 (unknown)	<0.001	0.7
Metabolite 5.2 (unknown)	<0.001	0.9
Spiroxamine-despropyl (M02)	0.002	3.0
Spiroxamine-N-oxide (M03)	0.006	8.6
Polar material (TLC-origin)	<0.001	1.0
Aqueous phase 1 (TLC not possible) the hydrolysis products of this phase were distributed into organic phase 3 and aqueous phase 3 as given below)	**0.020**	**28.5**
Organic (ethyl acetate) phase 2[1]	0.010	13.9
tert.-butylketone	0.001	1.7
Spiroxamine-N-formyl-desethyl (M04)	0.002	2.4
Spiroxamine-N-oxide (M03)	0.006	9.2
Polar material (TLC-origin)	<0.001	0.6
Aqueous phase 2 (TLC not possible)	**0.008**	**11.0**
Organic (dichloromethane) phase 3[1]	**0.013**	**18.5**
Hydrolysis product- tert.-butylketone (M15)	0.002	2.9
Hydrolysis product- tert.-butylcyclohexanol (M13)	0.002	2.4
Hydrolysis product- spiroxamine-hydroxy ketone (M16)	0.005	7.6
Hydrolysis product- diol (M14)	0.002	2.6
Hydrolysis product 1.5 (unknown)	<0.001	0.6
Hydrolysis product 1.6 (unknown)	<0.001	1.1
Hydrolysis product 1.7 (unknown)	<0.001	0.5
Polar material (TLC origin)	<0.001	0.8
Aqueous phase 3 (TLC not possible)	**0.007**	**10.0**
Non-extractable, bound residue	0.007	10.0
Total residue	0.070	100.0

1 – designation of unknown metabolites follows designation in the study report

Table CA 6.2.1/01-6 Summary of identified radioactive residues in wheat following two spray applications with [cyclohexyl-1-^{14}C]-spiroxamine

	Forage (0 DALA)		Forage (14 DALA)		Straw (61 DALA)		Grain (61 DALA)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Identified metabolites								
Spiroxamine, parent	6.03	76.0	5.74	53.8	8.76	25.1	0.010	14.3
Spiroxamine-N-oxide (M03)	0.63	8.0	0.96	9.0	7.68	22.0	0.012	17.8
Spiroxamine-N-formyl-desethyl (M04)	0.17	2.1	0.56	5.3	2.62	7.5	0.005	6.9
Spiroxamine-hydroxyl (M05)	0.25	3.2	0.54	5.1	0.84	2.4	0.001	1.6
Spiroxamine-desethyl (M01)					0.70	2.0	<0.001	0.5
Spiroxamine-despropyl (M02)	0.34	4.3	0.49	4.6	1.12	3.2	0.002	3.0
Spiroxamine-hydroxy-N-oxide glucoside (M20)			0.08	0.7	0.70	2.0		
Spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)[1]	0.02	0.2	0.21	2.0	0.67	1.9		
Spiroxamine-hydroxy-despropyl (M09)					0.11	0.3		
Total identified	**7.44**	**93.8**	**8.58**	**80.5**	**23.20**	**66.4**	**0.03**	**44.1**
Metabolites based on identified hydrolysis products								
Spiroxamine-hydroxy-despropyl (M09) plus metabolite 9 (based on tert.-butylketone)	0.161[2]	2.0	0.17[2]	1.6				
Conjugate of M14 (diol conjugate)	0.02[2]	0.2	0.11[2]	1.0	0.46	1.3		
Conjugate of M16 (spiroxamine-hydroxy-ketone conjugate)			0.12[2]	1.1	0.63	1.8		
Metabolite groups 11 and 14, based on tert.-butylketone					0.28	0.8		
Metabolite groups 11 and 14, based on spiroxamine-hydroxy ketone					0.24	0.7		
Metabolite 1, based on tert.-butylketone					2.02	5.8		
Metabolite 9, based on tert.-butylketone					0.9	0.31		
Identified hydrolysis products (exhaustive extraction)								
Tert.-butylketone (M15)					1.93	5.5	0.003	4.6
Spiroxamine-diol (M24)					0.07	0.2	0.002	2.6

	Forage (0 DALA)		Forage (14 DALA)		Straw (61 DALA)		Grain (61 DALA)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Identified metabolites								
Spiroxamine-hydroxy-ketone (M16)					0.35	1.0	0.005	7.6
Tert.-butylcyclohexanol (M13)							0.001	2.4
Total identified/characterised[3]	**7.62**	**96.0**	**8.98**	**84.2**	**30.08**	**83.81**	**0.041**	**61.3**

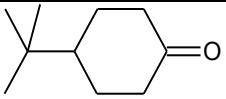
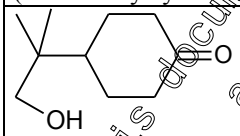
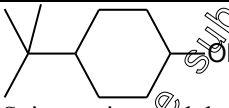
1 – glucoside conjugate (probably a spiroxamine-hydroxy-N-oxide malonyl glucoside) of the hydroxy-n-oxide
2 - Characterisation and assignment of components in forage based on comparison of chromatography of residues in straw. Individual components in forage not isolated
3 – Characterised residue includes identified residue from hydrolysis. Not included as primary plant metabolites

Hydrolysis of spiroxamine residues for method development

Spiroxamine was intensively metabolised in wheat and a large number of metabolites were formed, however, the identified and characterised metabolites were mainly based on one common moiety (tert.-butylketone) as shown in Table CA 6.2.1/01-7. This table provides a summarised overview and comparison for the quantity of tert.-butylketone (M15), spiroxamine-hydroxy ketone and spiroxamine-diol and was calculated from Table CA 6.2.1/01-4. As a result, tert.-butylketone represented 72.8% (25.42 mg/kg) of the TRR in straw. Significantly, smaller residues were based on spiroxamine-hydroxy ketone (10.1%, 3.54 mg/kg) and the diol was of minor importance (1.5%, 0.53 mg/kg) as were all other components.

As in straw, a large number of metabolites were formed in grain, and the identified and characterised metabolites were mainly based on tert.-butylketone as shown in Table CA 6.2.1/01-7. Table CA 6.2.1/01-7 provides a summarised overview and comparison for the quantity of tert.-butylketone (M15), spiroxamine-hydroxy ketone, spiroxamine-cyclohexanol and spiroxamine-diol and was calculated from Table CA 6.2.1/01-5. As a result, tert.-butylketone represented 48.6% (0.034 mg/kg) of the TRR in grain. Significantly, smaller residues were based on spiroxamine-hydroxy ketone (9.2%TRR, 0.006 mg/kg). The spiroxamine-cyclohexanol (2.4%TRR, 0.002 mg/kg) and the diol (2.6%, 0.002 mg/kg) were only of minor importance as were all other components.

Table CA 6.2.1/02-7 Hydrolysis products of cyclohexyl-labelled Spiroxamine residues in wheat straw and grain (information in this summary table is also replicated in CA 6.2.1/02)

Hydrolysis product	Straw mg/kg (%TRR)	Grain mg/kg (%TRR)
 Spiroxamine-ketone (M15) (4-tert.-butylcyclohexanone)	25.42 (72.8)	0.034 (48.6)
 Spiroxamine-hydroxy-ketone (M16) 4-(1,1-dimethyl-2-hydroxyethyl)-cyclohexanone	3.54 (10.1)	0.006 (9.2)
 Spiroxamine-cyclohexanol (M13)	-	0.002 (2.4)
 Spiroxamine-diol (M14) 4-(1,1-dimethyl-2-hydroxyethyl)-cyclohexanol	0.53 (1.5)	0.002 (2.6)
Identified hydrolysis products	29.49 (84.4)	0.044 (62.8)
Unknown metabolites and unknown hydrolysis products	2.4 (6.9)	0.017 (24.8)
TLC-origin	1.0 (2.9)	0.002 (2.4)
Solids (non-extractable residue)	2.0 (5.7)	0.007 (10.0)
Total residue	34.92 (100.0)	0.070 (100.0)

Storage stability

The storage stability of parent compound and metabolites was conducted using forage (day 14), straw and grain from the second container. For each analysis, two samples were extracted and investigated

separately. The storage conditions of the unextracted material was ≤ - 20°C in a deep freezer. For the extraction of forage and straw, prepacked portions were used which had been homogenised and prepared before storage. Grain was taken as needed from one storage container. Extractions for the storage stability were conducted from 32 to 566 days (forage), 1 to 518 days (straw) and 10 to 547 days (grain). It was concluded that parent compound and metabolites remained stable under these storage conditions.

III. Conclusions

[^{14}C-cyclohexyl]spiroxamine, formulated as a 500 EC formulation, was sprayed twice to spring wheat plants in a controlled vegetation area, at stem elongation and ear emergence. The applied rate was equivalent to 831 g a.s./ha (equivalent to 1.1N maximum seasonal rate). Forage was sampled immediately after the second application (day 0) and at day 14, with straw and grain sampled at harvest (day 61).

The total radioactive residue (TRR) in forage accounted for 7.93 mg/kg parent compound equivalents at day 0. The major residue found in forage (day 0) was parent spiroxamine (76%TRR, 6.03 mg/kg). Other metabolites identified were spiroxamine-N-oxide (M03) (8.0%TRR, 0.63 mg/kg), spiroxamine-N-formyl-desethyl (M04) (2.1%TRR, 0.17 mg/kg,), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (3.2%TRR, 0.25 mg/kg), spiroxamine-despropyl (M02) (4.3%TRR, 0.34 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (0.2% TRR, 0.02 mg/kg). Identified hydrolysis products included conjugates of diol and hydroxyl-despropyl (0.2-2.0% TRR, 0.02 - 0.16 mg/kg).

The total radioactive residue (TRR) in forage accounted for 10.67 mg/kg parent compound equivalents at day 14. The major residue found in forage (day 14) was parent spiroxamine (53.8%TRR, 5.74 mg/kg). Other metabolites identified were spiroxamine-N-oxide (M03) (9.0%TRR, 0.96 mg/kg), spiroxamine-N-formyl-desethyl (M04) (5.3%TRR, 0.56 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (5.1%TRR, 0.54 mg/kg), spiroxamine-despropyl (M02) (4.6%TRR, 0.49 mg/kg), spiroxamine-hydroxy-N-oxide glucoside (M20) (0.7%TRR, 0.08 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (2.0%TRR, 0.21 mg/kg). Identified hydrolysis products included conjugates of diol, conjugates of hydroxyl-ketone and hydroxyl-despropyl (1.0 - 1.6% TRR, 0.11 - 0.17 mg/kg).

At harvest (day 61), the TRR in straw accounted for 34.92 mg/kg parent equivalents. Parent spiroxamine and metabolite spiroxamine-N-oxide (M03) were the major residues in straw, accounting for 25.1% and 22% of the TRR (8.76 mg/kg and 7.68 mg/kg), respectively. Other metabolites identified were spiroxamine-N-formyl-desethyl (M04) (7.5%TRR, 2.62 mg/kg), spiroxamine-hydroxyl (M05) (2.4%TRR, 0.84 mg/kg), spiroxamine-desethyl (M01) (2.0%TRR, 0.70 mg/kg), spiroxamine-despropyl (M02) (3.2%TRR, 1.12 mg/kg), spiroxamine-hydroxy-despropyl(M09) (0.3%TRR, 0.11 mg/kg) and spiroxamine-hydroxy-N-oxide glucoside (M20) (2.0%TRR, 0.70 mg/kg). Identified hydrolysis products included conjugates of diol and conjugates of hydroxyl-ketone (1.3 - 1.8% TRR, 0.46 - 0.63 mg/kg). Identified hydrolysis products from exhaustive extraction included tert.-butylketone, diol (M14) and spiroxamine-hydroxy-ketone (M16) (0.2 - 5.5%TRR, 0.07 - 1.93 mg/kg).

The TRR in grain was very low (0.07 mg/kg). Parent spiroxamine and spiroxamine-N-oxide (M03) were the only significant residues in the grain, accounting for 14.3% (0.010 mg/kg) of the TRR and 17.8% (0.012 mg/kg) of the TRR. Metabolites spiroxamine-N-formyl-desethyl (M04), spiroxamine-hydroxyl (M05), spiroxamine-desethyl (M01), and spiroxamine-despropyl (M02) were detected at very low levels (<10% of the TRR, <0.01 mg/kg). Identified hydrolysis products from exhaustive extraction included tert.-butylketone, diol (M14), spiroxamine-hydroxy-ketone (M16) and tert.-butylcyclohexanol (2.4 - 7.6%TRR, 0.001 - 0.005 mg/kg).

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the spiroxamine-N-oxide, M03) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl, M05). The metabolites bearing an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide

glucoside (M20) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21). Some metabolites were formed by desalkylation (-spiroxamine, M01; spiroxamine-despropyl, M02) which were partly metabolised by formylation (spiroxamine-N-formyl-desethyl, M04). Only small amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

The proposed metabolic pathway for spiroxamine in cereals is shown in Figure CA 6.2-2.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009) IIA 6.2.1/05 and IIA 6.2.1/06. The study is considered compliant with OECD Guideline 501 – Metabolism in Crops, January 2007.

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the spiroxamine-N-oxide) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl). The metabolites bearing an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide glucoside and spiroxamine-hydroxy-N-oxide malonyl glucoside. Some metabolites were formed by desalkylation (spiroxamine-desethyl spiroxamine-despropyl) which were partly metabolised by formylation (spiroxamine-N-formyl-desethyl). Only low amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

Data Point:	KCA 6.2.1/02
Report Author:	[redacted]
Report Year:	1996
Report Title:	Metabolism of KWG 4168 in winter wheat
Report No:	PF4189
Document No:	M-006112-01-1
Guideline(s) followed in study:	US EPA Pesticides Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4: Nature of residues in plants and livestock
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary:

[1,3-dioxolane-4-^{14}C]-spiroxamine, formulated as a 500 EC formulation, was sprayed twice to winter wheat plants at BBCH 30 (stem elongation) and BBCH 51 (ear emergence) at a total rate of 1650 g a.s./ha (equivalent to 2.2N maximum seasonal rate). Forage was sampled immediately after the second application (day 0) and at day 14, with straw and grain sampled at harvest (day 56).

The total radioactive residue (TRR) in forage accounted for 15.81 mg/kg and 24.11 mg/kg parent equivalents at day 0 and day 14, respectively. At harvest (day 56), the TRR in straw accounted for 82.76 mg/kg parent equivalents and the TRR in grain accounted for 0.453 mg/kg parent equivalents.

Parent spiroxamine comprised the major component of the residue in day 0 forage accounting for 63.9%TRR (10.10 mg/kg). Other metabolites detected were Spiroxamine-N-oxide (M03) (11.1%TRR; 1.75 mg/kg), spiroxamine-N-formyl-desethyl (M04) (4.6%TRR, 0.73 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (4.8%TRR, 0.76 mg/kg), spiroxamine-despropyl (M02)

(3.3%TRR, 0.52 mg/kg), spiroxamine-hydroxy-despropyl (M09) (0.2%TRR, 0.03 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (0.2%TRR, 0.03 mg/kg).

Parent spiroxamine also comprised the major component of the residue in day 14 forage accounting for 41.2%TRR (9.93 mg/kg). Other metabolites detected were Spiroxamine-N-oxide (M03) (12.7%TRR, 3.06 mg/kg), Spiroxamine-N-formyl-desethyl (M04) (5.8%TRR, 1.40 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (7.1%TRR, 1.71 mg/kg), spiroxamine-despropyl (M02) (4.6%TRR, 1.11 mg/kg), spiroxamine-hydroxy-despropyl(M09) (0.6%TRR, 0.15 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (0.9%TRR,TRR 0.22 mg/kg).

Parent spiroxamine comprised the major component of the residue in day 56 straw accounting for 20.6%TRR (17.01 mg/kg). Other metabolites detected were spiroxamine-N-oxide (M03) (20.9%TRR, 17.26 mg/kg), spiroxamine-N-formyl-desethyl (M04) (9.7%TRR, 8.06 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (5.2%TRR, 4.32 mg/kg), spiroxamine-despropyl (M02) (4.2%TRR, 3.48 mg/kg), spiroxamine-hydroxy-despropyl (M09) (0.4%TRR, 0.35 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (3.1%TRR, 2.57 mg/kg).

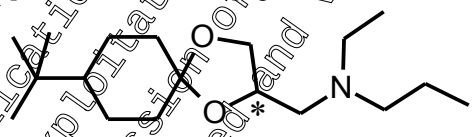
Only a small proportion of the TRR in 56-day grain could be identified. The identified metabolites were parent spiroxamine (2.8%TRR, 0.013 mg/kg) and spiroxamine-N-oxide (M03) (1.2%TRR, 0.005 mg/kg). The majority of radioactivity was released after intensive enzymatic hydrolysis using diastase. The residues were very polar in nature and could be partitioned into the aqueous phase (62.6% of TRR) when extraction was attempted with ethyl acetate. The aqueous phase could not be chromatographed by TLC due to the high matrix content.

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the Spiroxamine-N-oxide, M03) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl, M05). The metabolites bearing an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)). Some metabolites were formed by desalkylation (spiroxamine-desethyl, M01; spiroxamine-despropyl, M02) which were partly metabolised by formylation (Spiroxamine-N-formyl-desethyl, M04). Only small amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

I. Materials and methods

A. Test material

[1,3-dioxolane-4-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (MBq/mg):	1.23 (33.2 µCi/mg)
Lot/Batch No.:	Not stated
Purity:	Radiochemical purity > 99%
Storage condition:	Not stated
CAS No.:	118134-30-8

B. Study design

Test system

Soil

Table CA 6.2.1/02-1 Soil classification and physico-chemical properties

Soil Type (DIN 19682)	pH (KCl)	OM %	Sand %	Silt %	Clay %	Moisture holding capacity mL / 100g	CEC meq / 100 g
Loamy silt	6.63	1.14	17.1	71.8	11.1	Not reported	13

Test system

Two plant containers (each 1 m^2) with winter wheat, variety Orestis, were placed in the vegetation area of the Institute for Metabolism Research and Residue Analysis of Bayer AG, Monheim, Germany. One container was used for the metabolism study (experiment A) and one container for the storage stability investigations and validation of residue method (experiment B). The average temperature during the cultivation period ranged from 0.4°C (Feb. 1993) to 16.4°C (June 1993). The sunshine hours ranged from 57 (Jan. 1993) to 236 (May 1993) hours/month. The plant containers were fertilised with 1200 kg/ha potassium phosphate at Nov. 5 1992 and with 250 kg/ha calcium ammonium nitrate at Mar. 26, 1993. Treatment with other plant protection products was conducted as required, according to Good Agricultural Practice

Test design

The study started in April 1993 and from the QA statement, the final date for inspection for experimental work was July 1993. Definitive study dates were not stated in the report. The in-life phase was conducted in the vegetation area of the Institute for Metabolism Research and Residue Analysis Monheim, Bayer AG. The analytical phase and final reporting was conducted at the Institute for Metabolism Research and Residue Analysis of Bayer AG, Monheim, Germany.

Experimental conditions

[1,3-dioxolane-4-^{14}C]-spiroxamine, formulated as a 500 EC formulation, was applied twice as a spray application to winter wheat plants at BBCH 30 (stem elongation) and BBCH 51 (ear emergence) at a total rate of 1650 g a.s./ha.

A use on cereals is included in the representative GAP for spiroxamine. The rate employed in this study (total seasonal rate of 1650 g a.s./ha) is equivalent to 2.2 times the maximum seasonal use proposed for spiroxamine (750 g a.s./ha). This study is considered representative and appropriate for the objectives of establishing the nature and magnitude of spiroxamine in cereal crops.

Preparation of application solutions/applications

For planting container A, 165.1 mg of the 500 EC formulation was applied at the first and 164.9 mg at the second application. In total 330.0 mg of the formulation was applied which corresponded to 165.0 mg active ingredient or 202.95 MBq (5.478 mCi) ^{14}C-radioactivity equivalent to a field rate of1650 g a.s./ha. Each application was conducted using 40 mL of spray solution. Container B was treated in the same way as described for container A. A small amount of each of the four spray solutions was investigated by TLC to check the stability of the active ingredient

Sampling

The first forage sample (day 0) was taken immediately after the second application (May 25, 1993) from approximately 10% of the treated area from planting containers A and B. The combined sample was weighed and stored deep frozen. Six weeks later (July 6, 1993) the frozen plant material was homogenised in liquid N_2 and stored in different aliquots at -20°C or below.

The second forage sample (day 14) was taken 2 weeks after the second application (June 8, 1993) from approximately 30% of the treated area in the same way and stored deep frozen The plant material was homogenised in liquid N_2 (July 6, 1993) and stored in different aliquots at -20°C or below.

The winter wheat from the remaining treated area (approximately 60%) from containers A and B was harvested on day 56 (July 20, 1993) at maturity. The plants were separated into straw, grain and glumes. The glumes and the straw were combined and homogenised in liquid N_2. A total of 807.2 g homogenised straw was obtained and stored in aliquots of approximately 200 g. The homogenised straw and grain were stored at -20°C or below.

Sample preparation and extraction

Forage

An aliquot (50.0 g) of the homogenised forage from day 0 and day 14 was successively macerated with methanol (2 x 300 mL) and an equal volume of methanol/water 1:1 (v/v). The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and the solids (non-extractable residue). The methanol/water extract was evaporated to the aqueous remainder using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3 x 150 mL) resulting in the organic phase and aqueous phase. Aliquots from extracts, phases and solids were taken for radioactivity measurement.

Straw

An aliquot (60.0 g) of the homogenised straw from day 56 was successively macerated with methanol (2 x 450 mL) followed by 450 mL methanol/water 1:1 (v/v). The suspension was filtered by suction yielding the methanol/water extract and solid residue. The methanol/water extract was evaporated to an aqueous remainder using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3 x 300 mL) resulting in organic phase 1 and aqueous phase 1. Aliquots from all relevant extracts, phases, and solids were taken for radioactivity measurement.

Grain

An aliquot (20.0 g) of homogenised grain from day 56 was macerated with methanol/water 4:1 (v/v, 2 x 300 mL). The suspension was filtered by suction yielding the methanol/water extract and solid residue 1. The methanol/water extract was evaporated to an aqueous remainder using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3 x 100 mL) resulting in organic phase 1 and aqueous phase 1.

An aliquot (5.0 g) of solid residue 1 was further extracted by a repeated enzymatic hydrolysis procedure using diastase: 5.0 g solid residue 1 were combined with diastase (100 mg Diastase, Merck, no. 3604) and 55 mL citrate/NaOH-buffer (pH 6, Fixanal) containing 10 mg NaN_3 and stirred for six days in a closed 250 mL glass bulb at room temperature. The suspension was filtered by suction and the dissolved amount of solid residue 1 was determined by weighing of the remaining solids. The undissolved residue was repeatedly combined with diastase (100 mg) and buffer solution (55 mL) containing 10 mg NaN_3 and slowly stirred with a magnetic stirrer. The procedure was stopped (four extractions in total) when the final residue (solids, non-extractable residue) had decreased to 0.45 g, which was completely combusted. The aqueous enzyme extracts were combined and extracted with ethyl acetate (3 x 200 mL) resulting in organic phase 2 and aqueous phase 2. Aliquots from all relevant extracts, phases or solids were taken for radioactivity measurement by LSC and the radioactive residues of the combined organic phases (ethyl acetate) were analysed by radio-TLC.

Acidic hydrolysis of straw

An aliquot (5.0 g) of the homogenised straw from day 56 (*ca*. 509 kBq) was combined with 1N HCl (60 mL) and stirred under reflux for 1 hour. The suspension was filtered by suction, the filter cake dried at room temperature and aliquots were combusted. The filtrate was investigated by TLC following quantification by LSC.

Acidic hydrolysis of isolated metabolites

Approximately 8.7 kBq spiroxamine-desethyl (M01) and spiroxamine-hydroxyl (M05), obtained as a mixture following purification from the organic phase of straw by TLC, was dissolved in 500 µL methanol and 1N HCl, and heated in a Reacti-Vial for 1 hour at ca. 90°C. After cooling, the reaction mixture was analysed without neutralisation by radio-TLC. The same procedure was conducted using spiroxamine-despropyl (M02).

Radiochemical analysis

Radioactivity determination

The ^{14}C-radioactivity in liquid samples was determined in a liquid-scintillation counter PW4700 (Philips/Raytest), LKB 1219 Rackbeta (LKB-Wallac), LS 6000LL (Beckman Instruments) or LS 6500 (Beckman Instruments) with Instant-Scint-Gel (Packard), or Quicksafe A (Zinsser Analytic).

Solid samples were combusted in an OX 500 (Zinsser Analytic). The CO_2 produced by combustion was absorbed in a scintillation-cocktail [Oxysolve 0-400 (Zinsser Analytic)] and the radioactivity measured by LSC.

Thin layer chromatography (TLC)

The radioactive solutions were investigated by TLC using silica gel plates (silica gel 60_{F254}, 0.25 mm thickness, glass-coated, 20 cm x 20 cm, Merck, Darmstadt, Germany or silica gel 60_{F254}, 0.2 mm thickness, aluminium-coated, 20 cm x 20 cm, Merck, Darmstadt, Germany) and the following solvent systems (SS):

II: acetonitrile:water:25% ammonia 320:18:2 (v/v)
IV: acetonitrile:water:25% ammonia 80:18:2 (v/v)
V: chloroform:methanol:25% ammonia 65:28:8 (v/v)

The roman numerals of the solvent systems are according to those in the spring wheat study where 5 solvent systems were used in total.

The radioactive compounds were detected and evaluated quantitatively using a Linear Analyser (Rita 3200 or IM 3016, Raytest) or a Bio-Imaging Analyser (BAS 2000, Fuji). The reference compounds used in co-chromatography were visualised by UV-light (254 nm) or in an iodine chamber.

Mass spectroscopy (MS)

Aminodiol: GC/MS analyses were performed on a mass selective detector HP 5970 with a gas chromatograph 5880A (Hewlett-Packard). The capillary column used was a 12.5 m Ultra2 (Hewlett Packard). The oven temperature program was 80 °C for 1 minute, heating rate was 10 °C/min up to 280 °C. The final temperature was kept constant for 20 minutes. The samples were injected in the splitless mode at 280 °C. The helium pressure was 4 psi.

Spiroxamine-desethyl-aminodiol and spiroxamine-despropyl-aminodiol: For GC/MS analyses, the INCOS XL instrument by Finnigan with a Varian gas chromatograph was used. The capillary column used was a 30 m DB5MS (J&W Scientific) The oven temperature program was 60 °C for 1 minute, heating rate was 10°C/mm up to 310 °C or up to 280 °C. The final temperature was kept constant for 10 minutes The samples were injected in the splitless mode at 280 °C The helium pressure was 14 psi.

Spiroxamine-aminodiol-N-oxide: The electro-spray ionisation MS spectra (ESI) were obtained with a TSQ 7000 instrument by Finnigan. Sheath gas pressure 52 psi, capillary temperature 210 °C. The chromatographic and loop conditions for the MS experiments are given in the spectrum headers in the report. The flow rate was 200 µL/min. A radioactivity detector (Ramona 90, Raytest) was coupled between the HPLC (HP 1050, Hewlett Packard) and mass spectrometer. The delay time between the two instruments was approximately 20 sec. For the MS/MS experiments, argon was used as the collision gas (pressure in the collision chamber: 2.7 mT).

Derivatisation

Silylation was performed with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA). An excess of the reagent was added to an aliquot of the sample to be investigated spectroscopically. The mixture was heated to 70°C for 10 to 20 minutes. Injection into the GC was directly from the reaction mixture.

^{1}H-NMR-spectroscopy

The ^{1}H-NMR-spectra were recorded on a Bruker AC 300 spectrometer, the samples were dissolved in CD_3OD (Merck) for analysis.

II. Results and discussion

Total radioactive residue (TRR)

The total radioactive residue (TRR) in forage (day 0) accounted for 15.81 mg/kg and 24.11 mg/kg parent compound equivalents as determined by summation of the radioactivity in the dichloromethane phase, aqueous phase and in the solids at day 0 and day 14, respectively. The radioactivity in 0 day forage was easily extracted using methanol/water and partitioned almost completely into the dichloromethane phase (91.2% of the TRR, 14.42 mg/kg). Only 4.6% (0.72 mg/kg) of the TRR was found in the aqueous phase and 4.2% (0.66 mg/kg) in the solids. The radioactivity in day 14 forage was well extracted (88.6% of the TRR) using methanol/water and the major amount was partitioned into the dichloromethane phase (76.5% of the TRR, 18.45 mg/kg). Smaller amounts (12.1% of the TRR, 2.92 mg/kg) were present in the aqueous phase and in the solids (11.4%, 2.74 mg/kg). The radioactivity detected in the combined methanol/water extracts in 0 and 14 day forage (1335.7 kBq) was recovered nearly quantitatively in the organic and aqueous phases.

At harvest (day 56), the TRR in straw accounted for 82.76 mg/kg parent equivalents as determined by combustion. The majority of the radioactivity was extracted with methanol and methanol/water and partitioned into the organic phase (67.4% of the TRR, 55.75 mg/kg). Smaller amounts were found in the aqueous phase (17.2% of the TRR, 14.22 mg/kg) and in the solids (15.5% of the TRR, 12.80 mg/kg) which were not further extracted. No significant losses of radioactivity were observed during evaporation and partitioning. The radioactivity measured by LSC was normalised according to the combustion values of straw and solids.

At harvest (day 56), the TRR in grain accounted for 0.453 mg/kg parent equivalents as determined by combustion. Intensive efforts were made to solubilise the metabolites as quantitatively as possible without hydrolysing metabolite structures. Therefore, the extraction procedure commenced conventionally with methanol/water, and the remainder (17.87 g solid residue 1 resulting from 20.0 g grain) was hydrolysed enzymatically with diastase to solubilise the starch fraction. The radioactivity in the dichloromethane phase, obtained following evaporation of methanol, accounted for 7.0% (0.032 mg/kg) of the TRR. Slightly higher amounts (16.9% of the TRR, 0.077 mg/kg) partitioned into aqueous phase 1 and a high portion (76.1%) of the TRR remained in solid residue 1. Following enzymatic hydrolysis, only 4.7% (0.021 mg/kg) of the TRR partitioned into the ethyl acetate phase and 62.6% (0.283 mg/kg) into aqueous phase 2 and 8.7% (0.040 mg/kg) of the TRR remained in the solids (non-extractable residue). The quantitation of radioactivity in fractions was based on the combustion of grain (total), solid residue 1 (subtotal) and solids.

TRR and distribution of radioactivity are given in Table CA 6.2.1/02-2.

Table CA 6.2.1/02-2 Distribution of radioactive residues in wheat following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

Radioactive residues in wheat	Forage (0 DALA)		Forage (14 DALA)		Straw (56 DALA)		Grain (56 DALA)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR[1]	15.81	100	24.11	100	82.76	100	0.453	100.0
Organic phase 1 (dichloromethane)	14.42	91.2	18.45	76.5	55.75	67.4	0.032	7.0
Aqueous phase 1	0.72	4.6	2.92	12.1	14.22	17.2	0.077	16.9
Organic phase 2 (ethyl acetate)	-	-	-	-		-	0.021	4.7
Aqueous phase 2	-	-	-	-	-	-	0.283	62.6
Post extraction solids (PES)	0.66	4.2	2.74	11.4	12.8	15.5	0.040	8.7

1 - total radioactive residues given in mg parent substance equivalents per kg, determined by summation of all fractions after extraction for forage and by combustion for straw and grain.

Characterisation

Available synthesised compounds for characterisation were parent test substance (spiroxamine (KWG 4168)) and the following potential metabolites:

KWG 4557 (spiroxamine-desethyl) (M01)

KWG 4669 (spiroxamine-despropyl) (M02)

WAK6301/1 (spiroxamine-N-oxide) (M03)

WAK 6782 (spiroxamine-N-formyl-desethyl) (M04)

BNF 5567B (acetyl-spiroxamine-desethyl)

BNF 5567A (acetyl-spiroxamine-despropyl)

WAK 60712 (spiroxamine-amine)

WAK 5868 (spiroxamine-hydroxyl) (M05)

WAK 6084/1(spiroxamine-hydroxy-desethyl) (M41)

WAK 6079/1 (spiroxamine-hydroxy-despropyl) (M09)

WAK 5708 (spiroxamine-acid) (M06)

WAK 5756B (spiroxamine-desethyl acid) (M11)

BNF 5534A (spiroxamine-despropyl acid) (M12)

WAK 5428 (tert.-Butylcyclohexanone) (M15)

BNF 5544A (spiroxamine-hydroxy ketone) (M16)

BNF 5550A (spiroxamine-cyclohexanol) (M13)

WAK6482-4 (spiroxamine-diol) (M14)

WAK 6131-2-7 (spiroxamine-ketone acid) (M17)

WAK 5427 (spiroxamine-aminodiol) (M28)

WAK 6885 (spiroxamine-aminodiol-N-oxide) (M29)

Each metabolite was identified by co-chromatography with reference standards.

Residues in [^{14}C]spiroxamine treated wheat

Quantitation of metabolites was achieved by TLC using solvent system IV (dichloromethane phases) and solvent system V (aqueous phases). TLC was also used for comparison with reference compounds and for the identification of metabolites by co-chromatography with straw extracts from the spring wheat study (cyclohexyl-label) (CA 6.2.1/01). Results are summarised in Table CA 6.2.1/02-3.

Metabolites in forage (day 0)

Organo-extractable residue

The dichloromethane extract of forage (day 0) contained 91.2% (14.42 mg/kg) TRR and unchanged parent compound accounted for 63.9% TRR (10.10 mg/kg) in this extract. The main metabolite was spiroxamine-N-oxide (M03) and accounted for 11.1%TRR (1.75 mg/kg). Further metabolites were identified as follows by TLC comparison with the straw extract from spring wheat (CA 6.2.1/01): spiroxamine-N-formyl-desethyl (M04) (4.6%TRR, 0.73 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (4.8%TRR, 0.76 mg/kg), spiroxamine-despropyl (M02) (3.3%TRR, 0.52 mg/kg) and spiroxamine-hydroxy-despropyl(M09) (0.2%TRR, 0.03 mg/kg). Metabolite group 1 (2.2%TRR, 0.35 mg/kg), metabolite group 11 (0.5%TRR, 0.08 mg/kg) and metabolite group 14 (0.1%TRR, 0.02 mg/kg) were not further investigated.

Aqueous soluble residue

Only a small amount of radioactivity (4.6%, 0.72 mg/kg of the TRR) was present in the aqueous phase. Using TLC comparison, metabolites were assigned as follows: traces of spiroxamine (including metabolites 1-11, 0.8%TRR, 0.13 mg/kg), spiroxamine-hydroxy-N-oxide malonyl glucoside of the hydroxy-N-oxide (M21) (0.2%TRR, 0.03 mg/kg), metabolites 17+18 (unknown, 0.4%TRR, 0.06 mg/kg), metabolites 11a-16 (unknown, 2.5%TRR, 0.39 mg/kg), metabolites 21-23 (unknown, 0.7%TRR, 0.11 mg/kg).

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/02-3. The identified components of the terminal residue in Day 0 forage, are summarised in Table CA 6.2.1/02-5.

The sum of identified and characterised metabolites was 88.1% (13.93 mg/kg) of the TRR in forage (day 0) and 4.2%TRR (0.66 mg/kg) remained in the solids.

Metabolites in forage (day 14)

Organo-extractable residue

The dichloromethane phase of forage (day 14) contained 76.5% (18.45 mg/kg) TRR and unchanged parent compound accounted for 41.2% (9.93 mg/kg). The main metabolite was spiroxamine-N-oxide (M03) and accounted for 12.7% (3.06 mg/kg). Further metabolites were identified as follows by TLC comparison with the straw extract from spring wheat (CA 6.2.1/01): spiroxamine-N-formyl-desethyl (M04) (5.8%TRR, 1.40 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (7.1%TRR, 1.71 mg/kg), spiroxamine-despropyl (M02) (4.6%TRR, 1.11 mg/kg) and spiroxamine-hydroxy-despropyl(M09) (0.6%TRR, 0.15 mg/kg). Metabolite group 1 (2.3%TRR, 0.55 mg/kg), metabolite group 11 (0.6%TRR, 0.15 mg/kg) and metabolite group 14 (0.3%TRR, 0.07 mg/kg) were not further investigated.

Aqueous soluble residue

Only a small amount of radioactivity (12.1%, 2.92 mg/kg of the TRR) was present in the aqueous phase. Using TLC comparison, metabolites were assigned as follows: traces of spiroxamine (including metabolites 1-11, 0.8%TRR, 0.19 mg/kg), spiroxamine-hydroxy-N-oxide malonyl glucoside of the hydroxy-N-oxide (M21) (0.9%TRR, 0.22 mg/kg), metabolites 17+18 (unknown, 1.4%TRR, 0.34 mg/kg), metabolites 11a-16 (unknown, 6 7%TRR, 1 62 mg/kg), metabolites 21-23 (unknown, 2 3%TRR, 0 55 mg/kg).

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/02-3. The identified components of the terminal residue in day 14 forage, are summarised in Table CA 6.2.1/02-5.

The sum of identified and characterised metabolites was 72.9% (17.58 mg/kg) of the TRR in forage (day 14) and 11.4%TRR (2.74 mg/kg) remained in the solids.

Metabolites in straw

Organo-extractable residue

The dichloromethane extract of straw contained 67.4% (55.75 mg/kg) of the TRR, and unchanged parent compound accounted for 20.6% (17.01 mg/kg). The main metabolite was identified as spiroxamine-N-oxide (M03) and accounted for 20.9% (17.26 mg/kg). Further metabolites were identified as follows by TLC comparison with the straw extract from spring wheat (CA 6.2.1/01): spiroxamine-N-formyl-desethyl (M04) (9.7%TRR, 8.06 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (5.2%TRR, 4.32 mg/kg), spiroxamine-despropyl (M02) (4.2%TRR, 3.48 mg/kg) and spiroxamine-hydroxy-despropyl(M09) (0.4%TRR, 0.35 mg/kg). Metabolite group 1 (2.9%TRR, 2.43 mg/kg), metabolite group 11 (1.4%TRR, 1.15 mg/kg) and metabolite group 14 (0.6%TRR, 0.51 mg/kg) were not further investigated, however a complete hydrolysis of straw was conducted to investigate and quantify the relevant hydrolysis products obtained from the dioxolane labelled parent compound and its metabolites.

Aqueous soluble residue

The aqueous phase of straw contained 17.2% (14.22 mg/kg) of the TRR. Using TLC comparison, metabolites were assigned as follows: traces of spiroxamine (including metabolites 1-11, 0.4%TRR, 0.35 mg/kg), spiroxamine-hydroxy-N-oxide malonyl glucoside of the hydroxy-N-oxide (M21) (3.1%TRR, 2.57 mg/kg), metabolites 17+18 (unknown, 2.2%TRR, 1.86 mg/kg), metabolites 11a-16 (unknown, 7.0%TRR, 5.76 mg/kg), metabolites 21-23 (unknown, 4.5%TRR, 3.68 mg/kg). The aqueous phase was not further investigated, however, the metabolites were included in the characterisation by the complete hydrolysis of straw.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/02-3. The identified components of the terminal residue in straw, are summarised in Table CA 6.2.1/02-6.

The sum of identified and characterised metabolites was 63.7% (52.70 mg/kg) of the TRR in straw and 15.5% (12.80 mg/kg) remained in the solids.

Metabolites in grain

Organo-extractable residue

Organic phase 1 (dichloromethane) of grain was identified by comparison with the metabolite pattern from the dichloromethane phase of straw. The dichloromethane phase of grain contained only 7.0% (0 032 mg/kg) of the TRR, and unchanged parent compound accounted for 1 4% (0 006 mg/kg) of the TRR. The main metabolite was identified as spiroxamine-N-oxide (M03) and accounted for 0.7% (0.003 mg/kg) of the TRR

Organic phase 2 (ethyl acetate) Although a high proportion of solid residue was dissolved during the enzymatic treatment, only 4.7% (0.021 mg/kg) of the TRR was partitioned into ethyl acetate (organic phase 2). Following chromatography, unchanged parent compound accounted for 1.4% (0.006 mg/kg). of the TRR The main metabolite was identified as spiroxamine-N-oxide (M03) and accounted for 0.5% (0.002 mg/kg) of the TRR

Aqueous soluble residue

Aqueous phase 1 contained 16.9% (0.077 mg/kg) of the TRR. This phase could not be chromatographed by TLC due to the high matrix content.

The radioactivity remaining in aqueous phase 2 accounted for 62.6% (0.283 mg/kg) of the TRR and was characterised as being dissolved following enzymatic treatment but unextracted by ethyl acetate. Aqueous phase 2 could not be chromatographed by TLC due to the high matrix content, but probably contained some polar, water soluble aminodiols.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/02-4. The identified components of the terminal residue in straw, are summarised in Table CA 6.2.1/02-6.

The sum of characterised and identified metabolites was 89.6% (0.406 mg/kg) of the TRR and 8.7% (0.040 mg/kg) remained in the solids.

Table CA 6.2.1/02-3 **Summary of extractable radioactive residue fractions in wheat forage and straw following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine**

	Forage (0 DALA)		Forage (14 DALA)		Straw (56 DALA)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Organic (dichloromethane) phase[1]	**14.42**	**91.2**	**18.45**	**76.5**	**55.75**	**67.4**
Spiroxamine, parent[2]	10.10	63.9	9.93	41.2	17.01	20.6
Spiroxamine-N-oxide (M03)[3]	1.75	11.1	3.06	12.7	17.26	20.9
Spiroxamine-N-formyl-desethyl (M04)	0.73	4.6	1.40	5.8	8.06	9.7
Spiroxamine-hydroxyl (M05)[2] and Spiroxamine-desethyl (M01)[4]	0.76	4.8	1.71	7.1	4.33	5.2
Spiroxamine-despropyl (M02)[5]	0.52	3.3	1.11	4.6	3.48	4.2
Spiroxamine-hydroxy-despropyl(M09) plus metabolite 9	0.03	0.2	0.15	0.6	0.35	0.4
Metabolite group 1 (unknown)	0.35	2.2	0.55	2.3	2.43	2.9
Metabolite group 11[6]	0.08	0.5	0.15	0.6	1.15	1.4
Metabolite group 14[7]	0.02	0.1	0.07	0.3	0.51	0.6
Polar material (TLC origin)	0.08	0.5	0.31	1.3	1.18	1.4
Aqueous phase[1]	**0.72**	**4.6**	**2.92**	**12.1**	**14.22**	**17.2**
Metabolites 1-11 including traces of AS (aq phase)	0.13	0.8	0.19	0.8	0.35	0.4
Spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)[3]	0.03	0.2	0.22	0.9	2.57	3.1
Metabolites 11a - 16	0.39	2.5	1.62	6.7	5.76	7.0
Metabolites 17 + 18	0.06	0.4	0.34	1.4	1.86	2.2
Metabolites 21 - 23			0.55	2.3	3.68	4.5
Non-extractable, bound residue	0.66	4.2	2.74	11.4	12.8	15.5
Total residue	15.81	100.0	24.11	100.0	82.76	100.0

	Forage (0 DALA)		Forage (14 DALA)		Straw (56 DALA)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR

1 – designation of unknown metabolites follows designation in the study report
2 – hydrolysis results in aminodiol
3 – hydrolysis results in spiroxamine-aminodiol-N-oxide
4 – hydrolysis results in spiroxamine-spiroxamine-desethyl-aminodiol
5 – hydrolysis results in spiroxamine-spiroxamine-despropyl-aminodiol
6 – metabolite group 11 mainly based on tert.butylketone and hydroxy ketone separated into at least 6 components
7 – metabolite group 14 mainly based on tert.butylketone, spiroxamine hydroxy ketone and at least 2 unknown hydrolysis products

Table CA 6.2.1/02-4 Summary of extractable radioactive residue fractions in wheat grain following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

	Grain (56 DALA)	
	mg/kg	%TRR
Organic (dichloromethane) phase 1[1]	**0.032**	**7.0**
Spiroxamine, parent[2]	0.006	1.4
Non-polar unresolved radioactivity	0.004	0.9
Metabolites 4-9	0.005	1.1
Spiroxamine-N-oxide (M03)[3]	0.003	0.6
Metabolite group 11	0.001	0.3
Polar diffuse radioactivity	0.005	1.1
Origin material (TLC)	0.007	1.5
Aqueous phase1 1 (TLC not possible)	**0.077**	**16.9**
Organic (ethyl acetate) phase 1[1]	**0.021**	**4.7**
Spiroxamine, parent[2]	0.006	1.4
Non-polar unresolved radioactivity	0.009	1.9
Metabolites 4-9	0.001	0.2
Spiroxamine-N-oxide (M03)[3]	0.002	0.5
Metabolite group 11	0.001	0.3
Polar diffuse radioactivity	0.001	0.3
Origin material (TLC)	<0.001	0.1
Aqueous phase 2 (TLC not possible)	**0.283**	**62.6**
Non-extractable, bound residue	0.040	8.7
Total residue	0.453	100.0

1 – designation of unknown metabolites follows designation in the study report
2 – hydrolysis results in aminodiol
3 – hydrolysis results in spiroxamine-aminodiol-N-oxide

Table CA 6.2.1/02-5 Summary of identified radioactive residues in wheat forage following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

	Forage (Day 0)		Forage (Day 14)	
	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine, parent	63.9	10.10	41.2	9.93
Spiroxamine-N-oxide (M03)	11.1	1.75	12.7	3.06
Spiroxamine-N-formyl-desethyl (M04)	4.6	0.73	5.8	1.40
Spiroxamine-hydroxyl (M05) and Spiroxamine-desethyl (M01)	4.8	0.76	7.1	1.71
Spiroxamine-despropyl (M02)	3.3	0.52	4.6	1.11
Spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)[1]	0.2	0.03	0.9	0.22
Total identified	**87.9**	**13.89**	**72.3**	**17.43**

1 – glucoside conjugate (probably a spiroxamine-hydroxy-N-oxide malonyl glucoside) of the hydroxy-n-oxide

Table CA 6.2.1/02-6 Summary of identified radioactive residues in wheat straw and grain at maturity following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

	Straw (56 DALA)		Grain (56 DALA)	
Identified metabolites	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Spiroxamine, parent	20.6	17.01	2.8	0.013
Spiroxamine-N-oxide (M03)	20.9	17.26	1.2	0.005
Spiroxamine-N-formyl-desethyl (M04)	9.7	8.06	n.d.	n.d.
Spiroxamine-hydroxyl (M05) and Spiroxamine-desethyl (M01)	5.2	4.32	n.d.	n.d.
Spiroxamine-despropyl (M02)	4.2	3.48	n.d.	n.d.
Spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)[1]	3.1	2.57	n.d.	n.d.
Total identified	**63.7**	**52.70**	**4.0**	**0.018**

1 – glucoside conjugate (probably a spiroxamine-hydroxy-N-oxide malonyl glucoside) of the hydroxy-n-oxide
n.d. not detected

Hydrolysis of spiroxamine residues in straw for method development

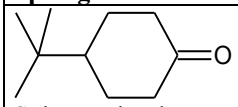
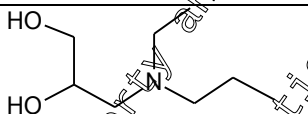
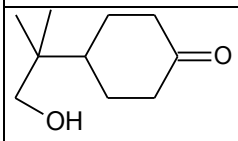
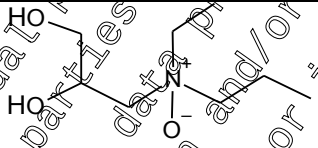
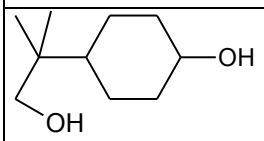
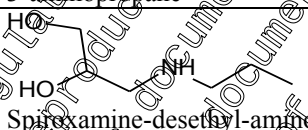
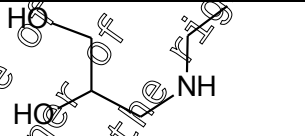
In the spring wheat study (CA 6.2.1/01) using [cyclohexyl-1-^{14}C]-spiroxamine, the majority of the TRR was attributed to parent spiroxamine, including identified metabolites bearing an intact ketal structure. A similar metabolite pattern was also expected from the winter wheat conducted with the dioxolane label (present study). Based on known metabolites, at least four major aminodiols derived from different N-substituents were expected following acidic hydrolysis.

Table CA 6.2.1/02-7 shows a quantitative comparison of the identified hydrolysis products obtained from straw using the cyclohexyl radiolabel (CA 6.2.1/01, Table CA 6.2.1/02-7) and the dioxolane radiolabel (this study).

As a result, the total of the identified hydrolysis products derived from the cyclohexyl label (CA 6.2.1/01) (84.4%) was in good agreement with the total of the corresponding hydrolysis products from the dioxolane label (75.7%). The main component derived from the dioxolane label was the aminodiol, which accounted for 36.5% of the TRR in straw. The other aminodiol moieties ranged from 10.7% to 16.8%.

The most abundant hydrolysis product was tert-butylcyclohexanone and as this represents the majority of the TRR in both winter and spring wheat (72.8% and 36.5% respectively, this was the preferred compound for the development of a total residue method in wheat.

Table CA 6.2.1/02-7 Hydrolysis products of cyclohexyl- and dioxolane-labelled spiroxamine residues in wheat straw (some information from this summary table replicated in CA 6.2.1/01)

[Cyclohexyl-1-^{14}C]-spiroxamine[1] Spring wheat	% of TRR	% of TRR	[1,3-Dioxolane-4-^{14}C]-spiroxamine Winter wheat[2]
 Spiroxamine-ketone (M15) (4-tert.-butylcyclohexanone)	(72.8)	(36.5)	 Spiroxamine-aminodiol (M28) N-ethyl-N-propyl-1,2-dihydroxy-3-aminopropane
 Spiroxamine-hydroxy-ketone (M16) 4-(1,1-dimethyl-2-hydroxyethyl)-cyclohexanone	(10.1)	(16.8)	 Spiroxamine-aminodiol-N-oxide (M29) N-ethyl-N-oxo-N-propyl-1,2-dihydroxy-3-aminopropane
 Spiroxamine-diol (M14) 4-(1,1-dimethyl-2-hydroxyethyl)-cyclohexanol	(1.5)	(11.7)	 Spiroxamine-desethyl-aminodiol (M30) N-propyl-N-1,2-dihydroxy-3-aminopropane
		(10.7)	 Spiroxamine-despropyl-aminodiol (M31) N-ethyl-N-1,2-dihydroxy-3-aminopropane
Identified hydrolysis products	(84.4)	(75.7)	
Unknown metabolites and unknown hydrolysis products	6.9	9.3	
TLC-origin	2.9	3.8	
Solids (non-extractable residue)	5.7	11.1	
Total residue	100.0	100.0	

1 - Calculated from identified metabolites and identified hydrolysis products as reported in CA 6.2.1/01
2 - After hydrolysis of homogenised straw using 1 N HCl under reflux for 1 h (this study)

Storage stability

Plant material from this winter wheat metabolism study was extracted either immediately after harvest or after short periods of storage at -20 °C or below. Intensive storage stability investigations were conducted in the first wheat metabolism study (CA 6.2.1/01) and no decomposition of parent compound or metabolites was observed during storage. Therefore, no extra extractions of additional samples for storage stability investigations were conducted in this second metabolism study.

III. Conclusions

[1,3-dioxolane-4-^{14}C]spiroxamine, formulated as a 500 EC formulation, was sprayed twice to winter wheat plants at BBCH 30 (stem elongation) and BBCH 51 (ear emergence) at a total rate of 1650 g a.s./ha (2.2N rate). Forage was sampled immediately after the second application (day 0) and at day 14, with straw and grain sampled at harvest (day 56).

The total radioactive residue (TRR) in forage accounted for 15.81 mg/kg and 24.11 mg/kg parent equivalents at day 0 and day 14, respectively. At harvest (day 56), the TRR in straw accounted for 82.76 mg/kg parent equivalents and the TRR in grain accounted for 0.453 mg/kg parent equivalents.

Parent spiroxamine comprised the major component of the residue in day 0 forage accounting for 63.9% TRR (10.10 mg/kg). Other metabolites detected were spiroxamine-N-oxide (M03) (11.1%TRR, 1.75 mg/kg), spiroxamine-N-formyl-desethyl (M04) (4.6%TRR, 0.73 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (4.8%TRR, 0.76 mg/kg), spiroxamine-despropyl (M02) (3.3%TRR, 0.52 mg/kg), spiroxamine-hydroxy-despropyl(M09) (0.2%TRR, 0.03 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (0.2%TRR, 0.03 mg/kg).

Parent spiroxamine comprised the major component of the residue in day 14 forage accounting for 41.2%TRR (9.93 mg/kg). Other metabolites detected were spiroxamine-N-oxide (M03) (12.7%TRR, 3.06 mg/kg), spiroxamine-N-formyl-desethyl (M04) (5.8%TRR, 1.40 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (7.1%TRR, 1.71 mg/kg), spiroxamine-despropyl (M02) (4.6%TRR, 1.11 mg/kg), spiroxamine-hydroxy-despropyl(M09) (0.6%TRR, 0.15 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (0.9%TRR,TRR 0.22 mg/kg).

Parent spiroxamine comprised the major component of the residue in day 56 straw accounting for 20.6%TRR (17.01 mg/kg). Other metabolites detected were spiroxamine-N-oxide (M03) (20.9%TRR, 17.26 mg/kg), spiroxamine-N-formyl-desethyl (M04) (9.7%TRR, 8.06 mg/kg), spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) (5.2%TRR, 4.32 mg/kg), spiroxamine-despropyl (M02) (4.2%TRR, 3.48 mg/kg), spiroxamine-hydroxy-despropyl(M09) (0.4%TRR, 0.35 mg/kg) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) (3.1%TRR, 2.57 mg/kg).

Only a low proportion of the TRR in 56 day grain could be identified. The identified metabolites were parent spiroxamine (2.8%TRR, 0.013 mg/kg) and spiroxamine-N-oxide (M03) (1.2%TRR, 0.005 mg/kg). The most prominent portion of radioactivity was released after intensive enzymatic hydrolysis using diastase. The residues were very polar and partitioned into the aqueous phase (62.6% of TRR) when extraction was attempted with ethyl acetate. The aqueous phase could not be chromatographed by TLC due to the high matrix content.

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the spiroxamine-N-oxide, M03) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl, M05). The metabolites bearing an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide malonyl glucoside (M21)). Some metabolites were formed by desalkylation (spiroxamine-desethyl, M01; spiroxamine-despropyl, M02) which were partly metabolised by formylation (spiroxamine-N-formyl-desethyl, M04). Only low amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

The proposed metabolic pathway for spiroxamine in cereals is shown in Figure CA 6.2-2.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.2.1/04. The study is considered compliant with OECD Guideline 501 – Metabolism in Crops, January 2007.

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the spiroxamine-N-oxide (MO3)) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl). The metabolites bearing an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide malonyl glucoside). Some metabolites were formed by desalkylation (spiroxamine-desethyl, spiroxamine-despropyl) which were partly metabolised by formylation (spiroxamine-N-formyl-desethyl). Only low amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

Data Point:	KCA 6.2.1/03
Report Author:	[redacted]
Report Year:	1996
Report Title:	Metabolism of [Cyclohexyl-1-14C] KWG 4168 in grapes
Report No:	PF4175
Document No:	M-006107-01-1
Guideline(s) followed in study:	US EPA Pesticides Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4, Nature of residues in plants and livestock
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

[Cyclohexyl-1-^{14}C]spiroxamine formulated as a 500 EC formulation, was sprayed twice to grapes 56 days before harvest (when flowering was finished) and 35 days before harvest at a total rate of 1600 g a.s./ha (2.7N maximum total seasonal application rate). Grapes were sampled immediately after the second application (day 0) and at day 35.

The total radioactive residue (TRR) in grapes was 3.41 mg/kg parent compound equivalents at harvest (day 35).

Parent spiroxamine comprised the major component of the residue in day 35 grapes accounting for 24.6% (0.84 mg/kg) followed by spiroxamine docosanoic acid ester (M35), accounting for 13.0% (0.44 mg/kg). Other minor metabolites detected were spiroxamine tetracosanoic acid ester (M36) (4.2%TRR; 0.14 mg/kg), spiroxamine-desethyl (M01) (1.1%TRR, 0.04mg/kg), spiroxamine-despropyl (M02) (0.5%TRR, 0.02 mg/kg) and spiroxamine-N-oxide (M03) (2.9%TRR, 0.10 mg/kg). The identified hydrolysis products (43.3% of TRR) exhibit a significant portion of the total radioactivity in grapes that can be attributed to glucoside conjugates.

It was also shown that spiroxamine was not translocated from leaves to berries of the grape vine plants.

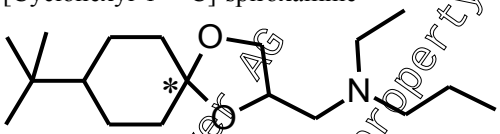
In grapes, the metabolism of spiroxamine proceeded via cleavage of the ketal structure. The cleavage product containing the cyclohexyl label (spiroxamine-ketone) was very transient as it could not be

detected in the free form. It was reduced to the corresponding alcohol (spiroxamine-cyclohexanol) that was also transient. This alcohol was conjugated with different plant endocons yielding either long chain fatty acid esters or different glucose derivatives.

I. Materials and methods

A. Test materials

[Cyclohexyl-1 -^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (MBq/mg)	6.81 (184 µCi/mg)
Lot/Batch No.:	Not stated
Purity:	Radiochemical purity ~98%
Storage condition:	Not stated
CAS No.:	118134-30-8

B. Study design

Test system

Soil

Table CA 6.2.1/03-1 Soil classification and physico-chemical properties

Soil Type (DIN 19682)	pH (KCl)	OM %	Sand %	Silt %	Clay %	Moisture holding capacity mL / 100g)	CEC meq / 100 g
Loamy silt	6.63	1.14	17.1	71.8	11.1	Not reported	15

Test system

Four plant containers (20 litres) with grape vine plants, variety Huxel, were placed in a vegetation hall. Containers contained Frimmersdorf (sandy loam soil). The average temperature during the cultivation period ranged from 6.3°C to 18.8°C. The sunshine hours ranged from 104 to 252 hours/month. Treatment with other plant protection products was conducted as required, according to Good Agricultural Practice.

Test design

The study started in July 1992 and from the QA statement, the final date for inspection for experimental work was April 1994. Definitive study dates were not stated in the report, however, storage stability analysis was conducted for up to 1442 days, so it is assumed that this covers the experimental phase from sampling to analysis. The in-life phase was conducted in the vegetation area (Building 6682) of the Institute for Metabolism Research and Residue Analysis Monheim, Bayer AG. The analytical phase and final reporting was conducted at the Institute for Metabolism Research and Residue Analysis of Bayer AG, Monheim, Germany.

Experimental conditions

[Cyclohexyl-1-^{14}C]-labelled spiroxamine, formulated as a 500 EC formulation, was applied twice as a spray application using a microsprayer.

The first treatment was made 56 days before harvest (when flowering was finished) using 25% (400 g a.s./ha) of the total amount. The second application was made 35 days before harvest using

75% (1200 g a.s./ha) of the total, in order to reflect the different size of the grapes. A total of 25 bunches of grapes distributed over 4 grape vine plants were sprayed from two opposite sides.

A use on grapes is included in the representative GAP for spiroxamine. The rate employed in this study (total seasonal rate of 1600 g a.s./ha) is equivalent to 2.7 times the maximum seasonal use proposed for spiroxamine (600 g a.s./ha). This study is considered representative and appropriate for the objectives of establishing the nature and magnitude of spiroxamine in fruit crops.

Preparation of application solutions/applications

First application: A stock solution was prepared dissolving the formulation ECW 9445A in 10.0 mL of water. Aliquots of 5 µL were measured by LS-counting (average 19951 Bq/5 µL, total amount 39.9 MBq/10 mL). The stock solution was applied in aliquots of 190 µL on each of the two sides, therefore a total of 2 x 190 µL = 1.52 MBq was applied per bunch of grapes, which was equivalent to 0 22 mg active ingredient (specific radioactivity 6.81 MBq/mg). For the 25 bunches of grapes on 4 vines a total of 5.6 mg a.s.(37 9 MBq) was applied.

Second application: A stock solution was prepared dissolving the formulation ECW 9445B in 13.5 mL of water. Aliquots of 3 µL were measured by LS-counting (average 45294 Bq/3 µL, total amount 203.8 MBq/13.5 mL). The stock solution was applied in aliquots of 250 µL on each of the two sides, therefore a total of 2 x 250 µL = 7.55 MBq was applied per bunch of grapes, which was equivalent to 1.11 mg active ingredient (specific radioactivity 6.81 MBq/mg). For the 25 bunches of grapes on 4 vines a total of 27.7 mg a.s.(188.7 MBq) was applied.

Translocation experiment

First application: On a separate vine, two bunches of grapes were protected by a plastic cover to prevent contamination during application. Two leaves, one ca. 15 cm above and one ca. 15 cm below each bunch of grapes, were labelled and 95 µL of the stock solution was applied by a syringe fitted with a brush tip to each leaf. For the 4 leaves a total of 0.22 mg a.s.(1.52 MBq) was applied.

Second application: The four leaves were treated analogously using 125 µL of the stock solution for each leaf amounting to a total of 1.11 mg a.s. (7.55 MBq).

Sampling

The first sample (day 0) was taken approximately 4 hours after the second application (July, 29, 1992). Two bunches of grapes of medium size were cut off and separated into grapes (253.3 g) and stalks (4.4 g).

The second sample (day 35) was taken at harvest (September 2,1992). Fifteen bunches of grapes were combined and separated into grapes (3102.8 g) and stalks (35.6 g). The grapes were deep frozen in aliquots. Eight bunches of grapes were separated into grapes (1795.6 g) and stalks (59.7 g) and kept in reserve.

The two bunches of grapes from the translocation experiment were also harvested at day 35 (September 2, 1992). They were separated into grapes (259.0 g) and stalks (11.0 g). The four treated leaves were homogenised in liquid nitrogen and stored.

All samples, which were not immediately extracted after sampling, were stored at -20°C or below.

Sample preparation and extraction

The grapes (253.3 g) from day 0 were successively macerated with methanol (2 x ca. 400 mL) and methanol/water 1:1 (v/v, ca. 400 mL). The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and the solids (non-extractable residue). Aliquots from the extract and solids were taken for radioactivity measurement.

An aliquot (500 g) of the grapes from day 35 was successively macerated with methanol (ca. 800 mL/400 mL) and methanol/water 1:1 (v/v, ca. 400 mL). The suspension was filtered by suction yielding

the methanol/water extract (combined filtrates) and the solids 1, which were further extracted. The methanol/water extract was evaporated to the aqueous remainder at ca. 40°C using a rotary evaporator. The aqueous remainder was extracted with dichloromethane (3 x ca. 500 mL) resulting in the organic phase (50 mL after concentration) and aqueous phase (229 mL). Aliquots from extracts, phases and solids were taken for radioactivity measurement.

Exhaustive extraction

Grapes (day 35): To an aliquot of solids 1, 1N HCl and toluene were added and heated under reflux for 1 hour. After cooling, the suspension was filtered by suction and the filter cake washed with a small amount of water and toluene. The combined filtrates were neutralised with $NaHCO_3$, the phases were separated, the aqueous phase was re-extracted with toluene (2 x 20 mL) and the toluene phases were combined. Aliquots from the phases and solids were taken for radioactivity measurement.

Translocation experiment

The four treated leaves of the translocation experiment (cut after sampling), were homogenised using liquid nitrogen and aliquots were used for the determination of the radioactivity by combustion. This was also done for grapes and stalks.

Repeated TLC separation of metabolites from organic phase 1

An aliquot of the organic phase 1 was applied to three silica gel coated aluminium plates and chromatographed using solvent system III. The complete radioactive zones being more polar than the active ingredient were cut out from each plate (divided into 3 zones) and eluted using methanol. The respective eluates 1-3 were combined, concentrated and re-chromatographed on a TLC plate under the same conditions.

Isolation of metabolites from the organic phase 1 (dichloromethane phase)

Metabolite 1 (spiroxamine tetracosanoic acid ester and spiroxamine docosanoic acid ester): An aliquot of the organic phase 1 was applied to ten Sep-Pak cartridges (Waters, no. 51900, silica gel). Each cartridge was eluted with dichloromethane (5 mL), the combined eluates were concentrated and applied to two silica gel coated aluminium plates and chromatographed using chloroform. The cartridge was also eluted with solvents of increasing polarity, but these eluates were not further used. The radioactive zones containing metabolite 1 were cut out and eluted using methanol. The eluate was concentrated and re-chromatographed under the same conditions. The radioactive zone was then analogously chromatographed by TLC using solvent system I, cut out and eluted using dichloromethane. The dichloromethane was evaporated under a stream of nitrogen and the residue investigated by spectroscopy.

Metabolite group 3 (based on cyclohexyl moieties): An aliquot of the organic phase 1 was applied to two silica gel coated aluminium plates and chromatographed using solvent system III. The radioactive zone of each plate containing metabolite 3 was cut out and eluted using methanol. The combined eluates were concentrated and re-chromatographed on a TLC plate under the same conditions. An aliquot of the isolated metabolite 3 was hydrolysed for characterisation.

Purification of aqueous phase 1

A glass column (ca. 10 mm diameter) was filled with XAD-4 resin (10 g, Sen/a no. 42838) and wetted with water. The resin was washed with methanol and water. An aliquot of aqueous phase 1 was applied and the column eluted with water followed by methanol. The radioactivity of the eluates was measured. The aqueous eluate was discarded as it contained only a small amount of radioactivity. The radioactivity of the methanol eluate was measured and used for TLC analysis.

The XAD-4 purification procedure was repeated analogously using 50 mL of aqueous phase 1 for the isolation of compounds by HPLC.

Alkaline hydrolysis of metabolite group 1 (spiroxamine tetracosanoic acid ester and spiroxamine docosanoic acid ester) from the dichloromethane phase

An aliquot of the metabolite group 1 isolated from organic phase 1 was concentrated under a stream of nitrogen, 1N NaOH and 1,4-dioxane were added and the solution stirred for 1 hour under reflux. After cooling, the solution was neutralised using 1N HCl, diluted with water and partitioned with dichloromethane. The radioactivity of both solutions was determined. The combined dichloromethane phases were concentrated, transferred to a silica-gel coated aluminium TLC plate and chromatographed using solvent system II. The radioactive zone of the TLC plate was cut out and eluted using methanol. An aliquot of the eluate was concentrated under a stream of nitrogen and investigated by mass spectroscopy.

Acidic hydrolysis of metabolite group 3 (based on cyclohexyl moieties)

An aliquot of the purified metabolite group 3 was concentrated under a stream of nitrogen, 1,4-dioxane and 1N HCl was added and the solution stirred in a reacti-vial for 1 hour at 100°C. After cooling, the solution was neutralised using 1N NaOH and investigated by TLC (Solvent systems II and III).

Total hydrolysis of aqueous phase 1

Experiment 1 (analytical): To an aliquot of aqueous phase 1, 1N HCl and toluene were added and heated for 1 hour at 100°C. The reflux condenser was connected with a glass bottle filled with methanol to trap possible volatile hydrolysis products. After cooling, the mixture was neutralised with $NaHCO_3$ and the phases were separated. The aqueous phase was partitioned with toluene (3x10 mL) and the toluene phases were combined. The reflux condenser was rinsed with methanol separately. The radioactivity was determined in the toluene phase, aqueous phase, in the methanol trap and methanol rinse.

Experiment 2 (isolation and identification of hydrolysis products): To an aliquot of the aqueous phase, 1N HCl and toluene were added and heated under reflux for 1 hour. After cooling, the mixture was neutralised with 1N NaOH and the phases were separated. The aqueous phase was partitioned with toluene (2 x 50 mL) and the toluene phases were combined. The radioactivity was determined in the toluene phase and aqueous phase. The toluene phase was concentrated and the radioactivity determined. Aliquots were applied to 8 silica gel coated aluminium TLC plates and chromatographed using solvent system II. The two main radioactive zones of each TLC plate were cut out, eluted with methanol and the respective eluates combined. The combined eluates were each re-chromatographed on one TLC plate (solvent system II). The upper zone was purified by HPLC and the isolated compound investigated by mass spectroscopy. The double zone was separated by TLC chromatography as described (solvent system II). The upper zone was investigated by mass spectroscopy. The lower zone was investigated and identified by TLC.

Total hydrolysis of grapes

Grapes (300 g) were macerated, 1N HCl and toluene were added and the mixture heated under reflux for 1 hour. After cooling, the mixture was neutralised with 1N NaOH, filtered by suction and the solids were washed with water. The phases were separated and the aqueous phase partitioned with toluene (2 x 200 mL). Aliquots from the filtrate and solids were taken for radioactivity measurement. The toluene phase was concentrated and the radioactivity measured.

Radiochemical analysis

Radioactivity determination

The ^{14}C-radioactivity in liquid samples was determined in a liquid-scintillation counter LKB 1219 Rackbeta (LKB-Wallac), LS 6000LL (Beckman Instruments) or LS 6500 (Beckman Instruments) with Instant-Scint-Gel (Packard), or Quicksafe A (Zinsser Analytic).

Solid samples were combusted in an OX 500 (Zinsser Analytic). The CO_2 produced by combustion was absorbed in a scintillation-cocktail [Oxysolve C-400 (Zinsser Analytic)] and the radioactivity measured

by LSC (PW 4700, Philips/Raytest) or LKB 1219 Rackbeta (LKB-Wallac), Beckman LS 6000LL (Beckman Instruments) or Beckman LS 6500 (Beckman Instruments).

Thin layer chromatography (TLC)

The radioactive solutions were investigated by TLC using silica gel plates (silica gel 60 $_{F254}$, 0.25 mm thickness, glass-coated, 20 cm x 20 cm, Merck, Darmstadt, Germany or silica gel 60 $_{F254}$, 0.2 mm thickness, aluminium-coated, 20 cm x 20 cm, Merck, Darmstadt, Germany) and the following solvent systems (SS):

I: dichloromethane:n-hexane 1:1 (v/v)
II: dichloromethane:ethyl acetate 9:2 (v/v)
III: ethyl acetate:2-propanol:water 5:3:1 (v/v/v)
IV: acetonitrile:water:25% ammonia 80:18:2 (v/v/v)
V: chloroform:methanol: 25% ammonia 65:28:8 (v/v/v).

The radioactive compounds were detected and evaluated quantitatively using a Linear Analyser (Rita 3200 or IM 3016, Raytest) or a Bio-Imaging Analyser (BAS 2000, Fuji). The reference compounds used in co-chromatography were visualised in an iodine chamber.

High performance liquid chromatography (HPLC)

For chromatographic comparison or for purification of metabolites by HPLC a HP 1050 Liquid Chromatograph (Hewlett Packard) with DAD-Detector (wave length 254 nm) and a radioactivity flow through monitor Ramona-5 (Raytest) with a solid scintillator glass cell were used. The separation was achieved with a RP-8 column (250 x 4 mm, 7 µm particle size) and a gradient elution mixture of water and methanol.

Mass spectroscopy (MS)

GC/MS

GC-MS analyses for metabolites were performed on two instruments. The first was a mass selective detector HP 5970 with a gas chromatograph 5880A (Hewlett-Packard). The capillary column used was a 15 m SE54 (CS-Chromatographie Service). The oven temperature program was 60 °C for 1 minute, heating rate was 10 °C/min up to 280 °C. The final temperature was kept constant for 20 minutes. The samples were injected in the splitless mode at 280 °C. The carrier gas was helium (4 psi). The second was the INCOS XL instrument by Finnigan with a Varian gas chromatograph. The capillary column used was a 30 m DB5MS (J&W Scientific). The oven temperature program was 60 °C for 1 minute, heating rate was 10 °C/min up to 300 °C. The final temperature was kept constant for 30 minutes. The samples were injected in the splitless mode at 250 °C. The pressure of the carrier gas helium was 10 psi.

GC/MS analyses for parent spiroxamine were performed on a mass selective detector HP 5970 with a gas chromatograph 5880A (Hewlett-Packard). The capillary column used was a 15 m DB1 (J&W Scientific). The oven temperature program was 80 °C for 1 minute, heating rate was10 °C/min up to 280 °C. The final temperature was kept constant for 20 minutes. The samples were injected in the splitless mode at 280 °C. The carrier gas was helium (12 psi).

Electro-spray ionisation MS spectra (ESI)

The electro-spray ionisation MS spectra (ESI) were obtained with a TSQ 7000 instrument by Finnigan. Sheath gas pressure 52 psi, capillary temperature: 210 °C. The flow rate was 500 µL/min.

Electron impact (EI)

Electron impact as well as chemical ionisation mass spectra obtained by direct evaporation were recorded on a FINNIGAN 8230 instrument. The ion source temperature was 200 °C, the electron energy

70 eV, the emission current was 1 mA (El) or 0.2 mA (Cl). Ammonia was used as reagent gas for chemical ionisation.

Direct chemical ionisation spectra (DCI)

Direct Chemical Ionisation spectra (DCI) using ammonia as reactant gas were recorded on a FINNIGAN 8230 instrument. The DCI-sample emitter was heated from 0 - 1 A at a rate of 4 mA/sec. The emission current was 200 mA (cathode). The source temperature was 210°C

^{1}H-NMR-spectroscopy

The ^{1}H-NMR-spectra were recorded on a Bruker AC 300 spectrometer, the samples were dissolved in CD_3OD (Merck) for analysis.

II. Results and discussion

Total radioactive residue (TRR)

Two bunches of grapes (253.3 g) were sampled immediately after the second application (day 0) and used for the determination of the total residue. The radioactivity was readily extracted using methanol/water. The TRR was 6.32 mg/kg parent compound equivalents as determined by summation of the radioactivity in the combined methanol/water extracts and in the solids.

The TRR at harvest (day 35) was determined from the extraction of 500 g grapes and was 3.41 mg/kg parent compound equivalents as determined by summation of the radioactivity in the combined methanol/water extracts and in the solids 1. The extracted radioactivity was distributed into organic phase 1 (44.6% of the TRR, 1.52 mg/kg) and aqueous phase 1 (46.9%, 1.60 mg/kg). The solids 1 amounted to 8.5% (0 29 mg/kg) and were further extracted using 1N HCl/toluene under reflux. As a result, 5.6% (0.19 mg/kg) was distributed into organic phase 2, a further 0.3% (0.01 mg/kg) into aqueous phase 2.The non-extractable residue 2.6% (0.09 mg/kg) remained in the solids (post extraction solids, PES).

For control purposes, the radioactivity determined before and following evaporation and partitioning was compared. The radioactivity in the combined methanol/water extracts was recovered quantitatively (>99%) in organic phase 1 and aqueous phase 1. The radioactivity determined in the dichloromethane phases and aqueous phases was normalised based on the combined methanol/water extracts and on the combustion values.

TRR and distribution of radioactivity are given in Table CA 6.2.1/03-2.

Table CA 6.2.1/03-2 Distribution of radioactive residues in grapes following two spray applications with [cyclohexyl-1-^{14}C]-spiroxamine

Radioactive residues in grapes	Grapes (0 DALA)		Grapes (35 DALA)	
	mg/kg	%TRR	mg/kg	%TRR
TRR[1]	6.32	100.0	3.41	100.0
Organic phase 1 (dichloromethane)[2]	-	-	1.52	44.6
Aqueous phase 1[2]	-	-	1.60	46.9
Organic phase 2 (toluene)[2]			0.19	5.6
Aqueous phase 2[2]			0.01	0.3
Post extraction solids (PES)	-	-	0.09	2.6

1 - total radioactive residues given in mg parent substance equivalents per kg, determined by summation of all fractions after extraction
2 - primary methanol/water extract was concentrated and partitioned between dichloromethane and water

Radioactive residues in grapes	Grapes (0 DALA)		Grapes (35 DALA)	
	mg/kg	%TRR	mg/kg	%TRR

3 - following primary extraction with methanol/water the solid (8.5% of TRR) was hydrolysed with 1 N HCl and partitioned between water and toluene

Characterisation

Available synthesised compounds for characterisation were parent test substance spiroxamine (KWG 4168) and the following potential metabolites:

KWG 4557 (spiroxamine-desethyl) (M01)

KWG 4669 (spiroxamine-despropyl) (M02)

WAK6301/1 (spiroxamine-N-oxide) (M03)

WAK 6782 (spiroxamine-N-formyl-desethyl) (M04)

BNF 5567B (acetyl-spiroxamine-desethyl)

BNF 5567A (acetyl-spiroxamine-despropyl)

WAK 60712 (spiroxamine-amine)

WAK 5868 (spiroxamine-hydroxyl) (M05)

WAK 6084/1(spiroxamine-hydroxy-desethyl) (M41)

WAK 6079/1 (spiroxamine-hydroxy-despropyl) (M09)

WAK 5708 (spiroxamine-acid) (M06)

WAK 5756B (spiroxamine-desethyl acid) (M11)

BNF 5534A (spiroxamine-despropyl acid) (M12)

WAK 5428 (tert.-butylcyclohexanone) (M15)

BNF 5544A (spiroxamine-hydroxy ketone) (M16)

BNF 5550A (spiroxamine-cyclohexanol) (M13)

WAK6482-4 (spiroxamine-diol) (M14)

WAK 6131-2-7 (spiroxamine-ketone acid) (M17)

WAK 5427 (spiroxamine-aminodiol) (M28)

WAK 6885 (spiroxamine-aminodiol-N-oxide) (M29)

WAK 6894 (spiroxamine-desethyl-aminodiol) (M30)

WAK 6893 (spiroxamine-despropyl-aminodiol) (M31)

PA 1344 (spiroxamine-tetracosanoic acid ester) (M36)

PA 1345 (spiroxamine-docosanoic acid ester) (M35)

Each metabolite was identified by co-chromatography with reference standards.

Residues in [^{14}C]spiroxamine treated grape

Metabolites in grapes (day 35)

Organo-extractable residue (organic phase 1)

The organic phase 1 (dichloromethane) of grapes contained 44 6% (1.52 mg/kg) of the TRR and 24 6% (0.84 mg/kg) and was unchanged parent compound. The main metabolite of organic phase 1 (metabolite 1) was extremely non-polar and largely resistant to acidic hydrolysis. GC/MS-investigation revealed that metabolite 1 consisted of two similar components spiroxamine tetracosanoic acid ester (M36) and spiroxamine docosanoic acid ester (M35) which accounted for 4.2% (0.14 mg/kg) and 8.7% (0.30 mg/kg) of the TRR, respectively. The remaining metabolites of organic phase 1 each accounted for <2.9% of the TRR (<0.10 mg/kg), including three identified metabolites with an intact ketal structure (spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-N-oxide (M03). The metabolite group 3 was characterised following acidic hydrolysis and it was concluded it was based on relatively polar cyclohexyl-moieties.

Aqueous soluble residue (aqueous phase 1)

The aqueous phase 1 of grapes accounted for 46.9% (1.60 mg/kg) of the TRR and was quantified by TLC following XAD-4 purification using solvent system V. However, defined metabolites were poorly resolved. This was similar when other TLC systems or HPLC were used, probably due to the chemical similarity of the sugar conjugates. Therefore, the components of aqueous phase 1 were listed as metabolite groups. Separate hydrolysis experiments showed that metabolite groups 11 and 13 were mainly based on *cis*-tert.-butylcyclohexanol, with the main component of group 11 designated as spiroxamine – cyclohexanol glucopyrosyl-pentose (M33), the main component of group 13 as spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) and the main component of metabolite group 12 based on the diol as spiroxamine-diol-diglycoside (M24).

After the main metabolites of aqueous phase 1 were identified as glucoside conjugates, a total acidic hydrolysis (of this phase) was conducted for further characterisation. The common cyclohexyl moieties were investigated and compared. The main hydrolysis product was *cis*-tert.-butylcyclohexanol (M13) (22.0%TRR, 0.75 mg/kg) and a significantly smaller amount was detected as *trans*-tert.-butylcyclohexanol (M13) (3.3%TRR, 0.11 mg/kg). This indicated that the *cis* isomer was conjugated with sugars to build up water soluble glycosides, whereas the *trans* isomer was preferably conjugated with the two non-polar fatty acids. The diol (M14) was also a major hydrolysis product of aqueous phase 1 and was detected in the toluene phase and aqueous phase of the hydrolysis experiment (13.0%TRR, 0.44 mg/kg). Tert.-butylketone was not detected after hydrolysis of aqueous phase 1 which meant that neither parent compound, nor the metabolites spiroxamine-desethyl, spiroxamine-despropyl, spiroxamine-N-oxide or any glycerol-derived conjugates of these compounds were in this phase, as they would have all been detected under the hydrolytic conditions as tert.-butylketone. Investigation of the hydrolysis products of aqueous phase 1 using solvent system V revealed further unknown cyclohexyl compounds being more polar than the diol (<1.5% TRR, <0.05 mg/kg).

Organo-extractable residue (organic phase 2)

The exhaustive extraction of solids 1 under acidic conditions yielded organic phase 2 (toluene) amounting to 5.6% (0.19 mg/kg) of the TRR and revealed only two compounds and traces of TLC-origin. The main component was identical with metabolite 1 (unhydrolysed) from organic phase 1. GC/MS analysis showed that spiroxamine docosanoic acid ester (M35) was present without the homologous metabolite (spiroxamine tetracosanoic acid ester (M36)) and accounted for 4 3% (0.15 mg/kg) of the TRR. The second component was identified as tertbutylketone (1.3%, 0.04 mg/kg), certainly a hydrolysis product.

Aqueous soluble residue (aqueous phase 2)

Aqueous phase 2 amounted to only 0.3% (0.01 mg/kg) of the TRR and was detected as polar radioactivity at the TLC-origin. The amount was low because the major portion of the radioactivity from the exhaustive extraction distributed into the toluene phase.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/03-3. The identified hydrolysis products of the aqueous 1 phase are

summarised in Table CA 6.2.1/03-4 and the identified components of the terminal residue in Day 35 grapes, are summarised in Table CA 6.2.1/03-5.

The sum of identified or characterised metabolites on the basis of identified metabolites or common moieties was 89 6% (3 06 mg/kg) of the TRR and 2 6% (0 09 mg/kg) remained in the solids.

Table CA 6.2.1/03-3 Summary of extractable radioactive residue fractions in grapes following two spray applications with [cyclohexyl-1 -^{14}C]-spiroxamine

	Grapes (35 DALA)	
	mg/kg	%TRR
Organic phase 1 (dichloromethane)[1]	**1.52**	**44.6**
Spiroxamine, parent	0.84	24.6
Spiroxamine tetracosanoic acid ester (M36)	0.14	4.2
Spiroxamine docosanoic acid ester (M35)	0.30	8.7
Metabolite group 2 (unknown)	0.04	1.1
Metabolite group 3 (based on cyclohexyl-moieties)	0.05	1.5
Spiroxamine-desethyl (M01)	0.04	1.1
Spiroxamine-despropyl (M02)	0.02	0.5
Spiroxamine-N-oxide (M03)	0.10	2.9
Aqueous phase 1[1]	**1.60**	**46.9**
Metabolite group 7	0.04	1.1
Metabolite group 8	0.04	1.1
Metabolite group 9	0.06	1.9
Metabolite group 10	0.08	2.4
Metabolite group 11, main component cyclohexanol-glucopyranosyl-pentose (M33)	0.65	19.1
Metabolite group 12, main component diol-diglycoside (M24)	0.50	14.8
Metabolite group 13, main component cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34)	0.13	3.8
Metabolite group 14	0.06	1.8
Polar material (TLC origin)	0.03	1.0
Organic phase 2 (toluene)	**0.19**	**5.6**
Spiroxamine docosanoic acid ester (M35)	0.15	4.3
4-tert.-butylcyclohexanone	0.04	1.3
Polar material (TLC origin)	<0.01	0.1
Aqueous phase 2 (TLC origin)	**0.01**	**0.3**
Non-extractable, bound residue	0.09	2.6
Total residue	3.41	100.0

1 – designation of unknown metabolites follows designation in the study report

Table CA 6.2.1/03-4 Hydrolysis products for the characterisation of aqueous phase 1 of grapes following two spray applications with [cyclohexyl-1 -^{14}C]-spiroxamine

	Grapes (Day 35)	
	% TRR	mg/kg
Aqueous phase 1	**46.9**	**1.60**
Identified hydrolysis products	**42.0**	**1.43**
Spiroxamine-cyclohexanol, isomer 1 (cis-4-tert.-butylcyclohexanol) (M13)	22.0	0.75
Spiroxamine-cyclohexanol, isomer 2 (trans-4-tert -butylcyclohexanol) (M13)	3.3	0.11
Spiroxamine-diol, (4-(hydroxytert.-butyl)cyclohexanol)[1] (M14)	13.0	0.44
Spiroxamine-hydroxy-ketone, (4-(hydroxytert.-butyl)cyclohexanone) (M16)	0.5	0.02
tert.-butylketone, (4-tert.-butylcyclohexanone)	ND	ND
Spiroxamine - cyclohexenol, (olefin derivative of tert.-butylcyclohexanol) (M37)	3.2	0.11
6 unknown hydrolysis products including TLC origin, each <1.5% & <0.05 mg/kg	4.9	0.17

ND- not detected
1 - resulting from the toluene phase and aqueous phase (11.7% + 1.3% = 13.0%)

Table CA 6.2.1/03-5 Summary of identified radioactive residues in grapes following two spray applications with [cyclohexyl-1 -^{14}C]-spiroxamine

	Grapes (Day 35)	
Identified metabolites	**% TRR**	**mg/kg**
Organic phases		
Spiroxamine, parent	24.6	0.84
Spiroxamine tetracosanoic acid ester (M36)	4.2	0.14
Spiroxamine docosanoic acid ester (M35)	13.0	0.44
Spiroxamine-desethyl (M01)	1.1	0.04
Spiroxamine-despropyl (M02)	0.5	0.02
Spiroxamine-N-oxide (M03)	2.9	0.10
Total identified (organic phases)	**46.3**	**1.58**
Aqueous phases		
Metabolite group 11[2], main component spiroxamine – cyclohexanol glucopyrosyl-pentose (M33)	19.1	0.65
Metabolite group 12[2], main component spiroxamine-diol-diglycoside (M24)	14.8	0.50
Metabolite group 13[2], main component spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34)	3.8	0.13
Total identified (aqueous phases)	**37.7**	**1.28**
Identified post-hydrolysis products (aqueous phase)[1]		
Cyclohexanol (M13) – (main pre-cursors M33, M34)	25.3	0.86
Diol (M14) - (main pre-cursor M24)	13.0	0.44
tert.-butylketone (M15)	1.3	0.04
Spiroxamine-hydroxy ketone (M16)	0.5	0.02
Spiroxamine - cyclohexenol (M37)	3.2	0.11
Total identified/characterised[3]	**89.6**	**3.05**

	Grapes (Day 35)	
Identified metabolites	**% TRR**	**mg/kg**

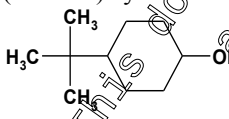
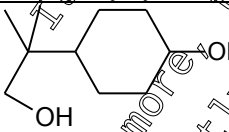
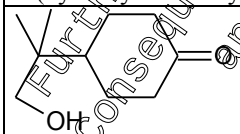
1 – Characterised residue includes identified residue from hydrolysis of aqueous phase. Not included as primary plant metabolites
2 – Identified components in the aqueous phase were multi-component in nature, so the %TRR represents the maximum residue of the main identified metabolite in each group.
3- Total identified/characterised is calculated from the sum of the identified components in the organic phases plus the identified components after hydrolysis of the aqueous phase/

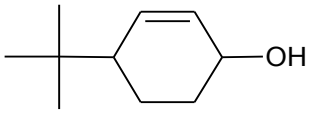
Total hydrolysis of spiroxamine residues in grapes

Grapes were exhaustively hydrolysed to assess the individual magnitude of cyclohexyl compounds for the possible development of a residue method. The grapes (30 g) were hydrolysed with a mixture of 1N HCl and toluene under reflux for 1 hour. The pattern of components was quantified by TLC using solvent system II for the toluene phase and solvent system V for the aqueous phase.

The amount of each common moiety is summarised in Table CA 6.2.1/03-6, showing that 34.6% of the TRR was based on cyclohexanol, 33.8% on tert.-butylketone, 10.1% on the diol and 0.4% on the spiroxamine-hydroxy ketone. However under these conditions, the proportion of cyclohexanol due to metabolites spiroxamine tetracosanoic acid ester (M36) plus spiroxamine docosanoic acid ester (M35) (14.7% of the TRR) would not be covered in a common moiety method. Note that the routine total spiroxamine residue method for grapes uses hydrolysis to the spiroxamine-aminodiol (M28) which captures the majority of residue from parent and Group A metabolites plus the majority of Group C metabolites with the cleaved dioxolane moiety.

Table CA 6.2.1/03-6 Hydrolysis products of complete grapes using cyclohexyl-labelled spiroxamine residues

30 g grapes (1N HCl/toluene/1h reflux) Hydrolysis Product or Common Moiety	% of TRR	mg eq/kg
(subtotal) cyclohexanol based compounds:	(34.6)	(1.07)
 Spiroxamine – cyclohexanol (M13) 4-tert.-butylcyclohexanol	19.8 (main met. org. phase)	0.61
spiroxamine tetracosanoic acid ester (M36) plus spiroxamine docosanoic acid ester (M35) (not hydrolysed, cyclohexanol = common moiety)	14.7	0.45
 Spiroxamine-ketone, tert.-butylketone (M15) 4-tert.-butylcyclohexanone	33.8	1.04
 Spiroxamine-diol (M14) 4-(hydroxy-tert.-butyl)cyclohexanol	10.1	0.31
 Spiroxamine-hydroxy-ketone (M16) 4-(hydroxy-tert.-butyl)cyclohexanone	0.4	0.01

30 g grapes (1N HCl/toluene/1h reflux) Hydrolysis Product or Common Moiety	% of TRR	mg eq/kg
 Spiroxamine-cyclohexenol (M37) olefin derivative of tert.-butylcyclohexanol	2.4	0.07
8 other hydrolysis products including TLC-origin	15.7	0.48
non-releasable by acid hydrolysis	3.0	0.09
Total	100.0	3.08

Translocation experiment

The translocation experiment was an additional minor supportive component to the guideline plant metabolism study, designed to estimate if a significant amount of radioactivity would be translocated from the leaves to the edible berries. The radioactivity in the two protected bunches of grapes was negligible (amounting to 0.04% of the radioactivity applied to the four leaves below and above the bunches), especially when compared to the direct residues by spraying. The concentration of radioactivity in the stems/stalks was even lower. The residue level in the soil of the planting containers was therefore not regarded as relevant and was not measured. Application to the grapes was made on the same day as the spray application to the primary metabolism study, with the first application onto leaves when the berries were very small (ca. 20% of their final size). The second application was conducted at Day 0. The radioactivity of the treated leaves remaining at harvest (35 days after the second treatment) amounted to 18% of the originally applied amount. From the low residues in stems/stalks and grapes it was concluded, that the amount of active ingredient or metabolites translocated from the leaves into grapes was low.

Storage stability

The storage stability of aged parent compound and metabolites was assessed by re-extraction and TLC check of a frozen grape sample after approximately 4 years storage at ≤-20°C. The extraction was conducted analogously to the metabolism experiment including the exhaustive extraction (release of non-extractable residues by acidic hydrolysis). The level and composition of radioactive Spiroxamine residues extracted after a 7-day storage period (Sep. 9, 1992) and after a 1442-day storage period (Aug. 14, 1996) was nearly identical. These data indicated the frozen storage stability of spiroxamine residues in grape samples for almost four years if stored at ≤-20°C

III. Conclusions

[Cyclohexyl-1-^{14}C]spiroxamine, formulated as a 500 EC formulation, was sprayed twice to grapes 56 days before harvest (when flowering was finished) and 35 days before harvest at a total rate of 1600 g a.s./ha (2.7N maximum total seasonal application rate). Grapes were sampled immediately after the second application (day 0) and at day 35.

The total radioactive residue (TRR) in grapes amounted to 3.41 mg/kg parent compound equivalents at harvest (day 35).

Parent spiroxamine comprised the major component of the residue in day 35 grapes accounting for 24.6% (0.84 mg/kg) followed by spiroxamine-docosanoic acid ester (M35), which accounted for 13.0% (0.44 mg/kg). Other minor metabolites detected were spiroxamine tetracosanoic acid ester (M36) (4.2%TRR; 0.14 mg/kg), spiroxamine-desethyl (M01) (1.1%TRR, 0.04mg/kg), spiroxamine-despropyl (M02) (0.5%TRR, 0.02 mg/kg) and spiroxamine-N-oxide (M03) (2.9%TRR, 0.10 mg/kg). The identified post hydrolysis products (43.3% of TRR) exhibit a significant portion of the total radioactivity in grapes that can be attributed to glucoside conjugates.

It was also shown that spiroxamine was not translocated from leaves to berries of the grape vine plants.

In grapes, the metabolism of spiroxamine proceeded via cleavage of the ketal structure. The cleavage product containing the cyclohexyl label (spiroxamine-ketone) was very transient as it could not be detected in the free form. It was reduced to the corresponding alcohol (spiroxamine-cyclohexanol) that was also transient. This alcohol was conjugated with different plant endocons yielding either long chain fatty acid esters or different glucose derivatives.

The proposed metabolic pathway of spiroxamine in grapes is shown in Figure CA 6.2-4.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.2.1/02. The study is considered compliant with OECD Guideline 501 – Metabolism in Crops, January 2007.

In grapes, the metabolism of spiroxamine proceeded via cleavage of the ketal structure. The cleavage product containing the cyclohexyl label (spiroxamine-ketone) was very transient as it could not be detected in the free form. It was reduced to the corresponding alcohol (spiroxamine-cyclohexanol) that was also transient. This alcohol was conjugated with different plant endocons yielding either long chain fatty acid esters or different glucose derivatives. It was also shown that spiroxamine was not translocated from leaves to berries of the grape vine plants.

Data Point:	KCA 6.2.1/04
Report Author:	[redacted]
Report Year:	1996
Report Title:	Metabolism of [1,3-Dioxolane-4-14C]KWG 4168 in grapes
Report No:	PF4176
Document No:	M-006104-01-1
Guideline(s) followed in study:	US EPA Pesticides Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4, Nature of residues in plants and livestock
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary:

[1,3-dioxolane-4-^{14}C]-spiroxamine, formulated as a 500 EC formulation, was sprayed twice to grapes 56 days before harvest (when flowering was finished) and 35 days before harvest at a total rate of 1600 g a.s./ha (2.7N maximum total seasonal application rate). Grapes were sampled immediately after the second application (day 0) and at day 35.

The total radioactive residue (TRR) in grapes was 13.08 mg/kg parent compound equivalents at harvest (day 35).

Parent spiroxamine comprised the major component of the residue in day 35 grapes accounting for 45.6% (5.96 mg/kg) followed by spiroxamine-aminodiol (M28), which accounted for 36.3% (4.75 mg/kg). Other metabolites detected were spiroxamine-N-oxide (M03) (4.7%TRR; 0.61 mg/kg), spiroxamine-hydroxyl (M05) (0.3% TRR, 0.04 mg/kg), spiroxamine-desethyl (M01) (2.1%TRR, 0.27 mg/kg), spiroxamine-despropyl (M02) (1.5%TRR, 0.20 mg/kg), spiroxamine-aminodiol-N-oxide

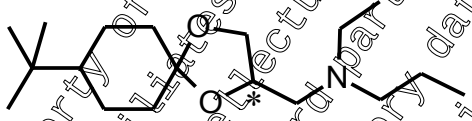
(M29) (0.1%TRR, 0.01 mg/kg), spiroxamine-desethyl-aminodiol (M30) (1.1%TRR, 0.14 mg/kg) and spiroxamine-despropyl-aminodiol (M31) (1.2%TRR, 0.16 mg/kg).

Unchanged spiroxamine and spiroxamine-aminodiol (M28) were identified as the major components of the residue in grapes after application of dioxolane-labelled spiroxamine. Three further metabolites with the aminodiol moiety were identified in grapes at low levels (spiroxamine-aminodiol-N-oxide (M29), spiroxamine-desethyl-aminodiol (M30), and spiroxamine-despropyl-aminodiol (M31)). The main metabolic reactions were hydrolytic ketal cleavage, desalkylation and oxidation. The aminodiols predominantly remained unconjugated as indicated by a similar composition of residues following aqueous/organic extraction and acid hydrolysis of the grapes.

I. Materials and methods

A. Test material

[1,3-dioxolane-4-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (MBq/mg)	4.29 (116 µCi/mg)
Lot/Batch No.:	Not stated
Purity:	Radiochemical purity ≥ 99%
Storage condition:	Not stated
CAS No.:	118134-30-8

B. Study design

Test system

Soil

Table CA 6.2.1/03-1 Soil classification and physico-chemical properties

Soil Type (DIN 19682)	pH (KCl)	OM %	Sand %	Silt %	Clay %	Moisture holding capacity mL / 100g)	CEC meq / 100 g
Loamy sand	5.9	1.38	75.8	19.0	5.2	Not reported	5

Test system

Three plant containers (35 litres) with grape vine plants, variety Müller-Thurgau, were placed in a vegetation hall, presumed to be the same as described in previous studies where the hall was covered with a glass roof and open walls (fence-shielded) to simulate outdoor conditions.. Containers contained Lagenfeld (sandy loam soil). The average temperature during the cultivation period ranged from 6.5°C (April 1994) to 20.9°C (July 1994). The sunshine hours ranged from 169 (April 1994) to 285 (July 1994) hours/month. The plant containers were fertilised with 2800 kg/ha calcium carbonate on March 29, 1994 and with 400 kg/ha potassium nitrate and phosphate on April 18, 1994. Treatment with other plant protection products was conducted as required, according to Good Agricultural Practice.

Test design

The study started in July 1994 and from the QA statement, the final date for inspection for experimental work was July 1995. Definitive study dates were not stated in the report, however, storage stability analysis was conducted for up to 634 days, so it is assumed that this covers the experimental phase from

sampling to analysis. The in-life phase was conducted in the vegetation area of the Institute for Metabolism Research and Residue Analysis Monheim, Bayer AG. The analytical phase and final reporting was conducted at the Institute for Metabolism Research and Residue Analysis of Bayer AG, Monheim, Germany.

Experimental conditions

[1,3-dioxolane-4-^{14}C]- labelled spiroxamine, formulated as a 500 EC formulation, was applied twice as a spray application using a retouch microsprayer.

The first treatment was conducted 56 days before harvest (when flowering was finished) using 25% (400 g a.s./ha) of the total amount onto small grapes. The second application was conducted 35 days before harvest using 75% (1200 g a.s./ha) of the total, in order to reflect the different size of the grapes. A total of 12 bunches of grapes distributed over 3 grape vine plants were sprayed from two different sides.

A use on grapes is included in the representative GAP for spiroxamine. The rate employed in this study (total seasonal rate of 1600 g a.s./ha) is equivalent to approximately 2.7 times the maximum seasonal use proposed for spiroxamine (600 g a.s./ha). This study is considered representative and appropriate for the objectives of establishing the nature and magnitude of spiroxamine in cereal crops.

Preparation of application solutions/applications

First application: A stock solution was prepared dissolving the formulation ECW 10102A in 5.0 mL of water. Aliquots (10 µL) were measured by LS-counting (average 44618 Bq/10 µL, total amount 22.3 MBq/5 mL). The stock solution was applied in aliquots of 145 µL on each of the two sides, therefore a total of 2 x 145 µL = 1.29 MBq was applied per bunch of grapes, which was equivalent to 0.30 mg active ingredient (specific radioactivity 4.29 MBq/mg). For the twelve bunches of grapes on 3 vines a total of 3 6 mg a.s.(155 MBq) was applied.

Second application: A stock solution was prepared dissolving the formulation ECW 10102B in 8.3 mL of water. An aliquot of 100 µL was diluted with 900 µL of water for LS measurement (1:10 dilution, 7515 Bq/10 µL, total amount 62.4 MBq/8.3 mL). The stock solution was applied in aliquots of 260 µL on each of the two sides, therefore a total of 2 x 260 µL = 3.91 MBq was applied per bunch of grapes, which was equivalent to 0.91 mg active ingredient (specific radioactivity 4.29 MBq/mg). For the twelve bunches of grapes on 3 vines a total of 10.9 mg a.s. (46.9 MBq) was applied.

Sampling

The first sample (day 0) was taken approximately 3 hours after the second application (August 11, 1994). One bunch of grapes of medium size was cut off and separated into grapes (15.1 g) and stalks (1.0 g).

The second sample (day 35) was taken at harvest (September 15,1994). Seven bunches of grapes were combined and separated into grapes (265.8 g) and stalks (10.9 g). An aliquot (100.0 g) of the grapes was directly used for the extraction.

The remaining four bunches of grapes were rather small and were kept in reserve for possible metabolite isolation (35.6 g grapes, 1.62 g stalks).

All samples which were not immediately extracted after sampling, were stored at -20°C or below.

Sample preparation and extraction

The grapes (15.1 g) from day 0 were successively macerated with methanol (2 x ca. 50 mL) and methanol/water 1:1 (v/v, ca. 50 mL). The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and the solids (non-extractable residue). Aliquots from the extract and solids were taken for radioactivity measurement.

An aliquot (100 0 g) of the grapes from day 35 was successively macerated with methanol (ca. 200 ml/100 mL) and methanol/water 1:1 (v/v, ca. 100 mL) The suspension was filtered by suction yielding the methanol/water extract (combined filtrates) and the solids (non-extractable residue). The methanol/water extract was evaporated to the aqueous remainder at ca. 40 °C using a rotary evaporator. The aqueous remainder was partitioned with dichloromethane (3 x ca. 200 mL) resulting in the organic phase (20 mL after concentration) and aqueous phase (45 mL). Aliquots from extracts, phases and solids were taken for radioactivity measurement.

Isolation of metabolites from the dichloromethane phase

An aliquot of the dichloromethane phase was applied to six silica gel coated aluminium TLC plates and developed using solvent system II. The radioactive zones were cut out and eluted with methanol. The eluates containing the same zones were combined, concentrated and chromatographed using solvent system III, except the parent compound, which was re-chromatographed using solvent system II. The radioactive zones were cut out separately and eluted with methanol. The methanol was evaporated under a stream of nitrogen and each residue was investigated by mass spectroscopy

Isolation of aminodiol from the aqueous phase

An aliquot of the aqueous phase of grapes (day 35) was diluted with an alkaline buffer solution (sodium borate, 3 mL, pH 10) and transferred to a phenylboronic acid cartridge (no. 1211-3018, JCT). The cartridge was eluted using further buffer solution and the eluate was discarded. The cartridge was then eluted with 0.01 N HCl dissolved in methanol. This clean-up procedure was repeated using 5 cartridges in parallel and the eluates were combined. The eluates were concentrated using a rotary evaporator at approximately 40°C and the residual solution transferred to a silica-gel coated aluminium TLC plate and chromatographed using solvent system IV. The aminodiol zone of the TLC plate was cut out and eluted with 25% ammonia/methanol 1:10 (v/v). The eluate was concentrated and chromatographed a second time analogously. The final eluate was investigated by mass spectroscopy.

Alkaline hydrolysis of metabolite group 1 from the dichloromethane phase

An aliquot of the purified metabolite group 1 was concentrated under a stream of nitrogen, 1N NaOH was added and the solution stirred in a reacti-vial for 2 hours at 100°C. After cooling, the solution was neutralised using 1N HCl and analysed by TLC.

Acidic hydrolysis of isolated metabolites

Spiroxamine-desethyl (M01) and spiroxamine-hydroxyl (M03), obtained as a mixture following purification from the organic phase by TLC, were concentrated under a stream of nitrogen, 1N HCl was added and the mixture heated in a reacti-vial for 1 hour at 100°C. After cooling, the reaction mixture was neutralised by 1N NaOH and analysed by radio-TLC.

Total hydrolysis of Grapes

Grapes (10.0 g) were macerated, 1N HCl was added and the mixture heated under reflux for 1 hour. After cooling, the solution was neutralised with 1N NaOH, filtered by suction and the solids were washed with water. Aliquots from the filtrate and solids were taken for radioactivity measurement.

Radiochemical analysis

Radioactivity determination

The ^{14}C-radioactivity in liquid samples was determined in a liquid-scintillation counter LKB 1219 Rackbeta (LKB-Wallac), LS 6000LL (Beckman Instruments) or LS 6500 (Beckman Instruments) with Instant-Scint Gel (Packard) or Quicksafe A (Zinsser Analytic).

Solid samples were combusted in an OX 500 (Zinsser Analytic). The CO_2 produced by combustion was absorbed in a scintillation-cocktail [Oxysolve C-400 (Zinsser Analytic)] and the radioactivity measured

by LSC (PW 4700, Philips/Raytest) or LKB 1219 Rackbeta (LKB-Wallac), Beckman LS 6000LL (Beckman Instruments) or Beckman LS 6500 (Beckman Instruments).

Thin layer chromatography (TLC)

The radioactive solutions were investigated by TLC using silica gel plates (silica gel 60 $_{F254}$, 0.25 mm thickness, glass-coated, 20 cm x 20 cm, Merck, Darmstadt, Germany or silica gel 60 $_{F254}$, 0.2 mm thickness, aluminium-coated, 20 cm x 20 cm, Merck, Darmstadt, Germany) and the following solvent systems (SS):

I: dichloromethane:ethyl acetate 9:2 (v/v)
II: ethyl acetate:2-propanol:water 5:3:1 (v/v/v)
III: acetonitrile:water:25% ammonia 80:18:2 (v/v/v)
IV: chloroform:methanol: 25% ammonia 65:28:8 (v/v/v)

The radioactive compounds were detected and evaluated quantitatively using Bio-Imaging Analyser (BAS 2000, Fuji) and a TINA software package (Raytest). The reference compounds used in co-chromatography were visualised in an iodine chamber.

Mass spectroscopy (MS)

LC/MS-ESI

The electro-spray ionisation MS spectra (ESI) were obtained with a Finnigan TSQ 7000 instrument. Sheath gas pressure: 52 psi, capillary temperature: 210 °C. The flow rate was 200 µL/min. A radioactivity detector (Ramona 90, Raytest) was coupled between the HPLC instrument (HP 1050, Hewlett Packard) and mass spectrometer. The delay time between the two instruments was approximately 20 sec. For the MS/MS experiments, argon was used as the collision gas (pressure in the collision chamber: 2.7 mT).

GC-MS/EI

Parent spiroxamine: GC/MS analyses were performed on a mass selective detector HP 5970 with a gas chromatograph 5880A (Hewlett-Packard). The capillary column used was a 15 m SE 54 (J&W Scientific). The oven temperature program was 60 °C for 1 minute, heating rate was 10 °C/min up to 280 °C. The final temperature was kept constant for 20 minutes. The samples were injected in the splitless mode at 280 °C. The helium carrier gas pressure was 4 psi.

Aminodiol: GC/MS analyses were performed on a INCOS XL instrument by Finnigan with a Varian gas chromatograph. The capillary column used was a 30 m DB5MS (J&W Scientific). The oven temperature program was 60 °C for 1 minute, heating rate was 10 °C/min up to 310 °C. The final temperature was kept constant for 10 minutes. The samples were injected in the splitless mode at 250 °C. The helium pressure was 14 psi.

Derivatisation

Silylation was performed with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA). An excess of the reagent was added to an aliquot of the sample to be investigated spectroscopically. The mixture was heated to 70°C for 10 to 20 minutes. Injection into the GC was directly from the reaction mixture.

^{1}H-NMR-spectroscopy

The ^{1}H-NMR-spectra were recorded on a Bruker AC 300 spectrometer, the samples were dissolved in CD_3OD (Merck) for analysis.

II. Results and discussion

Total radioactive residue (TRR)

One bunch of grapes (15.1 g) was sampled immediately after the second application (day 0) and was used for method development. The radioactivity was easily extracted using methanol/water. The TRR

amounted to 33.8 mg/kg parent compound equivalents as determined by summation of the radioactivity in the methanol/water extract and in the solids.

The TRR at harvest (day 35) was determined from the extraction of 100.0 g grapes and amounted to 13.08 mg/kg parent compound equivalents as determined by summation of the radioactivity in the combined methanol/water extracts and in the solids. The extracted radioactivity was distributed into a dichloromethane phase (56.9% of the TRR, 7.44 mg/kg) and an aqueous phase 40.2% (5.26 mg/kg). Only 2.9% (0.38 mg/kg) of the TRR was found in the solids. The radioactivity detected in the combined methanol/water extracts was recovered nearly quantitatively in the organic and aqueous phases following evaporation and partitioning. The radioactivity determined in the dichloromethane phase and aqueous phase was normalised to the original radioactivity found in the methanol/water extract.

TRR and distribution of radioactivity are given in Table CA 6.2.1/04-2.

Table CA 6.2.1/04-2 Distribution of radioactive residues in grapes following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

Radioactive residues in grapes	Grapes (0 DALA)		Grapes (35 DALA)	
	mg/kg	%TRR	mg/kg	%TRR
TRR[1]	33.8	100.0	13.08	100.0
Dichloromethane phase[2]	-	-	7.44	56.9
Aqueous phase[2]	-	-	5.26	40.2
Post extraction solids (PES)	-	-	0.38	2.9

1 - total radioactive residues given in mg parent substance equivalents per kg, determined by summation of all fractions after extraction
2 - primary methanol/water extract was concentrated and partitioned between dichloromethane and water

Characterisation

Available synthesised compounds for characterisation were parent test substance spiroxamine (KWG 4168) and the following potential metabolites:

KWG 4557 (spiroxamine-desethyl) (M01)

KWG 4669 (spiroxamine-despropyl) (M02)

WAK6301/1 (spiroxamine-N-oxide) (M03)

WAK 6782 (Spiroxamine-N-formyl-desethyl) (M04)

BNF 5567B (acetyl-spiroxamine-desethyl)

BNF 5567A (acetyl-spiroxamine-despropyl)

WAK 6072 (spiroxamine-amine)

WAK 5868 (spiroxamine-hydroxyl) (M05)

WAK 6084/1(spiroxamine-hydroxy-desethyl) (M41)

WAK 6079/1 (spiroxamine-hydroxy-despropyl) (M09)

WAK 5708 (spiroxamine-acid) (M06)

WAK 5736B (spiroxamine-desethyl acid) (M11)

BNF 5534A (spiroxamine-despropyl acid) (M12)

WAK 5428 (tert.-Butylcyclohexanone) (M15)

BNF 5544A (spiroxamine-hydroxy ketone) (M16)

BNF 5550A (spiroxamine-cyclohexanol) (M13)

WAK6482-4 (spiroxamine-diol) (M14)

WAK 6131-2-7 (spiroxamine-ketone acid) (M17)

WAK 5427 (spiroxamine-aminodiol) (M28)

WAK 6885 (spiroxamine-aminodiol-N-oxide) (M29)

WAK 6894 (spiroxamine-desethyl-aminodiol) (M30)

WAK 6893 (spiroxamine-despropyl-aminodiol) (M31)

PA 1344 (spiroxamine tetracosanoic acid ester) (M36)

PA 1345 (spiroxamine docosanoic acid ester) (M35)

Each metabolite was identified by co-chromatography reference standards.

Residues in [^{14}C]-spiroxamine treated grape

Metabolites in grapes (day 35)

Organo-extractable residue

The dichloromethane phase of grapes contained 56.9% (7.44 mg/kg) of the TRR and unchanged parent compound accounted for 45.6% (5.96 mg/kg) in this extract. All other metabolites of this phase were below ≤4.7% of the TRR (≤0.61 mg/kg) and were mainly due to compounds bearing an intact ketal structure as spiroxamine-hydroxyl (M05), spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-N-oxide (M03). A small aliquot of the aminodiol (M28) (1.2% of the TRR, 0 16 mg/kg) was detected in the dichloromethane phase.

Aqueous soluble residue

The aqueous phase of grapes accounted for 40.2% (5.26 mg/kg) of the TRR and was essentially based on the aminodiol (M28), which accounted for 36.3% (4.75 mg/kg) of the TRR. The corresponding desalkyl-aminodiols and the spiroxamine-aminodiol-N-oxide (M29) were also identified, however each of them accounted for ≤1.2% (≤0.16 mg/kg). Several minor unidentified metabolites (each ≤0.4%, ≤0.05 mg/kg) were also found in the aqueous phase.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in Table CA 6.2.1/04-3. The identified components of the terminal residue in Day 35 grapes, are summarised in Table CA 6.2.1/04-4.

The sum of the identified metabolites was 94.1% (12.31 mg/kg) of the TRR in grapes and 2.9% (0.38 mg/kg) remained associated with the solids.

Table CA 6.2.1/04-3 Summary of extractable radioactive residue fractions in grapes following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

	Grapes (35 DALA)	
	mg/kg	%TRR
Organic (dichloromethane) phase[1]	**7.44**	**56.9**
Spiroxamine, parent[2]	5.96	45.6
Metabolite group 1 (unknown consisting of several minor non-polar components)	0.20	1.5
Spiroxamine-hydroxyl (M05)[2]	0.04	0.3
Spiroxamine-desethyl (M01)[4]	2.1	0.27
Spiroxamine-despropyl (M02)[5]	1.5	0.20
Spiroxamine-N-oxide (M03)[3]	4.7	0.61
Spiroxamine-aminodiol (M28)	1.2	0.16
Aqueous phase[1]	**5.26**	**40.2**
Spiroxamine-aminodiol (M28)	4.75	36.3
Metabolite 7 (unknown)	0.03	0.2
Spiroxamine-aminodiol-N-oxide (M29)	0.01	0.1
Spiroxamine-desethyl-aminodiol (M30)	0.14	1.1
Metabolite 10 (unknown)	0.03	0.2
Spiroxamine-despropyl-aminodiol (M31)	0.16	1.2
Metabolite 12 (unknown)	0.03	0.2
Metabolite 13 (unknown)	0.01	0.1
Metabolite 14 (unknown)	0.04	0.3
Polar material (TLC origin)	0.05	0.4
Non-extractable, bound residue	0.38	2.9
Total residue	13.08	100.0

1 – designation of unknown metabolites follows designation in the study report

Table CA 6.2.1/04-4 Summary of identified radioactive residues in grapes following two spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine

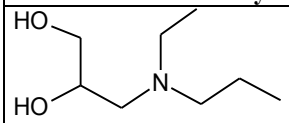
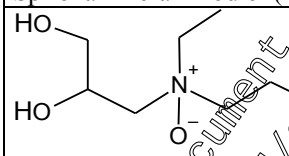
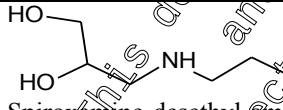
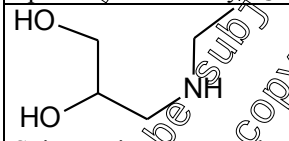
	Grapes (Day 35)	
Identified metabolites	**% TRR**	**mg/kg**
Spiroxamine, parent	45.6	5.96
Spiroxamine-hydroxyl (M05)	0.3	0.04
Spiroxamine-desethyl (M01)	2.1	0.27
Spiroxamine-despropyl (M02)	1.5	0.20
Spiroxamine-N-oxide (M03)	4.7	0.61
Spiroxamine-aminodiol (M28)	37.5	4.91
Spiroxamine-aminodiol-N-oxide (M29)	0.1	0.01
Spiroxamine-desethyl-aminodiol (M30)	1.1	0.14
Spiroxamine-despropyl-aminodiol (M31)	1.2	0.16
Total identified	**94.1**	**12.31**

Total hydrolysis of spiroxamine residues in grapes

A total acidic hydrolysis of grapes was conducted to characterise the TRR in grapes and to assess the importance of individual hydrolysis products (common moieties) for the development of a residue method. The experiment was conducted using grapes at 0 DALA. It was obvious that the aminodiol would be the major component since first it was the main metabolite and second would be obtained additionally from the parent compound. For comparison reasons, the dichloromethane phase and the aqueous phase from the metabolism study were shown. The metabolite pattern of the aqueous phase was very similar to the hydrolysis product pattern, indicating, that only a relatively low percentage of the aminodiols was conjugated.

The results are given in Table CA 6.2.1/04-5 and show that 79.1% (7.50 mg/kg) of the TRR was based on the aminodiol moiety and the relative intensities of the other identified aminodiols were in good agreement to the identified metabolites in grapes.

Table CA 6.2.1/04-5 Hydrolysis products of complete grapes using dioxolane-labelled spiroxamine residues

10.0 g grapes hydrolysed (1N HCl/1h reflux) Hydrolysis Product	% of TRR	mg eq/kg
 Spiroxamine-aminodiol (M28)	79.1	7.50
 Spiroxamine-aminodiol-N-oxide (M29)	1.9	0.18
 Spiroxamine-desethyl-aminodiol (M30)	5.9	0.56
 Spiroxamine-despropyl-aminodiol (M31)	6.5	0.62
4 unknown hydrolysis products including TLC-origin	4.0	0.38
non-releasable by acid hydrolysis	2.6	0.25
Total	100.0	9.49

Storage stability

The storage stability of parent compound and metabolites was assessed by re-extraction and TLC separation of a frozen grape sample after 21 months of storage at ≤ -20°C. The later extraction was conducted analogously to that conducted in the metabolism experiment. The composition of radioactive spiroxamine residues extracted directly after sampling (Sep. 15, 1994) and after a 634 day storage period (Oct. 6, 1996) was nearly identical although small differences in absolute concentrations were noted. These data indicated the storage stability of spiroxamine residues in grape samples for almost two years (21 months) if stored at ≤ -20°C.

III. Conclusions

[1,3-dioxolane-4-^{14}C]spiroxamine, formulated as a 500 EC formulation, sprayed twice to grapes 56 days before harvest (when flowering was finished) and 35 days before harvest at a total rate of 1600 g a.s./ha (2.7N maximum total seasonal application rate). Grapes were sampled immediately after the second application (day 0) and at day 35.

The total radioactive residue (TRR) in grapes amounted to 13.08 mg/kg parent compound equivalents at harvest (day 35).

Parent spiroxamine comprised the major component of the residue in day 35 grapes accounting for 45.6% (5.96 mg/kg) followed by aminodiol (M28), which accounted for 36.3% (4.75 mg/kg). Other metabolites detected were spiroxamine-N-oxide (M03) (4.7%TRR, 0.61 mg/kg), spiroxamine-hydroxyl (M05) (0.3% TRR, 0.04 mg/kg), spiroxamine-desethyl (M01) (2.1%TRR, 0.27 mg/kg), spiroxamine-despropyl (M02) (1.5%TRR, 0.20 mg/kg), spiroxamine-aminodiol-N-oxide (M29) (0.1%TRR, 0.01 mg/kg), spiroxamine-desethyl-aminodiol (M30) (1.1%TRR, 0.14 mg/kg) and spiroxamine-despropyl-aminodiol (M31) (1.2%TRR, 0.16 mg/kg).

Unchanged spiroxamine and spiroxamine-aminodiol (M28) were identified as the dominant residues in grapes after application of dioxolane-labelled spiroxamine. Three further metabolites with the aminodiol moiety were identified in grapes at low levels (spiroxamine-aminodiol-N-oxide (M29); spiroxamine-desethyl-aminodiol (M30); and spiroxamine-despropyl-aminodiol (M31)). The main metabolic reactions were hydrolytic ketal cleavage, desalkylation and oxidation. The aminodiols predominantly remained unconjugated as indicated by a similar composition of residues following aqueous/organic extraction and acid hydrolysis of the grapes.

The proposed metabolic pathway for spiroxamine in grapes is shown in Figure CA 6.2-4.

Assessment and conclusion by applicant:

Acceptable study to address data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.2.1/03. The study is considered compliant with OECD Guideline 501 – Metabolism in Crops, January 2007.

Unchanged spiroxamine and spiroxamine-aminodiol were identified as the major components of the residue in grapes after application of dioxolane-labelled spiroxamine. Three further metabolites with the aminodiol moiety were identified in grapes at low levels (spiroxamine-aminodiol-N-oxide, spiroxamine-desethyl-aminodiol and spiroxamine-despropyl-aminodiol). The main metabolic reactions were hydrolytic ketal cleavage, desalkylation and oxidation. The aminodiols predominantly remained unconjugated as indicated by a similar composition of residues following aqueous/organic extraction and acid hydrolysis of the grapes.

Data Point:	KCA 6.2.1/05
Report Author:	[redacted]
Report Year:	2000
Report Title:	Metabolism of [14C]-KWG 4168 in banana (In-life phase)
Report No:	FM775
Document No:	M-026783-01-1
Guideline(s) followed in study:	US EPA Pesticides Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4, Nature of residues in plants and livestock
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Note to reviewer: CA 6.2.1/05 details the in-life phase of both cyclohexyl and 1,3-dioxalane spiroxamine on bananas. This phase is fully detailed in the study summary presented under CA 6.2.1/06.

Data Point:	KCA 6.2.1/06
Report Author:	[redacted]
Report Year:	2002
Report Title:	Metabolism of [cyclohexyl-1-14C]KWG4168 in banana (analytical part)
Report No:	MR-140/01
Document No:	M-030423-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

[Cyclohexyl-1-^{14}C]-spiroxamine, formulated as a 800 EC formulation, was sprayed three times to bananas bunches when small fruit were present, when medium size fruit were present and just before harvest (0 days) to simulate a total seasonal rate of 3.2 kg a.s./ha (equivalent to 0.8N maximum intended use rate). Peel and pulp were sampled immediately after the last application (day 0).

The total radioactive residue (TRR) accounted for 0.444 mg/kg in pulp and 4.77 mg/kg in peel and was effectively extracted with a methanol/water mixture. The extracted radioactivity (92.2% of the TRR from pulp and 95.3% from peel) was partitioned into a dichloromethane phase and an aqueous phase 1. The dichloromethane phase contained 52.2% (0.232 mg/kg) of the TRR in pulp and 55.9% (2.67 mg/kg) of the TRR in peel. The aqueous phase 1 contained 40.0% (0.178 mg/kg) of the TRR in pulp and 39.4% (1.88 mg/kg) in peel.

Unchanged parent spiroxamine was the predominant compound in pulp accounting for 44.9% (0.200 mg/kg) of the TRR. Identified metabolites with intact ketal-structure were spiroxamine-N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in small amounts, each ≤1.1% (≤0.005 mg/kg) of the TRR. Cyclohexanol conjugated with a hexose-disaccharide accounted for 10.4% (0.046

mg/kg) of the TRR. A diol-disaccharide (hexose-pentose) accounted for 9.2% (0.041 mg/kg) of the TRR and a small amount of a second cyclohexanol-disaccharide (hexose-pentose) was identified at a level of 3.2% (0.014 mg/kg) of the TRR.

Unchanged parent spiroxamine was the predominant compound in peel accounting for 42.2% (2.02 mg/kg) of the TRR. Identified metabolites with intact ketal-structure were Spiroxamine-N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in small amounts ≤4.9% (≤0.23 mg/kg) of the TRR. Cyclohexanol conjugated with a hexose-disaccharide accounted for 9.1% (0.43 mg/kg) of the TRR. Three minor metabolites were identified as isomers of cyclohexanol-disaccharides (hexose-pentose) in the range of 3.5% to 7.4% of the TRR (0.17 to 0.35 mg/kg) and, in addition, a diol disaccharide (hexose-pentose) at 5.5% of the TRR (0.26 mg/kg).

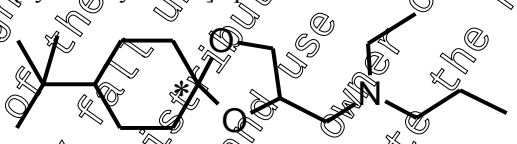
In total, 70.0% (0.311 mg/kg) of the TRR was identified in pulp and 81.2% (3.87 mg/kg) in peel. In addition, 22.1% (0.098 mg/kg) of the TRR in pulp and 14.1% (0.67 mg/kg) in peel, was characterised.

The main metabolic step in banana was the cleavage of the ketal structure generating the intermediate cyclohexanone. The ketone was reduced to the major aglycon cyclohexanol. Analogous metabolism was observed for the diol representing the cyclohexanol-structure however, with an oxidised t-butyl-group. Both major intermediates were intensively conjugated to glycosides. Based on the results of grapes and banana it was concluded that glucose-conjugates were the most important monosaccharides which were further conjugated. A minor metabolic step was the oxidation of the parent compound at the tertiary amine moiety to yield spiroxamine-N-oxide (M03). Another minor metabolic step was the desalkylation of the tertiary amine moiety to generate spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02).

I. Materials and methods

A. Test materials

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (MBq/mg):	3.23 (87.3 µCi/mg)
Lot/Batch No.:	Not stated
Purity:	Radiochemical purity > 98% (sum of isomers) before and after formulation, and before application; 96.1 – 97.5% after application
Storage condition:	Not stated
CAS No.:	118134-30-8

B. Study design

Test system

Soil

A sandy loam soil from Litchfield Ranch, Watsonville, CA, USA was used. The physico-chemical properties were not determined. The soil had no previous exposure to spiroxamine.

Test system

Two 10 gallon (38 L) plant containers with one banana tree (variety dwarf Cavendish) each were placed in a greenhouse in Watsonville, CA, USA. They were used for separate metabolism studies with cyclohexyl and dioxolane labelled spiroxamine. They were set up within separate sections of the

greenhouse approximately 70 feet (21 m) apart. Each section was enclosed using plastic sheeting. Fans were installed near the top in each section to provide circulation and to vent airborne radiocarbon to the outside of the greenhouse. Temperature was daily measured using a data logger. The average maximum temperature was 89.7°F (32.1°C); the average minimum temperature was 67.1°F (19.5°C). Normal sunlight conditions in the greenhouse were used. No additional light was required.

The banana plants were fertilised five times by adding Sorba-Spray ZKP (16% P_2O_5, 9% K_2O, 1% Zn liquid concentrate) to the irrigation water before and after treatment with Spiroxamine (10.2 quarts/acre = 23.8 L/ha in total). Treatment with other plant protection products to control aphids and spider mites were conducted as required according to Good Agricultural Practice. The banana trees were irrigated by applying water directly to the soil surface.

Test design

The experimental work was conducted between June 1999 and December 2001. The in-life phase was conducted at the field test site of Plant Sciences, Inc., Watsonville, USA and the test site of PTRL West, Inc., Richmond, USA. The analytical phase and final reporting was conducted at the Institute for Metabolism Research and Residue Analysis of Bayer AG, Monheim, Germany.

Experimental conditions

Three foliar applications were made to a bunch of banana fruits (30 - 50 fruits) at the following growth stages: small fruit, medium size fruit and just before harvest (pre-harvest interval, PHI: 0 days). The experimental application rate was derived from the maximum seasonal aerial rate of 3.2 kg as/ha and the size of a banana bunch (25 cm x 25 cm = 0.0625 m^2). Thus, a bunch is, in principal, exposed to 3.2 x 10^6 mg x 10^{-4} m^{-2} x 0.0625 m^2 = 20 mg. Assuming that a similar amount would reach the bunch from the side due to wind movement caused by aerial application, the total amount increases to 40 mg as/bunch/season. In the metabolism study, 14 mg of the test substance were used for each individual application resulting in a total of 42 mg. The formulated radiolabelled test substance was mixed with 8.5 mL of water and sonicated for 1 min resulting in a homogeneous spray mixture. This mixture was sprayed to the banana fruits from two sides using an atomizer type sprayer.

In an overdose experiment, three tagged bananas near the bottom of the bunch received an extra dose of approximately 1 mg of radiolabelled test substance at each application using a small brush attached to a syringe.

In the following Table CA 6.2.1/05-1 the schedule of events for the in-life phase is shown.

A use on fruit is included in the representative GAP for spiroxamine. The rate employed in this study (total seasonal rate of 3.2 kg as/ha) is equivalent to 5.3 times the maximum seasonal use proposed for spiroxamine (600 g as/ha) on grapes and stated to be 0.8 times the maximum seasonal rate on bananas. This study is considered representative and appropriate for the objectives of establishing the nature and magnitude of spiroxamine in fruit crops.

Table CA 6.2.1/05-1 Schedule of events in the in-life phase of the metabolism study of spiroxamine in bananas

Days after initial treatment	Date	Event
0	06/29/1999	Banana trees were set up within their enclosures inside the greenhouse.
0	06/29/1999	Application #1. Green fruit, small size. cyclohexyl label tree height: ~ 80" (2 m), fruit length: 6.5-7" (16.5–18 cm), width 1-1.5" (2.5–4 cm) dioxolane label tree height: ~ 73" (1.9 m),

Days after initial treatment	Date	Event
		fruit length: 6.5-7" (16.5–18 cm), width 1-1.5" (2.5–4 cm)
17	07/16/1999	Application #2. Green fruit, medium size. cyclohexyl label tree height: ~ 80" (2 m), fruit length: 6.5-7" (16.5–18 cm), width 1-2" (2.5–5 cm) dioxolane label tree height: ~ 73" (1.9 m), fruit length: 6.5-7" (16.5–18 cm), width 1-2"" (2.5–5 cm)
71	09/08/1999	Application #3. Mature fruit, yellow or light yellow. A significant amount of fruit was cracked (due to hot weather). cyclohexyl label tree height: ~ 80" (2 m), fruit length: 6.5-7" (16.5–18 cm), width 1.5-3" (4–7.5 cm) dioxolane label tree height: ~ 73" (1.9 m), fruit length: 6.5-7" (16.5–18 cm), width 1-2"" (2.5–5 cm)
71	09/08/1999	Harvest of mature Bananas. The harvest for each label was started as soon as the surface of the fruit was dry. Uncracked bananas were peeled immediately after each harvest.
71	09/08/1999	Samples transported to PTRL West, Richmond, CA, USA
90	09/27/1999	Samples sent to Bayer AG via World Courier for analysis
92	09/29/1999	Samples arrived at Bayer AG, Leverkusen, Germany

Preparation of application solutions/applications

The test substance was provided by Bayer AG as an 800 EC formulation in separate premeasured amounts (normal dose and overdose) for each application.

Approximately 24 hours before each application the designated normal dose and overdose samples were mixed with 8 mL and 0.5 mL of water respectively. Each sample was sonicated for ~ 1 min to obtain a homogeneous mixture. The homogeneity and the total dpm for each normal dose was verified by LSC analysis of triplicate 2 µL. Pre-application purity for normal dose solutions was established by taking a small sample, 10 or 20 µL for each label and adding 250 µL of acetonitrile prior to TLC analysis. A second sample was taken for the post-application purity determination of the normal doses. These post-application purity samples were placed in a small vial and transported to the field with the corresponding dose solution in separate coolers with substitute ice. Following application these post-application purity samples were placed on dry ice and transported back to PTRL West. The samples were diluted with 250 µL of acetonitrile for post-application TLC analysis. All dose solutions were prepared in the same vials as used for shipping the test substances.

Sampling

Mature banana fruits were harvested immediately after the third application as soon as the fruits were dry. Intact fruits were separated from cracked (split) fruits at the time they were cut. After weighing the intact fruits were peeled and the peel (278 g) and pulp (364 g) samples were again weighed and stored frozen. Care was taken during the peeling process to avoid touching the pulp with contaminated gloves.

Samples were transported in the PTRL van using separate coolers with dry ice for each label. (Samples treated with the alternative dioxolane labelled Spiroxamine were sampled and transported at the same time). At PTRL West samples were inventoried and stored in freezers. Samples were shipped via World Courier from PTRL West, CA, USA, to Bayer AG, Leverkusen, Germany in separate containers with dry ice for each label. Samples arrived in good condition with dry ice still present.

Sample preparation and extraction

The pulp sample was thawed at room temperature and cut in 2 – 4 mm slices and the main portion of mixed slices was stored frozen again at – 20°C. A subsample was twice extracted with methanol, followed by methanol/water (1/1, v/v) by a Polytron high-speed stirrer. The filtered and combined extracts were concentrated to the aqueous remainder using a rotary evaporator. The extracted residues were then partitioned between water and dichloromethane. Aliquots of the solid filter cake (containing non-extractable residues) were combusted and trapped in LSC cocktail and the radioactivity content of the liquid phases was determined by LSC. Aliquots of the organic fraction were also investigated by radio-TLC. Aliquots of the aqueous fraction were investigated by radio-HPLC.

The peel sample was homogenised by a Polytron homogeniser in presence of liquid nitrogen to yield a fine powder. A subsample was extracted with methanol and methanol/water followed by water-dichloromethane partitioning as done with the pulp sample.

For purification of non-polar metabolites aliquots of the dichloromethane extract were subjected as a chromatographic zone to semi-preparative TLC with silica gel coated aluminium plates and acetonitrile/water/25% ammonia (80/18/2, v/v/v) as developing solvent mixture. After cutting out of the respective zone and extraction of the radioactivity with methanol the semi-chromatography was repeated using the same solvent mixture. Cutting out of the radioactive band, desorption with methanol and evaporation of methanol yielded radioactive residues that could be subjected to analytical co-chromatographic TLC and to hydrolysis (addition to 1 N HCl and toluene in a screw-capped tube, 1 hour, 100°C) followed by co-chromatography.

For purification of polar metabolites aliquots of the aqueous phase (after partitioning with dichloromethane) were subjected to semi-preparative HPLC with several runs. Selected factions (representing major radioactive peaks) were collected, combined and concentrated. The resulting concentrate was radioassayed, re-chromatographed and hydrolysed. Hydrolysis was conducted under the same conditions as described before.

For hydrolysis of chromatographically purified residue fractions partitioned into the dichloromethane phase these were heated with 1 N HCl and toluene (1 hour, 100°C) in a screw-cap tube. After cooling to room temperature the phases were separated, the aqueous phase partitioned twice with toluene and the combined toluene phases radioassayed (analysed for radioactivity) and investigated by radio-TLC with reference standards. Polar residues of the aqueous phase after partitioning with dichloromethane were hydrolysed in the same way.

Radiochemical analysis

Radioactivity determination

Determination of ^{14}C-radioactivity was conducted by liquid scintillation counting (LSC) using the counters Beckman LS 6500 (Beckman Instruments) and PW 4700 (Philips/Raytest). All counters made use of an external standard for quench correction. Aliquots of liquid samples were mixed to Quicksafe A + 5% water scintillation cocktail (Zinsser Analytic) for scintillation counting. 50 – 200 mg aliquots of solid samples were first combusted in an OX 500 Oxidiser (Zinsser Analytic) equipped with the sample robot/05-3 Robox 200 (Zinsser Analytic). The formed $^{14}CO_2$ was absorbed in the basic scintillation cocktail Oxysolve C-400 (Zinsser Analytic). Counting was generally stopped after a maximum period of 10 minutes or until the 2-sigma counting error of the cpm value had reached 0.7%. Usually 3 – 5 replicates were measured from each sample to achieve a reproducibility of ≤ 1 – 2%.

Thin layer chromatography (TLC):

Separation of organo-soluble radioactive residue components was performed by radio-thin layer chromatography (TLC) employing silica gel plates (60 F_{254} , 20 x 20 cm glass plates coated with 0.25 mm separation layer or 60 $_{F254}$, 20 x 20 cm aluminium plates coated with 0.2 mm silica gel; Merck, Darmstadt). Four different solvent mixtures were used for development: (I) acetonitrile/ water/25%

ammonia (80/18/2, v/v/v), (II) chloroform/methanol/25% ammonia (65/28/8, v/v/v), (III) ethyl acetate/2-propanol/water (5/3/1, v/v/v) and (IV) dichloromethane/ethyl acetate (9/2, v/v). Quantitative radio detection was performed by a Bio-Imaging Analyser (BAS 2000, Fuji) and a TINA 2.09g software package (Raytest). Non-radioactive reference standards were visualized by spot darkening in an iodine chamber.

The limit of quantification was derived from the smallest peaks that still have been detected. It was set to 0.003 mg eq/kg for the pulp metabolites in the dichloromethane phase and to 0.03 mg eq/kg for the peel metabolites in dichloromethane phase.

The active substance and some of the metabolites contain asymmetric centres in the molecule and, therefore, form diastereomers. For this reason, these substances were observed occasionally as double peaks (if the chromatographic conditions ensured sufficient resolution power).

High performance liquid chromatography

Separation of water-soluble radioactive metabolites was performed by radio-high-performance liquid chromatography (radio-HPLC) using a HP 1050 HPLC instrument (Hewlett Packard) operating with an UV/VIS detector (adjusted to 254 nm) and the flow-through radio monitor Ramona 2000 (Raytest). A LiChrosorb RP 18 e column (5 µm particle size) served for separation of peaks. The column was gradient-eluted with the solvents water and methanol at 40°C (flow: 1 mL/min).

The limit of quantification was derived from the smallest HPLC peaks still detectable. Thus, it was set to 0.005 mg eq/kg for pulp metabolites in the aqueous phase and to 0.01 mg eq/kg for peel metabolites in the aqueous phase.

The recovery during HPLC separation was determined by comparison of injected radioactivity and the eluted radioactivity collected in one fraction. Employing the aqueous fraction originating from the pulp sample the recovery accounted for ≥ 99%.

High performance liquid chromatography/mass spectroscopy (LC/MS-ESI)

Identification of metabolites was performed by liquid chromatography/mass spectrometry using the combination of a HP 1100 HPLC instrument (Hewlett Packard) and a TSQ 7000 MS instrument (Finnigan). Electrospray (ESI) was used for ionisation. A LiChrospher 60 RP Select B column (250 x 2 mm, particle size: 5 µm) served for separation. A radioactivity detector (Ramona 90, Raytest) was coupled via a flow splitter between HPLC and MS instruments. The split ratio was 30/170 (MS/UV+^{14}C). A gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used as eluent. The flow rate was adjusted to 200 µL/min. For additional MS/MS experiments, argon was used as the collision gas.

Gas chromatography/mass spectroscopy (GC/MS)

For identification of the parent substance and a reference standard a combination of a Varian gas chromatograph equipped with a 15 m DBXLB capillary column (J&W Scientific) and an INCOS XL mass spectrometer (Finnigan) was used. Electron impact mode was selected for ionisation. Helium was used as carrier gas and a temperature program ranged from 60 to 310°C. Splitless injection mode was used.

Nuclear magnetic resonance

^{1}H NMR spectra were measured by an DMX 600 NMR spectrometer (Bruker) at 600 MHz.

II. Results and discussion

Total radioactive residue (TRR)

The total radioactive residues (TRR) in bananas sampled immediately after the third application of cyclohexyl-labelled spiroxamine accounted for 0.444 mg parent equivalents/kg (mg eq/kg) in pulp and 4.77 mg eq/kg in peel of intact bananas.

The extracted radioactivity in pulp was distributed into a dichloromethane phase (52.2% of the TRR, 0.232 mg/kg) and an aqueous phase 1 (40.0% of the TRR, 0.178 mg/kg). The unextracted radioactivity in the solids accounted for 7.8% (0.035 mg/kg) of the TRR. The radioactivity detected in the combined methanol/water extract was recovered nearly quantitatively (98.9%) in the dichloromethane phase and aqueous phase 1 following evaporation and partitioning. The radioactivity determined in the dichloromethane phase and aqueous phase 1 was normalised to the original radioactivity found in the methanol/water extract.

The dichloromethane phase in peel accounted for 55.9% (2.67 mg/kg) of the TRR and the aqueous phase 1 represented 39.4% (1.88 mg/kg) of the TRR. The recovery of radioactivity corresponded to 98.2%. Only 4.7% (0.22 mg/kg) of the TRR was found in the solids.

TRR and distribution of radioactivity are given in Table CA 6.2.1/05-2.

Table CA 6.2.1/05-2 Distribution of radioactive residues in mature bananas following three spray applications with [cyclohexyl-1-^{14}C]-spiroxamine, directly after the last application

Radioactive residues in banana	Pulp		Peel	
	mg/kg	%TRR	mg/kg	%TRR
TRR[1]	0.444	100	4.77	100.0
Dichloromethane phase[2]	0.232	52.2	2.67	55.9
Aqueous phase[2]	0.178	40.0	1.88	39.4
Post extraction solids (PES)	0.035	7.8	0.22	4.7

1 - total radioactive residues given in mg parent substance equivalents per kg, determined by summation of all fractions after extraction
2 - primary methanol/water extract was concentrated and partitioned between dichloromethane and water

Characterisation

Available synthesised compounds for characterisation were parent test substance spiroxamine (KWG 4168) and the following potential metabolites:

FHW0104H (spiroxamine-desethyl) (M01)

WAK 5868/2 (spiroxamine-hydroxyl) (M05)

WAK 6174 (spiroxamine-despropyl) (M02)

WAK 6301/1 (spiroxamine-N-oxide) (M03)

WAK 5428 (spiroxamine-ketone) (M15)

BNF 5550A (spiroxamine-cyclohexanol) (M13)

BNF 5569B (spiroxamine-hydroxy ketone) (M16)

WAK 6482-4 (spiroxamine-diol) (M14)

WAK 6850-3A (spiroxamine-cyclohexanol)

Each metabolite was identified by co-chromatography with reference standards.

Residues in [^{14}C]spiroxamine treated banana

A summary of the extractable radioactive residue fractions in pulp and peel following three spray applications is shown in Table CA 6.2.1/05-3.

Pulp

Organo-extractable residue

The dichloromethane phase of pulp accounted for 52.2% TRR (0.232 mg eq/kg). The main portion was due to unchanged parent compound (KWG 4168, spiroxamine) accounting for 44.9% of TRR (0.200 mg/kg), whereas each individual metabolite in the organic phase represented ≤ 1.6% of TRR (≤ 0.007 mg eq/kg). Besides the parent compound the minor metabolites designated as spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-N-oxide (M03) contained the intact ketal structure. Each of these metabolites accounted for ≤1.1% of TRR (≤0.005 mg eq/kg). Three TLC regions were quantified as diffuse radioactivity amounting to 2.9% (0.013 mg/kg) of the TRR in total.

Aqueous soluble residue

The aqueous phase of pulp (following partitioning with dichloromethane) contained 40.0% of TRR (0.178 mg eq/kg). Ten metabolites were quantified by radio-HPLC ranging from 1.1% (0.005 mg eq/kg) to 10.4% of TRR (0.046 mg eq/kg). Nine of them were identified or characterised as conjugates of metabolites containing the cyclohexyl ring arising from hydrolysis of the ketal structure of the parent compound.

Cyclohexanol conjugated with a hexose-disaccharide was the main metabolite of the aqueous phase 1 accounting for 10.4% (0.046 mg/kg) of the TRR in pulp. In pulp, a diol-disaccharide (hexose-pentose) accounted for 9.2% (0.041 mg/kg) of the TRR and a small amount of a second cyclohexanol-disaccharide (hexose-pentose) was identified at a level of 3.2% (0.014 mg/kg) of the TRR.

Finally, 70.0% of TRR (0.311 mg eq/kg) could be identified and 22.1% of TRR (0.098 mg eq/kg) characterised by the extraction, partitioning and chromatographic analysis.

Peel

Organo-extractable residue

The dichloromethane phase of peel contained 55.9% of TRR (2.67 mg eq/kg). The main component (42.2% of TRR, 2.02 mg/kg) was identified as unchanged parent compound. In addition, the following metabolites could be identified: spiroxamine-desethyl (M01) (2.4% of TRR, 0.11 mg eq/kg), spiroxamine-despropyl (M02) (2.4% of TRR, 0.11 mg eq/kg) and spiroxamine-N-oxide (M03) (4.9% of TRR, 0.23 mg eq/kg).

A small amount of radioactivity (1.8%, 0.08 mg/kg of the TRR) was designated to a group of metabolites (OP1) being more lipophilic than the parent compound. This metabolite group was isolated and hydrolysed for further characterisation. The nature of the minor metabolite OP2 (0.5% of the TRR) was not further investigated.

Aqueous soluble residue

The aqueous phase of peel contained 39.4% of TRR (1.88 mg eq/kg). Fourteen metabolites or metabolite fractions could be quantified ranging from 0.2% (0.01 mg eq/kg) to 7.4% of TRR (0.35 mg eq/kg). They were identified or characterised as conjugates of that part of the parent molecule containing the cyclohexyl ring arising from hydrolysis of the ketal structure of the parent compound.

Cyclohexanol conjugated with a hexose-disaccharide was the main metabolite of the aqueous phase 1 accounting for 9.1% (0.43 mg/kg) in peel. Three minor metabolites were identified as isomers of cyclohexanol-disaccharides (hexose-pentose) in the range of 3.5% to 7.4% of the TRR (0.17 to 0.35 mg/kg) and, in addition, a diol disaccharide (hexose-pentose) at 5.5% of the TRR (0.26 mg/kg).

A total of 81.2% of TRR (3.87 mg eq/kg) in peel could be identified and 14.1% of TRR (0.67 mg eq/kg) characterised by the extraction, partitioning and chromatographic analysis.

A summary of identified radioactive residues in peel and pulp of mature bananas following three spray applications to the fruits with spiroxamine is presented in Table CA 6.2.1/05-5.

Table CA 6.2.1/05-3 **Summary of extractable radioactive residue fractions in pulp and peel following three spray applications with [cyclohexyl-1-^{14}C]-spiroxamine, sampling directly after the last application**

	Peel		Pulp	
	mg/kg	%TRR	mg/kg	%TRR
Organic (dichloromethane) phase	**0.232**	**52.2**	**2.67**	**55.9**
Spiroxamine, parent	0.200	44.9	2.02	42.2
Non-polar metabolite group 1 (based on ketone)	0.002	0.5	0.08	1.8
Diffuse radioactivity 1	0.007	1.6	0.02	0.4
Spiroxamine-desethyl (M01)	0.005	1.1	0.11	2.4
Spiroxamine-despropyl (M02)	0.002	0.5	0.11	2.4
Spiroxamine-N-oxide (M03)	0.003	0.8	0.23	4.9
Diffuse radioactivity 2	0.002	0.4	0.03	0.5
Metabolite OP2 (unknown)	0.005	1.2	0.03	0.5
Diffuse radioactivity 3	0.004	0.8	0.03	0.5
Polar material (TLC origin)	0.001	0.3	0.01	0.3
Aqueous phase	**0.178**	**40.0**	**1.88**	**39.4**
Diol and spiroxamine-hydroxy ketone-conjugates	-	-	0.09	1.8
Diol-conjugate	0.022	4.8	0.03	0.6
Diol-conjugate	0.015	3.5	0.05	1.0
Diol-conjugate	0.007	1.5	0.03	0.7
Diol-conjugate	0.013	3.0	-	-
Diol-[hexose-pentose]	0.041	9.2	0.26	5.5
Minor double peak (no.3)	0.008	1.9	-	-
Spiroxamine-hydroxy ketone- and diol- and cyclohexanol-conjugates	-	-	0.13	2.7
Cyclohexanol-conjugate	0.005	1.1	0.07	1.4
Cyclohexanol-conjugate	0.006	1.4	0.01	0.2
Cyclohexanol-conjugate	-	-	0.07	1.5
Cyclohexanol-conjugate	-	-	0.2	0.01
Cyclohexanol-[hexose-pentose] (A)	-	-	0.17	3.5
Cyclohexanol-[hexose-pentose] (B)	-	-	0.18	3.7
Cyclohexanol-[hexose-pentose] (C)	0.014	3.2	0.35	7.4
Cyclohexanol-[hexose-hexose]	0.046	10.4	0.43	9.1
Non-extractable, bound residue	0.035	7.8	0.22	4.7
Total residue	**0.444**	**100.0**	**4.77**	**100.0**

Hydrolysis of polar metabolites of spiroxamine in bananas partitioned into the aqueous phase

The metabolite pattern of the aqueous phases of pulp and peel consisting of numerous polar glycosides could not be separated adequately by TLC. Therefore, these conjugates were hydrolysed by HCl and the hydrolysis products were partitioned between the aqueous phase (after neutralization) and toluene. Identification of hydrolysis products (partitioned into toluene) could be achieved by radio-TLC and co-chromatography with reference standards. The summarised amounts of each aglycon (exocon) following hydrolysis are presented in Table CA 6.2.1/05-4

Acid hydrolysis of these polar conjugates released the two postulated major aglycons, i.e. spiroxamine-cyclohexanol (M13) and spiroxamine – diol (M14). Spiroxamine-cyclohexanol accounted for 14.7% of TRR (0.065 mg/kg) in pulp and 26.6% of TRR (1.27 mg/kg) in peel. Spiroxamine-diol (M14) accounted for 20.8% of TRR in pulp (0.092 mg/kg) and 9.4% of TRR (0.45 mg/kg) in peel.

The portions of aglycons resulting from hydrolysis of the complete aqueous phases were in good agreement with the sum of individual aglycons resulting from hydrolysis of purified polar residue fractions representing conjugates.

Table CA 6.2.1/05-4 Hydrolysis products of polar spiroxamine conjugates in bananas treated with [cyclohexyl-1-^{14}C]-spiroxamine

Hydrolysis products of bananas (hydrolysed aqueous phases)	Pulp		Peel	
	%TRR	mg eq/kg	% TRR	mg eq/kg
Spiroxamine-cyclohexanol (M13)	14.7	0.065	26.6	1.27
Spiroxamine-diol (M14)	20.8	0.092	9.4	0.45
Spiroxamine-hydroxy ketone (M16)	n.d.	n.d.	1.6	0.03
Subtotal identified aglycons	(35.5)	(0.158)	(37.6)	(1.79)
Unknown hydrolysis products	4.5	0.020	1.8	0.09
Total	40.0	0.178	39.4	1.88

Table CA 6.2.1/05-5 Summary of identified radioactive residues in pulp and peel of mature bananas following three spray applications to the fruits with [cyclohexyl-1-^{14}C]-spiroxamine

	Pulp		Peel	
Identified metabolites	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Spiroxamine, parent compound	44.9	0.200	42.2	2.02
Spiroxamine-desethyl (M01)	1.1	0.005	2.4	0.11
Spiroxamine-despropyl (M02)	0.5	0.002	2.4	0.11
Spiroxamine-N-oxide (M03)	0.8	0.003	4.9	0.23
Diol-[hexose-pentose]	9.2	0.041	5.5	0.26
Cyclohexanol-[hexose-hexose]	10.4	0.046	9.1	0.43
Cyclohexanol-[hexose-pentose] (A)	-	-	3.5	0.17
Cyclohexanol-[hexose-pentose] (B)	-	-	3.7	0.18
Cyclohexanol-[hexose-pentose] (C)	3.2	0.014	7.4	0.35
Total identified	**70.1**	**0.311**	**81.2**	**3.87**
Identified hydrolysis products				
Spiroxamine-cyclohexanol (M13) (aglycon of further conjugates of aq. phase 1)	2.5	0.011	3.7	0.18
Spiroxamine-diol (M14) (aglycon of further conjugates of aq. phase 1)	12.8	0.057	4.2	0.20
Spiroxamine-hydroxy ketone (M16) (aglycon of further conjugates of aq. phase 1)	-	-	1.3	0.06
Total identified/characterised[1]	**85.4**	**0.379**	**90.4**	**4.31**

1 – Characterised residue includes identified residue from hydrolysis. Not included as primary plant metabolites

Storage stability

The storage stability of parent compound and metabolites was assessed by re-extraction and TLC of frozen pulp and peel samples of bananas following 2-years of storage at ≤- 20°C. The later extraction was conducted analogously to the metabolism experiment. The composition of radioactive Spiroxamine residues extracted shortly after harvest (Oct. 12/13, 1999) and after a 2-year storage period (Nov. 26, 2001) was nearly identical. These data indicated the storage stability of Spiroxamine residues in banana samples for two years if stored at ≤- 20°C.

III. Conclusions

[Cyclohexyl-1-^{14}C]spiroxamine, formulated as a 800 EC formulation, was sprayed three times to bananas bunches when small fruit were present, when medium size fruit were present and just before harvest (0 days) to simulate a total seasonal rate of 3.2 kg a.s./ha (equivalent to 0.8N maximum intended use rate). Peel and pulp were sampled immediately after the last application (day 0).

The total radioactive residue (TRR) accounted for 0.444 mg/kg in pulp and 4.77 mg/kg in peel and was effectively extracted with a methanol/water mixture. The extracted radioactivity (92.2% of the TRR from pulp and 95.3% from peel) was partitioned into a dichloromethane phase and an aqueous phase 1. The dichloromethane phase contained 52.2% (0.232 mg/kg) of the TRR in pulp and 55.9% (2.67 mg/kg) of the TRR in peel. The aqueous phase 1 contained 40.0% (0.178 mg/kg) of the TRR in pulp and 39.4% (1.88 mg/kg) in peel.

Unchanged parent spiroxamine was the predominant compound in pulp accounting for 44.9% (0.200 mg/kg) of the TRR. Identified metabolites with intact ketal-structure were spiroxamine-N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in small amounts, each ≤1.1% (≤0.005 mg/kg) of the TRR. Cyclohexanol conjugated with a hexose-disaccharide accounted for 10.4% (0.046 mg/kg) of the TRR. A diol-disaccharide (hexose-pentose) accounted for 9.2% (0.041 mg/kg) of the TRR and a small amount of a second cyclohexanol-disaccharide (hexose-pentose) was identified at a level of 3.2% (0.014 mg/kg) of the TRR.

Unchanged parent spiroxamine was the predominant compound in peel accounting for 42.2% (2.02 mg/kg) of the TRR. Identified metabolites with intact ketal-structure were spiroxamine-N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in small amounts ≤4.9% (≤0.23 mg/kg) of the TRR. Cyclohexanol conjugated with a hexose-disaccharide accounted for 9.1% (0.43 mg/kg) of the TRR. Three minor metabolites were identified as isomers of cyclohexanol-disaccharides (hexose-pentose) in the range of 3.5% to 7.4% of the TRR (0.17 to 0.35 mg/kg) and, in addition, a diol disaccharide (hexose-pentose) at 5.5% of the TRR (0.26 mg/kg).

In total, 70.0% (0.311 mg/kg) of the TRR was identified in pulp and 81.2% (3.87 mg/kg) in peel. In addition, 22.1% (0.098 mg/kg) of the TRR in pulp and 14.1% (0.67 mg/kg) in peel, was characterised.

The main metabolic step in banana was the cleavage of the ketal structure generating the intermediate cyclohexanone. The ketone was reduced to the major aglycone cyclohexanol. Analogous metabolism was observed for the diol representing the cyclohexanol-structure however, with an oxidised t-butyl-group. Both major intermediates were intensively conjugated to glycosides. Based on the results of grapes and banana it was concluded that glucose-conjugates were the most important monosaccharides which were further conjugated. A minor metabolic step was the oxidation of the parent compound at the tertiary amine moiety to yield spiroxamine-N-oxide (M03). Another minor metabolic step was the desalkylation of the tertiary amine moiety to generate spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02).

The proposed metabolic pathway for spiroxamine in fruits is shown in Figure CA 6.2-4.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.2.1/05 and IIA 6.2.1/06. The study is considered compliant with OECD Guideline 501 – Metabolism in Crops, January 2007.

The main metabolic step in banana was the cleavage of the ketal structure generating the intermediate cyclohexanone. The ketone was reduced to the major aglycone cyclohexanol. Analogous metabolism was observed for the diol representing the cyclohexanol-structure however with an oxidised t-butyl-group. Both major intermediates were intensively conjugated to glycosides. Based on the results of grapes and banana it was concluded that glucose-conjugates were the most important monosaccharides which were further conjugated. A minor metabolic step was the oxidation of the parent compound at the tertiary amine moiety to yield spiroxamine-N-oxide. Another minor metabolic step was the desalkylation of the tertiary amine moiety to generate spiroxamine-desethyl and spiroxamine-despropyl.

Data Point:	KCA 6.2.1/07
Report Author:	[redacted]
Report Year:	2001
Report Title:	Metabolism of [1,3-dioxolane-4-14C]KWG4168 in banana (analytical part)
Report No:	MR-139/01
Document No:	M-088457-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Note to reviewer: CA 6.2.1/05 details the in-life phase of both cyclohexyl and 1,3-dioxalane spiroxamine on bananas. These are fully detailed in the study summary presented under CA 6.2.1/06.

Executive summary

[1,3-dioxolane-4-^{14}C]spiroxamine, formulated as a 800 EC formulation, was sprayed three times to bananas bunches when small fruit were present, when medium size fruit were present and just before harvest (0 days) to simulate a total seasonal rate of 3.2 kg a.s./ha (equivalent to 0.8N maximum intended use rate). Peel and pulp were sampled immediately after the last application (day 0).

The total radioactive residue (TRR) accounted for 0.553 mg/kg in pulp and 6.60 mg/kg in peel and was effectively extracted with a methanol/water mixture. The extracted radioactivity (98.0% of the TRR from pulp and 96.8% from peel) was partitioned into a dichloromethane phase and an aqueous phase. The dichloromethane phase contained 63.0% (0.349 mg/kg) of the TRR in pulp and 55.0% (3.63 mg/kg) of the TRR in peel. The aqueous phase contained 35.0% (0.194 mg/kg) of the TRR in pulp and 41.8% (2.76 mg/kg) in peel.

Unchanged parent spiroxamine comprised the predominant residue in pulp accounting for 60.0% (0.333 mg/kg) of the TRR. The predominant metabolite was spiroxamine-aminodiol (M28) accounting for 30.2% (0.167 mg/kg) of the TRR. Identified metabolites with intact ketal-structure were spiroxamine -N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in small

amounts, each ≤1.2% (≤0.007 mg/kg) of the TRR. Small amounts of spiroxamine-desethyl-aminodiol (M30) and spiroxamine-despropyl-aminodiol (M31) were also detected at ≤0.6% (≤0.003 mg/kg) of the TRR.

Unchanged parent spiroxamine also comprised the predominant residue in peel accounting for 42.3% (2.79 mg/kg) of the TRR. The most abundant metabolite was spiroxamine-aminodiol (M28) accounting for 36.1% (2.38 mg/kg). Identified metabolites with intact ketal-structure were spiroxamine -N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) each ≤ 4.2% (≤0.28 mg/kg) of the TRR. Small amounts of spiroxamine-desethyl-aminodiol (M30) and spiroxamine-despropyl-aminodiol (M31) were also detected at 0.9% (0.06mg/kg) of the TRR.

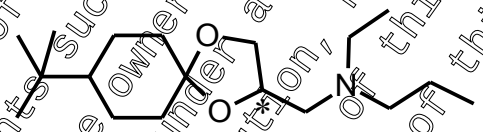
In total, 94.9% (0.525 mg/kg) of the TRR was identified in pulp and 91.0% (6.01 mg/kg) in peel. In addition, 3.1% (0.017 mg/kg) of the TRR in pulp and 5.8% (0.38 mg/kg) in peel, was characterised.

In summary, unchanged parent spiroxamine and the aminodiol moiety were identified as the major components of the residue in banana peel and pulp following application of dioxolane-labelled spiroxamine. The main metabolic process involved the cleavage of the ketal ring structure, leading to formation of the spiroxamine-aminodiol. Other minor processes involved the oxidation of the nitrogen of the tertiary amine group resulting in the formation of the N-oxide and the desalkylation of the amino group, forming the desethyl and despropyl aminodiol moieties.

I. Materials and methods

A. Test material

[1,3-dioxolane-4-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (MBq/mg)	1.61 (43.5 µCi/mg)
Lot/Batch No.:	Not stated
Purity:	Radiochemical purity > 98% (sum of isomers) before and after formulation, and before application; 96.6 to 97.5% after application
Storage condition:	Not stated
CAS No.:	118134-30-8

B. Study design

Test system

Soil

A sandy loam soil from Litchfield Ranch, Watsonville, CA, USA was used. The physico-chemical properties were not determined. The soil had not had any previous exposure to spiroxamine.

Test system

The study design of the in-life study phase, spray application, sampling and shipment to the Bayer AG for analysis is already described in the previous study summary employing the alternative cyclohexyl label (see CA 6.2.1.06 for full application details). Therefore, only the analytical portion and the results are presented in the following description.

Test design

The experimental work was conducted between June 1999 and March 2001. The in-life phase was conducted at the field test site of Plant Sciences, Inc., Watsonville, USA and the test site of PTRL West, Inc., Richmond, USA. The analytical phase and final reporting was conducted at the Institute for Metabolism Research and Residue Analysis of Bayer AG, Monheim, Germany.

Sample preparation and extraction

The pulp sample was thawed at room temperature and cut in 2 – 4 mm slices. Whereas the main sample was again stored frozen at – 20°C. A subsample was twice extracted with methanol followed by methanol/water (1/1, v/v) by means of a Polytron high-speed stirrer. The filtered and combined extracts were concentrated to the aqueous remainder using a rotary evaporator. The extracted residues were then partitioned between water and dichloromethane. Aliquots of the solid filter cake (containing non-extractable residues) were combusted and trapped in LSC cocktail and the radioactivity content of the liquid phases was determined by LSC. Aliquots of the organic fraction were also investigated by radio-TLC. Aliquots of the aqueous fraction were investigated by radio-HPLC.

The peel sample was homogenised in the presence of liquid nitrogen to yield a fine powder. A subsample was extracted with methanol and methanol/water followed by water-dichloromethane partitioning as done with the pulp sample.

For purification of non-polar metabolites aliquots of the dichloromethane extract were subjected to semi-preparative TLC with silica gel coated aluminium plates and acetonitrile/water/25% ammonia (80/18/2, v/v/v) as developing solvent mixture. After cutting out of the respective zone and extraction of the radioactivity with methanol, the semi-chromatography was repeated using the same solvent mixture. Cutting out of the radioactive band, desorption with methanol and evaporation of methanol yielded radioactive residues that could be subjected to analytical co-chromatographic TLC and to hydrolysis followed by co-chromatography.

For hydrolysis of chromatographically purified residue fractions partitioned into the dichloromethane phase these were heated with 1 N HCl and toluene (1 hour, 100°C) in a screw-cap tube. After cooling to room temperature the phases were separated, the aqueous phase twice extracted with toluene and the combined toluene phases radioassayed (analysed for radioactivity) and investigated by radio-TLC with reference standards. Polar residues of the aqueous phase after partitioning with dichloromethane were hydrolysed in the same way. Subsamples of complete pulp and peel samples were subjected to the same hydrolysis conditions.

Radiochemical analysis

Radioactivity determination

Determination of ^{14}C-radioactivity was conducted by liquid scintillation counting (LSC). Aliquots of liquid samples were mixed to Quickszafe A + 5% water scintillation cocktail (Zinsser Analytic) for scintillation counting. Aliquots (50 – 200 mg) of solid samples were first combusted in an OX 500 Oxidiser (Zinsser Analytic). The formed $^{14}CO_2$ was absorbed in the basic scintillation cocktail Oxysolve C-400 (Zinsser Analytic).

Thin layer chromatography (TLC):

Separation of organo-soluble radioactive residue components was performed by radio-thin layer chromatography (TLC) employing silica gel plates (60 F_{254} , 20 x 20 cm glass plates coated with 0.25 mm separation layer or 60 F_{254}, 20 x 20 cm aluminium plates coated with 0.2 mm silica gel; Merck). Three different solvent mixtures were used for development: (I) acetonitrile/ water/25% ammonia (80/18/2, v/v/v), (II) chloroform/methanol/25% ammonia (65/28/8, v/v/v) and (III) ethyl acetate/2-propanol/water (5/3/1, v/v/v). Quantitative radio detection was performed by a Bio-Imaging Analyser (BAS 2000, Fuji) and a TINA 2.09 software package (Raytest). In case of the semi-preparative isolation of metabolites from the dichloromethane phase of peel, 200 µL-subsamples were applied as a

chromatographic zone. Non-radioactive reference standards were visualized by spot darkening in an iodine chamber.

The active substance and some of the metabolites contain asymmetric centres in the molecule and, therefore, form diastereomers. For this reason, these substances were observed occasionally as double peaks (if the chromatographic conditions ensure sufficient resolution power).

Gas chromatography/mass spectroscopy (GC/MS)

For identification of the parent substance and a reference standard a combination of a Varian gas chromatograph equipped with a 15 m DBXLB capillary column (J&W Scientific) and an INCOS XL mass spectrometer (Finnigan) was used. Electron impact mode was selected for ionisation. Helium was used as carrier gas and a temperature program ranged from 60 to 310°C. Splitless injection mode was used.

II. Results and discussion

Total radioactive residue (TRR)

The total radioactive residues (TRR) in bananas sampled immediately after the third application of dioxolane-labelled spiroxamine accounted for 0.553 mg parent equivalents/kg (mg eq/kg) in pulp and 6.60 mg eq/kg in peel of intact bananas.

In pulp, the extracted radioactivity was distributed into a dichloromethane phase (63.0% of the TRR, 0.349 mg/kg) and an aqueous phase (35.0% of the TRR, 0.194 mg/kg). Only 2.0% (0.011 mg/kg) of the TRR was found in the solids. The radioactivity detected in the combined methanol/water extract was recovered nearly quantitatively (99.6%) in the dichloromethane phase and aqueous phase following evaporation and partitioning. The radioactivity determined in the dichloromethane phase and aqueous phase was normalised to the original radioactivity found in the methanol/water extract.

In peel, the extraction procedure and partitioning was conducted as described for pulp. The dichloromethane phase accounted for 55.0% (3.63 mg/kg) of the TRR and the aqueous phase represented 41.8% (2.76 mg/kg) of the TRR. The radioactivity was quantitatively recovered (99.2%). Only 3.2% (0.21 mg/kg) of the TRR was found in the solids.

TRR and distribution of radioactivity are given in Table CA 6.2.1/07-1.

Table CA 6.2.1/07-1 Distribution of radioactive residues in mature bananas following three spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine, directly after the last application

Radioactive residues in banana	Pulp		Peel	
	mg/kg	%TRR	mg/kg	%TRR
TRR[1]	0.553	100.0	6.60	100.0
Dichloromethane phase[2]	0.349	63.0	3.63	55.0
Aqueous phase[2]	0.194	35.0	2.76	41.8
Post-extraction solids (PES)	0.011	2.0	0.21	3.2

1 - total radioactive residues given in mg parent substance equivalents per kg, determined by summation of all fractions after extraction
2 - primary methanol/water extract was concentrated and partitioned between dichloromethane and water

Characterisation

Available synthesised compounds for characterisation were parent spiroxamine (KWG 4168) and the following potential metabolites:

FHW01041 (spiroxamine-desethyl) (M01)

WAK5868/2 (spiroxamine-hydroxyl) (M05)

WAK6174 (spiroxamine-despropyl) (M02)

WAK6301/1 (spiroxamine -N-oxide) (M03)

WAK5427 (spiroxamine-aminodiol) (M28)

WAK6885 (spiroxamine-aminodiol-N-oxide) (M29)

WAK6893 (spiroxamine-despropyl-aminodiol) (M31)

WAK6894 (spiroxamine-desethyl-aminodiol) (M30)

Each metabolite was identified by co-chromatography reference standards.

Residues in [^{14}C]spiroxamine treated banana

A summary of the extractable radioactive residue fractions in pulp and peel following three spray applications is shown in Table CA 6.2.1/07-2. A summary of the identified metabolites is presented in Table CA 6.2.1/07-3.

Pulp

Organo-extractable residue

The dichloromethane phase of pulp accounted for 63.0% of TRR (0.349 mg eq/kg). The main portion was due to unchanged parent spiroxamine accounting for 60.0% of TRR (0.333 mg/kg), whereas each individual metabolite in the organic phase represented ≤1.0% of TRR (≤0.006 mg eq/kg). Besides the parent compound the minor metabolites designated as KWG spiroxamine-desethyl (M01), spiroxamine-despropyl (M02), and spiroxamine -N-oxide (M03) contained the intact ketal structure. A small amount of aminodiol (M28) (1.0%, 0.006 mg/kg of the TRR) was also observed in the organic phase.

Aqueous soluble residue

The aqueous phase of pulp (following partitioning with dichloromethane) contained 35.0% of TRR (0.194 mg eq/kg). Almost the complete radioactivity was represented by aminodiol (M28) (30.2%, 0.167 mg eq/kg) originating from hydrolysis of the ketal structure of the parent compound. Small amounts of spiroxamine-desethyl-aminodiol (M30) and spiroxamine-despropyl-aminodiol (M31) (each 0.6% of TRR, 0.003 mg eq/kg) and spiroxamine-N-oxide (M03) (0.7% of TRR, 0.004 mg eq/kg) could also be detected. Due to matrix influence and relatively low residues three TLC regions were quantified as diffuse radioactivity accounting for 2.4% (0.013 mg/kg) of the TRR in total.

As a result, the distribution of parent compound, identified metabolites and unknown components are summarised in table CA 6.2.1/07-2. The identified components of the terminal residue in pulp, are summarised in Table CA 6.2.1/07-3.

In total, 94.9% of TRR (0.525 mg eq/kg) in pulp was identified and 3.1% of TRR (0.017 mg eq/kg) characterised by the extraction, partitioning and chromatographic analysis.

Peel

Organo-extractable residue

The dichloromethane phase of peel contained 55.0% of TRR (3.63 mg eq/kg). The main component (42.3% of TRR, 2.79 mg/kg) was identified as unchanged parent compound. In addition, the following metabolites could be identified: spiroxamine-desethyl (M01) (2.7% of TRR, 0.18 mg eq/kg), spiroxamine-despropyl (M02) (2.9% of TRR, 0.19 mg eq/kg), spiroxamine -N-oxide (M03) (3.6% of TRR, 0.24 mg eq/kg) and aminodiol (M28) (1.1% of TRR, 0.07 mg eq/kg).

A small amount of radioactivity (1.7%, 0.11 mg/kg of the TRR) was designated to a group of metabolites (MG1) being more lipophilic than the parent compound. This metabolite group was isolated and hydrolysed for further characterisation. The two main components were identified as spiroxamine-desethyl-aminodiol (M30) (0.6%, 0.04 mg/kg of TRR) and spiroxamine-despropyl-aminodiol (M31)

(0.5%, 0.03 mg/kg of TRR). Three unknown hydrolysis products accounted for ≤0.3% (≤0.02 mg/kg) each.

Aqueous soluble residue

The aqueous phase of peel (41.8% of TRR, 2.76 mg eq/kg) contained aminodiol (M28) as the predominant metabolite amounting to 36.1% of the TRR, 2.38 mg eq/kg. Small amounts of spiroxamine-desethyl-aminodiol (M30), spiroxamine-despropyl-aminodiol (M31) (each 0.9% of TRR, 0.06 mg eq/kg) and spiroxamine-N-oxide (M03) (0.6% of TRR, 0.04 mg eq/kg) were also detected. Other individual metabolites or groups of them were below 0.8% of TRR (0.06 mg eq/kg). It was concluded that spiroxamine-aminodiol-N-oxide (M29) was not present as a metabolite, however was confirmed as a hydrolysis product from spiroxamine-N-oxide (M03).

In total, 91.0% of TRR (6.01 mg/kg) in peel was identified and 5.8% (0.38 mg/kg) of TRR was characterised by the extraction procedure, partitioning and TLC-analysis.

Table CA 6.2.1/07-2 Summary of extractable radioactive residue fractions in pulp and peel following three spray applications with [1,3-dioxolane-4-^{14}C]-spiroxamine, sampling directly after the last application

	Peel		Pulp	
	mg/kg	%TRR	mg/kg	%TRR
Organic (dichloromethane) phase	**0.349**	**63.0**	**3.63**	**55.0**
Spiroxamine, parent	0.333	60.0	2.79	42.3
Spiroxamine-desethyl (M01)	0.005	0.9	0.18	2.7
Spiroxamine-despropyl (M02)	0.002	0.4	0.19	2.9
Spiroxamine -N-oxide (M03)	0.003	0.5	0.24	3.6
Spiroxamine-aminodiol (M28)	0.006	1.0	0.07	1.1
TLC-origin	0.001	0.1	0.01	0.2
Metabolite Group 1 (MG1)[1]	-	-	0.11	1.7
Diffuse activity	-	-	0.03	0.5
Metabolite U1 (unknown)	-	-	0.01	0.2
Aqueous phase	**0.194**	**35.0**	**2.76**	**41.8**
Spiroxamine-N-oxide (M03)	0.004	0.7	0.04	0.6
Spiroxamine-aminodiol (M28)	0.167	30.2	2.38	36.1
Diffuse activity 1	0.007	1.2	0.04	0.7
Spiroxamine-desethyl-aminodiol (M30)	0.003	0.6	0.06	0.9
Diffuse activity 2	0.003	0.6	0.05	0.7
Spiroxamine-despropyl-aminodiol (M31)	0.003	0.6	0.06	0.9
Diffuse activity 3	0.003	0.6	0.06	0.8
TLC-origin	0.003	0.6	0.03	0.4
Metabolite U2 (unknown)			0.04	0.6
Non-extractable, bound residue	0.011	2.0	0.21	3.2
Total residue	**0.553**	**100.0**	**6.60**	**100.0**

1 - More lipophilic than the parent compound. After hydrolysis the two main components were identified as spiroxamine-desethyl-aminodiol (M30) (0.6%TRR, 0.04 mg/kg), spiroxamine-despropyl-aminodiol (M31) (0.5%TRR, 0.03 mg/kg) and three unknown hydrolysis products (each ≤0.3%TRR, ≤0.02 mg/kg)

Table CA 6.2.1/07-3 Summary of identified metabolites radioactive residues in the pulp and peel of mature bananas following three spray applications to the fruits with [1,3-dioxolane-4-^{14}C]-spiroxamine

	Pulp		Peel	
Identified metabolites	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Spiroxamine, parent compound	60.0	0.333	42.3	2.79
Spiroxamine-desethyl (M01)	0.9	0.005	2.7	0.18
Spiroxamine-despropyl (M02)	0.4	0.002	2.9	0.19
Spiroxamine -N-oxide (M03)	1.2	0.007	4.2	0.28
Spiroxamine-aminodiol (M28)	31.2	0.173	37.2	2.45
Spiroxamine-desethyl-aminodiol (M30)	0.6	0.003	0.9	0.06
Spiroxamine-despropyl-aminodiol (M31)	0.6	0.003	0.9	0.06
Total identified	**94.9**	**0.525**	**91.0**	**6.01**

Hydrolysis of spiroxamine residues in pulp and peel of bananas

Subsamples (10 g) of pulp and peel samples were also subjected to acidic hydrolysis. A total of 98.9% of TRR of pulp and 97.4% of TRR of peel was released into the acidic filtrate following hydrolysis. The total radioactive residues of both samples were higher than in the metabolism study probably due to the inhomogeneity of residues in smaller samples employed in the hydrolysis experiment. Aliquots of neutralised filtrate were analysed by quantitative radio-TLC using reference standards for chromatographic comparison. The composition of the hydrolysis products in peel and pulp is presented in Table CA 6.2.1/07-4.

In pulp hydrolysate spiroxamine-aminodiol (M28) was the predominant compound accounting for 85.8% of TRR (0.718 mg eq/kg). Spiroxamine - -desethyl-aminodiol (M30) accounted for 1.8% of TRR (0.015 mg eq/kg) and spiroxamine-despropyl-aminodiol (M31) to 1.4% of TRR (0.011 mg eq/kg). spiroxamine-aminodiol-N-oxide (M29) was only detected in traces (0.7% of TRR, 0.006 mg eq/kg). A small amount of non-polar radioactivity (3.3%, 0.028 mg/kg) was due to unhydrolysed parent spiroxamine. Three unknown minor hydrolysis products were at or below 1.8% (0.015 mg/kg) of the TRR.

In peel hydrolysate spiroxamine-aminodiol (M28) also proved to be the main compound accounting for 77.8% of TRR (5.44 mg eq/kg). Spiroxamine-aminodiol-N-oxide (M29) accounted for 2.7% of TRR (0.19 mg eq/kg), spiroxamine-desethyl-aminodiol to 4.7% of TRR (M30) (0.33 mg eq/kg) and spiroxamine-despropyl-aminodiol (M31) to 4.2% of TRR (0.30 mg eq/kg).

Table CA 6.2.1/07-4 Hydrolysis products of Spiroxamine residues in banana pulp and peel treated with [1,3-dioxolane-4-^{14}C]-spiroxamine

Identified metabolites	Pulp		Peel	
	%TRR	mg/kg	%TRR	mg/kg
Spiroxamine-aminodiol (M28)	85.8[1]	0.718	77.8	5.44
Spiroxamine-aminodiol-N-oxide (M29)	0.7	0.006	2.7	0.19
Spiroxamine-desethyl-aminodiol (M30)	1.8	0.015	4.7	0.33
Hydrolysis product 1 (unknown)	1.8	0.015	2.6	0.19
Spiroxamine-despropyl-aminodiol (31)	1.4	0.011	4.2	0.30
Hydrolysis product 2 (unknown)	1.2	0.010	0.8	0.06
Hydrolysis product 3 (unknown)	1.2	0.010	1.4	0.10
Hydrolysis product 4 (unknown)	-	-	1.1	0.08
Total	98.9	0.827	97.4	6.81

1 - sum equivalent to 89.1% of TRR, because 3.3% was due to unhydrolysed parent spiroxamine

Storage stability

The storage stability of parent compound and metabolites was assessed by re-extraction and TLC of frozen pulp and peel samples of bananas following approximately 1.5 years of storage at ≤ -20°C. The later extraction was conducted analogously to the metabolism experiment. The composition of radioactive spiroxamine residues extracted shortly after harvest (Oct. 12/13, 1999) and after a 1.5-year storage period (March 23, 2001) was nearly identical. These data indicated the storage stability of Spiroxamine residues in banana samples for two years if stored at ≤ -20°C.

III. Conclusions

[1,3-dioxolane-4-^{14}C]-spiroxamine, formulated as a 800 EC formulation, was sprayed three times to bananas bunches when small fruit were present, when medium size fruit were present and just before harvest (0 days) to simulate a total seasonal rate of 3.2 kg a.s./ha (equivalent to 0.8N maximum intended use rate). Peel and pulp were sampled immediately after the last application (day 0).

The total radioactive residue (TRR) accounted for 0.556 mg/kg in pulp and 6.60 mg/kg in peel and was effectively extracted with a methanol/water mixture. The extracted radioactivity (98.0% of the TRR from pulp and 96.8% from peel) was partitioned into a dichloromethane phase and an aqueous phase. The dichloromethane phase contained 63.0% (0.349 mg/kg) of the TRR in pulp and 55.0% (3.63 mg/kg) of the TRR in peel. The aqueous phase contained 35.0% (0.194 mg/kg) of the TRR in pulp and 41.8% (2.76 mg/kg) in peel.

Unchanged parent spiroxamine was the predominant compound in pulp accounting for 60.0% (0.333 mg/kg) of the TRR. The predominant metabolite was spiroxamine-aminodiol (M28) accounting for 30.2% (0.167 mg/kg) of the TRR. Identified metabolites with intact ketal-structure were spiroxamine-N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in small amounts, each ≤1.2% (≤0.007 mg/kg) of the TRR. Small amounts of spiroxamine- desethyl-aminodiol (M30) and spiroxamine-despropyl-aminodiol (31) were also detected at ≤0.6% (≤0.003 mg/kg) of the TRR.

Unchanged parent spiroxamine was main constituent of the residue in peel accounting for 42.3% (2.79 mg/kg) TRR. The predominant metabolite was spiroxamine-aminodiol (M28) accounting for 36.1% (2.38 mg/kg). Identified metabolites with intact ketal-structure were spiroxamine-N-oxide (M03), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) each ≤ 4.2% (≤0.28 mg/kg) of the TRR. Small amounts of spiroxamine-desethyl-aminodiol (M30) and spiroxamine-despropyl-aminodiol (M31) were also detected at 0.9% (0.06mg/kg) of the TRR.

In total, 94.9% (0.525 mg/kg) of the TRR was identified in pulp and 91.0% (6.01 mg/kg) in peel. In addition, 3.1% (0.017 mg/kg) of the TRR in pulp and 5.8% (0.38 mg/kg) in peel, was characterised.

In summary, unchanged parent spiroxamine and the aminodiol moiety were identified as the major components of the residue in banana peel and pulp following application of dioxolane-labelled spiroxamine. The main metabolic process involved the cleavage of the ketal ring structure, leading to formation of the aminodiol. Other minor processes involved the oxidation of the nitrogen of the tertiary amine group resulting in the formation of the N-oxide and the desalkylation of the amino group, forming the desethyl and despropyl aminodiol moieties.

The proposed metabolic pathway for spiroxamine in fruits is shown in Figure CA 6.2-4.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU-RAR Annex B7 (2009), IIA 6.2.1/05 and IIA 6.2.1/07. The study is considered compliant with OECD Guideline 501 – Metabolism in Crops, January 2007.

Unchanged parent spiroxamine and the aminodiol moiety were identified as the major components of the residue in banana peel and pulp following application of dioxolane-labelled spiroxamine. The main metabolic process involved the cleavage of the ketal ring structure, leading to formation of the aminodiol. Other minor processes involved the oxidation of the nitrogen of the tertiary amine group resulting in the formation of the N-oxide and the desalkylation of the amino group, forming the desethyl and despropyl aminodiol moieties.

Applicant conclusion on plant metabolism

The metabolism of spiroxamine in plants is summarised in Figure CA 6.2-2.

The metabolism studies summarised in this dossier show that the metabolic pathway of spiroxamine was broadly similar in crops. The metabolism has been extensively investigated in three crops; wheat, grapes and banana, representing cereal/grass and fruit crop groups as outlined in OECD 501, Metabolism in crops (Adopted 8 January 2007). Some differences were however noted between metabolism in cereal crops and in fruiting crops.

Spiroxamine is extensively metabolised in wheat. Oxidation occurred preferentially in the tertiary amine group (formation of the spiroxamine-N-oxide) and also to a minor extent in the tert.-butyl group of the molecule (spiroxamine-hydroxyl). The metabolites bearing an hydroxylated tert.-butyl group were further conjugated in different ways (e.g. metabolites spiroxamine-hydroxy-N-oxide malonyl glucoside). Some metabolites were formed by desalkylation (spiroxamine-desethyl, spiroxamine-despropyl) which were partly metabolised by formylation (spiroxamine-N-formyl-desethyl). Only low amounts of the parent compound or metabolites were cleaved at the spiro position indicating the stability of this position.

In grapes, the metabolism of spiroxamine proceeded via cleavage of the ketal structure. The cleavage product containing the cyclohexyl label (spiroxamine-ketone) was very transient as it could not be detected in the free form. It was reduced to the corresponding alcohol (spiroxamine-cyclohexanol) that was also transient. This alcohol was conjugated with different plant endocons yielding either long chain fatty acid esters or different glucose derivatives. It was also shown that spiroxamine was not translocated from leaves to berries of the grape vine plants. After application of dioxolane radiolabelled spiroxamine, unchanged spiroxamine and spiroxamine-aminodiol were identified as the major components of the residue in grapes. Three further metabolites with the aminodiol moiety were identified in grapes at low levels (spiroxamine-aminodiol-N-oxide, Spiroxamine-desethyl-aminodiol and Spiroxamine-despropyl-aminodiol). The main metabolic reactions were hydrolytic ketal cleavage, desalkylation and oxidation. The aminodiols predominantly remained unconjugated as indicated by a similar composition of residues following aqueous/organic extraction and acid hydrolysis of the grapes.

A similar pattern of metabolism was seen in bananas. The main metabolic step in banana was the cleavage of the ketal structure generating the intermediate cyclohexanone. The ketone was reduced to the major aglycon cyclohexanol. Analogous metabolism was observed for the diol representing the cycohexanol-structure however, with an oxidised t-butyl-group. Both major intermediates were intensively conjugated to glycosides. In addition, bananas sprayed with dioxolane-labelled spiroxamine saw unchanged parent spiroxamine and the aminodiol moiety identified as the major components of the residue in banana peel and pulp. Here again, cleavage of the ketal ring structure, lead to the formation of the spiroxamine – aminodiol.

Based on the results of grapes and banana it was concluded that glucose-conjugates were the most important monosaccharides which were further conjugated. A minor metabolic step was the oxidation of the parent compound at the tertiary amine moiety to yield spiroxamine-N-oxide. Another minor metabolic step was the desalkylation of the tertiary amine moiety to generate spiroxamine-desethyl and spiroxamine-despropyl.

Parent spiroxamine is the major component of the residue in primary crops cereals and fruits as well as in rotational crops.

Plant residue definition for monitoring: spiroxamine (parent only)

Plant residue definition for risk assessment in <u>cereals and rotational crops</u>: proposed as sum of spiroxamine and metabolites containing the tert.-butylcyclohexanone moiety, expressed as spiroxamine

Plant residue definition for risk assessment in fruits: proposed as sum of spiroxamine and metabolites containing the aminodiol (N-ethyl-N-propyl-1,2-dihydroxy-3-amino-propane), expressed as spiroxamine

Please refer to CA 6.7.1.

Figure CA 6.2-2 Metabolic pathway of spiroxamine in cereals (majority Group A metabolites)

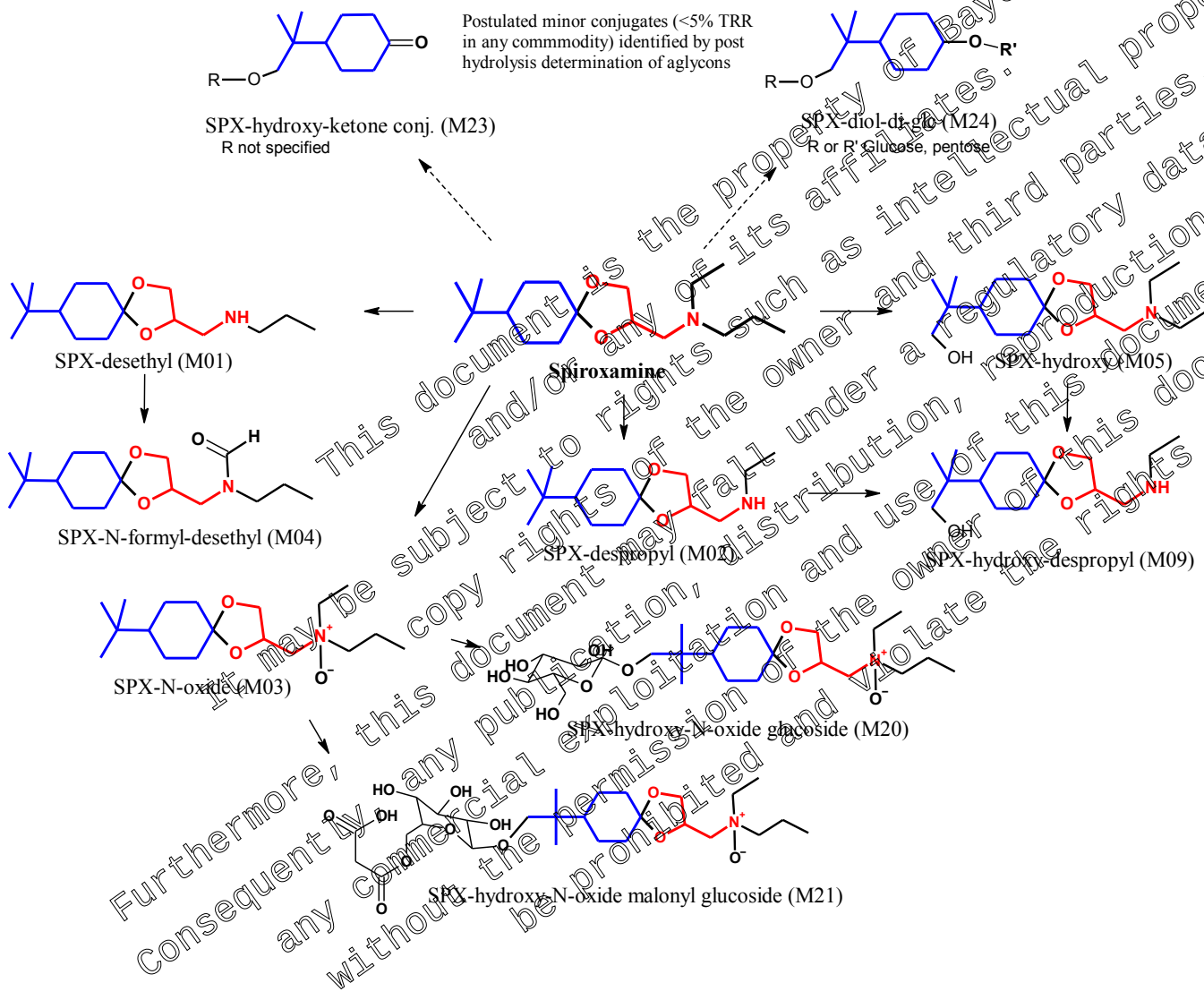


Figure CA 6.2-3 Hydrolysis to common moiety for residue analysis of total spiroxamine in cereals (Group A metabolites)

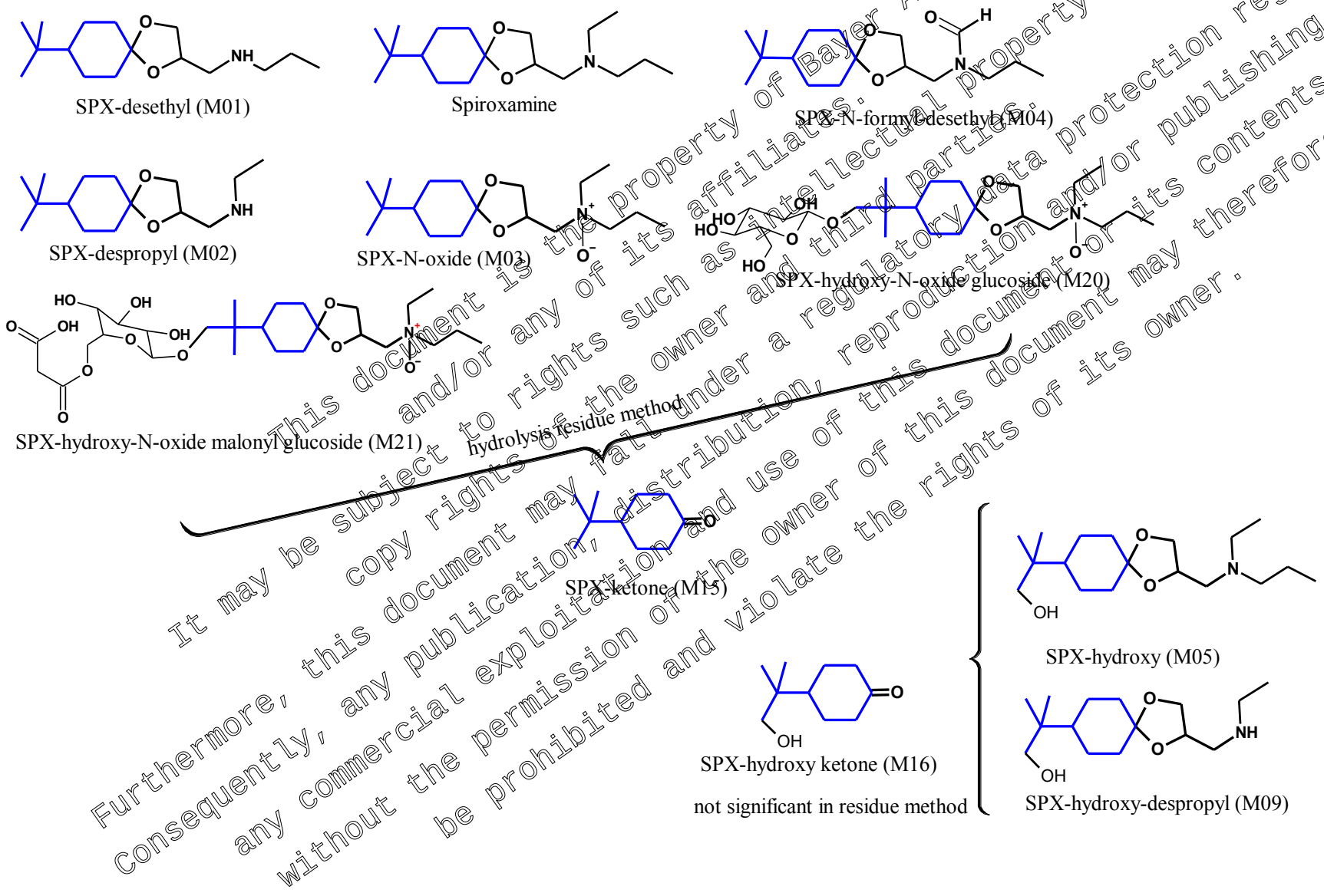


Figure CA 6.2-4 Metabolic pathway of spiroxamine in grapes (and other fruit crops)

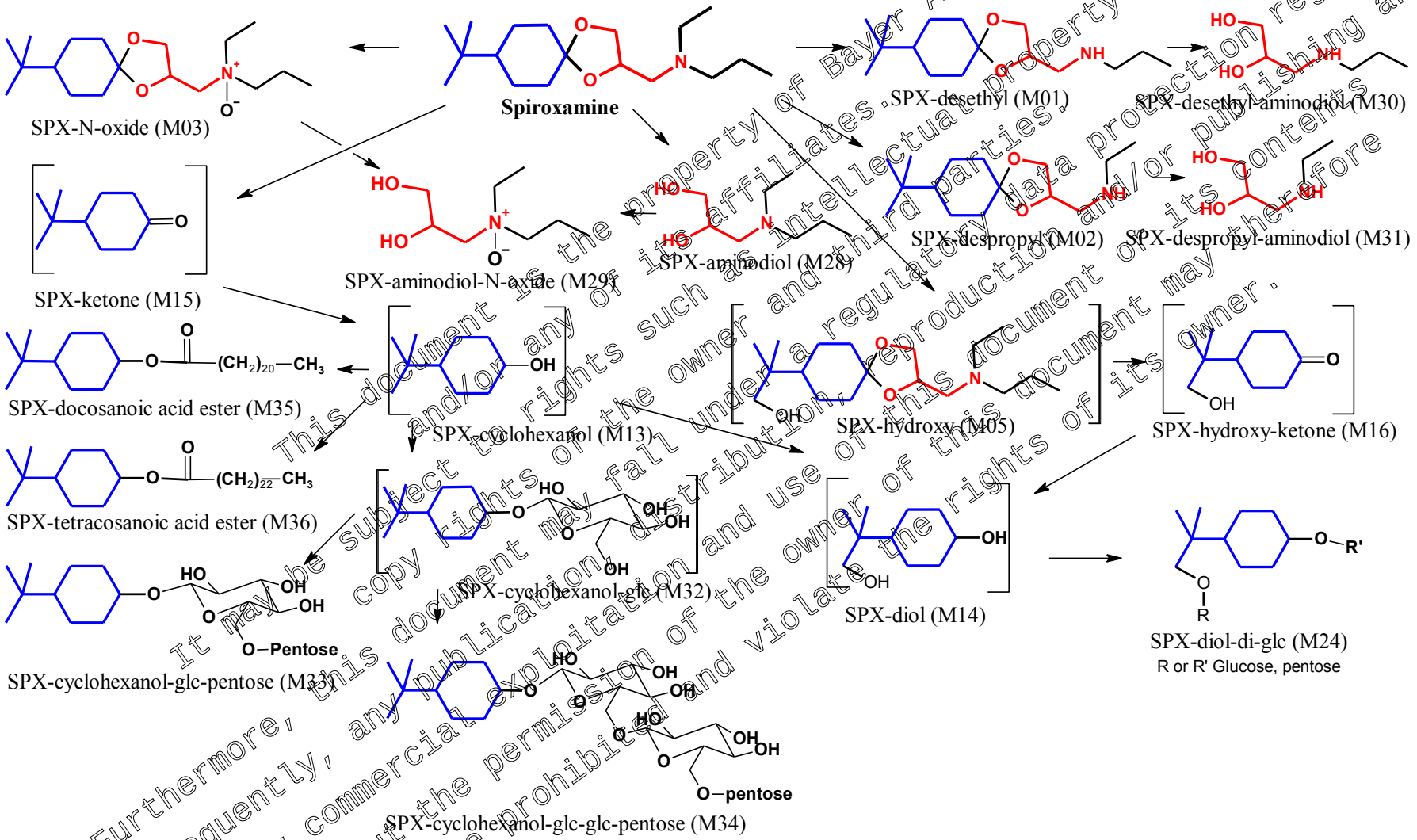
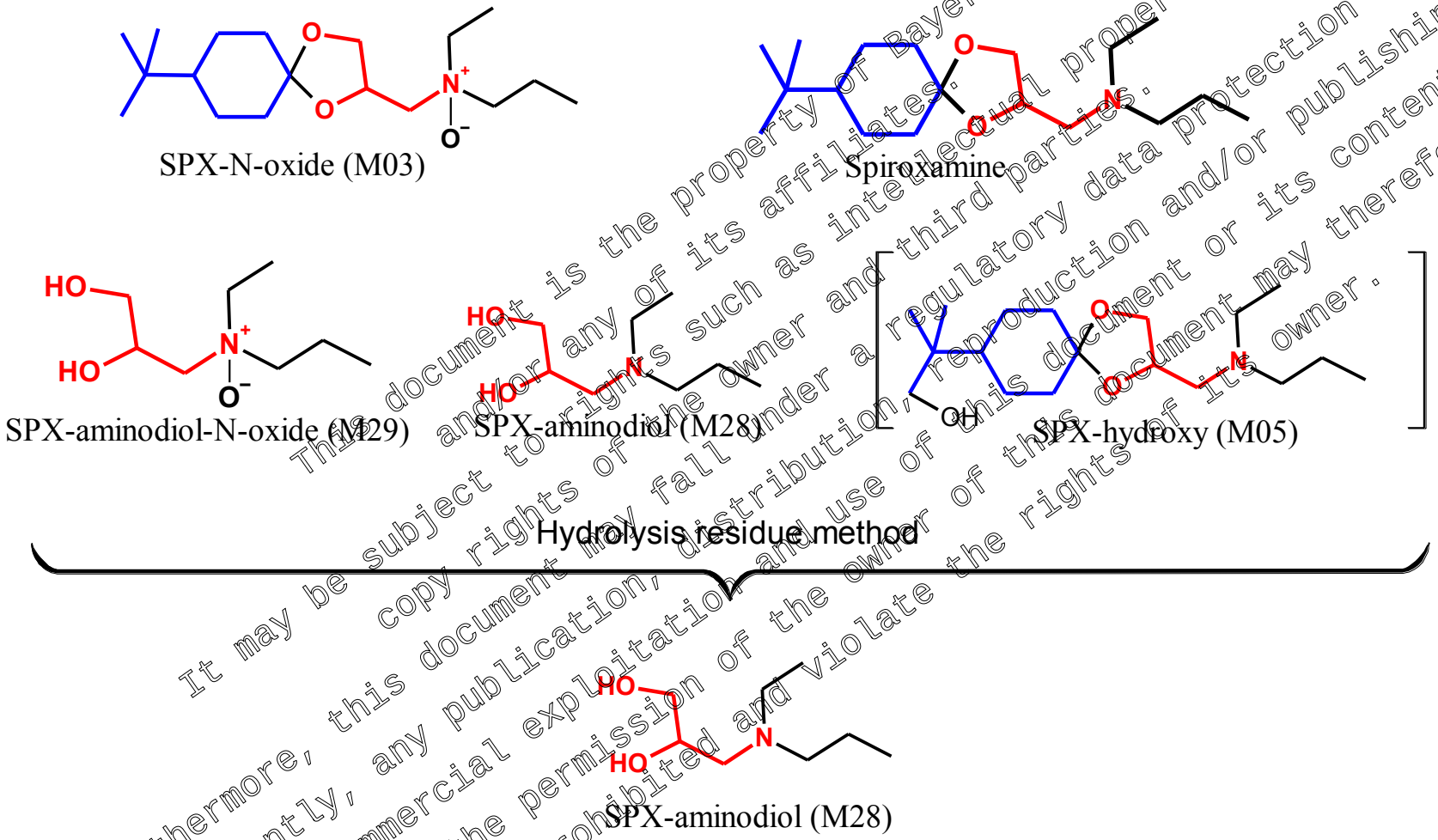


Figure CA 6.2-5 Hydrolysis to common moiety for residue analysis of total spiroxamine in grapes (using Group C aminodiol moiety)



CA 6.2.2 Poultry

Data Point:	KCA 6.2.2/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	[Cyclohexyl-1-14C] KWG 4168: Absorption, distribution, excretion and metabolism in laying hens
Report No:	PF4068
Document No:	M-006038-01-1
Guideline(s) followed in study:	U.S. EPA FIFRA Guideline, Subdivision 0, No. 171-4(b)
Deviations from current test guideline:	Yes OECD 503 (2007) requires that birds (hens) are dosed for at least 7 days. The birds in this study were dosed on a single occasion or over 3 consecutive days. The guideline also recommends that 10 birds are dosed. Only 6 per treatment group were dosed in this study. Plateau levels of residues in eggs were not demonstrated.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

Two groups of six laying hens (*Gallus gallus domesticus*, strain white leghorn) were administered [^{14}C]-spiroxamine (labelled in the 1-position of the cyclohexyl-ring) in either a single oral dose of 10 mg/kg bodyweight (~15.7 mg/hen/day – group 1) or three consecutive daily doses at a rate of 10 mg/kg bw (~15.3 mg/hen/day – group 2).

In the test group that received a single dose, birds were sacrificed 24 hours after dosing. Radioactivity was absorbed from the intestinal tract following a mean lag time of 15 minutes with a mean absorption half life $t_{1/2a}$ of 1.8 hours. The mean plasma curve showed a broad range of concentration 3 – 6 hours after dosing. The experimentally determined peak occurred at 4 hours (2.22 µg/mL). Elimination was monophasic with a plasma half-life ($t_{1/2e}$) of 5.4 hours, with a mean residence time (MRT) of 10.6 hours.

In the group that received 3 consecutive daily doses, the birds were sacrificed 5 hours after the last dose (which coincided with one hour after the determined maximum concentration in plasma as measured from the single dose). The following tissues were collected from treated animals: kidney, liver, skin, muscle (leg and breast) and subcutaneous fat. Eggs, excreta and plasma were collected throughout the dosing period and also analysed.

In the multiple dose group, an average of 74% of the total radioactivity was recovered in excreta. Only 0.2% of the administered dose was recovered in eggs. A further 9.9% of the total radioactive residue (TRR) was recovered in tissues giving a total recovery of 84% of the administered radioactive dose.

Highest tissue residues were seen in liver, kidney and fat with mean levels of 17.48, 12.91 and 12.35 mg/kg respectively. Levels in un-laid eggs were 6.54 mg/kg with a maximum concentration in eggs of 1.38 mg/kg seen at sacrifice. Residues in breast and leg muscle were 2.19 and 2.65 mg/kg respectively and residual/average residues in skin were 5.79 mg/kg.

Radioactive residues in tissues were solubilised and extracted with a mixture of acetonitrile and tetrahydrofuran. Recovery of radioactivity was quantitative and complete. Residues in liver were purified and structural elucidation of isolated metabolites was achieved by LC-MS/MS and by co-

chromatography and comparison of metabolites isolated from a rat metabolism study that had previously been spectroscopically identified. The metabolites in other tissue extracts were then identified by co-chromatography with liver extracts.

In poultry, metabolism of spiroxamine proceeded via two pathways: oxidation of the t-butyl moiety forming the carboxylic acid or alternatively, via desalkylation of the amino group forming the spiroxamine desethyl or spiroxamine despropyl metabolites. Unchanged parent spiroxamine was also seen in edible tissues and eggs.

I. Materials and methods

A. Test materials

[cyclohexyl-1-^{14}C]-spiroxamine

* Denotes radiolabel position

Specific activity (µCi/mg)	98 (3.63 MBq/mg)
Lot/Batch No.:	KML 2239
Radiochemical purity:	>99% (sum of isomers) by radio HPLC
CAS No.:	118134-30-8

Non radio-labelled spiroxamine

Lot/Batch No.:	920522ELB01
Purity:	99.0%
CAS No.:	118134-30-8

Test system: Laying hen

Species:	Laying hen *(Gallus gallus domesticus)*
Strain:	White leghorn
Justification:	Laying hens are recognised as a model for poultry (broiler and egg production) to comply with the corresponding residue studies
Number / sex:	Twelve laying hens (six in each treatment group)
Age / body weight:	The hens were approximately 6 to 8 months of age at the time of dosing and the average bodyweight at dosing was 1530 to 1570 g for the hens in the treated groups at the time of study initiation.
Acclimatisation:	Hens were purchased from J. Brinkschulte, Gut Averfeld, D-48308, Munster, Germany. Eighteen hens were delivered initially and hens were selected for treatment group based on egg production. Hens were acclimatised to laboratory conditions for 3 weeks prior to the study starting.

B. Study design

The study was conducted between November 24 1993 and April 26 1995.

The study was conducted at [redacted]

Experimental conditions

Laying hens (*Gallus gallus domesticus,* strain white leghorn) were administered [^{14}C]spiroxamine (labelled in the 1-position of the cyclohexyl-ring) with either a single oral dose of 10 mg/kg bodyweight (group 1) or for three consecutive daily doses at a rate of 10 mg/kg bw (~15.3 mg/hen/day – group 2). Based on the average feed consumption of 50g feed per day and a hen weighing approximately 1.5 kg bodyweight, this equated to an exaggerated concentration of approximately 300 mg/kg in the diet. Treatments were administered orally via gelatin capsules. Six birds were each administered either a single oral or three consecutive oral daily doses of radiolabelled spiroxamine. Samples of untreated tissues were also taken from a third group of control hens that had been used as companion animals in another hen metabolism study.

Hens were housed individually stainless steel metabolism cages that allowed the separate quantitative collection of excreta and eggs. The maximum and minimum ambient temperature was recorded daily (22 ± 3°C) and the relative humidity was set at 54 ± 10°C. During the test period, the birds were offered food (Hőveler laying hen complete feed) approximately 200 g/day and the hens were fed in the morning. The feed was withdrawn in the evening prior to each administration in order to ensure a uniform absorption of the test compound within the animal group. Fresh potable drinking water was provided *ad libitum*.

Blood was taken from the wing vein of hens in the single dose group (group 1) at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dosing. Blood was collected into heparinised capillary tubes.

Egg production was checked during the acclimation period and all hens were deemed to be in good egg production. During the test period, eggs were collected twice daily. The number and weight of eggs were recorded for all hens.

In the test group that received a single dose, birds were sacrificed 24 hours after dosing. In the group that received 3 consecutive daily doses, the birds were sacrificed 5 hours after the last dose (which coincided with one after the determined maximum concentration in plasma as measured from a single dose) and the following tissues were collected from treated animals: kidney, liver, skin, muscle and fat. Eggs, excreta and plasma were collected throughout the dosing period and also analysed. Samples were stored frozen at -20°C.

The total radioactive residues (TRRs) in eggs and tissues were determined.

Dose preparation and dosing

[^{14}C]-spiroxamine (labelled in the 1-position of the cyclohexyl-ring) was evaluated.

The test item was delivered as a pure liquid. In order to prepare a stock solution, the whole amount was dissolved in 15 mL of acetonitrile. The solution was calibrated by radioactivity measurement. The total amount of radioactivity was 16166 µCi (2.59 x 10^{10} dpm or 598.14 MBq) corresponding to 164.96 mg. The radioactivity concentration was 1077.7 µCi/mL (2.39 x 109 dpm/mL or 39.87 MBq/mL) corresponding to 11.0 mg/mL.

An aliquot of 100 mg (9800 µCi) was pipetted into an Erlenmeyer flask and diluted with 9 parts of the authentic unlabelled compound to give 1000 mg with a specific radioactivity of 9.8 µCi/mg (2.18 x 10^{7} dpm/mg). The solvent was removed under a gentle stream of nitrogen at room temperature.

Capsules were prepared the day before the first administration and stored refrigerated (5°C) until used. The birds were dosed according to their body weights.

Group 1: the hens received a mean dose of 15.7 mg (153.9 µCi) per animal with a group mean bodyweight of 1.57 kg.

Group 2: the hens received a mean dose of 15.3 mg (150.0 µCi) per animal with a group mean bodyweight of 1.53 kg.

The capsules (lubricated with corn oil) were placed in the oesophagus of the bird with forceps and the beak was held shut. Swallowing was encouraged by gentle massage of the throat towards the crop. No adverse dose-related symptoms were observed.

Sacrifice and tissue collection

The test animals were humanely sacrificed after being anaesthetised with CO_2. The birds that received a single dosed were sacrificed after 24 hours, whilst the birds receiving three consecutive doses were sacrificed 5 hours after the last dose. The following tissues were collected at necropsy: Liver, kidney, subcutaneous fat, muscle (breast and leg) and skin. Eggs, plasma and excreta collected throughout the dosing period were also analysed.

Total radioactivity was determined in samples of dose solutions, excreta, plasma, eggs (pooled samples of whites and yolks) and tissues (muscle, liver, kidney skin and fat).

Sample analysis

Preparation of samples and radiochemical analysis

Blood/plasma

Blood was collected into heparinised capillary tubes, which were centrifuged at 12,000g for 10 minutes to obtain the plasma in a haemocrit centrifuge. Radioactivity content in the plasma samples was determined by liquid scintillation counting (LSC).

Eggs

For sampling, the egg shells were discarded and the whites and yolks thoroughly mixed. The weights were recorded. One aliquot of each egg mix was solubilised with BTS-450 tissue solubiliser (Beckman instruments) and measured in duplicate by LSC. The remaining egg mix was stored frozen (-20°C) for metabolite analysis.

Excreta

Excreta fractions were collected individually at room temperature in 24 hour intervals. The containers were changed and cleaned prior to each dose administration. The rinsing water was not analysed.

All fractions were freeze-dried and homogenised. After recording the dry weight, an aliquot of each fraction was combusted in triplicate. The resulting $^{14}CO_2$ was trapped and radioactivity content measured by LSC. The remaining sample was stored at ambient temperature for (optional) metabolite analysis.

Tissues

In group 1 (single oral dose), only the liver, subcutaneous fat and breast and leg muscle were prepared. The tissue samples from the birds in this group were combined prior to weighing and processing as detailed below.

In group 2 (multiple oral doses), kidney, liver, breast and leg muscle, skin (minus fat), subcutaneous fat and eggs from the ovary and oviduct were prepared. Tissue samples (with the exception of skin and subcutaneous fat) were minced in the presence of dry ice. Radioactivity content in tissue samples was determined by combustion of sample aliquots in triplicate. In order to prepare the tissue samples for metabolite analysis, the homogenised tissue from each sample type was divided into four sub-samples and stored wet at approximately -20°C. The two different muscle types were combined giving a composite muscle sample. Fat and skin sample aliquots were taken without homogenisation. Aliquots were directly solubilised with tissue solubiliser and then assayed in duplicate by LSC.

Combustion analysis:

Combustion analysis was carried out using a Packard Tri-Carb Tissue Oxidiser (Model 306). The resultant $^{14}CO_2$ was absorbed in Carbosorb and Scintillation fluid (Permafluor E+) was added. The efficiency of the oxidiser was determined prior to sample analysis.

Liquid scintillation counting (LSC):

Liquid samples were counted in a liquid scintillation counter (Beckman LS 6000 LL, Philips PW 4700, LKB Rack Beta 1219 or LKB Rack Beta 1214) using commercial scintillation cocktails (Instant Scint-Gel, Quick-Safe A). Counts per minute (cpm) were converted to dpm using a set of commercially prepared quenched standards.

High performance liquid chromatography (HPLC):

A Hewlett Packard 1050 Liquid Chromatograph was used equipped with a variable wavelength detector. Radioactivity was detected using a Ramona (Raytest) flow-through detector.

Mass spectroscopy (MS):

Radioactive residues were analysed on a Finnegan TSQ 7000 instrument (ESI). A radioactivity detector was coupled between the HPLC instrument and the mass spectrometer. The delay time between the two instruments was approximately 20 seconds.

Characterisation of radioactivity

Tissue samples were extracted with a sequence of solvents to characterise and quantify the distribution of radioactive residues in the sample.

Extraction of eggs

The combined egg samples (63.75 g) were filtered under suction. A small amount of de-foaming agent was added before the sample was exhaustively extracted with mixtures of acetonitrile and tetrahydrofuran (7:3, v/v) followed by acetonitrile and sodium chloride solution (7:3 v/v). The sample was extensively homogenised with an Ultra-Turrax tissue homogeniser at each step. At each stage, the extracts were suction filtered with de-foaming agent added. The post-extraction solid (PES) was dried under vacuum and residual radioactivity determined by combustion. Radioactivity in the extracts was determined by LSC.

The combined acetonitrile phases were further purified after addition of NaCl and pH adjustment to 3.5 by partitioning with n-heptane. The acetonitrile phase was then extracted with acetonitrile/ethanol (1:1 v/v) followed by methanol/ethanol (1:1 v/v). These extracts were combined. The heptane phase was partitioned with acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v) and the acetonitrile phase was combined with other acetonitrile phases. After concentration, 20 mL water and 1 mL of methanol were added and the pH adjusted to 6.9. This extract represented more than 97% of the TRR in egg and was suitable for analysis by HPLC where the profile was compared to liver extracts.

Extraction of liver

Macerated liver (29.84 g) was extracted in a similar procedure as that described for eggs. However, two additional steps were necessary after the two previously described extraction steps. The third step comprised extraction with acetonitrile, 0.5% sodium chloride solution and 25% NH_4OH (70:30:1 v/v/v), followed by acetonitrile and 0.5% sodium chloride (7:3 v/v). The residue was finally extracted under alkaline conditions with a mixture of methanol/ethanol/25% ammonia. Over 98% of the TRR could be extracted by this solvent regime. Small losses occurred during purification for HPLC, but over 93% TRR was subjected to HPLC analysis.

Extraction of muscle

Composite muscle sample (65.73 g) was extracted in a similar procedure as that described for eggs. Over 96% TRR was extractable. Small losses occurred during purification for HPLC, but over 93% TRR was subjected to HPLC analysis.

Extraction of fat

Subcutaneous fat sample (22.12 g) was extracted in a similar procedure as that described for eggs except the ratio of acetonitrile/tetrahydrofuran used was 1:1 v/v. The acetonitrile/tetrahydrofuran phase was mixed with water and acidified with acetic acid and subsequently partitioned twice with n-heptane. The first heptane phase was re-partitioned with acetonitrile/tetrahydrofuran/water/acetic acid (55:55:20:2 v/v/v/v). All acetonitrile phases were combined and concentrated for HPLC. Over 99% TRR was readily organo-extractable and almost 92% TRR was chromatographed by HPLC.

II. Results and discussion

Total radioactive residues (TRR)

Refer to Table CA 6.2.2/01-1 to CA 6.2.2/01-3

Laying hens were dosed with 10 mg spiroxamine /kg bodyweight (~15.3 – 16.7 mg/hen/day). The radiochemical purity for the test item was >99%.

Following a single oral dose of radiolabelled spiroxamine, residue levels in plasma reached a maximum concentration after approximately 4 – 6 hours (Table CA 6.2.2/01-1). Kinetic assessment determined that radioactivity was eliminated monophasically, with an elimination half-life ($t_{1/2e}$) of 5.4 hours and a mean residence time (MRT) of 10.6 hours.

After three consecutive daily doses of spiroxamine, approximately 74% of the administered radioactivity was eliminated in the excreta with over 30% eliminated in the first 24 hours (Table CA 6.2.2/01-2). A further 0.22% TRR was recovered in the eggs and approximately 9.94% TRR was estimated in tissues, giving an overall recovery of administered dose of 84%. Cage washings were not assayed and almost certainly accounted for the remainder of the administered dose.

A plateau concentration in whole eggs was not demonstrated (Table CA 6.2.2/01-3). After three consecutive daily does, the concentration of radioactive residues reached a maximum of 1.378 mg/kg. Whole eggs from all three days dosing were pooled and blended for further analysis of radioactive residues. The average concentration in eggs was therefore 0.848 mg/kg.

Residue levels in eggs and edible tissues taken at sacrifice 5 hours after the final dose to reflect peak plasma concentration are shown in Table CA 6.2.2/01-4. Highest tissue residues were seen in the liver (average residue 17.48 mg/kg), kidney (12.91 mg/kg) and subcutaneous fat (12.35 mg/kg). Residue levels in other tissues and skin are lower, with an average concentration of 5.79 mg/kg in skin, between 2.19 and 2.65 mg/kg seen in muscle (breast and leg respectively) and 1.38 mg/kg seen as a maximum concentration in whole eggs.

Table CA 6.2.2/01-1 Concentration of spiroxamine residues (µg/mL) in plasma in laying hens after a single oral dose of spiroxamine1 (10 mg/kg bodyweight)

Time (hours)	Mean concentration (µg/mL) (CV)[2]
0.25	0.086 (115)
0.50	0.334 (73)
1.00	0.636 (64)
2.00	1.765 (32)
3.00	2.126 (30)
4.00	2.215 (32)

Time (hours)	Mean concentration (µg/mL) (CV)[2]
6.00	2.051 (39)
8.00	1.783 (33)
24.00	0.264 (124)

1 - Birds received 15.7 mg of spiroxamine per day
2 – coefficient of variance expressed as percentage

Table CA 6.2.2/01-2 Summary of recovery of the recovery of radioactivity after 3 consecutive daily doses of spiroxamine[1] (10 mg/kg bodyweight)

	Time after first dose[1]	Average% Total administered radioactivity (CV)[2]
Excreta	24 48 53	30.45 (10.7) 31.08 (14.4) 12.27 (37.1)
Subtotal		73.81 (9.1)
Eggs (0 – 53h)		0.22 (15.1)
Total excreted		**74.03 (9.1)**
Estimated residue in tissues[3]		9.94 (11.8)
Total recovery		**83.97 (7.3)**

1 - Birds received 15.3 mg of spiroxamine per day
2 – coefficient of variance
3 – calculated from the body weight assuming 10, 12 and 4% of the body weight for total muscle, dissectible body fat and skin (minus subcutaneous fat) and including the sum of the organs prepared in total.

Table CA 6.2.2/01-3 Mean concentration of radioactive residue in pooled egg samples after 3 consecutive daily doses of spiroxamine1 (10 mg/kg bodyweight)

Time after first administration (h)	Dose number	Spiroxamine equivalent concentration (mg/kg)	Number of eggs
0	1	-	
24	2	0.216	n = 6
48	3	0.949	n = 5
53		1.378	n = 6
Pool for analysis		0.848	

1 - Birds received 15.3 mg of spiroxamine per day

Table CA 6.2.2/01-4 Summary of the excretion and recovery of radioactivity from laying hens after 3 daily doses of spiroxamine[1] (10 mg/kg bodyweight)

Sample	TRR expressed as mg [^{14}C] spiroxamine/kg
Liver	17.48
Kidney	12.91
Subcutaneous fat	12.35
Leg muscle	2.65
Breast muscle	2.19
Skin	5.79
Eggs (oviduct)	6.54
Eggs	1.38 (maximum concentration at sacrifice)

1 - Birds received 15.3 mg of spiroxamine per day

Characterisation

Based on the above results, in order to characterise and identify the nature of the radioactive residues, all tissues and pooled eggs were extracted. A summary of the extraction regimes is presented in Table CA 6.2.2/01-5 to Table CA 6.2.2/01-8.

Radioactive components in extracts were quantified and identified using HPLC and LC-MS/MS.

Available reference standards for characterisation were spiroxamine (KWG 4168) itself as the parent test substance and the following potential metabolites:

It should be noted that the reference compounds used for characterisation and identification of radioactive residues were typically isolated in their radioactive form from either rat or goat urine from other metabolism studies and spectroscopically identified. This was considered necessary as neither the parent compound or its metabolites demonstrated adequate UV absorbance.

The following reference materials were structurally elucidated in this manner:

ECW 80511 (spiroxamine-acid) (M06)

ECW 8046 (spiroxamine-acid isomeric version of ECW 80511) (M06)

ECW 8044 (spiroxamine-desethyl acid) (M11)

ECW 8042 (spiroxamine-despropyl acid) (M12)

ECW 8085 (spiroxamine-ketone-sulphate)

ECW 8081 (spiroxamine-cyclohexanol-glucuronide) (M22)

ECW 8096 (spiroxamine-8-hydroxy acid) (M08)

ECW 8079 and ECW 80822 (spiroxamine-desethyl-sulphate) (M26)

ECW 80862 (spiroxamine-despropyl-sulphate) (M27)

ECW 8076 (spiroxamine-sulphate) (M25)

WAK 6301 (spiroxamine-N-oxide) (M03)

KWG 4537 (spiroxamine-desethyl) (M01)

WAK 6174 (spiroxamine-despropyl) (M02)

Eggs

Table CA 6.2.2/01-5 and Table CA 6.2.2/01-9.

Total radioactive residues in pooled whole eggs were 0.848 mg/kg. Radioactive residues were almost completely extracted with acetonitrile-based solvent mixtures (98.6% TRR based on the normalised balance). Less than 2% TRR remained associated with the PES. The acetonitrile phases were combined and further purified with NaCl (pH was adjusted to 3.5 by partitioning with n-heptane).

After partitioning, almost 95% TRR (normalised) was recovered in the first 3 acetonitrile partitions, with a further 4.3% TRR released by acetonitrile/ethanol/methanol. The acetonitrile phases were combined and concentrated prior to HPLC analysis.

The concentrated egg extract was chromatographically compared to the liver extract (see below for details). A total of 70.9% TRR (0.600 mg/kg) was identified. Parent spiroxamine accounted for 11.8% TRR (0.100 mg/kg). The main metabolite determined was the spiroxamine-acid moiety (M06) accounting for 37.4%TRR (0.317 mg/kg). This metabolite was formed via oxidation of the t-butyl moiety forming the carboxylic acid. Two other metabolites were formed in eggs as a result of desalkylation, the spiroxamine-desethyl (M01) accounting for 11.5% TRR (0.097 mg/kg) and the spiroxamine-despropyl (M02) accounting for 10.2% TRR (0.086 mg/kg).

A total of 5 unidentified smaller metabolites were detected in whole egg extracts. The largest of these accounted for 9.2% TRR (0.078 mg/kg). No further work was conducted to identify these residues.

Liver

Table CA 6.2.2/01-6 and Table CA 6.2.2/01-9.

The total radioactive residue in liver was 17.45 mg/kg. Similarly to eggs, radioactive residues were almost completely extracted with acetonitrile-based solvent mixtures (98.6% TRR based on the normalised balance). Less than 2% TRR remained associated with the PES. The acetonitrile phases were combined and further purified with NaCl (pH was adjusted to 3.5 by partitioning with n-heptane).

After partitioning, almost 87% TRR was recovered in the first 3 acetonitrile partitions, with a further 6.5% TRR released by acetonitrile/ethanol/methanol. 5% TRR remained associated with the heptane fraction. The acetonitrile phases were combined and concentrated prior to HPLC analysis.

Some small losses occurred during the purification and concentration phases meaning 93% TRR was analysed by HPLC. A total of 64.8% TRR (11.326 mg/kg) was identified. The spiroxamine-acid metabolite (M06) was identified by co-chromatography with the reference compounds ECW 8046 and ECW 80511 (isomeric versions of spiroxamine-acid, M06). The chromatogram revealed that only one isomer (ECW 8046) was present in the liver of poultry (8.5% TRR; 1.486 mg/kg). Three other metabolites present in poultry liver were also seen in the excreta profile. As larger amount were found in excreta, these were initially isolated and purified from excreta extracts and then a chromatographic comparison was made with the liver extracts. Purified fractions were analysed by ESI/MS. Structural elucidation showed the presence of parent spiroxamine (13.3% TRR, 2.324 mg/kg) and the two metabolites formed by desalkylation; the spiroxamine-desethyl (M01) accounting for 21.3% TRR (3.723 mg/kg) and the spiroxamine-despropyl (M02) accounting for 21.7% TRR (3.793 mg/kg).

A total of 11 unidentified smaller metabolites were detected in liver extracts. The largest of these accounted for 5.9% TRR (1.031 mg/kg). No further work was conducted to identify these residues.

Muscle

Table CA 6.2.2/01-7 and Table CA 6.2.2/01-9.

Total radioactive residue in the combined leg and breast muscle sample was 2.42 mg/kg. As with other tissues, radioactive residues were almost completely extracted with acetonitrile-based solvent mixtures (96.9% TRR based on the normalised balance). Approximately 3% TRR remained associated with the

PES. The acetonitrile phases were combined and further purified with NaCl (pH was adjusted to 3.5 by partitioning with n-heptane).

After partitioning, almost 87% TRR was recovered in the first 3 acetonitrile partitions, with a further 9.7% TRR released by acetonitrile/ethanol/methanol. 3.3% TR remained associated with the heptane phase. The acetonitrile phases were combined and concentrated prior to HPLC analysis.

The concentrated combined muscle extract was chromatographically compared to the liver extract. A total of 75.7% TRR was identified. Parent spiroxamine accounted for 17.8% TRR (0.430 mg/kg). The main metabolite determined was the spiroxamine-acid moiety, (M06) accounting for 37.3% TRR (0.901 mg/kg). Two other metabolites formed in muscle were spiroxamine-desethyl (M01) accounting for 9.3% TRR (0.225 mg/kg) and spiroxamine-despropyl (M02) accounting for 11.3% TRR (0.273 mg/kg).

A total of 6 unidentified metabolites were detected in muscle extracts. The largest of these accounted for 6.0% TRR (0.145 mg/kg). No further work was conducted to identify these residues.

Subcutaneous fat

Table CA 6.2.2/01-8 and Table CA 6.2.2/01-9.

Total radioactive residue in the subcutaneous fat sample was 12.35 mg/kg. Again radioactive residues were exhaustively extracted with acetonitrile-based solvent mixtures (99.9% TRR based on the normalised balance) which almost completely solubilised the tissue sample. The acetonitrile phases were combined and further purified with NaCl (pH was adjusted to 3.5 by partitioning with n-heptane).

After partitioning, 90% TRR (normalised) remained in the initial heptane phase, with a further 8.3% TRR released by additional heptane after partition with acetonitrile/THF. The heptane phases were combined and concentrated prior to HPLC analysis. Some small losses occurred and 91.6% TRR was subjected to chromatographic analysis.

The same metabolites seen in liver and muscle were also determined in the fat sample but in different proportions. Unchanged parent spiroxamine formed the majority of the residue accounting for 77.4% TRR (9.56 mg/kg). The desalkylated metabolites, spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) were present accounting for 8.4% TRR (1.04 mg/kg) and 3.4% TRR (0.420 mg/kg), respectively. Small amounts of spiroxamine-acid (M06) (1.7% TRR, 0.210 mg/kg) were also detected.

A single unidentified metabolite was detected in fat accounting for 0.6% TRR (0.074 mg/kg). No further work was conducted to identify this residue.

Table CA 6.2.2/01-5 Summary of the extractability and characterisation of radioactivity in pooled eggs from laying hens after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 0.848 mg/kg	
	% of initial radioactivity	Normalised balance
Initial extracts		
Acetonitrile/tetrahydrofuran (7:3 v/v)	96.45 (0.818)	83.84
Acetonitrile/tetrahydrofuran (7:3 v/v)	11.51 (0.098)	10.01
Acetonitrile/0.5% NaCl (7:3 v/v)	4.16 (0.035)	3.62
Acetonitrile/0.5% NaCl (7:3 v/v)	1.27 (0.011)	1.10
Post extraction solid (PES)	1.65 (0.014)	1.43
Total	115.04 (0.976)	100.00
Extracts 1 – 4 combined, pH adjusted and extracted with heptane		
Heptane after partition	0.22 (0.002)	0.23
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	58.61 (0.497)	60.31
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	32.63 (0.277)	33.58
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	0.72 (0.006)	0.75
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	0.00 (0.00)	0.00
Acetonitrile/ethanol (1:1 v/v) + methanol/ethanol (1:1 v/v)	4.21 (0.036)	4.33
Methanol/ethanol/ammonium hydroxide (50:50:5 v/v/v)	0.00 (0.00)	0.00
Sodium chloride solution	0.79 (0.007)	0.81
Total	97.19 (0.824)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg

Table CA 6.2.2/01-6 Summary of the extractability and characterisation of radioactivity in liver from laying hens after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 17.48 mg/kg	
Initial extracts		
Acetonitrile/tetrahydrofuran (7:3 v/v)	115.58 (20.20)	92.71
Acetonitrile/0.5% NaCl (7:3 v/v)	5.66 (0.989)	4.54
Acetonitrile/0.5% NaCl (7:3 v/v)	1.02 (0.178)	0.82
Acetonitrile/0.5% NaCl (7:3 v/v)	0.23 (0.040)	0.19
Acetonitrile/0.5% NaCl/ammonium hydroxide (70:30:1 v/v/v)	0.21 (0.037)	0.17
Acetonitrile/0.5% NaCl (7:3 v/v)	0.15 (0.026)	0.12
Post extraction solid (PES)	1.81 (0.316)	1.45
Total	124.66 (21.79)	100.00
Extracts 1 – 6 combined, pH adjusted and extracted with heptane		
Heptane after partition	4.96 (0.867)	5.06
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	83.51 (14.60)	85.22
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	1.10 (0.192)	1.12
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	0.49 (0.086)	0.50
Acetonitrile/ethanol (1:1 v/v) + methanol/ethanol (1:1 v/v)	6.41 (1.120)	6.54

Extract	% TRR (mg/kg)[1]	
	Total residue 17.48 mg/kg	
Methanol/ethanol/ammonium hydroxide (50:50:5 v/v/v)	0.93 (0.163)	0.95
Sodium chloride solution	0.60 (0.105)	0.61
Total	97.99 (17.13)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.2/01-7 Summary of the extractability and characterisation of radioactivity in composite muscle from laying hens after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 2.42 mg/kg	
Initial extracts		
Acetonitrile/tetrahydrofuran (7:3 v/v)	92.23 (2.23)	75.13
Acetonitrile/tetrahydrofuran (7:3 v/v)	16.42 (0.397)	13.37
Acetonitrile/0.5% NaCl (7:3 v/v)	8.08 (0.196)	6.58
Acetonitrile/0.5% NaCl (7:3 v/v)	2.24 (0.054)	1.82
Post extraction solid (PES)	3.79 (0.092)	3.09
Total	122.76 (2.971)	100.00
Extracts 1 – 4 combined, pH adjusted and extracted with heptane		
Heptane after partition	3.24 (0.078)	3.26
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	85.32 (2.065)	85.93
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	0.59 (0.014)	0.60
Acetonitrile/methanol/water/acetic acid (30:55:24:1 v/v/v/v)	0.11 (0.004)	0.11
Acetonitrile/ethanol (1:1 v/v) + methanol/ethanol (1:1 v/v)	9.62 (0.233)	9.69
Methanol/ethanol/ammonium hydroxide (50:50:5 v/v/v)	0.04 (0.001)	0.04
Sodium chloride solution	0.37 (0.009)	0.37
Total	99.29 (2.40)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.2/01-8 Summary of the extractability and characterisation of radioactivity in subcutaneous fat from laying hens after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 12.35 mg/kg	
Initial extracts		
Acetonitrile/tetrahydrofuran (1:1 v/v)	113.94 (14.07)	99.39
Acetonitrile/0.5% NaCl (7:3 v/v)	0.58 (0.072)	0.51
Post extraction solid (PES)	0.11 (0.014)	0.10
Total	114.64 (14.16)	100.00
Extract 1 was partitioned twice with heptane		
Heptane after partition	88.73 (10.96)	90.14
Acetonitrile/tetrahydrofuran/water/acetic acid (55:55:20:2 v/v/v/v)	1.51 (0.186)	1.54

Extract	% TRR (mg/kg)[1]	
	Total residue 12.35 mg/kg	
Heptane-1	6.56 (0.810)	6.67
Heptane-2	1.63 (0.201)	1.65
Total	98.43 (12.16)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.2/01-9 Summary of identified and characterised residues in tissues from laying hens after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Metabolite	Percent of total radioactive residues - % TRR (mg/kg)			
	Liver (17.45 mg/kg)	Muscle (2.42 mg/kg)	Fat (12.35 mg/kg)	Eggs (0.848 mg/kg)
% TRR extracted	98.6	96.9	99.9	98.6
Spiroxamine-desethyl (M01)	21.3 (3.723)	9.3 (0.225)	8.4 (1.038)	11.5 (0.097)
Spiroxamine-despropyl (M02)	21.7 (3.793)	11.3 (0.273)	3.4 (0.420)	10.2 (0.086)
Spiroxamine-acid (M06)	8.5 (1.486)	3.3 (0.901)	1.7 (0.210)	37.4 (0.317)
Spiroxamine	13.3 (2.324)	17.8 (0.430)	77.4 (9.562)	11.8 (0.100)
Unknown metabolites	28.2[1] (4.929)	17.6 (0.425)	0.6[3] (0.074)	[4]
% TRR identified	64.8 (11.326)	75.7 (1.829)	90.9 (11.230)	70.9 (0.600)

1 – 11 unidentified metabolites in liver with a maximum of 5.9% TRR (1.031 mg/kg)
2 - 6 unidentified metabolites in muscle with a maximum of 6.0% TRR (0.145 mg/kg)
3 - 1 unidentified metabolite in fat accounting for 0.6% TRR (0.074 mg/kg)
4 - 5 unidentified metabolites in eggs with a maximum of 9.2% TRR (0.078mg/kg)

Storage stability:

Storage intervals for tissues were not specifically stated, however, the report does state that metabolite stability in edible tissues under the prevailing storage conditions were assessed separately and would only be reported if any changes in the metabolic pattern compared with the initial metabolite traces was noted. Samples were analysed without any significant delay. As no further information was included, it is assumed that sample extracts did not deteriorate under the specific storage conditions used in this study indicating no chemical instability.

III. Conclusions

The metabolism of spiroxamine was investigated in laying hens. Two groups of six hens were administered [^{14}C]spiroxamine (labelled in the 1-position of the cyclohexyl-ring) with either a single oral dose of 10 mg/kg bodyweight (~15.7 mg/hen/day) or for three consecutive daily doses at a rate of 10 mg/kg bw (~15.3 mg/hen/day).

In the test group that received a single dose, birds were sacrificed 24 hours after dosing. Radioactivity was absorbed from the intestinal tract following a mean lag time of 15 minutes with a mean absorption half life $t_{1/2a}$ of 1.8 hours. The mean plasma curve showed a broad range of concentration 3 – 6 hours after dosing. The experimentally determined peak occurred at 4 hours (2.22 µg/mL). Elimination was monophasic with a plasma half-life ($t_{1/2e}$) of 5.4 hours, with a mean residence time (MRT) of 10.6 hours.

In the group that received 3 consecutive daily doses, the birds were sacrificed 5 hours after the last dose (which coincided with one hour after the determined maximum concentration in plasma as measured from the single dose). The following tissues were collected from treated animals: kidney, liver, skin,

muscle (leg and breast) and subcutaneous fat. Eggs, excreta and plasma were collected throughout the dosing period and also analysed.

On average 74% of the total radioactivity was recovered in excreta. Only 0.2% of the administered dose was recovered in eggs. A further 9.9% of the total radioactive residue (TRR) was recovered in tissues giving a total recovery of 84% of the administered radioactive dose.

Highest tissue residues were seen in liver, kidney and fat with mean levels of 17.48, 12.91 and 12.35 mg/kg respectively. Levels in un-laid eggs were 6.54 mg/kg with a maximum concentration in eggs of 1.38 mg/kg seen at sacrifice. Residues in breast and leg muscle were 2.19 and 2.65 mg/kg respectively and residual average residues in skin were 5.79 mg/kg.

Radioactive residues in tissues were solubilised and extracted typically with mixtures of acetonitrile and tetrahydrofuran. Residues in liver were purified and structural elucidation of isolated metabolites was achieved by LC-MS/MS and by co-chromatography and comparison of metabolites isolated from a rat metabolism study that had previously been spectroscopically identified. The metabolites in other tissue extracts were then identified by co-chromatography with liver extracts.

In poultry, metabolism of spiroxamine proceeded via two pathways: oxidation of the t-butyl moiety forming the carboxylic acid or alternatively, via desalkylation of the amino group forming the spiroxamine desethyl or spiroxamine despropyl metabolites. Unchanged parent spiroxamine was also seen in edible tissues and eggs.

The proposed metabolic pathway for spiroxamine in livestock is shown in Figure CA 6.2-3.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7, 2009, IIA 6.2.2/01. The dosing was only conducted for 5 days and a plateau of residues in eggs was not established. However, the study is considered to be acceptable as the analysis is scientifically robust and it is acknowledged that the study was conducted at a hugely exaggerated dose level compared to realistic dietary exposure of laying hens to spiroxamine derived plant residues.

At least 74% of the administered radioactivity was recovered in poultry excreta and there was very little transfer into edible tissues and eggs.

Metabolism of spiroxamine in poultry proceeded via two pathways: oxidation of the t-butyl moiety forming the spiroxamine-acid or alternatively, via desalkylation of the amino group forming the spiroxamine-desethyl or spiroxamine-despropyl metabolites. Unchanged parent spiroxamine was also seen in edible tissues and eggs.

CA 6.2.3 Lactating ruminants

Data Point:	KCA 6.2.3/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	[Cyclohexyl-1-14C] KWG 4168: Absorption, distribution, excretion, and metabolism in a lactating goat
Report No:	PF4073
Document No:	M-006039-01-1
Guideline(s) followed in study:	U.S. EPA FIFRA Guideline, Subdivision 0, No. 171-4(b)
Deviations from current test guideline:	Yes OECD 503 (2007) requires that ruminants are dosed for at least 5 days. The animal in this study was dosed for only 3 consecutive days. Plateau level of residue in milk was not demonstrated during this time.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A single lactating goat (*Capra hircus* – 'Bunte Deutsche Edelziege') was orally dosed with [^{14}C]-spiroxamine (labelled in the 1-position of the cyclohexyl-ring) on three consecutive days at a rate of 10 mg/kg body weight. Assuming a daily total feed consumption of 4% of the body weight at most, this dose corresponded to an exaggerated concentration of 250 mg/kg in the feed.

Milk was collected twice daily from the animals just before dosing each morning and approximately 8 hours later. Urine and faeces were collected 24 hours after the first and second dose administration and 7 hours after the third. The test animals were sacrificed approximately seven hours after the final dose followed immediately be exsanguination. Liver, kidney, muscle (flank, round and loin), and fat (omental, subcutaneous and peri-renal) samples were collected from the treated goat.

Total radioactivity was determined in samples of dose solutions, excreta, plasma, milk and edible tissues (liver, kidney, muscle and fat). The total radioactive residue (TRRs) in milk and edible tissues were determined.

Radioactivity was absorbed from the intestinal tract following a mean lag time of 14 minutes with a mean absorption half life $t_{1/2a}$ of approximately 4 hours. This was followed by a relatively slow distribution process. The mean plasma curve showed a broad maximum concentration. The experimentally determined peak occurred at 6.6 hours (1.86 µg/mL). Elimination from plasma showed a plasma half-life ($t_{1/2e}$) of approximately 6 hours, with a mean residence time (MRT) of 14 hours.

The goat was sacrificed 7 hours after the last dose (which coincided with one hour after the determined maximum concentration in plasma and was equivalent to 55 hours after the first dose). The following tissues were collected from the treated animal: kidney, liver, muscle (flank, loin and round) and fat (peri-renal, subcutaneous and omental). Milk, excreta and plasma were collected throughout the dosing period and also analysed.

An average of 75% of the total radioactivity was recovered in excreta (63% in the urine and a further 12% eliminated in the faeces). It was concluded from the high concentration in the liver, that a significant amount of the faecal radioactivity was absorbed prior to elimination in the bile. Only 0.23%

of the administered dose was recovered in milk. A further 3.4% of the total radioactive residue (TRR) was recovered in tissues giving a total recovery of 79% of the administered radioactive dose.

Highest tissue residues were seen in the excretory organs, liver and kidney with mean levels of 22.07 and 14.21 mg/kg respectively. The level in milk was 0.93 mg/kg (maximum concentration at sacrifice). Residues in muscle and fat were quantitatively similar. In muscle, residue levels in flank, loin and round muscle were 1.11, 0.93 and 1.06 mg/kg respectively and levels in peri-renal, subcutaneous and omental fat were 0.51, 1.04 and 0.42 mg/kg.

Radioactive residues in tissues were solubilised and extracted with mixtures of acetonitrile and water. Recovery of radioactivity was quantitative and complete. After purification, the extracts were co-chromatographed in two different HPLC systems with comparison of metabolites isolated from a rat metabolism study that had previously been spectroscopically identified. By this method, metabolites in edible tissues and milk were identified.

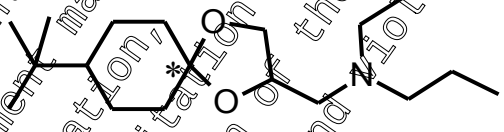
In ruminants, spiroxamine was extensively metabolised. The primary route of metabolism involved oxidation in the t-butyl moiety to spiroxamine acid. Metabolism via this route formed three metabolites: the parent compound acid (which exists as two isomeric forms) and also formed a glucuronide conjugate and the two N-desalkylated compounds, spiroxamine despropyl acid and spiroxamine desethyl acid (both of which exist as two isomeric forms). Other metabolites which were determined are related to oxidation of the parent compound to the alcohol which then formed the sulphated form of the acid. This moiety further metabolised to the corresponding desmethyl and despropyl moieties, all seen in both isomeric forms. Other very minor metabolites were determined, namely the glucuronide of the t-butylcyclohexanol and the metabolite being oxidised to the carboxylic acid to the carboxylic acid in the t-butyl moiety and simultaneously monohydroxylated in the position of the cyclohexyl ring also bearing the t-butyl group.

Unchanged parent spiroxamine was only seen in very small amounts in the kidney and liver and was not detected in other edible tissues or milk.

I. Materials and methods

A. Test material

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (µCi/mg):	9.87 (0.365 MBq/mg)
Lot/Batch No.:	KML 2038
Radiochemical purity:	>98% (sum of isomers) by radio HPLC
CAS No.:	118134-30-8

Test System: Lactating goat

Species:	Lactating goat *(Capra hircus)*
Strain:	Bunte Deutsche Edelziege
Justification:	Lactating goats are recognised as a model for dairy cows and beef cattle to comply with the corresponding residue studies
Number / sex:	Single lactating female

Age / body weight:	The goat was approximately 18 to 20 months old at the time of dosing and bodyweight at day 1 was 43.0 kg decreasing to 42.6 kg at sacrifice.
Acclimatisation:	The goat was purchased from Ziegenzuchverband Baden-Württemberg e.V., Stuttgart, Germany. It was acclimatised to laboratory conditions for 1 week prior to the study starting. Daily maximum and minimum temperatures and humidity were recorded. Fresh feed (hay and ruminant feed) was offered with approximately 500g of ruminant feed plus one apple offered per day. Hay way offered *ad libitum*. Food consumption was recorded. The animals had ad libitum access to fresh potable water. Animals were milked twice daily during the acclimation and study phase.

B. Study design

The study dates are not specifically stated in the report, however from the QA statement, the protocol was inspected in July 1992 and the final report was issued in July 1995, so theoretically, some samples may have been stored for up to 3 years.

The study was conducted at [redacted]

Experimental conditions

A lactating goat (*Capra hircus*, strain Bunte Deutsche Edelziege) was dosed orally with [^{14}C]spiroxamine (labelled in the 1-position of the cyclohexyl ring) for three consecutive days at a rate of 10 mg/kg body weight. Based on the average feed consumption of 4% of the bodyweight per day and the goat weighing approximately 43 kg bodyweight, this equated to an exaggerated concentration of approximately 250 mg/kg in the diet. Doses were administered orally via gelatin capsules.

During both the acclimation and treatment period, the goat was house in an electro-polished stainless steel metabolism cage that allowed the separate quantitative collection of urine and faeces. The maximum and minimum ambient temperature was recorded daily (23 ± 2°C) and the relative humidity was 73 ± 15%. During the whole residence time, the goat was fed with hay and ruminant feed (ssniff S for sheep). Approximately 500g of ruminant feed and 1 apple was offered each day Hay was provided *ad libitum*. Fresh potable drinking water was also provided *ad libitum*.

Blood was taken from the ear vein of the goat at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after the first dose administration. Blood was collected into heparinised capillary tubes.

During the test period, the goat was milked twice daily (immediately prior to dose administration in the morning and approximately 8 hours later). The milk weights were recorded. One aliquot was taken from each fraction, quantified by liquid scintillation counting (LSC). The remaining milk was stored frozen (approximately -20°C) for metabolite analysis. Urine and faeces were collected 24 hours after the first and second dose administration and 7 hours after the third. Urine was collected over dry ice and stored frozen whish faeces were collected and stored at ambient temperature.

The goat was sacrificed 7 hours after the last dose (which coincided with one hour after the determined maximum concentration in plasma and was equivalent to 55 hours after the first dose). The following tissues were collected from the treated animal: kidney, liver, muscle (flank, loin and round) and fat (peri-renal, subcutaneous and omental). Milk, excreta and plasma were collected throughout the dosing period and were also analysed. Samples were stored frozen at -20°C.

The total radioactive residue (TRR) in milk and tissues were determined.

Dose preparation and dosing

[^{14}C]spiroxamine (labelled in the 1-position of the cyclohexyl-ring) was evaluated.

The test item was delivered as a solution in approximately 50 mL acetonitrile containing 0.25% triethylamine. The solution was calibrated by radioactivity measurement. The total amount of radioactivity was 1602 mg with 0.365 MBq/mg (2.19 x 10^7 dpm/mg). The solvent was removed under a gentle stream of nitrogen at room temperature.

Appropriate amounts of the dose were weighed. For each of the three administrations, 430 mg of the compound was distributed to 2 gelatin capsules. The capsules were filled the day prior to the first administration and were stored refrigerated (5°C) until usage.

Based on the specific radioactivity of the compound and the weight of the goat at administration (43 kg), the lactating goat received a total radioactive dose of 471 MBq or 2.83 x 10^{10} dpm, corresponding to 1290 mg. This therefore corresponded to a mean actual dose of 10 mg per kg body weight dosage.

The gelatin capsules filled with the liquid test item were administered through a flexible stomach tube (diameter 0.8 cm, length 80 cm) that was lubricated with corn oil and inserted directly into the rumen. No adverse dose-related symptoms were observed.

Sacrifice and tissue collection

The goat was sacrificed 7 hours after the last dose (which coincided with one hour after the determined maximum concentration in plasma and was equivalent to 55 hours after the first dose). The following tissues were collected from treated animals: kidney, liver, muscle (flank, loin and round) and fat (peri-renal, subcutaneous and omental). Milk, excreta and plasma were collected throughout the dosing period and also analysed.

Total radioactivity was determined in samples of dose solutions, excreta, plasma, milk and tissues (muscle, liver, kidney and fat).

Sample analysis

Preparation of samples and radiochemical analysis

Blood/plasma

Blood was collected into heparinised capillary tubes, which were centrifuged at 12,000g for 10 minutes to obtain the plasma in a haemocrit centrifuge. Radioactivity content in the plasma samples was determined by liquid scintillation counting (LSC).

Milk

The goat was milked twice daily, immediately before dosing in the morning and approximately 8 hours later. A representative aliquot was taken from each sample and radioactivity content determined by LSC in duplicate. The remaining milk was stored frozen at -20°C for metabolite profiling.

Urine

The urine fractions were collected as quantitatively as possible under dry-ice cooling at 24 hour intervals after the first and second doses and 7 hours after the third at termination of the study. The collection vessels were changed and the collection funnel was rinsed with deionised water into a separate collection vessel at the end of each collection period. A sub-sample of each urine and rinse sample was taken from all fractions. After recording the total volumes, the samples were radioassayed in duplicate by LSC. The remaining urine samples were stored frozen at -20°C for (optional) metabolite profiling.

Faeces

The faeces fractions were collected as quantitatively as possible at room temperature at 24 hour intervals after the first and second doses and 7 hours after the third at termination of the study. The collecting grid was cleaned prior to each dose administration. No samples of the rinsing water were taken for

radioactivity measurement. The faeces were freeze-dried and homogenised. After recording the total dry weight, one aliquot of each fraction was prepared and combusted in triplicate. The absorbed $^{14}CO_2$ was measured by LSC. The remaining faeces samples were stored frozen at room temperature for (optional) metabolite profiling.

Tissues

The following tissues and organs were dissected: liver, kidneys, muscle (flank, round and loin) and fat (peri-renal, omental and subcutaneous).

After recording of weights, tissue samples were transferred into ice-cooled vessels. Liver, kidney and muscle samples were minced 4-5 times through a Mőkle-boy mincing machine in the presence of dry-ice. One sample of the resulting minced tissue sample pulp was weighed, freeze-dried, weighed again and prepared for combustion analysis in triplicate to determine radioactivity content.

One sample of each type of fat was taken without homogenisation. The samples were solubilised for radioassay using BTS 450 tissue solubiliser. Each samples was measured by LSC in duplicate.

Samples for metabolite analysis were prepared in their wet state and stored frozen (-20°C).

Combustion analysis

Combustion analysis was carried out using a Packard Tri-Carb Tissue Oxidiser (Model 306). The resultant $^{14}CO_2$ was absorbed in Carbosorb and Scintillation fluid (Permafluor E+) was added. The efficiency of the oxidiser was determined prior to sample analysis.

Liquid scintillation counting (LSC)

Liquid samples were counted in a liquid scintillation counter (Beckman LS 6000 LL, Philips PW 4700, LKB Rack Beta 1219 or LKB Rack Beta 1214) using commercial scintillation cocktails (Instant Scint-Gel, Quick-Safe A). Counts per minute (cpm) were converted to dpm using a set of commercially prepared quenched standards.

High performance liquid chromatography (HPLC)

A Hewlett Packard 1050 Liquid Chromatograph was used equipped with a variable wavelength detector. Radioactivity was detected using a Ramona (Raytest) flow-through detector.

Mass spectroscopy (MS)

GC/MS analyses were performed on a mass selective detector HP 5970 with a gas chromatograph 5880A (Hewlett-Packard).

Mass spectra obtained by direct evaporation (electron impact and chemical ionisation) were recorded on a Finnegan 8230 instrument.

Other radioactive residues were analysed on a Finnegan TSQ 7000 instrument (ESI). A radioactivity detector was coupled between the HPLC instrument and the mass spectrometer. The delay time between the two instruments was approximately 20 seconds.

Nuclear magnetic resonance spectroscopy (NMR)

The ^1H NMR spectrum was recorded on a Bruker AC 300. Methanol (CD_3OD, Merck) was used as the solvent.

Derivatisation

Trimethylsilyl derivatives were prepared by reaction with N-methyl-N-(trimethylsilyl)trifluoro-acetamide (MSTFA, Pierce) at 70°C for 10 minutes. The measurement of the mass spectra was conducted directly from the reaction mixture.

Enzyme treatment

Selected samples were incubated with a β-glucosidase/sulphatase enzyme mixture (*Helix pomatia*, Serva). 10µL of aqueous samples was dissolved in 1mL methanol and 100 µL standard citrate buffer, (pH5) were mixed with 5µL β-glucosidase/sulphatase and incubated for 4 hours at 37°C. Enzyme activity was assessed by taking an aliquot of the reaction mixture and incubating with an aqueous solution of phenolphthalein glucuronide sodium salt under identical conditions. After addition of glycine buffer, a red colour developed indicating the presence of enzyme activity.

Characterisation of radioactivity

Tissue samples were extracted with a sequence of solvents to characterise and quantify the distribution of radioactive residues in the sample.

Extraction of milk

The combined milk sample was slowly mixed with 2L of acetonitrile under constant stirring. After addition of the acetonitrile solution, the solution was stirred for a further 5 hours before it was centrifuged for 10 minutes at 8000 rpm and the clear supernatant separated. The sediment was exhaustively extracted with mixtures of acetonitrile and heptane under homogenisation an Ultra-Turrax tissue homogeniser. The post-extraction solid (PES) was dried under vacuum and residual radioactivity determined by combustion. Radioactivity in the extracts was determined by LSC.

The combined acetonitrile phases were further purified by partitioning with acetonitrile/methanol, followed by pure methanol and finally partitioning against acetonitrile/methanol/water with the latter phase extracting almost all of the available radioactivity (97.6% TRR). The sample was concentrated prior to HPLC analysis.

Extraction of liver

Macerated liver (51.22g) was added to a suction filter. To the liver homogenate, 150 mL of acetonitrile and 0.5 g of de-foaming agent were added and the slurry treated for 5 minutes with a tissue homogenised (polytron). The extract was then filtered under a slight vacuum before a mixture of acetonitrile:water containing the de-foaming agent (7:3 v/v) was added to the extracted residue. The homogenisation was repeated and the filtrate removed. This last step was repeated 3 times and all filtrates and The post-extraction solid (PES) were quantified for radioactivity content.

Extraction of kidney

Macerated kidney (41.89 g) was extracted in the same way as liver. A second kidney sample (54.91 g) was extracted for further isolation and identification of metabolites which could not be identified by co-chromatography from the first extract.

Extraction of muscle

Composite muscle sample (74.36 g round muscle, 20.18g flank muscle and 11.16g loin muscle) was extracted in a similar manner to that described for liver and kidney. The only difference was that a successive treatment with heptane, acetonitrile:methanol (1:1 v/v) and methanol was used.

Extraction of fat

Subcutaneous fat sample (22.12 g) was extracted in a similar procedure as that described for eggs except the ratio of acetonitrile/tetrahydrofuran used was 1:1 v/v. The acetonitrile/tetrahydrofuran phase was mixed with water and acidified with acetic acid and subsequently partitioned twice with n-heptane. The first heptane phase was re-partitioned with acetonitrile/tetrahydrofuran/water/acetic acid (55:55:20:2 v/v/v/v). All acetonitrile phases were combined and concentrated for HPLC. Over 99% TRR was readily organo-extractable and almost 92% TRR was chromatographed by HPLC.

Extraction of urine

Urine (from the 24 - 48 hour dose interval) was purified by a combination of an anion exchange cartridge (SAX) followed by a reverse phase cartridge (C8). Both cartridges were pre-conditioned with organic solvents, water and a pH 7.25 buffer. The urine was drawn-through the columns under gentle vacuum followed by a water rinse. The C8 column was eluted with 10 mL acetonitrile followed by 10 mL methanol whilst the SAX-cartridge was eluted with a 1:1 mixture of methanol and 25% H_3PO_4. The C8 eluate was taken for further isolation of metabolites. It was taken to dryness at 60°C and redissolved in a mixture of methanol/water (1:4 v/v). This sample was then subjected to further purification via HPLC which is described in the results section.

In a second purification procedure the SAX-elutes were further purified by a RP-18 cartridge. These were successfully rinsed with water/methanol/acetonitrile (18:1:1 v/v/v) acetonitrile and methanol. The bulk of the radioactivity and the more unipolar metabolites were found in the latter two eluates and were further purified.

II. Results and discussion

Total radioactive residues (TRR)

Refer to Table CA 6.2.3/01-1 to CA 6.2.3/01-4.

A lactating goat was dosed with 10 mg spiroxamine /kg bodyweight (which corresponded to an exaggerated concentration of 250 mg/kg in the feed). The radiochemical purity for the test item was >98%.

Analysis of plasma showed that total spiroxamine residues showed a broad maximum concentration with a calculated peak concentration after 6.6 hours of 1.86 µg/mL (Table CA 6.2.3/01-1). Kinetic assessment determined that radioactivity was eliminated from the plasma, with an elimination half-life ($t_{1/2e}$) of 6 hours and a mean residence time (MRT) of 14 hours.

After three consecutive daily doses of spiroxamine, approximately 63% of the administered radioactivity was excreted in the urine with a further 12% TRR eliminated in the faeces. Approximately 20% of the dose was recovered in the in the first 24 hours and a further 39% in the second 24 hour period (Table CA 6.2.3/01-2). Only 0.23% TRR was recovered in milk and approximately 3.39% TRR was estimated in tissues, giving an overall recovery of administered dose of 78.48%. Cage washings were not assayed and almost certainly contributed towards much of the remainder of the administered dose.

A plateau concentration in milk was not demonstrated (Table CA 6.2.3/01-3). After three consecutive daily does, the concentration of radioactive residues reached a maximum of 0.930 mg/mL. Overall, 0.23% TRR was recovered in milk.

Residue levels in milk and edible tissues were determined after sacrifice (7 hours after the final dose to reflect peak plasma concentration) and are shown in Table CA 6.2.3/01-4. Highest tissue residues were seen in the liver (22.07 mg/kg) and kidney (14.21 mg/kg). Residue levels in muscle and fat were lower, with an average composite concentration of 1.035 mg/kg in muscle and 0.654 mg/kg in composite body fat.

Table CA 6.2.3/01-1 Concentration of spiroxamine residues (µg/mL) in plasma in a lactating ruminant after dosing with spiroxamine (10 mg/kg bodyweight)

Time after first administration (hours)	Mean concentration (µg/mL)
0.25	0.059
0.50	0.576
1.00	0.987
2.00	1.246
3.00	1.510
4.00	1.740
6.00	1.918
8.00	1.776
24.00	0.611

Table CA 6.2.3/01-2 Summary of recovery of the recovery of radioactivity after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight per day)

	Time after first dose	Average% Total administered radioactivity
Urine (+ funnel rinse)	0	
	24	17.07
	48	32.29
	53	13.56
Subtotal		62.92
Faeces (+ funnel rinse)	0	
	24	3.23
	48	6.62
	53	2.09
Subtotal		11.94
Milk	0	
	8	0.042
	24	0.034
	32	0.058
	48	0.039
	55	0.052
Subtotal		0.225
Total excreted		**75.09**
Estimated residue in tissues[1]		3.39
Total recovery		**78.48**

1 – calculated from the body weight assuming 30 and 12 of the body weight for total muscle and dissectible body fat respectively. (body weight at sacrifice 42.6 kg).

Table CA 6.2.3/01-3 Mean concentration of radioactive residue in milk samples after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)

Time after first administration (h)	Dose number	Spiroxamine equivalent concentration (mg/mL)	% TRR	Cumulative % TRR
0	1	-	-	-
8		0.655	0.042	0.042
24		0.305	0.034	0.076
24	2	-	-	-
32		0.864	0.058	0.134
48		0.329	0.039	0.173
48	3	-	-	-
55		0.930	0.052	0.226

Table CA 6.2.3/01-4 Summary of the radioactive residues in the edible tissues of a lactating ruminant after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Sample	Spiroxamine equivalent concentration (mg/kg)	TRR expressed as mg [^{14}C] spiroxamine/kg
Liver	22.072	1.94
Kidney	14.206	0.16
Round muscle	1.059	
Flank muscle	1.114	
Loin muscle	0.931	
Total body musculature[1]	1.035[2]	1.03
Peri-renal fat	0.505	
Subcutaneous fat	1.039	
Omental fat	0.419	
Total dissectible body fat[1]	0.654[2]	0.26
Estimated % TRR in edible tissues	-	3.39

1 – calculated from the body weight assuming 30% and 12% of the body weight for total muscle and dissectible body fat respectively (body weight at sacrifice 42.6 kg).
2 – mean concentration of the three different types of muscle or fat

Characterisation

Based on the above results, in order to characterise and identify the nature of the radioactive residues, all tissues and pooled milk samples were extracted. A summary of the extraction regimes is presented in Table CA 6.2.3/01-5 to Table CA 6.2.3/01-9. Both uncorrected and normalised data are presented.

Radioactive components in extracts were quantified and identified using HPLC and LC-MS/MS.

Available reference standards for characterisation were spiroxamine (KWG 4168) itself as the parent test substance and the following potential metabolites:

It should be noted that the reference compounds used for characterisation and identification of radioactive residues were typically isolated in their radioactive form from either rat or goat urine from other metabolism studies and spectroscopically identified. This was considered necessary as neither the parent compound or its metabolites demonstrated adequate UV absorbance.

The following reference materials were structurally elucidated in this manner:

ECW 80511 and ECW 8046 (spiroxamine-acid) (M06)

ECW 8044 and ECW 8045 (spiroxamine-desethyl acid) (M11)

ECW 8042 (spiroxamine-despropyl acid) (M12)

ECW 8085

ECW 8081 (spiroxamine-cyclohexanol-glucuronide) (M22)

ECW 8096 (spiroxamine-8-hydroxy acid) (M08)

ECW 8079 and ECW 80822 (spiroxamine-desethyl-sulphate) (M26)

ECW 80862 (spiroxamine-despropyl-sulphate) (M27)

ECW 8076 (spiroxamine-sulphate) (M25)

WAK 6301 (spiroxamine-N-oxide) (M03)

Milk

Table CA 6.2.3/01-5 and Table CA 6.2.3/01-11.

Total radioactive residues in a pooled milk sample were 0.929 mg/kg. Radioactive residues were almost completely extracted with acetonitrile based solvent mixtures (>99% TRR based on the normalised balance). Less than 1% TRR remained associated with the PES. The acetonitrile phases were combined extracted with heptane.

After partitioning, over 96% TRR (normalised) was recovered in the acetonitrile/methanol partitions. The acetonitrile phases were combined and concentrated and re-extracted with acetonitrile/methanol/water. This recovered almost all of the radioactivity in the sample and was applied to HPLC analysis. Separation of peaks was achieved by applying a gradient from pH 8 to 6.8 in a water/acetonitrile system on an ODS-Hypersil column. Triethylamine was added to the solvent to improve peak shapes.

The concentrated milk extract was chromatographically compared to the radioactive reference materials isolated from a previous rat metabolism study. A total of 77.8% TRR (0.723 mg/kg) was identified. No parent spiroxamine was detected in milk. The major metabolite determined was spiroxamine-acid (M06) accounting for 53.3%TRR (0.496 mg/kg). This metabolite was formed via oxidation of the t-butyl moiety forming the carboxylic acid. This metabolite goes on to form three other metabolites; spiroxamine-desethyl acid (M11) accounting for 6.5% TRR, 0.051 mg/kg, spiroxamine-hydroxy acid (M07) (10.9% TRR, 0.101 mg/kg) and the sulphated form of the acid (spiroxamine – sulphate, M25) (8.1% TRR, 0.075 mg/kg).

A total of 9 unidentified smaller metabolites were detected in whole egg extracts. The largest of these accounted for 8.3% TRR (0.077 mg/kg) and they totalled 19.9% TRR (0.185 mg/kg). No further work was conducted to identify these residues.

Therefore, 77.8% TRR in milk was identified (0.723 mg/kg) and a total of 97.7% TRR was characterised (0.908 mg/kg).

Liver

Table CA 6.2.3/01-6 and Table CA 6.2.3/01-11.

The total radioactive residue in liver tissue was 22.072 mg/kg. Similarly to milk, radioactive residues were almost completely extracted with acetonitrile-based solvent mixtures (>98% TRR based on the normalised balance). Less than 2% TRR remained associated with the PES. The acetonitrile phases were combined extracted with heptane.

After partitioning, almost 87% TRR (normalised) was recovered in the first 3 acetonitrile partitions, with a further 6.5% TRR released by acetonitrile/ethanol/methanol. 5% TRR remained associated with the heptane fraction. The acetonitrile phases were combined and concentrated prior to HPLC analysis.

The extracts were successfully quantitatively re-extracted with acetonitrile/water/ethanol and methanol/ethanol after the addition of sodium chloride. The remaining heptane phase (approximately 72% TRR) was successfully re-partitioned with acetonitrile/methanol/water. The acetonitrile containing phases were combined and concentrated.

Some small losses occurred during the purification and concentration phases meaning 92.3% TRR was analysed by HPLC. A total of 76.3% TRR (16.85 mg/kg) was identified. Spiroxamine-acid metabolite (M06) was identified by co-chromatography with reference compounds. This accounted for 19.6% TRR; 4.33 mg/kg). A number of other metabolites were detected in liver: spiroxamine - 8-hydroxy acid (M08) (1.2% TRR, 0.26 mg/kg), spiroxamine-desethyl sulphate (M26), (1.9% TRR, 0.42 mg/kg), spiroxamine-sulphate (M25) (2.2% TRR, 0.49 mg/kg) and spiroxamine – acid glucuronide, the glucose conjugate of the parent carboxylic acid moiety (M19) (32.7% TRR, 7.22 mg/kg). Structural elucidation showed the presence of parent spiroxamine (5.0% TRR, 1.10 mg/kg) and two metabolites formed by desalkylation; spiroxamine-desethyl acid (M11) accounting for 3.8% TRR (0.84 mg/kg) and spiroxamine-despropyl acid (M12) accounting for 3.5% TRR (0.77 mg/kg).

A total of 10 unidentified smaller metabolites were detected in liver extracts. The largest of these accounted for 6.4% TRR (1.41 mg/kg). Unidentified components totalled 18.1% TRR (4.01 mg/kg) No further work was conducted to identify these residues.

Therefore, 76.3% TRR in liver was identified (16.85 mg/kg) and a total of 94.4% TRR was characterised (20.86 mg/kg).

Kidney

Table CA 6.2.3/01-7 and Table CA 6.2.3/01-11

The total radioactive residue in kidney tissue was 14.206 mg/kg. As in other samples, radioactive residues were almost completely extracted with acetonitrile-based solvent mixtures (>99% TRR based on the normalised balance). Less than 1% TRR remained associated with the PES. The acetonitrile phases were combined extracted with heptane.

Almost 57% TRR remained in the heptane phase after partition, so this heptane phase was concentrated, redissolved and partitioned again against acetonitrile/methanol/water. All organic acetonitrile containing phases from the two separate heptane extractions were combined and concentrated for HPLC.

Some small losses occurred during the purification and concentration phases meaning 94.8% TRR (13.47 mg/kg) was analysed by HPLC. A total of 68.0% TRR (9.66 mg/kg) was identified. Spiroxamine-acid (M06) was identified by co-chromatography with reference compounds. This accounted for 10.4% TRR; 1.48 mg/kg). A number of other metabolites were detected in kidney: spiroxamine - 8-hydroxy acid (M08) (2.3% TRR, 0.33 mg/kg), spiroxamine-desethyl-sulphate (M26) (3.2% TRR, 0.45 mg/kg), spiroxamine-sulphate (M25) (1.6% TRR, 0.23 mg/kg) and spiroxamine acid glucuronide – the glucose conjugate of the parent carboxylic acid moiety (M19) (13.3% TRR, 1.89 mg/kg). Structural elucidation showed the presence of trace amounts of parent spiroxamine (0.2% TRR, 0.03 mg/kg) and the two metabolites formed by desalkylation; the spiroxamine-desethyl (M11) accounting for 5.8% TRR (0.82 mg/kg) and the spiroxamine-despropyl (M12) accounting for 9.0% TRR (1.28 mg/kg). Trace amounts of the glucuronide of the t-butylcyclohexanol moiety (M22) were also detected (0.4% TRR, 0.06 mg/kg).

It should be noted that the kidney was extracted a second time as initial HPLC traces showed the presence of 2 peaks that did not match the reference materials. The two unidentified peaks were isolated and purified prior to LC-MS. The ESI mass spectra of both fractions was identical to the ESI mass spectrum of the metabolite spiroxamine-hydroxy acid (M07) that was isolated from urine. It was concluded that the two compounds isolated from the kidney were the two isomeric forms of the metabolite being oxidised in the t-butyl moiety to form the carboxylic acid and simultaneously at a

second methyl group to give the alcohol which was also isolated and identified in urine. This moiety as spiroxamine-hydroxy acid (M07) accounted for 16.0% TRR (2.27 mg/kg).

A total of 8 unidentified smaller metabolites were detected in kidney extracts. The largest of these accounted for 6.0% TRR (0.85 mg/kg). Unidentified components totalled 26.6% TRR (3.78 mg/kg) No further work was conducted to identify these residues.

Therefore, 68.0% TRR in kidney was identified (9.66 mg/kg) and a total of 94.6% TRR was characterised (13.44 mg/kg).

Muscle

Table CA 6.2.2/01-8 and Table CA 6.2.2/01-11.

The total radioactive residue in the composite muscle sample was 1.035 mg/kg. As with other tissues, radioactive residues were almost completely extracted with acetonitrile-based solvent mixtures (>99% TRR based on the normalised balance). Less than 1% TRR remained associated with the PES. The acetonitrile phases were combined and partitioned with n-heptane.

Approximately 57% TRR remained in the heptane phase after partition, so this heptane phase was concentrated, redissolved and portioned again against acetonitrile/methanol/water. All organic acetonitrile containing phases from the two separate heptane extractions were combined and concentrated for HPLC.

Some very small losses occurred during the purification and concentration phases meaning 97.4% TRR (1.01 mg/kg) was analysed by HPLC. A total of 79.7% TRR (0.824 mg/kg) was identified. The major metabolite in muscle tissues was spiroxamine-acid metabolite (M06) and was identified by co-chromatography with the reference compounds. This accounted for 48.3% TRR (0.500 mg/kg).

A number of other metabolites were detected in muscle including spiroxamine acid glucuronide (M19) (7.9% TRR, 0.082 mg/kg), spiroxamine-hydroxy acid (M07) (also seen in liver and kidney) accounting for 10.3% TRR (0.106 mg/kg), and the two metabolites formed by desalkylation; the spiroxamine-desethyl (M11) accounting for 6.4% TRR (0.066 mg/kg) and the spiroxamine-despropyl (M12) accounting for 6.8% TRR (0.07 mg/kg). No parent spiroxamine was detected in muscle.

A total of 7 unidentified smaller metabolites were detected in composite muscle extracts. The largest of these accounted for 6.6% TRR (0.068 mg/kg). Unidentified components totalled 17.9% TRR (0.183 mg/kg). No further work was conducted to identify these residues.

Therefore, 79.7% TRR in composite muscle was identified (0.824 mg/kg) and a total of 97.6% TRR was characterised (1.007 mg/kg).

Fat

Table CA 6.2.2/01-9 and Table CA 6.2.2/01-11.

The total radioactive residue in the composite fat sample was 0.654 mg/kg. Again, radioactive residues were exhaustively extracted with acetonitrile-based solvent mixtures (>97% TRR based on the normalised balance) which almost completely solubilised the tissue sample with only 0.61% remaining associated with the PES. The heptane phases were combined and partitioned with methanol/water. The acetonitrile phases were combined, concentrated and re-partitioned with heptane. The resulting radioactivity was almost exclusively retained in the acetonitrile/methanol/water phase.

The acetonitrile phases were combined and concentrated prior to HPLC analysis. Some small losses occurred and 97.1% TRR was subjected to chromatographic analysis.

By co-injection with reference mixtures, the following metabolites were detected in fat. A total of 73.1% TRR (0.498 mg/kg) was identified. The major metabolite in fat tissue was the spiroxamine-acid metabolite and was identified by co-chromatography with the reference compounds. This accounted for (30.5% TRR, 0.199mg/kg). A number of other metabolites were detected in fat

including spiroxamine – acid glucuronide (M19)– the glucose conjugate of the parent carboxylic acid moiety (15.4% TRR, 0.101 mg/kg), spiroxamine-hydroxy acid (M07) which was also seen in liver, kidney and muscle, accounting for 9.7% TRR (0.063 mg/kg), and the two metabolites formed by desalkylation; the spiroxamine-desethyl (M11) accounting for 4.3% TRR (0.028 mg/kg) and the spiroxamine-despropyl (M12) accounting for 6.2% TRR (0.041 mg/kg). No parent spiroxamine was detected in fat.

A total of 8 unidentified smaller metabolites were detected in composite fat extracts. The largest of these accounted for 7.4% TRR (0.048 mg/kg). Unidentified components totalled 24.4% TRR (0.158 mg/kg) No further work was conducted to identify these residues.

Therefore, 73.1% TRR in composite fat was identified (0.723 mg/kg) and a total of 97.5% TRR was characterised (0.656 mg/kg).

Urine

Table CA 6.2.3/01-10

The total radioactivity in the composite urine sample (24 – 48 hrs) was 103782998 dpm.

The urine was purified by a combination of an anion exchange cartridge (SAX) followed by a reverse phase cartridge (C8). Most of the TRR in urine (78.90%) was recovered in the eluate from the C8 cartridge.

The urine was particularly used as a source of radiolabelled reference materials that other tissue extracts were compared against via co-chromatography. The urine was also used to aid the structural elucidation of an unknown component in tissue extracts that did not co-chromatograph with other reference materials. The metabolite was isolated from eluate fractions and subjected to MS and ^{1}H-NMR. The unknown metabolite was demonstrated to have the same chemical structure of the metabolite spiroxamine-hydroxy acid (KNO 2212, M07).

Further quantitative extraction and analysis of urine was not conducted.

Table CA 6.2.3/01-5 Summary of the extractability and characterisation of radioactivity in composite milk sample after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 0.929 mg/kg	
	% of initial radioactivity	Normalised balance
Initial extracts		
3L Acetonitrile	94.13 (0.874)	95.84
Acetonitrile	2.49 (0.023)	2.53
Acetonitrile	0.65 (0.006)	0.66
Acetonitrile/0.5% NaCl (1:1 v/v)	0.35 (0.003)	0.36
Heptane	0.06 (0.001)	0.06
Heptane	0.04 (<0.001)	0.04
Heptane	0.01 (<0.001)	0.01
Post extraction solid (PES)	0.50 (0.005)	0.51
Total	98.22 (0.912)	100.00
Extracts 1 – 4 combined, pH adjusted and extracted with heptane		
Heptane after partition	1.93 (0.018)	1.87
Acetonitrile/methanol (1:1 v/v)	87.04 (0.809)	84.56

Extract	% TRR (mg/kg)[1]	
	Total residue 0.929 mg/kg	
	% of initial radioactivity	Normalised balance
Acetonitrile/methanol (1:1 v/v)	8.03 (0.075)	7.80
Acetonitrile/methanol (1:1 v/v)	4.16 (0.039)	4.05
Methanol	0.67 (0.006)	0.65
Residue	1.10 (0.010)	1.07
Total	102.93 (0.956)	100.00
Concentrated MeCN extracts pH adjusted and redissolved in heptane. Partitioned against MeCN:MeOH:H2O (3/5.6/2/4 v/v/v)		
Heptane	0.70 (0.007)	0.76
Acetonitrile/methanol/water (3:5.6:2.4 v/v/v)	91.48 (0.850)	99.24
Total	92.18 (0.856)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.3/01-6 Summary of the extractability and characterisation of radioactivity in liver from a lactating ruminant after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 22.072 mg/kg	
	% of initial radioactivity	Normalised balance
Initial extracts		
Acetonitrile	57.39 (12.67)	57.31
Acetonitrile/water (7:3 v/v)	27.47 (6.06)	27.43
Acetonitrile/water (7:3 v/v)	9.29 (2.05)	9.28
Acetonitrile/water (7:3 v/v)	2.68 (0.592)	2.68
Acetonitrile/water (7:3 v/v)	1.33 (0.294)	1.33
Post extraction solid (PES)	1.98 (0.437)	1.98
Total (not normalised)	100.14 (22.10)	100.00
Extracts 1 – 3 combined and extracted with heptane		
Heptane after partition	71.63 (15.81)	72.75
Acetonitrile/methanol/ethanol (1:1:1 v/v/v)	14.17 (3.13)	14.39
Methanol/ethanol (1:1 v/v)	7.91 (1.75)	8.03
Sodium chloride solution	4.75 (1.05)	4.82
Total	98.45 (21.73)	100.00
Heptane phase concentrated and redissolved in heptane. Partitioned 4 times against MeCN/MeOH/H2O		
Acetonitrile/methanol/water (1:1:1 v/v/v) (ext 1 & 2)	101.00 (22.29)	97.02
Acetonitrile/methanol/water (1:1:1 v/v/v) (ext 3 & 4)	1.69 (0.373)	1.63
Heptane	1.41 (0.311)	1.35
Total	104.10 (22.98)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.3/01-7 **Summary of the extractability and characterisation of radioactivity in kidney from a lactating ruminant after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)**

Extract	% TRR (mg/kg)[1]	
	Total residue 14.206 mg/kg	
	% of initial radioactivity	Normalised balance
Initial extracts		
Acetonitrile	70.93 (10.08)	66.53
Acetonitrile/water (7:3 v/v)	26.89 (3.82)	25.22
Acetonitrile/water (7:3 v/v)	6.43 (0.913)	6.03
Acetonitrile/water (7:3 v/v)	1.36 (0.193)	1.28
Acetonitrile/water (7:3 v/v)	0.37 (0.053)	0.35
Post extraction solid (PES)	0.64 (0.091)	0.60
Total (not normalised)	106.62 (15.15)	100.00
Extracts 1 – 5 combined and extracted with heptane		
Heptane after partition	56.46 (8.02)	56.92
Acetonitrile/methanol/ethanol (1:1:1 v/v/v)	25.72 (3.65)	25.92
Methanol/ethanol (1:1 v/v)	12.86 (1.83)	12.96
Sodium chloride solution	4.17 (0.592)	4.20
Total	99.20 (14.09)	100.00
Heptane phase concentrated and redissolved in heptane. Partitioned 4 times against MeCN/MeOH/H_2O		
Acetonitrile/methanol/water (1:1:1 v/v/v) (ext 1 & 2)	102.84 (14.61)	97.82
Acetonitrile/methanol/water (1:1:1 v/v/v) (ext 3 & 4)	1.54 (0.219)	1.46
Heptane	0.76 (0.108)	0.72
Total	105.14 (14.94)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.3/01-8 Summary of the extractability and characterisation of radioactivity in composite muscle from a lactating ruminant after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 1.035 mg/kg	
	% of initial radioactivity	Normalised balance
Initial extracts		
Acetonitrile	67.00 (0.693)	68.36
Acetonitrile/water (7:3 v/v)	20.37 (0.211)	20.78
Acetonitrile/water (7:3 v/v)	6.77 (0.070)	6.90
Acetonitrile/water (7:3 v/v)	2.37 (0.025)	2.42
Acetonitrile/water (7:3 v/v)	0.73 (0.008)	0.75
Post extraction solid (PES)	0.77 (0.008)	0.79
Total (not normalised)	98.01 (1.014)	100.00
Extracts 1 – 5 combined and extracted with heptane		
Heptane after partition	56.17 (0.581)	57.04
Acetonitrile/methanol (1:1 v/v)	31.16 (0.323)	31.64
Acetonitrile/methanol (1:1 v/v)	6.84 (0.070)	6.94
Acetonitrile/methanol (1:1 v/v)	2.16 (0.022)	2.19
Methanol	0.88 (0.009)	0.89
Sodium chloride solution	1.27 (0.013)	1.29
Total	98.48 (1.019)	100.00
Heptane phase concentrated and redissolved in heptane. Partitioned 4 times against MeCN/MeOH/H_2O		
Acetonitrile/methanol/water (1:1:1 v/v/v) (ext 1 & 2)	103.82 (1.075)	96.03
Acetonitrile/methanol/water (1:1:1 v/v/v) (ext 3 & 4)	3.34 (0.035)	3.09
Heptane	0.95 (0.010)	0.88
Total	108.10 (1.12)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.3/01-9 Summary of the extractability and characterisation of radioactivity in composite fat from a lactating ruminant after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)[1]	
	Total residue 0.654 mg/kg	
	% of initial radioactivity	Normalised balance
Initial extracts		
Heptane	1.79 (0.012)	1.65
Heptane	0.74 (0.005)	0.68
Heptane	0.23 (0.002)	0.21
Acetonitrile/methanol (1:1 v/v)	49.66 (0.325)	45.70
Acetonitrile	0.77 (0.005)	0.71
Acetonitrile/methanol (1:1 v/v)	0.79 (0.005)	0.73
Acetonitrile/methanol (1:1 v/v)	41.61 (0.272)	38.29

Extract	% TRR (mg/kg)[1]	
	Total residue 0.654 mg/kg	
	% of initial radioactivity	Normalised balance
Acetonitrile/methanol (1:1 v/v)	0.94 (0.006)	0.87
Acetonitrile	2.38 (0.016)	2.19
Acetonitrile/methanol (1:1 v/v)	0.77 (0.005)	0.71
Acetonitrile/0.5% NaCl (7:3 v/v)	5.06 (0.033)	4.66
Acetonitrile/0.5% NaCl (7:3 v/v)	2.57 (0.017)	2.37
Acetonitrile/0.5% NaCl (7:3 v/v)	0.69 (0.005)	0.64
Residue	0.66 (0.004)	0.61
Total	108.67 (0.711)	100.00
The heptane phases (1 & 2) were partitioned with MeOH/H2O (8:2 v/v)		
Methanol/water (8:2 v/v)	26.19 (0.171)	29.06
Methanol/water (8:2 v/v)	26.03 (0.170)	25.51
Heptane 1	63.95 (0.418)	70.94
Heptane 2	75.98 (0.497)	74.48
Acetonitrile phases combined and concentrated. Re-partitioned with heptane		
Heptane 1	0.33 (0.002)	0.34
Heptane 2	0.03 (<0.001)	0.04
Acetonitrile/methanol/water (3:5:2 v/v/v)	96.10 (0.628)	99.62
Total	96.47 (0.631)	100.00

1 – values in parentheses not given in the report. These have been manually calculated using the % TRR given as a function of the total residue in mg/kg.

Table CA 6.2.3/01-10 Summary of the extractability and characterisation of radioactivity in urine from a lactating ruminant after 3 consecutive daily doses of spiroxamine (10 mg/kg bodyweight)

Extract	% TRR (mg/kg)	
	Initial radioactivity: 103782998 dpm	
	% of initial radioactivity	Normalised balance
C8 eluate	77.32	78.90
Urine	20.67	21.10
SAX/C8-rinse	0.73	0.75
SAX-rinse	0.47	0.48
Total	97.99	100.00

Table CA 6.2.3/01-11 Summary of identified and characterised residues in tissues and milk from a lactating ruminant after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Metabolite	Percent of total radioactive residues in the tissue/milk -% TRR (mg/kg)				
	Liver (22.072 mg/kg)	Kidney (14.206 mg/kg)	Muscle (1.035 mg/kg)	Fat (0.654 mg/kg)	Milk (0.929)
% TRR extracted	98.0	99.4	99.2	99.4	99.5
Spiroxamine-despropyl acid (M12)	3.5 (0.77)	9.0 (1.28)	6.8 (0.07)	6.2 (0.041)	-
Spiroxamine-desethyl acid (M11)	3.8 (0.84)	5.8 (0.82)	6.4 (0.066)	4.3 (0.028)	5.5 (0.051)
Spiroxamine-hydroxy acid (M07)	1.7 (0.38)	16.0 (2.27)	10.3 (0.106)	9.7 (0.063)	10.9 (0.101)
Spiroxamine-despropyl-sulphate (M27)	4.7 (1.04)	5.8 (0.82)	-	3.5 (0.043)	-
Spiroxamine - 8-hydroxy acid (M08)	1.2 (0.26)	2.3 (0.33)	-	-	-
Spiroxamine-acid (M06)	19.6 (4.33)	10.4 (1.48)	48.3 (0.500)	30.5 (0.199)	53.3 (0.496)
Spiroxamine-desethyl-sulphate (M26)	1.9 (0.42)	3.2 (0.45)	-	3.5 (0.023)	-
Spiroxamine-acid glucuronide (M19)	32.7 (7.22)	13.2 (1.89)	7.9 (0.082)	15.4 (0.101)	-
Spiroxamine-sulphate (M25)	2.2 (0.49)	1.6 (0.23)	-	-	8.1 (0.075)
Spiroxamine-cyclohexanol-glucuronide (M22)	-	0.4 (0.06)	-	-	-
Spiroxamine	5.0 (1.10)	0.2 (0.03)	-	-	-
Unknown metabolites	18.1 (4.01)[1]	26.6 (3.78)[2]	17.9 (0.183)[3]	24.4 (0.158)[4]	19.9 (0.185)[5]
% TRR identified	76.3 (16.85)	68.0 (9.66)	79.7 (0.824)	73.1 (0.498)	77.8 (0.723)
Total characterised	94.4 (20.86)	94.6 (13.44)	97.6 (1.007)	97.5 (0.656)	97.7 (0.908)

1 – 10 unidentified metabolites in liver with a maximum of 6.4% TRR (1.41 mg/kg)
2 - 8 unidentified metabolites in kidney with a maximum of 6.0% TRR (0.85 mg/kg)
3 - 7 unidentified metabolite in muscle with a maximum of 6.6% TRR (0.068 mg/kg)
4 - 8 unidentified metabolite in fat with a maximum of 7.4% TRR (0.048 mg/kg)
4 - 9 unidentified metabolites in milk with a maximum of 8.3% TRR (0.077 mg/kg)

Storage stability:

Storage intervals for tissues were not specifically stated, however, the report does state that metabolite stability in edible tissues under the prevailing storage conditions were assessed separately and would only be reported if any changes in the metabolic pattern compared with the initial metabolite traces was noted. Samples were analysed without any significant delay. As no further information was included, it is assumed that sample extracts did not deteriorate under the specific storage conditions used in this study indicating no chemical instability.

III. Conclusions

The metabolism of spiroxamine was investigated in a lactating ruminant. A single lactating goat was dosed with [^{14}C]spiroxamine (labelled in the 1-position of the cyclohexyl-ring) for three consecutive daily doses at a rate of 10 mg/kg bw, corresponding to an exaggerated concentration of 250 mg/kg in the feed.

Radioactivity was absorbed from the intestinal tract following a mean lag time of 14 minutes with a mean absorption half life $t_{1/2a}$ of approximately 4 hours. This was followed by a relatively slow distribution process. The mean plasma curve showed a broad maximum concentration. The experimentally determined peak occurred at 6.6 hours (1.86 µg/mL). Elimination from plasma showed a plasma half-life ($t_{1/2e}$) of approximately 6 hours, with a mean residence time (MRT) of 14 hours.

The goat was sacrificed 7 hours after the last dose (which coincided with one hour after the determined maximum concentration in plasma and was equivalent to 58 hours after the first dose). The following tissues were collected from the treated animal: kidney, liver, muscle (flank, loin and round) and fat (peri-renal, subcutaneous and omental). Milk, excreta and plasma were collected throughout the dosing period and also analysed.

An average of 75% of the total radioactivity was recovered in excreta (63% in the urine and a further 12% eliminated in the faeces). It was concluded from the high concentration in the liver, that a significant amount of the faecal radioactivity was absorbed prior to elimination in the bile. Only 0.23% of the administered dose was recovered in milk. A further 3.4% of the total radioactive residue (TRR) was recovered in tissues giving a total recovery of 79% of the administered radioactive dose.

Highest tissue residues were seen in the excretory organs, liver and kidney with mean levels of 22.07 and 14.21 mg/kg respectively. Levels in milk were 0.93 mg/kg (maximum concentration at sacrifice). Residues in muscle and fat were quantitatively similar. In muscle, residue levels in flank, loin and round muscle were 1.11, 0.93 and 1.06 mg/kg respectively and levels in peri-renal, subcutaneous and omental fat were 0.51, 1.04 and 0.42 mg/kg.

Radioactive residues in tissues were solubilised and extracted with mixtures of acetonitrile and water. Recovery of radioactivity was quantitative and complete. After purification, the extracts were co-chromatographed in two different HPTC systems with comparison of metabolites isolated from a rat metabolism study that had previously been spectroscopically identified. By this method, metabolites in edible tissues and milk were identified.

In ruminants, spiroxamine is extensively metabolised. In all tissues and milk, the major metabolites occur following oxidation in the t-butyl moiety to spiroxamine-acid, forming the parent carboxylic acid (M06) and the two desalkylated components; spiroxamine-despropyl acid (M12) and spiroxamine-desethyl acid (M11). Spiroxamine-acid was also identified as its glucuronide conjugate (M19). The corresponding metabolites, which are oxidised in the t-butyl group to the alcohol, were only found in sulphated form. They also occur in the fully alkylated form spiroxamine – sulphate (M25), as the spiroxamine-despropyl-sulphate moiety (M27) and as the spiroxamine-desethyl-sulphate moiety (M26). Further minor metabolites are the spiroxamine – cyclohexanol-glucuronide (M22) and the metabolite being oxidised to the carboxylic acid in the t-butyl moiety and simultaneously monohydroxylated in that position of the cyclohexyl ring also was been identified which was oxidised twice in the t-butyl moiety: one methyl group is oxidised to the carboxylic acid while a second methyl

group is oxidised to the alcohol. The N-terminus still bears both alkyl chains, giving spiroxamine-hydroxy acid (M07). This is the only metabolite which did not occur in the excreta of the rat.

The proposed metabolic pathway for spiroxamine in livestock is shown in Figure CA 6.2-3.

Assessment and conclusion by applicant:

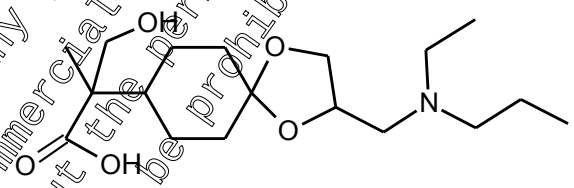
Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.2.3/01. The dosing was only conducted for 3 days and a plateau of residues in milk was not established. However, the study is considered to be acceptable as the analysis is scientifically robust and it is acknowledged that the study was conducted at an exaggerated dose level compared to realistic dietary exposure of lactating ruminants to spiroxamine derived plant residues.

In lactating ruminants metabolism was extensive. The primary route is via oxidation of the t-butyl moiety forming the carboxylic acid which then desalkylates at the amino group forming the spiroxamine despropyl acid and spiroxamine desethyl acid metabolites. Unchanged parent spiroxamine is also seen in very small quantities in edible tissues (liver and kidney).

Data Point:	KCA 6.2.3/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	KWG 4168: Evaluation of the significance of the goat metabolite KNO 2212
Report No:	MR-1017/95
Document No:	M-010086-01-1
Guideline(s) followed in study:	None stated
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

In the edible tissues and milk from the goat study discussed in CA 6.2.3/01, the spiroxamine-hydroxy acid metabolite was identified (KNO 2212; M07):



This was the only metabolite that was unique to the goat and not detected in the rat studies. The relative amounts of this metabolite in milk and tissues is shown in Table CA 6.2.3/02-1.

Table CA 6.2.3/02-1 Summary of total spiroxamine-hydroxy acid residues in tissues and milk from a lactating goat after 3 daily doses of spiroxamine (10 mg/kg bodyweight)

Metabolite	Percent of total radioactive residues -% TRR (mg/kg)				
	Fat	Liver	Kidney	Milk	Muscle
Total radioactive residue (mg/kg)	0.65	22.07	14.21	0.93	1.04
Spiroxamine-hydroxy acid (M07)	9.7 (0.063)	1.7 (0.375)	16.0 (2.275)	11.1 (0.103)	10.3 (0.107)

It was noted in the paper that the dose level in the goat metabolism study was approximately 15X higher when compared to the 1X level in the ruminant feeding study and approximately 130 times higher when compared to a dose per day basis. Given the rapid excretion of radioactive material from the goat, this latter figure was proposed as a more realistic estimation of residues in tissues and milk.

The goat was sacrificed close to peak plasma level, i.e. 7 hours after the final dose and levels in tissues were determined to be approximately 3 times lower in the goat that was sacrificed at 24 hours post-dose. Therefore a realistic estimate of spiroxamine-hydroxy acid residue in the kidney at a 15X overdose might be approximately 0.05 mg/kg. It was noted that in the goat study, the dose was administered via a daily bolus dose rather than taken up over a continuous 24 hour period in the diet. This would mean that in reality, similar peak concentrations are very unlikely to be reached. The concentration of spiroxamine-hydroxy acid in the kidney can also be estimated from the feeding study by using the percentage ratio of the spiroxamine-acid metabolite (M06, which is measured by the residue method) and spiroxamine-hydroxy acid. Based on an average residue of 45 µg/kg (0.045 mg/kg) as determined in the kidney of the feeding study (CA 6.4.2/01) and a contribution of 10.4% of the spiroxamine-acid and 16.0% of the spiroxamine-hydroxy acid, the latter metabolite would be expected to occur at a concentration of 68 µg/kg (0.068 mg/kg) in the kidney of the cattle feeding study, which is in good correlation with the value determined in the present ruminant metabolism study.

The elevated concentration of spiroxamine-hydroxy acid in the kidney compared to other tissues evaluated at peak plasma level was explained by its increased polarity as a result of the introduction of two very polar groups into the metabolite; the carboxylic acid and the hydroxy functions. Increasing the polarity in such a way is likely to result in increased renal metabolic activity as the metabolite will be readily excreted and therefore unlikely to accumulate in milk or tissues. The metabolite in question was also seen as one of the major metabolites in ruminant urine, thus supporting the proposal that it was readily excreted and of only transient nature in the kidney.

Applicant overall conclusions on livestock metabolism

The metabolism of spiroxamine in livestock is summarised in Figure CA 6.2-3.

The metabolism studies described within, show that the metabolic pathway of spiroxamine is similar in laying hens and lactating ruminants although metabolism was found to be more extensive in the ruminant. The available studies provide a good overall assessment of the route of metabolism to satisfy the requirements for Annex I renewal. In both species, the spiroketal structure remains largely intact.

In poultry, metabolism of spiroxamine proceeded via two pathways: oxidation of the t-butyl moiety forming the spiroxamine-acid or alternatively, via desalkylation of the amino group forming the spiroxamine desethyl or spiroxamine despropyl metabolites. Unchanged parent spiroxamine was also seen in edible tissues and eggs.

In lactating ruminants metabolism was extensive. The primary route was via oxidation of the t-butyl moiety forming the carboxylic acid which then desalkylates at the amino group forming the spiroxamine despropyl acid and spiroxamine desethyl acid metabolites. Unchanged parent spiroxamine is also seen in very small quantities in edible tissues (liver and kidney). Additionally glucuronide and sulphate conjugates are also seen in ruminants.

Therefore, the **residue definition for monitoring in livestock meat, fat, milk and eggs is proposed as:**

Ruminants: Spiroxamine-acid (M06), expressed as spiroxamine

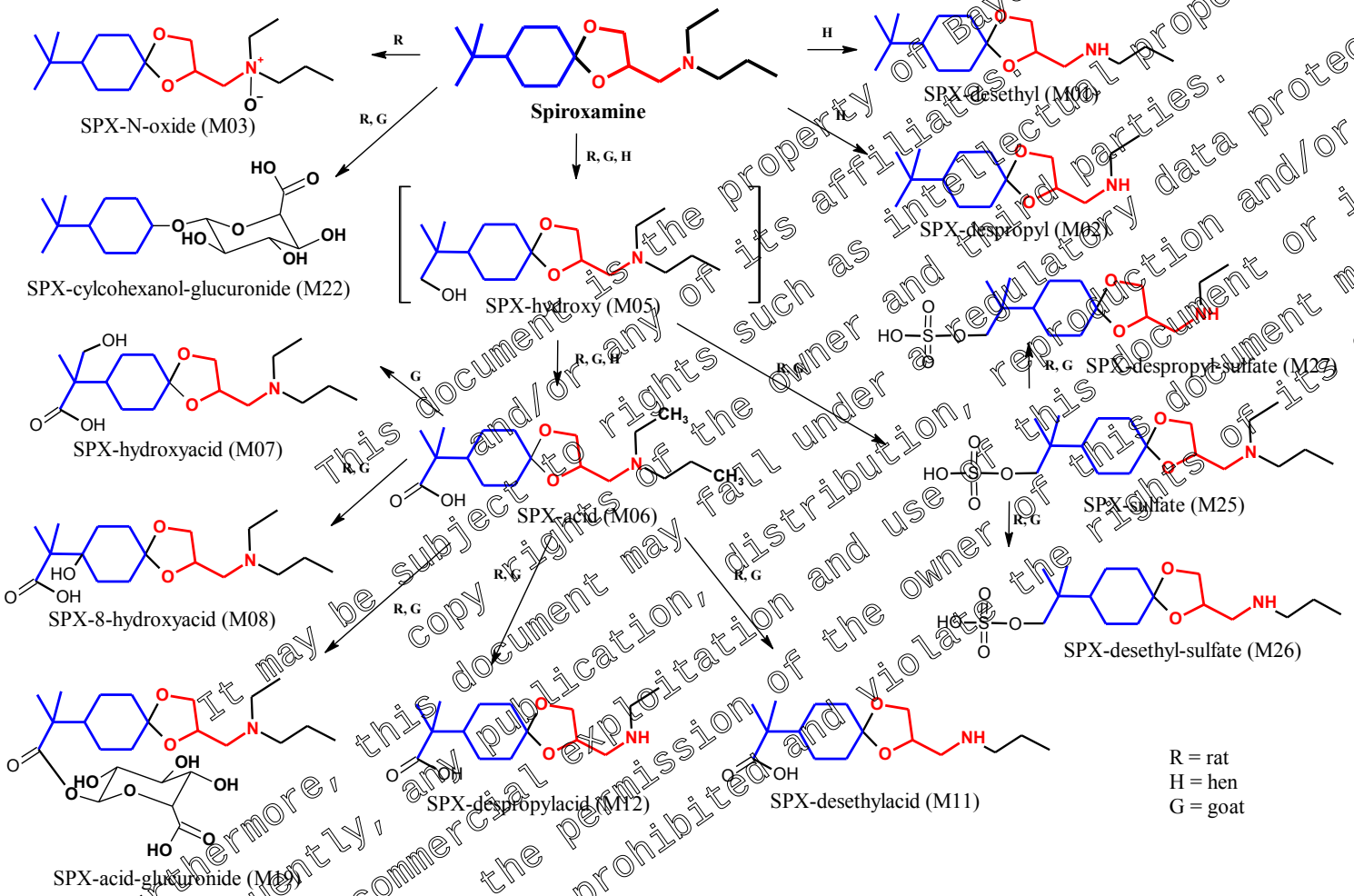
Poultry: Sum of spiroxamine and spiroxamine-acid (M06), expressed as spiroxamine

And for **risk assessment purposes is proposed as:**

Ruminants: Sum of spiroxamine, spiroxamine-acid (M06), its glucuronide conjugate (M19) and spiroxamine-hydroxy acid (M07), expressed as spiroxamine (sum of isomers)

Poultry: Sum of spiroxamine, desethyl-spiroxamine (M01), despropyl-spiroxamine (M02) and spiroxamine-acid (M06) expressed as spiroxamine (sum of isomers)

Figure CA 6.2-3 Proposed metabolic pathway of spiroxamine in livestock (including rats for information)



CA 6.2.4 Pigs

Qualitatively, the metabolism of spiroxamine is very similar in the rat and ruminant. According to the EU data requirements [Commission Regulation (EU) No 283/2013] a significant difference in metabolic pattern is a pre-requisite for a pig metabolism study and it is not justified to make a data requirement in this case. Therefore, no further data on metabolism in pigs are required.

CA 6.2.5 Fish

According to SANCO/11187/2013, fish metabolism data quantify total residues and characterise the chemical nature of residues which may occur in edible tissues of fish exposed to pesticides. They are required when pesticide use may lead to significant residues (generally considered to be >0.1 mg/kg of the total diet (dry weight basis) in fish feed).

Of the crops presented from the representative uses of spiroxamine for Renewal of Approval, only cereals and their processed fractions may form part of a commercial fish diet.

Additionally, the potential of pesticide residues to accumulate in fish tissue is determined to a significant extent by the lipophilicity of the active substance. The accumulation of compounds of relatively low lipophilicity (log Pow <3) via the diet is known to be negligible as far as reported residues in fish are taken into account. Fish metabolism studies are therefore required only for active substances where the log Pow is greater than or equal to 3.

The log Pow of spiroxamine is 2.79 (diastereomer A) and 2.98 (diastereomer B) at 25°C. Therefore metabolism studies in fish are not required. Additionally, it is understood that the conclusions of the SCoPAFF meeting of 24/25 November 2014 still stand, stating that in the absence of agreed test guidelines to be published in the respective Commission Communications, the data requirements regarding fish could still be waived.

However, spiroxamine was exposed to fish in bio-concentration studies conducted as part of the ecotoxicology dossier for spiroxamine (refer to MCA Section 8). A short summary of the study is discussed here:

Data Point:	KCA 6.2.5/01
Report Author:	[redacted]
Report Year:	1997
Report Title:	[Cyclohexyl-1-14C] KWG 4168: Metabolism in the edible parts of bluegill sunfish
Report No:	PF4215
Document No:	M-006169-01-1
Guideline(s) followed in study:	US EPA 165-4 Pesticide Accumulation in Fish
Deviations from current test guideline:	Not conducted to a specific guideline
Previous evaluation:	yes, evaluated and classified DAR (1997), RAR (2016), RAR (2017) Validation not possible (The study as such is acceptable considering the respective EPA test method. However, the cited test is not designed to determine a BCF.)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The purpose of the study was to gain information on the nature of the residue in the edible parts of the fish and to quantify metabolites. A 42-day study was conducted to evaluate the bio-concentration of [cyclohexyl-1-^{14}C]-spiroxamine by bluegill sunfish (*Lepomis macrochirus*). Mean water concentrations of 20 and 200 µg spiroxamine/L, respectively, were maintained for a 28-day exposure period. The purpose of the study was to gain information on the nature and magnitude of the residue in the edible parts of the fish.

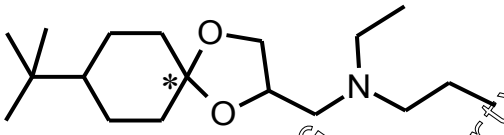
The radioactivity was almost completely extracted from the edible tissues with mixtures of acetonitrile and tetrahydrofuran. After purification, 3 metabolites besides the unchanged parent compound were identified: Spiroxamine-acid (M06) accounting for 12.4% TRR (0.072 mg/kg) at the low dose and 15.4% (0.70 mg/kg) at the high dose, spiroxamine-acid glucuronide (M19) was seen at the high dose accounting for 18.4% TRR (0.83 mg/kg) and spiroxamine-sulphate (M25), which was only seen in edible fish tissue from the low dose, accounting for 5.4% TRR (0.031 mg/kg). Concentration of metabolites was almost exactly an order of magnitude lower in the group of fish dosed at 20 µg [^{14}C]spiroxamine/L, suggesting a good linear dose response.

Therefore, metabolism of spiroxamine in fish is similar to other livestock species. The primary route is via oxidation of the t-butyl moiety forming the carboxylic acid which then forms a glucuronide conjugate. Unchanged parent spiroxamine is also seen in very small quantities in edible tissues

I. Materials and methods

A. Test materials

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (µCi/mg)	2.44 (90.28 KBq/mg) for low a.s. concentration 0.247 (9.14 KBq/mg) for high a.s. concentration
Lot/Batch No.:	Lager Nr. 8686/B
Radiochemical purity:	>99% by radio HPLC
CAS No.:	118134-30-8

Test system: Bluegill sunfish

Species:	Bluegill sunfish *(Lepomis macrochirus)*
Lot:	1293
Acclimatisation:	All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. No mortality was noted in this period and any unsuitable fish (i.e. injured, deformed) were eliminated from inclusion in the test prior to random assignment to study groups. Fish were fed once daily ad libitum with standard fish feed (Kronen-FB 50E) that was analysed for unwanted contaminants.

B. Study design

The test was started June 7, 1994 and exposure ended July 5, 1994. The in-life phase concluded July 19, 1994.

The study was conducted at [redacted]

Experimental conditions

Nominal concentrations of 20 µg/L and 200 µg/L spiroxamine were used in this study, justified as representative of 1 to 10% of the acute LD_{50} from fish toxicity tests.

The dose solutions were dispensed to each 100 L test aquarium via a Hamilton 250 µL syringe controlled by an EPSON HX20 computer. Flow meters were introduced for water flow control. Aerated water was dosed into the aquaria at an average rate of 25 L/hour/aquarium during the 28 day exposure period, which meant the 100L was replaced approximately 6 times in each 24 hour period. Stock solutions ([cyclohexyl-1-^{14}C]spiroxamine in triethyleneglycol) were dosed at a rate of 50 µL every 72 seconds. Water was maintained at a mean temperature of 22°C (± 0.3°C) by adding diluent water that was electronically thermostatted.

Uptake phase

The uptake phase was initiated by transferring groups of 58 randomly selected acclimated fish to each of the control and test acquaria. The fish were observed every 24 hours where possible for mortality and/or adverse behaviour.

Depuration phase

On day 28 of the exposure period, the [^{14}C] spiroxamine dose material was stopped. The acquaria were cleaned mechanically and filled with uncontaminated diluent water for a further 14 days. Water conditions were monitored and recorded.

Sampling

Fish were sampled throughout the uptake and depuration phase. At each sampling interval, 4 fish from each group were collected and processed individually. The fish were dissected into edible (body, muscle, skin, skeleton) and viscera/non-edible (head, fins, internal organs). After weighing, samples were frozen, lyophilised, re-weighed and homogenised. Radioactivity content was determined by combustion to $^{14}CO_2$ followed by liquid scintillation counting (LSC).

Water was also sampled at each sampling interval. Three replicate 3 mL aliquots from each aquarium were removed by pipette. The concentration of radioactivity in each was determined by LSC.

Extraction and sample processing

For the high dose group, combined fish samples (28.92 g) were filtered under suction. After addition of each extraction solution (firstly tetrahydrofuran:acetonitrile, 1:1 v/v, 2 x acetonitrile: 0.5% NaCl, 8:2 v/v, then 2 x acetonitrile: 0.5% NaCl, 7:3 v/v) the sample was homogenised for three minutes using a Polytron homogeniser. Then the solution was filtered by applying a controlled vacuum. The volumes of the filtrates were determined and their radioactivity content was measured by LSC of replicate aliquots. The extracted residue was dried under vacuum and the radioactivity determined after combustion. Further purification was conducted by liquid partition and elution via a hydrophobic polyaromatic resin (Amberlite XAD-4, Sigma).

For the low dose group, the combined samples (33.34 g) were extracted as described for the high dose group, however, some of the liquid partition steps were omitted, since they proved to be unnecessary.

Measurement of radioactivity

Liquid samples were radioassayed by the following liquid scintillation counters; Beckman LS 6000 LL and LS 6500, Philips PW 4700 and LKB Rack Beta 1219 Spectral each using appropriate quench correction.

Solid samples i.e. post-extraction solids were weighed and combusted to $^{14}CO_2$ in a Packard 307 Instruments Oxidiser. Radioactivity was trapped in carbosorb and scintillation fluid (Permafluor E+) was added.

II. Results and discussion

Total radioactive residues (TRR)

The concentration of total radioactivity in the edible parts of the fish after administration of 20 or 200 µg/L [^{14}C]-spiroxamine is summarised in Table CA 6.2.5/01, with average values presented in Table CA 6.2.5/01-2. Since the overall concentration is very low, all available samples from the 28-day bio-concentration and the 14-day depuration period (*i.e.* from day 1 to day 42) were combined separately for the two dose groups to gain as much material as possible for the metabolite identification. The metabolism was only investigated in the edible parts of the fish.

Table CA 6.2.5/01-1 Concentration of [^{14}C]spiroxamine radioactive residue in edible parts of the fish

Time after dosing [days]	Dose level 20 µg/L			Dose level 200 µg/L		
	Concentration edible fish [mg/kg]	Concentration viscera [mg/kg]	Concentration whole fish [mg/kg][1]	Concentration edible fish [mg/kg]	Concentration viscera [mg/kg]	Concentration whole fish [mg/kg][1]
1	0.485	2.530	1.419	3.482	20.203	11.098
3	0.911	3.565	2.171	3.700	26.197	15.071
7	0.636	2.722	1.625	8.171	25.650	16.164
10	0.369	1.905	1.186	2.164	11.814	7.410
14	0.462	3.156	1.678	3.567	16.562	9.801
21	0.576	3.396	2.124	2.640	18.382	11.323
28	0.369	2.789	1.450	4.104	27.946	15.022
29	0.340	2.141	1.135	3.011	14.535	8.228
31	0.097	0.453	0.249	0.649	1.959	1.227
35	0.081	0.327	0.186	0.457	0.540	0.332
38	0.049	0.252	0.142	0.064	0.712	0.357
42	0.071	0.223	0.141	0.060	0.409	0.223

1 – residue in whole fish calculate from edible part and viscera

Table CA 6.2.5/01-2 Mean concentration of [^{14}C]spiroxamine radioactive residue in edible parts of the fish (values corrected for outliers)

	Dose level	
	20 µg [^{14}C]spiroxamine/L	200 µg [^{14}C]spiroxamine/L
Edible parts:	0.58 mg/kg	4.52 mg/kg
Non edible parts:	3.72 mg/kg	22.8 mg/kg
Whole fish:	1.64 mg/kg	13.4 mg/kg

Characterisation

Based on the above results, in order to characterise and identify the nature of the radioactive residues, all edible and inedible fish tissue samples were extracted. A summary of the extractability is presented in Table CA 6.2.5/01-3.

Table CA 6.2.5/01-3 [^{14}C]spiroxamine: Extraction yields of the total radioactivity from the edible tissues and percentage of the radioactivity subjected to chromatographic analysis

Dose [µg/L]	Extraction rate [%]	% of extractable radioactivity analysed by HPLC
20	84.20	80.02
200	91.36	86.39

Radioactive components in extracts were quantified and identified using HPLC and were confirmed by LC-MS/MS.

Available reference standards for characterisation were spiroxamine (KWG 4168) itself as the parent test substance and the following potential metabolites:

It should be noted that the reference compounds used for characterisation and identification of radioactive residues were typically isolated in their radioactive form from either rat or goat urine from other metabolism studies and spectroscopically identified. This was considered necessary as neither the parent compound or its metabolites demonstrated adequate UV absorbance.

The following reference materials were structurally elucidated in this manner:

ECW 80511 and ECW 8046 (spiroxamine-acid) (M06)

KNO 2212 (spiroxamine-hydroxy acid) (M07)

KNO 2222/KNO 22243 (spiroxamine-desethyl acid) (M11)

KNO 2218 (spiroxamine-despropyl acid) (M12)

KNO 1634A (spiroxamine-acid glucuronide) (M19)

KNO 22302 (spiroxamine-sulphate) (M25)

Using mixtures of acetonitrile, tetrahydrofuran and 0.5% sodium chloride, 84.2 % of the total radioactive residue (TRR) could be extracted from low dose edible fish tissue and 91.4% TRR from high dose tissues. These were purified and partitioned before being applied to HPLC. Two different HPLC systems were used to characterise residues. The distribution and magnitude of radioactive residues is presented in Table CA 6.2.5/01-4.

Unchanged parent spiroxamine accounted for 8.4% TRR (0.049 mg/kg) in edible tissues from the low dose regime and 22.2% TRR (1.00 mg/kg) at the high dose. Spiroxamine – acid (M06) accounted for 12.4% TRR (0.072 mg/kg) at the low dose and 15.4% (0.70 mg/kg) at the high dose. The corresponding acid glucuronide conjugate (M19) was seen at the high dose accounting for 18.4% TRR (0.83 mg/kg). Spiroxamine – sulphate (M25) was only seen in edible fish tissue from the low dose, accounting for 5.4% TRR (0.031 mg/kg). Concentration of metabolites was almost exactly an order of magnitude lower in the group of fish dosed at 20 µg [14C]spiroxamine/L, suggesting a good linear dose response.

Table CA 6.2.5/01-4 Relative distribution and concentration (mg a.s. equiv/kg) of metabolites in the edible parts Bluegill sunfish dosed with [cyclohexyl-1-^{14}C]spiroxamine

Metabolite	20 µg [^{14}C]spiroxamine/L		200 µg [^{14}C]spiroxamine/L	
	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine – acid glucuronide (M19)	-	-	18.4	0.83
Spiroxamine – sulphate (M25)	5.4	0.031	-	-
Spiroxamine – acid (M06)	12.4	0.072	15.4	0.70
Spiroxamine	8.4	0.049	22.2	1.00
Total identified	26.2	0.152	56.0	2.53

III. Conclusions

A 42-day study was conducted to evaluate the bio-concentration of [cyclohexyl-1-^{14}C]-spiroxamine by bluegill sunfish (*Lepomis macrochirus*). Mean water concentrations of 20 and 200 µg spiroxamine/L, respectively, were maintained for a 28-day exposure period. The purpose of the study was to gain information on the nature and magnitude of the residue in the edible parts of the fish.

The radioactivity was almost completely extracted from the edible tissues with mixtures of acetonitrile and tetrahydrofuran. After purification, 3 metabolites besides the unchanged parent compound were identified: Spiroxamine-acid (M06) accounting for 12.4% TRR (0.072 mg/kg) at the low dose and 15.4% (0.70 mg/kg) at the high dose, spiroxamine-acid glucuronide (M19) was seen at the high dose accounting for 18.4% TRR (0.83 mg/kg) and spiroxamine-sulphate (M25), which was only seen in edible fish tissue from the low dose, accounting for 5.4% TRR (0.031 mg/kg). Concentration of metabolites was almost exactly an order of magnitude lower in the group of fish dosed at 20 µg [14C]spiroxamine/L, suggesting a good linear dose response.

Therefore, metabolism of spiroxamine in fish is similar to other livestock species. The primary route is via oxidation of the t-butyl moiety forming the carboxylic acid which then forms a glucuronide conjugate. Unchanged parent spiroxamine is also seen in very small quantities in edible tissues.

Assessment and conclusion by applicant:

Acceptable study to address the data point, although the study did not follow a recognised test guideline. Study previously submitted and accepted in the EU: RAR Annex B9 (2009), IIA, 8.2.6.1/02. The results demonstrate that metabolism in fish is similar to that seen in other livestock species and as such the results are considered to provide additional supplementary information.

CA 6.3 Magnitude of residue trials in plants

In this submission for renewal of approval of spiroxamine, the representative uses and critical GAPs for application of spiroxamine (Spiroxamine EC 500, 500 g/L (grapes) and Spiroxamine plus Prothioconazole EC 460, 300 + 160 g/L cereals) to edible crops are defined as shown in Table CA 6.3-1. Full details are provided in Document D-1 of this submission.

Table CA 6.3-1 Critical GAP for spiroxamine on representative crops

Crop	Region	Indoor / outdoor	Application				PHI days
			Method (BBCH stage)	Rate g a.s./ha	Water L/ha	Maximum number (interval)	
Grapes table / wine	Table SEU Wine NEU/SEU	Outdoor	Foliar spray (BBCH 13 – 85)	300	150-1000	2 (10 days)	Table 14 Wine 35
Grapes table / wine	Table SEU Wine NEU/SEU	Outdoor	Foliar spray (BBCH 53 – 85)	300	150-1000	2 (10 days)	Table 14 Wine 35
Fall back GAP Grapes table / wine	Table SEU Wine NEU/SEU	Outdoor	Foliar spray (BBCH 13 – 19)	300	150-1000	1	n.a.
Barley / oats	NEU/SEU	Outdoor	Foliar spray (BBCH 30 – 61)	375	100 - 400	2 (14 – 21 days)	n.a.
Wheat / rye / triticale	NEU/SEU	Outdoor	Foliar spray (BBCH 30 – 69)	375	100 - 400	2 (14 – 21 days)	n.a.

CA 6.3.1 Grapes

Spiroxamine is supported for application to grapes according to the more critical GAP detailed in Table CA 6.3-1, involving up to 2 applications at 300 g a.s./ha.

The available residue reports supporting the more critical GAP for spiroxamine on grapes are presented below.

The use of spiroxamine for application to grapes according to the 'fall back' GAP detailed in Table CA 6.3-1, involving 1 early season application at 300 g a.s./ha to growth stages BBCH 13-19 is

a pre-flowering / pre-fruit set situation and therefore before consumable commodities are on the vines. A non-significant residue situation can be presumed and consumer risk would be covered under the risk-envelope scenario from the critical GAP. To demonstrate this, trials conducted on grapes during growing season 2020 and summarised under CA 6.3.1/18 included application in support of the 'fall back' GAP and confirmed for parent spiroxamine that all residues were below the analytical LOQ of 0.01 mg/kg from four trials in northern Europe and four trials in southern Europe.

Residue trials conducted after the previous EU approval evaluation from 2016 onwards employed residue methods measuring both spiroxamine itself with a fully chiral method and total spiroxamine by means of a common moiety approach. Residues of spiroxamine diastereomers A and B and their enantiomers A1: A2, B1 : B2 were measured and reported as mg/kg both individually and as a sum. The summaries in this section present these data but do not include enantiomer or A / B diastereomer ratios. The ratios are discussed and presented under Section CA 6.7.1 in order to consider the impact of isomer ratios on consumer risk assessment.

The residues data presented here does include trials conducted at the nominal individual application rate of 300 g a.s./ha but to provide a complete dataset from the large number of existing trials conducted at higher or lower application individual rates (from 200 to 400 g a.s./ha), all such trials are included. As these rates are outside of the usually applied ±25% rule, the accepted approach of proportionality or scaling has been used to adjust any residues reported above the analytical LOQ to the nominal rate.

The proportionality approach for scaling of residues with examples is described in detail within various guidance documents, notably the following:

- ENV/JM/MONO(2011)50/REV1, 07 September 2016, OECD Guidance Document on Crop Field Trials, Second Edition, Series on Pesticides - No. 66, Series on Testing & Assessment - No. 164
- EFSA Supporting publication 2018:EN-1503, 06 November 2018, Recommendations on the use of the proportionality approach in the framework of risk assessment for pesticide residues
- EFSA September 2015, Residues trials and MRL calculations - Proposals for a harmonised approach for the selection of the trials and data used for the estimation of MRL, STMR and HR

The representative use GAP considered in this dossier is for grapes with two applications at 300 g a.s./ha with a nominal 14 to 35 day interval for both NEU and SEU residue zones. In any studies / trials where three or more applications were used, expert judgement of the magnitude of residues reported in trials on grapes concludes that the residues in mature grapes at normal commercial harvest would not be significantly impacted by additional applications made to crops.

Therefore it is appropriate for the purposes of scaling residues that all trials, regardless of whether they involved two or three applications, can be scaled to the nominal rate of 300 g a.s./ha per application.

Further consideration for the scaling of residues relates to the approach taken where multiple applications or more than one application is involved. The guidance includes two approaches for this situation.

EFSA 2018 provides the following information:

In case of multiple applications, scaling is in principle applicable if the dose rate in the individual applications deviate from the application rate defined in the GAP by a constant factor. If this is not the case, the application of the proportionality principle might not be suitable. On a case-by-case basis the application of the proportionality to the seasonal application rate may be acceptable, but should be supported by a reasoned case, i.e. considering the persistence of the compound, its systemicity (based on metabolism data, decline residue trials), the time interval between applications and whether it can be demonstrated that the successive applications contribute significantly to the residues (residue

transfer rates). If the last application is shown to contribute the most to the terminal residues, i.e. significant and rapid degradation of the residues along with the PHI after last application (decline residue trials), the scaling factor can be calculated on the basis of the dose rate at the last application only (See Case B – Appendix A).

Case B – Appendix A

EFSA assessment: In that case of multiple applications, the proportionality concept was applied to the last single application rate and not to the maximum seasonal application rate. Indeed, in the case of "Substance C", the decline residue trials showed a significant and rapid degradation of the residues with the PHI and it was concluded that the last treatment contributed the most to the terminal residue. The scaling was therefore applied to the dose rate corresponding to the last application and not to the seasonal application rate.

For the trials presented here on grapes, spiroxamine (total isomers) data in grapes have been scaled using the actual reported (nominal) last application rate in comparison to the critical GAP rate. The alternative approach of using the seasonal loading or the average seasonal rate to take account of trials with two or three applications, compared to the critical GAP rate would be expected to give almost identical data and therefore the selected approach is considered to be appropriate.

Finally, the scaled data includes those trials conducted at the nominal GAP rate and therefore within ±25% of the GAP. This is compliant with the cited above guidance and the following text from OECD 2016 paragraph 32 justifies this approach. In reality the scaled data used for MRL purposes from trials conducted at the nominal rate is approximately equal to the reported values but the scaling of these data (either slightly higher or slightly lower) is consistent with OECD and EFSA guidance.

OECD 2016

32. All data points under consideration, i.e. data points corresponding to application rates within/outside the acceptable range of ± 25% of the nominal application rate, should be adjusted to the nominal (1x) application rate to prevent issues of bias.

An example calculation from the grapes lower rate data set is shown here.

Trial reference: CA 6.3.1/14, M-629509-01-1, 16-2111-01

Final application rate: 200 g a.s./ha

Critical GAP final application rate: 300 g a.s./ha

Reported grape residue 13 DALA: 0.15 mg/kg spiroxamine (total isomers)

Scaling factor: 300 / 200 = 1.5

Scaled residue for MRL purposes: 0.15 x 1.5 = 0.225 mg/kg spiroxamine (total isomers) or 0.23 mg/kg to two decimal places.

Data Point:	KCA 6.3.1/14
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of fluopyram & spiroxamine SE 275 in Italy, Greece and Spain
Report No:	16-2111
Document No:	M-629509-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Eight trials conducted during season 2016 are available to evaluate the magnitude of spiroxamine in/on grapes (bunches of grapes) after two foliar spray applications at 1.004 L/ha of fluopyram and spiroxamine SE 275 in southern Europe. The designated PHI was 14 days (valid for table grapes). Samples were analysed for both spiroxamine (comprising A1 enantiomer, A2 enantiomer, B1 enantiomer, B2 enantiomer, with total spiroxamine as sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Eight residue trials on grapes were conducted in southern Europe (Greece, Italy and Spain) with all eight performed as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/14-1 and Table CA 6.3.1/14-2 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a nominal product rate of 1.0 L/ha corresponding to 200 g a.s./ha. The water application rates were between 600 and 1261 L/ha. Residues above the analytical LOQ have been scaled proportionally to a 300 g a.s./ha application rate in line with the critical GAP.

Spray intervals were between 7 and 11 days with the final treatment being made at growth stage BBCH 83-85. The critical GAP for the use of spiroxamine on grapes is supported in terms of timing.

The trials were conducted using a SE 275 g/L formulation containing 75 g/L of fluopyram and 200 g/L of spiroxamine (actual content 197.7 g/L spiroxamine).

Samples of grape bunches were taken on the day of the final treatment (prior and subsequent to the application), and then on several occasions, including at 13/14 days after last application (DALA, PHI for table grapes). Control samples were taken on the day of final treatment (prior to application), and 13/14 DALA.

Samples of grapes (bunches of grapes) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine (as aminodiol).

Spiroxamine parent enantiomers

Residue analysis of samples of grapes for the determination of the four enantiomers of spiroxamine was conducted using the validated analytical method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol:water 3:1 (v/v) using a high-speed blender. After filtration, spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

The quantification was carried out by internal standardization using the stable d7 ISTD of spiroxamine, which is also separated into its four enantiomers.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. All final extracts were analysed within 6 days after the extraction was completed; nevertheless, the stability of the final extracts was demonstrated during the validation of the method 01480 for at least 12 days at 6°C.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as aminodiol) in/on grapes was conducted using the validated method no. 01089/M001, report reference M-592369-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetone/water (2/1; v/v) using a high-speed blender. After filtration, an aliquot of the extract is heated (90°C) under acidic conditions yielding aminodiol representing spiroxamine and all spiroxamine metabolites containing the aminodiol common moiety.

After dilution with acetonitrile and filtration, the samples were subjected to LC-MS/MS with electrospray ionization in positive ionization mode (column used SeQuant ZIC-HILIC, 150 x 2.1 mm, 5µm particle size) without further clean-up.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. The samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

All final extracts were analysed within 2 days after the preparation was completed; nevertheless, the stability of the final plant extracts was demonstrated during the validation of the method 01089/M001 for at least 2 weeks at 6°C.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/14-5, the maximum storage time between date of sampling and date of last extraction was 430 days for the four enantiomers of spiroxamine and 500 days for the analysis of total spiroxamine (via aminodiol).

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.1/14-3 and Table CA 6.3.1/14-4).

The limits of quantification (LOQ) for the A1 and -A2 enantiomer were 0.00271 mg/kg and for the B1 and -B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via aminodiol) was 0.01 mg/kg.

The limit of quantification (LOQ) for total spiroxamine (via aminodiol) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples with the exception of Italian trials 16-2111-03 and 16-2111-04 in both analyses, spiroxamine as sum of its four enantiomers and total spiroxamine as common moiety aminodiol. The ratio of the residues found in the control samples between spiroxamine as sum of its four enantiomers and the total residues analysed as the common moiety aminodiol suggested that a previous application to the crops with a spiroxamine containing product was likely. Any possible such applications were not reported or noted in the trial raw data for these sites. These trials are not considered for MRL purposes.

All eight of the trials were conducted as residue decline trials, with grapes sampled at 13 to 15 (PHI for table grapes) DALA and also at 25-29 DALA.

Results are summarised below from the six acceptable trials (Italian trials excluded due to potential contamination).

Residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) found 0 DALA in bunches of grapes ranged from 0.09 to 1.0 mg/kg, declining to between 0.04 and 0.27 mg/kg, 13-15 DALA. Results reported as <0.01 mg/kg are not considered for scaling so are not included.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found 0 DALA in bunches of grapes ranged from 0.56 to 1.1 mg/kg, declining to between 0.06 and 0.54 mg/kg, 13-15 DALA.

Table CA 6.3.1/14-1 Residue trials with fluopyram and spiroxamine SE 275 in grapes – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/14 (16-2111) 16-2111-01 Greece, 57011 Vathylakos Grape (Sinsaut) 2016	1 2	0.200 0.200	600 600	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 13 26	0.051 0.11 0.084 0.050 0.046 0.024	0.051 0.11 0.083 0.050 0.046 0.023	0.036 0.087 0.062 0.036 0.032 0.014	0.034 0.082 0.058 0.034 0.030 0.013	0.17 0.38 0.29 0.17 0.15 0.074	0.23 0.37 0.33 0.23 0.21 0.18
CA 6.3.1/14 (16-2111) 16-2111-02 Greece, 64008 Kariani Grape (Cabernet Sauvignon) 2016	1 2	0.200 0.200	600 600	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 13 28	0.069 0.10 0.12 0.12 0.053 0.032	0.068 0.19 0.12 0.12 0.053 0.032	0.055 0.15 0.099 0.091 0.040 0.024	0.053 0.15 0.093 0.085 0.038 0.022	0.25 0.67 0.44 0.42 0.18 0.11	0.29 0.74 0.46 0.49 0.24 0.16
CA 6.3.1/14 (16-2111) 16-2111-03 Italy, 40053 Bazzano (Albana) 2016	1 2	0.200 0.200	800 800	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 14 28	<0.00271 0.016 0.007 0.003 <0.00271 0.011	<0.00271 0.015 0.007 0.003 <0.0271 0.011	<0.00229 0.014 0.005 <0.00229 <0.00229 0.007	<0.00229 0.013 0.004 <0.00229 <0.00229 0.007	<0.01 0.058 0.022 0.011 <0.01 0.035	<0.01 0.055 0.026 0.012 0.012 0.25
CA 6.3.1/14 (16-2111) 16-2111-04 Italy, 48018 Faenza (Trebbiano) 2016	1 2	0.200 0.200	800 800	BBCH 81 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 8 14 29	0.009 0.023 0.014 0.016 0.004 0.011	0.009 0.023 0.014 0.015 0.004 0.011	0.007 0.006 0.018 0.011 0.011 0.008	0.006 0.018 0.011 0.011 0.003 0.007	0.030 0.082 0.050 0.053 0.015 0.037	0.27 0.33 0.23 0.27 0.15 0.25

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/14 (16-2111) 16-2111-05 Greece, 60100 Agios Spyridonas (Crimson) 2016	1 2	0.200 0.200	1000 1000	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 14 25	0.056 0.11 0.047 0.043 0.033 0.028	0.056 0.11 0.046 0.042 0.034 0.028	0.038 0.081 0.032 0.027 0.019 0.014	0.038 0.081 0.033 0.027 0.019 0.014	0.19 0.38 0.16 0.14 0.10 0.084	0.25 0.54 0.22 0.21 0.16 0.16
CA 6.3.1/14 (16-2111) 16-2111-06 Greece, 58400 Piperia (Opsimos Edesissis) 2016	1 2	0.200 0.200	1000 1000	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 6 13 26	0.008 0.079 0.021 0.017 0.009 0.012	0.008 0.079 0.021 0.017 0.009 0.012	0.006 0.062 0.015 0.011 0.006 0.007	0.006 0.062 0.015 0.012 0.006 0.007	0.029 0.28 0.073 0.057 0.030 0.037	0.039 0.37 0.085 0.082 0.043 0.062
CA 6.3.1/14 (16-2111) 16-2111-07 Spain, 41720 Los Palacios y Villafranca (Red Globe) 2016	1 2	0.205[1] 0.200	1261[1] 1200	BBCH 83 BBCH 83	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 6 13 26	0.034 0.072 0.058 0.036 0.035 0.019	0.034 0.072 0.058 0.035 0.035 0.019	0.015 0.046 0.031 0.019 0.019 0.010	0.016 0.044 0.030 0.017 0.021 0.010	0.10 0.23 0.18 0.11 0.11 0.057	0.23 0.44 0.44 0.27 0.36 0.28
CA 6.3.1/14 (16-2111) 16-2111-08 Spain, 21720 Ronciana del Condado (Beba) 2016	1 2	0.200 0.200	1000 992	BBCH 83 BBCH 83	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 8 15 29	0.012 0.097 0.054 0.025 0.007 0.005	0.012 0.096 0.054 0.025 0.007 0.005	0.007 0.078 0.036 0.016 0.005 0.003	0.007 0.076 0.035 0.016 0.004 0.003	0.039 0.35 0.18 0.082 0.024 0.016	0.092 0.43 0.28 0.17 0.054 0.042

1 - Overdosage (just above 5%) no impact

Table CA 6.3.1/14-2 Residue trials with fluopyram and spiroxamine SE 275 in grapes – scaled residue results for southern Europe (field)

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,4]					Scaled total residue of spiroxamine (via aminodiol, mg/kg)[1,4]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/14 (16-2111) 16-2111-01 Greece, 57011 Vathylakos Grape (Sinsaut) 2016	1 2	0.200 0.200	600 600	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 13 26	0.08 0.17 0.13 0.08 0.07 0.04	0.08 0.17 0.12 0.08 0.07 0.03	0.05 0.13 0.09 0.05 0.05 0.02	0.05 0.12 0.09 0.05 0.05 0.02	0.26 0.57 0.44 0.26 <u>0.23</u> 0.11	0.35 0.86 0.50 0.35 0.32 0.27
CA 6.3.1/14 (16-2111) 16-2111-02 Greece, 64008 Kariani Grape (Cabernet Sauvignon) 2016	1 2	0.200 0.200	600 600	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 13 28	0.10 0.15 0.18 0.18 0.08 0.05	0.10 0.29 0.18 0.18 0.08 0.05	0.08 0.15 0.14 0.14 0.06 0.04	0.08 0.23 0.14 0.13 0.06 0.03	0.38 1.00 0.66 0.63 <u>0.27</u> 0.17	0.44 1.11 0.69 0.74 0.36 0.24
CA 6.3.1/14 (16-2111) 16-2111-03 Italy, 40053 Bazzano (Albana) 2016	1 2	0.200 0.200	800 800	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 14 28	<0.00271 0.02 0.01 0.005 <0.0271 0.02	<0.00271 0.02 0.01 0.005 <0.0271 0.02	<0.00229 0.02 0.007 <0.00229 <0.00229 0.01	<0.00229 0.02 0.006 <0.00229 <0.00229 0.01	<0.01 0.09 0.03 0.02 <0.01 0.05	<0.01 0.08 0.04 0.02 0.02 0.38
CA 6.3.1/14 (16-2111) 16-2111-04 Italy, 48018 Faenza (Trebbiano) 2016	1 2	0.200 0.200	800 800	BBCH 81 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 8 14 29	0.01 0.03 0.02 0.02 0.006 0.02	0.01 0.03 0.02 0.02 0.006 0.02	0.01 0.009 0.03 0.02 0.02 0.01	0.009 0.03 0.02 0.02 0.008 0.01	0.05 0.12 0.08 0.08 0.02 0.06	0.41 0.50 0.35 0.41 0.23 0.38
CA 6.3.1/14 (16-2111) 16-2111-05 Greece, 60100 Agios Spyridonas (Crimson) 2016	1 2	0.200 0.200	1000 1000	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 14 25	0.08 0.17 0.07 0.06 0.05 0.04	0.08 0.17 0.07 0.06 0.05 0.04	0.06 0.12 0.05 0.04 0.03 0.02	0.06 0.12 0.05 0.04 0.03 0.02	0.29 0.57 0.24 0.21 <u>0.15</u> 0.13	0.38 0.81 0.33 0.32 0.24 0.24

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,4]					Scaled total residue of spiroxamine (via aminodiol, mg/kg)[1,4]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/14 (16-2111) 16-2111-06 Greece, 58400 Piperia (Opsimos Edesissis) 2016	1 2	0.200 0.200	1000 1000	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 6 13 26	0.01 0.12 0.03 0.03 0.01 0.02	0.01 0.12 0.03 0.03 0.01 0.02	0.009 0.01 0.02 0.02 0.009 0.01	0.009 0.09 0.02 0.02 0.009 0.01	0.04 0.42 0.11 0.09 0.05 0.06[2]	0.06 0.56 0.13 0.12 0.06 0.09
CA 6.3.1/14 (16-2111) 16-2111-07 Spain, 41720 Los Palacios y Villafranca (Red Globe) 2016	1 2	0.210[3] 0.200	1261[3] 1200	BBCH 83 BBCH 83	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 6 13 26	0.05 0.11 0.09 0.05 0.05 0.03	0.05 0.11 0.09 0.05 0.05 0.03	0.03 0.07 0.05 0.03 0.03 0.02	0.02 0.07 0.05 0.03 0.03 0.02	0.15[2] 0.35 0.27 0.15 0.17 0.09	0.35 0.66 0.66 0.41 0.54 0.42
CA 6.3.1/14 (16-2111) 16-2111-08 Spain, 21720 Ronciana del Condado (Beba) 2016	1 2	0.200 0.200	1000 992	BBCH 81 BBCH 83	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 8 15 29	0.02 0.13 0.08 0.04 0.01 0.008	0.02 0.14 0.08 0.024 0.01 0.008	0.01 0.12 0.05 0.02 0.008 0.005	0.01 0.12 0.05 0.02 0.006 0.005	0.06 0.53 0.27 0.12 0.04 0.02	0.14 0.65 0.42 0.26 0.08 0.06

1 – The proportionality (scaling) concept was applied to the last single application rate
2 - Higher value used for MRL on table grapes from later PHI
3 - Overdosage (just above 5%) no impact on final application scaling
4 - Values reported below the LOQ not appropriate to scale up

Table CA 6.3.1/14-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/14	01480	A1 enantiomer	Grape (bunch)	0.00271 0.0271 0.271 Overall	4 4 1 9	89; 93; 94; 96 95; 96; 97; 97 100 Mean: 95; RSD: 3.2
CA 6.3.1/14	01480	A2 enantiomer	Grape (bunch)	0.00271 0.0271 0.271 Overall	4 4 1 9	93; 93; 97; 98 94; 95; 96; 96 98 Mean: 96; RSD: 2.0
CA 6.3.1/14	01480	B1 enantiomer	Grape (bunch)	0.00229 0.0229 0.229 Overall	4 4 1 9	96; 96; 97; 98 97; 98; 99; 99 101 Mean: 98; RSD: 1.7
CA 6.3.1/14	01480	B2 enantiomer	Grape (bunch)	0.00229 0.0229 0.229 Overall	4 4 1 9	91; 92; 96; 96 94; 95; 96; 96 100 Mean: 95; RSD: 2.8

Table CA 6.3.1/14-4 Procedural recovery data for the determination of total spiroxamine (via aminodiol)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/14	01089/M001	Total spiroxamine (via aminodiol)	Grape (bunch)	0.01 0.10 1.0 Overall	3 4 3 10	94; 97; 100 93; 96; 96; 99 90; 92; 93 Mean: 95, RSD: 3.3

Table CA 6.3.1/14-5 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/14	Grapes	341 and 430 (enantiomers of spiroxamine) 411 and 500 days (spiroxamine via aminodiol)

III. Conclusions

A total of six valid residue trials conducted in 2016 in southern Europe are available to evaluate the residues of spiroxamine (sum of A1, A2, B1 and B2 enantiomers) and the total residue of spiroxamine (via aminodiol) in/on grape bunches after two applications of fluopyram and spiroxamine SE 275 to vines at 13 to 15 days pre-harvest (table grapes).

Residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) found 0 DALA in bunches of grapes ranged from 0.09 to 1.0 mg/kg, declining to between 0.04 and 0.27 mg/kg, 13-15 DALA. Results reported as <0.01 mg/kg are not considered for scaling so are not included.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found 0 DALA in bunches of grapes ranged from 0.56 to 1.1 mg/kg, declining to between 0.06 and 0.54 mg/kg, 13-15 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) found 0 DALA in bunches of grapes ranged from 0.09 to 1.0 mg/kg, declining to between 0.04 and 0.27 mg/kg, 13-15 DALA. Results reported as <0.01 mg/kg are not considered for scaling so are not included.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found 0 DALA in bunches of grapes ranged from 0.56 to 1.1 mg/kg, declining to between 0.06 and 0.54 mg/kg, 13-15 DALA.

Data Point:	KCA 6.3.1/15
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Determination of the residues of AE C656948 and spiroxamine in/on grape (red and white varieties) after spray application of fluopyram & spiroxamine SE 275 in Northern France and Austria
Report No:	16-2078
Document No:	M-627030-01-1
Guideline(s) followed in study:	Data Requirements/Guidelines Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during season 2016 are available to evaluate the magnitude of spiroxamine in/on grapes (bunches of grape) after two foliar spray applications of fluopyram and spiroxamine SE 275 at 1.0 L/ha in northern Europe. The designated PHI was 14 days (SEU table grapes PHI). Therefore these trials are not considered for MRL purposes for data from NEU. The data are included for this evaluation as the chiral data for enantiomers is useful for the assessment of isomer ratio.

Residues were analysed for both spiroxamine (comprising A1 enantiomer, A2 enantiomer, B1 enantiomer, B2 enantiomer, the total residue of spiroxamine as sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as

spiroxamine equivalents. Residues of fluopyram were also analysed, however are not reported in this summary.

Four residue trials on grapes were conducted in northern Europe (northern France and Austria) with all four performed as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/15-1 and Table CA 6.3.1/15-2. As noted above, no values are considered for MRL purposes or risk assessment as the northern Europe data are not relevant for the representative use on table grapes (SEU only).

Two spray applications of spiroxamine were made at a nominal product rate of 1.0 L/ha corresponding to 200 g a.s./ha. The water application rates were between 200 and 873 L/ha. Residues above the analytical LOQ have been scaled proportionally to a 300 g a.s./ha application rate in line with the critical GAP.

Spray intervals were between 10-11 days with the exception of the Austrian trial (16-2078-04) which had an interval of 14 days due to a mistake in the schedule. It is not expected to impact the validity of the study with the trial producing no significantly lower residues in comparison to the other three trials. The final treatments were made at growth stage BBCH 85 supporting the critical GAP for the use of spiroxamine on grapes.

The trials were conducted using a SE 275 g/L formulation containing 75 g/L of fluopyram and 200 g/L of spiroxamine (actual content 197.7 g/L spiroxamine).

Samples of grapes (bunches of grapes) were taken on the day of the final treatment (prior to and subsequent to the application), and then on several occasions, including at 13/14 days after last application (DALA, PHI for table grapes). Control samples were taken on the day of final treatment (prior to application), and 13/14 DALA.

Samples of grapes (bunches of grapes) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine (as aminodiol).

Spiroxamine parent enantiomers

Residue analysis of samples of grapes for the determination of the four enantiomers of spiroxamine was conducted using the validated analytical method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol:water 3:1 (v/v) using a high-speed blender. After filtration, spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. All final extracts were analysed within 6 days after the extraction was completed; nevertheless, the stability of the final extracts was demonstrated during the validation of the method 01480 for at least 12 days at 6°C.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as aminodiol) in/on grapes was conducted using the validated method no. 01089/M001, report reference M-592369-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetone/water (2/1; v/v) using a high-speed blender. After filtration, an aliquot of the extract is heated (90°C) under acidic conditions yielding aminodiol representing all spiroxamine metabolites containing the aminodiol common moiety.

After dilution with acetonitrile and filtration, the samples were subjected to LC-MS/MS with electrospray ionization in positive ionization mode (column used SeQuant ZIC-HILIC, 150 x 2.1 mm, 5µm particle size) without further clean-up.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. The samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

All final extracts were analysed within 2 days after the preparation was completed; nevertheless, the stability of the final plant extracts was demonstrated during the validation of the method 01089/M001 for at least 2 weeks at 6°C + 3°C.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/15-5, the maximum storage time between date of deep-freezing and date of last extraction was 408 days for the four enantiomers of spiroxamine and 476 days for the analysis of spiroxamine (via aminodiol).

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.1/15-3 and Table CA 6.3.1/15-4).

The limits of quantification (LOQ) for the A1 and -A2 enantiomer were 0.00271 mg/kg, and for the B1 and -B2 enantiomer, 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via aminodiol) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as residue decline trials, with grapes sampled at 13 to 15 (PHI for table grapes) DALA and also at 28-29 DALA for three trials.

Residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) found 0 DALA in bunches of grapes ranged from 0.14 to 0.8 mg/kg declining to between 0.06 and 0.27 mg/kg, 13-14 DALA.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found 0 DALA in bunches of grapes ranged from 0.14 to 0.84 mg/kg, declining to between 0.08 and 0.27 mg/kg, 13-14 DALA.

Table CA 6.3.1/15-1 Residue trials with fluopyram and spiroxamine SE 275 in grapes – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/15 (16-2078) 16-2078-01 Northern France, 37210 Vernou sur Brenne Grape (Chenin) 2016	1 2	0.218[1] 0.200	873 800	BBCH 85 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 14 28	0.011 0.054 0.024 0.019 0.016 0.020	0.011 0.055 0.025 0.018 0.016 0.019	0.007 0.041 0.017 0.013 0.010 0.012	0.007 0.041 0.017 0.013 0.010 0.012	0.035 0.19 0.083 0.063 0.052 0.062	0.036 0.18 0.081 0.060 0.051 0.034
CA 6.3.1/15 (16-2078) 16-2078-02 Northern France, 71390 Chenôves Grape (Pinot noir) 2016	1 2	0.200 0.200	500 500	BBCH 83 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 13 29	0.016 0.10 0.031 0.022 0.030 0.020	0.015 0.10 0.033 0.022 0.031 0.019	0.011 0.081 0.020 0.014 0.019 0.012	0.011 0.080 0.020 0.014 0.018 0.012	0.052 0.37 0.10 0.072 0.098 0.063	0.039 0.37 0.11 0.081 0.12 0.075
CA 6.3.1/15 (16-2078) 16-2078-04 Austria, 7122 Gols Grape (Zweigelt) 2016	1 2	0.200 0.200	600 600	BBCH 81 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 13 28	0.045 0.16 0.075 0.072 0.055 0.039	0.045 0.15 0.074 0.074 0.055 0.039	0.028 0.11 0.050 0.046 0.033 0.022	0.027 0.11 0.051 0.045 0.033 0.022	0.14 0.53 0.25 0.24 0.18 0.12	0.15 0.56 0.29 0.29 0.18 0.17

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/15 (16-2078) 16-2078-05 Northern France, 49260 Le Puy Notre Dame Grape (Chenin) 2016	1 2	0.200 0.200	600 600	BBCH 85 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 14	0.010 0.026 0.015 0.017 0.012	0.010 0.026 0.015 0.018 0.012	0.007 0.019 0.010 0.012 0.008	0.006 0.019 0.010 0.011 0.008	0.033 0.090 0.051 0.059 0.040	0.037 0.094 0.056 0.068 0.048

1– The plot was overdosed by 9%

Table CA 6.3.1/15-2 Residue trials with fluopyram and spiroxamine SE 275 in grapes –scaled residue results for northern Europe

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1]					Scaled total residue of spiroxamine (via aminodiol, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/15 (16-2078) 16-2078-01 Northern France, 37210 Vernou sur Brenne Grape 2016	1 2	0.218[2] 0.200	873 800	BBCH 85 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 14 28	0.02 0.08 0.04 0.03 0.02 0.03	0.02 0.08 0.04 0.03 0.02 0.05	0.01 0.06 0.03 0.02 0.02 0.02	0.01 0.06 0.03 0.02 0.02 0.02	0.05 0.29 0.12 0.10 0.08 0.09	0.05 0.27 0.12 0.09 0.08 0.05
CA 6.3.1/15 (16-2078) 16-2078-02 Northern France, 71390 Chenôves Grape 2016	1 2	0.200 0.200	500 500	BBCH 83 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 13 29	0.02 0.15 0.05 0.03 0.05 0.03	0.02 0.15 0.05 0.03 0.07 0.03	0.02 0.12 0.03 0.02 0.03 0.02	0.02 0.12 0.03 0.02 0.03 0.02	0.08 0.56 0.15 0.11 0.15 0.10	0.06 0.56 0.23 0.12 0.18 0.11
CA 6.3.1/15 (16-2078) 16-2078-04 Austria, 7122 Gols Grape 2016	1 2	0.200 0.200	600 600	BBCH 81 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 13 28	0.07 0.24 0.11 0.11 0.08 0.06	0.07 0.23 0.11 0.11 0.08 0.09	0.04 0.17 0.08 0.07 0.05 0.03	0.04 0.17 0.08 0.07 0.05 0.03	0.21 0.80 0.38 0.36 0.27 0.18	0.23 0.84 0.44 0.44 0.27 0.26
CA 6.3.1/15 (16-2078) 16-2078-05 Northern France, 49260 Le Puy Notre Dame Grape 2016	1 2	0.200 0.200	600 600	BBCH 85 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes	-0 0 3 7 14	0.02 0.04 0.02 0.03 0.02	0.02 0.04 0.02 0.03 0.02	0.01 0.03 0.02 0.02 0.01	0.01 0.03 0.02 0.02 0.01	0.05 0.14 0.08 0.09 0.06	0.06 0.14 0.08 0.10 0.07

1 - The proportionality (scaling) concept was applied to the last single application rate
2 - Overdosage, no significant impact on final application scaling

Table CA 6.3.1/15-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/15	01480	A1 enantiomer	Grapes	0.00271 0.0271 0.271 Overall	3 3 1 7	90; 91; 94 89; 90; 92 101 Mean: 92, RSD: 4.5
CA 6.3.1/15	01480	A2 enantiomer	Grape bunches	0.00271 0.0271 0.271 Overall	3 3 1 7	88; 89; 91 88; 91; 91 99 Mean: 91, RSD: 4.2
CA 6.3.1/15	01480	B1 enantiomer	Grape bunches	0.00229 0.0229 0.229 Overall	3 3 1 7	86; 89; 91 90; 91; 92 99 Mean: 91, RSD: 4.4
CA. 6.3.1/15	01480	B2 enantiomer	Grape bunches	0.00229 0.0229 0.229 Overall	3 3 1 7	86; 88; 90 89; 89; 90 103 Mean: 91, RSD: 6.2

Table CA 6.3.1/15-4 Procedural recovery data for the determination of total spiroxamine (via aminodiol)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/15	01089/M001	Total spiroxamine (via aminodiol)	Grape bunches	0.01 0.10 1.0 Overall	3 3 1 7	101; 103; 104 91; 96; 98 92 Mean: 98, RSD: 5.3

Table CA 6.3.1/15-5 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/15	Grapes	349 to 408 (four enantiomers of spiroxamine) 417 to 476 days (spiroxamine via aminodiol)

III. Conclusions

A total of four residue trials conducted in 2016 in northern Europe are available to evaluate the residues of spiroxamine (comprising of A1, A2, B1 and B2 enantiomers and the total residue as sum of the four enantiomers), and the total residue of spiroxamine (via aminodiol) in/on grape (bunch of grapes) after two applications of fluopyram and spiroxamine SE 275 to vines at 13 or 14 days pre-harvest (table grapes).

All four trials were conducted as residue decline trials. Residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) found 0 DALA in bunches of grapes ranged from 0.14 to 0.8 mg/kg, declining to between 0.06 and 0.27 mg/kg, 13-14 DALA.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found 0 DALA in bunches of grapes ranged from 0.14 to 0.84 mg/kg, declining to between 0.08 and 0.27 mg/kg, 13-14 DALA.

The data are included for this evaluation as the chiral data for enantiomers is useful for the assessment of isomer ratio, however no values are considered for MRL purposes or risk assessment as the northern Europe data are not relevant for the representative use on table grapes (SEU only).

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) found 0 DALA in bunches of grapes ranged from 0.14 to 0.8 mg/kg, declining to between 0.06 and 0.27 mg/kg, 13-14 DALA.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found 0 DALA in bunches of grapes ranged from 0.14 to 0.84 mg/kg, declining to between 0.08 and 0.27 mg/kg, 13-14 DALA.

The data are included for this evaluation as the chiral data for enantiomers is useful for the assessment of isomer ratio, however no values are considered for MRL purposes or risk assessment as the northern Europe data are not relevant for the representative use on table grapes (SEU only).

Data Point:	KCA 6.3.1/16
Report Author:	[redacted]
Report Year:	2018
Report Title:	Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 500 in Italy, Greece and France (South)
Report No:	16-2047
Document No:	M-626160-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during season 2016 are available to evaluate the magnitude of spiroxamine in/on grapes (bunches of grapes and berries) after two foliar spray applications at nominally 0.6 L/ha of Spiroxamine EC 500 in southern Europe. The designated PHI was 14 days (valid for table grapes) with additional sampling at 35 days (PHI for wine grapes). Residues were analysed both for spiroxamine (comprising A1 enantiomer, A2 enantiomer, B1 enantiomer, B2 enantiomer, the total residue of

spiroxamine as sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Four residue trials on grapes were conducted in southern Europe (Italy, Greece and southern France), with all four performed as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/16-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a nominal product rate of 0.6 L/ha corresponding to 300 g a.s./ha. The water application rates were between 700 and 800 L/ha.

Spray intervals were 10 days with the final treatment being made at growth stage BBCH 79-85. The critical GAP for the use of spiroxamine on grapes is supported in terms of rates and timings.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 495 g/L spiroxamine).

Samples of grape bunches were taken on the day of the final treatment (prior and subsequent to the application), and then on several occasions, including at 14 days after last application (DALA, PHI for table grapes) and 35 DALA (PHI for wine grapes). Berries were also sampled at 14 and 35 DALA. Control samples were taken on day of the final treatment day prior to the last treatment, 14 and 35 DALA.

Samples of grapes (bunches of grapes and berries) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine (as aminodiol).

Spiroxamine parent enantiomers

Residue analysis of samples of grapes for the determination of the four enantiomers of spiroxamine was conducted using the validated analytical method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.0029 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water 3:1 (v/v) using a high-speed blender. After filtration, spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. All final extracts were analysed within 6 days after the extraction was completed; nevertheless, the stability of the final extracts was demonstrated during the validation of the method 01480 for at least 12 days at 6°C.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as aminodiol) in/on grapes was conducted using the validated method no. 01089/M001, report reference M-592369-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetone/water (2/1; v/v) using a high-speed blender. After filtration, an aliquot of the extract is heated (90°C) under acidic conditions yielding aminodiol representing all spiroxamine metabolites containing the aminodiol common moiety.

After dilution with acetonitrile and filtration, the samples were subjected to LC-MS/MS with electrospray ionization in positive ionization mode (column used SeQuant ZIC-HILIC, 150 x 2.1 mm, 5µm particle size) without further clean-up.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. The samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

All final extracts were analysed within 2 days after the preparation was completed; nevertheless, the stability of the final plant extracts was demonstrated during the validation of the method 01089/M001 for at least 2 weeks at 6°C + 3°C.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/16-4, the maximum storage time between date of deep-freezing and date of last extraction was 415 days for the four enantiomers of spiroxamine and 496 days for the analysis of spiroxamine (via aminodiol).

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.1/16-2 and Table CA 6.3.1/16-3).

The limits of quantification (LOQ) for the A1 and -A2 enantiomer were 0.00271 mg/kg and for the B1 and -B2 enantiomer, 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via aminodiol) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control specimens with the exception of the Greek trial (16-2047-03). Here, residues in all control samples were found in both the analyses of spiroxamine as the sum of its four enantiomers and via the common moiety aminodiol. This is due to a previous, unscheduled application with a spiroxamine-containing product to the trial plots. However, the residues of the parent compound in the treated samples in this trial were still quite similar to those in one of the remaining trials, i.e. in the "same population" as the other trials.

All four of the trials were conducted as residue decline trials, with berries and bunches of grapes sampled at 14 (PHI for table grapes), to 21 DALA and 35 (PHI for wine grapes) DALA.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in grapes ranged from 0.15 to 0.73 mg/kg, declining to between 0.043 and 0.17 mg/kg, 14 DALA and 0.027 to 0.12 mg/kg, 35 DALA.

The total residues of spiroxamine (as common moiety aminodiol) found 0 DALA in grapes ranged from 0.15 to 0.96 mg/kg, declining to between 0.17 and 0.46 mg/kg, 13-14 DALA and 0.026 to 0.54 mg/kg, 35 DALA.

Table CA 6.3.1/16-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via ammodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.1/16 (16-2047) 16-2047-01 Italy, 76123 Andria Grape (Malvasia White variety) 2016	1 2	0.300 0.300	800 800	BBCH 81 BBCH 83	Bunch of grapes	-0	0.038	0.038	0.026	0.027	0.13	0.14
						0	0.20	0.21	0.16	0.15	0.72	0.69
					Bunch of grapes	7	0.060	0.059	0.039	0.042	0.20	0.20
						14	0.053	0.053	0.031	0.036	0.17	0.17
					Bunch of grapes	14	0.052	0.050	0.032	0.035	0.17	0.16
						21	0.031	0.031	0.018	0.019	0.10	0.12
					Berries	28	0.027	0.027	0.016	0.017	0.087	0.098
					Bunch of grapes	35	0.031	0.030	0.017	0.018	0.096	0.12
						35	0.022	0.022	0.013	0.014	0.072	0.088
					Bunch of grapes							
					Bunch of grapes							
					Berries							
					Bunch of grapes							

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.1/16 (16-2047) 16-2047-02 Italy, 95033 C. da Baronessa; Biancavilla (CT) Grape (Nerello Mascalese) 2016	1 2	0.300 0.300	700 700	BBCH 79 BBCH 79	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Berries Bunch of grapes Bunch of grapes Berries Bunch of grapes	-0 0 7 14 14 21 28 35 35	0.008 0.043 0.015 0.013 0.009 0.008 0.008 0.009 0.005	0.008 0.044 0.014 0.013 0.009 0.008 0.008 0.008 0.005	0.005 0.033 0.009 0.008 0.006 0.005 0.005 0.005 0.003	0.005 0.034 0.010 0.009 0.006 0.005 0.005 0.005 0.003	0.025 0.15 0.048 0.043 0.030 0.026 0.025 0.027 0.017	0.026 0.15 0.037 0.042 0.029 0.025 0.019 0.026 0.018

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.1/16 (16-2047) 16-2047-03 Greece, GR - 575 00 K. Scholario, Thessaloniki Grape (Victoria table white) 2016	1 2	0.300 0.300	800 800	BBCH 85 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Berries Bunch of grapes Bunch of grapes Bunch of grapes Berries Bunch of grapes	-0 0 7 14 14 21 28 35 35 35	0.046 0.21 0.045 0.056 0.056 0.046 0.040 0.043 0.025	0.046 0.22 0.044 0.058 0.054 0.046 0.039 0.043 0.025	0.023 0.15 0.025 0.028 0.029 0.023 0.017 0.018 0.012	0.023 0.15 0.026 0.029 0.031 0.024 0.017 0.018 0.012	0.14 0.73 0.14 0.17 0.17 0.14 0.11 0.12 0.075	0.40 0.96 0.32 0.46 0.39 0.38 0.38 0.54 0.29

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.1/16 (16-2047) 16-2047-04 Southern France, 30290 Saint Victor la Coste Grape (Syrah Grape Red) 2016	1 2	0.300 0.300	200 200	BBCH 85 BBCH 85	Bunch of grapes	-0	0.009	0.008	0.005	0.005	0.027	0.028
					Bunch of grapes	0	0.079	0.078	0.078	0.058	0.28	0.27
					Bunch of grapes	7	0.019	0.018	0.018	0.011	0.061	0.062
					Bunch of grapes	14	0.011	0.012	0.012	0.006	0.036	0.036
					Bunch of grapes	14	0.015	0.014	0.014	0.008	0.046	0.049
					Bunch of grapes	21	0.019	0.019	0.019	0.011	<u>0.060</u>[2]	0.073
					Berries	28	0.023	0.013	0.013	0.007	0.042	0.049
					Bunch of grapes	35	0.014	0.013	0.013	0.007	<u>0.042</u>	0.042
					Bunch of grapes	35	0.011	0.011	0.011	0.006	0.035	0.043
					Bunch of grapes							
					Bunch of grapes							
					Berries							
					Bunch of grapes							

1 - Underlined value to be used to support MRL for table grapes; Double underlined value to be used to support MRL for wine grapes
2 - Higher value used for MRL on table grapes from later PHI

Table CA 6.3.1/16-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/16	01480	A1 enantiomer	Grapes	0.00271 0.0271 0.271 Overall	3 3 1 7	97; 99; 112 96; 97; 99 95 Mean: 99, RSD: 5.8
CA 6.3.1/16	01480	A2 enantiomer	Grapes	0.00271 0.0271 0.271 Overall	3 3 1 7	94; 95; 116 88; 96; 97 98 Mean: 98, RSD: 8.9
CA 6.3.1/16	01480	B1 enantiomer	Grapes	0.00229 0.0229 0.229 Overall	3 3 1 7	93; 95; 113 95; 95; 96 97 Mean: 98, RSD: 7.0
CA 6.3.1/16	01480	B2 enantiomer	Grapes	0.00229 0.0229 0.229 Overall	3 3 1 7	96; 98; 118 96; 97; 97 96 Mean: 100, RSD: 8.1

Table CA 6.3.1/16-3 Procedural recovery data for the determination of total spiroxamine (via aminodiol)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/16	01089/M001	Total spiroxamine (via aminodiol)	Grape (berries)	0.01 0.10 1.0 Overall	3 3 1 7	92; 95; 103 90; 90; 105 84 Mean: 94, RSD: 8.0
CA 6.3.1/16	01089/M001	Total spiroxamine (via aminodiol)	Grape (bunches)	0.01 0.10 1.0 Overall	3 3 1 7	92[1] (124); 99[1] (131); 100 92; 93; 95 96 Mean: 95, RSD: 3.4

1 - These recoveries were corrected for the residue in the corresponding control samples. Numbers in brackets are the recover values before correction

Table CA 6.3.1/16-4 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/16	Grapes	360 to 415 (four enantiomers of spiroxamine) 439 to 496 days (spiroxamine via aminodiol)

III. Conclusions

A total of four residue trials conducted in 2016 in southern Europe are available to evaluate the residues of spiroxamine (comprising of spiroxamine A1, A2, B1 and B2 enantiomers and the total residue as sum of the four enantiomers), and the total residue of spiroxamine (via aminodiol) in/on grape (bunch

of grapes and berry) after two applications of spiroxamine 500 EC to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

All four trials were conducted as residue decline trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in grapes ranged from 0.15 to 0.73 mg/kg, declining to between 0.043 and 0.17 mg/kg, 14 DALA and 0.027 to 0.12 mg/kg, 35 DALA.

The total residues of spiroxamine (as common moiety aminodiol) found 0 DALA in grapes ranged from 0.15 to 0.96 mg/kg, declining to between 0.17 and 0.46 mg/kg, 13-14 DALA and 0.026 to 0.54 mg/kg, 35 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

All four trials were conducted as residue decline trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in grapes ranged from 0.15 to 0.73 mg/kg, declining to between 0.043 and 0.17 mg/kg, 14 DALA and 0.027 to 0.12 mg/kg, 35 DALA.

The total residues of spiroxamine (as common moiety aminodiol) found 0 DALA in grapes ranged from 0.15 to 0.96 mg/kg, declining to between 0.17 and 0.46 mg/kg, 13-14 DALA and 0.026 to 0.54 mg/kg, 35 DALA.

Data Point:	KCA 6.3.1/17
Report Author:	[redacted], [redacted]
Report Year:	2018
Report Title:	Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 300 in France (North), Belgium and Germany
Report No:	16-2193
Document No:	M-623069-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD Guideline for the testing of chemicals, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during season 2016 are available to evaluate the magnitude of spiroxamine in/on grape (bunches of grapes and berries) after two foliar spray applications at nominally 0.6 L/ha of

Spiroxamine EC 500 in northern Europe. The designated PHI was 34/35 days (PHI for wine grapes), with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for data from NEU. Residues were analysed both for spiroxamine (comprising A1 enantiomer, A2 enantiomer, B1 enantiomer, B2 enantiomer, the total residue of spiroxamine as sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Four residue trials on grapes were conducted in northern Europe (northern France, Belgium and Germany) with all four performed as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/17-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a nominal product rate of 0.6 L/ha corresponding to 300 g a.s./ha. The water application rates were between 200 and 864 L/ha.

Spray intervals were 10 days with the final treatment being made at growth stage BBCH 83-85. The critical GAP for the use of spiroxamine on grapes is supported in terms of rates and timings.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 495 g/L spiroxamine).

Samples of grape bunches were taken on the day of the final treatment (prior and subsequent to the application), in addition to several points thereafter, including 34/35 days after application (DALA, PHI for wine grapes). Berries were also sampled at 14 and 35 DALA. Control samples were taken on day of the final treatment day prior to the last treatment, 14 and 35 DALA.

Samples of grapes (bunches of grapes and berries) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine as the sum of the four enantiomers and also for the total residue of spiroxamine (as aminodiol).

Spiroxamine parent enantiomers

Residue analysis of samples of grapes for the determination of the four enantiomers of spiroxamine was conducted using the validated analytical method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol:water 3:1 (v/v) using a high-speed blender. After filtration, spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used: YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. All final extracts were analysed within 6 days after the extraction was completed; nevertheless, the stability of the final extracts was demonstrated during the validation of the method 01480 for at least 12 days at 6 °C.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as aminodiol) in/on grapes was conducted using the validated method no. 01089/M001, report reference M-592369-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetone/water (2/1; v/v) using a high-speed blender. After filtration, an aliquot of the extract is heated (90°C) under acidic conditions yielding aminodiol representing all spiroxamine metabolites containing the aminodiol common moiety.

After dilution with acetonitrile and filtration, the samples were subjected to LC-MS/MS with electrospray ionization in positive ionization mode (column used SeQuant ZIC-HILIC, 150 x 2.1 mm, 5µm particle size) without further clean-up.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. The samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

All final extracts were analysed within 2 days after the preparation was completed; nevertheless, the stability of the final plant extracts was demonstrated during the validation of the method 01089/M001 for at least 2 weeks at 6°C + 3°C.

The samples were stored deep frozen within 24 hours of sampling at ≤ -18°C. As shown in Table CA 6.3.1/17-4, the maximum storage time between date of deep-freezing and date of last extraction was 413 days for the four enantiomers of spiroxamine and 476 days for the analysis of spiroxamine (via aminodiol).

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.1/17-2 and Table CA 6.3.1/17-3).

The limits of quantification (LOQ) for the A1 and -A2 enantiomer were 0.00271 mg/kg, for the B1 and -B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via aminodiol) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as residue decline trials, with berries and bunches of grapes sampled at 34/35 (PHI for wine grapes) DALA.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in grapes ranged from 0.39 to 0.81 mg/kg, declining to between 0.079 and 0.18 mg/kg, 34/35 DALA.

The total residues of spiroxamine (as common moiety aminodiol) found 0 DALA in grapes ranged from 0.45 to 0.94 mg/kg, declining to between 0.08 and 0.21 mg/kg, 35 DALA.

Table CA 6.3.1/17-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of 4 spiroxamine enantiomer	
CA 6.3.1/17 (16-2193) 16-2193-01 Northern France, 37270 Athée sur Cher Grape (Chardonnay) 2016	1	0.300	200	BBCH 79	Grapes bunches	-0	0.023	0.024	0.016	0.016	0.078	0.099
	2	0.300	200	BBCH 83	Grapes bunches	0	0.12	0.12	0.098	0.097	0.44	0.48
					Grapes bunches	7	0.080	0.081	0.053	0.053	0.27	0.30
					Grapes bunches	14	0.034	0.034	0.022	0.021	0.11	0.15
					Berries	14	0.027	0.027	0.017	0.017	0.087	0.097
					Grapes bunches	21	0.028	0.027	0.017	0.017	0.089	0.12
					Grapes bunches	28	0.025	0.024	0.015	0.015	0.079	0.11
					Grapes bunches	34	0.024	0.023	0.015	0.015	0.079	0.10
					Berries	34	0.022	0.021	0.014	0.014	0.071	0.082
CA 6.3.1/17 (16-2193) 16-2193-02 Belgium, 4257 Rosoux-Crenwick Grape (Regent) 2016	1	0.281[1]	808 - 864	BBCH 73	Grapes bunches	-0	0.048	0.047	0.022	0.022	0.14	0.23
	2	0.300		BBCH 79	Grapes bunches	0	0.12	0.12	0.078	0.081	0.39	0.45
					Grapes bunches	7	0.061	0.061	0.030	0.030	0.18	0.26
					Grapes bunches	14	0.043	0.043	0.021	0.020	0.13	0.20
					Berries	14	0.070	0.070	0.032	0.032	0.20	0.36
					Grapes bunches	21	0.032	0.033	0.015	0.014	0.094	0.15
					Grapes bunches	28	0.046	0.045	0.019	0.018	0.13	0.28
					Grapes bunches	35	0.032	0.033	0.013	0.013	0.091	0.18
					Berries	35	0.037	0.038	0.015	0.015	0.10	0.18
CA 6.3.1/17 (16-2193) 16-2193-03 Germany, 55218 Ingelheim Grape 2016 (Müller-Thurgau)	1	0.300	600	BBCH 81	Grapes bunches	-0	0.043	0.044	0.027	0.027	0.14	0.16
	2	0.300	600	BBCH 83	Grapes bunches	0	0.23	0.23	0.17	0.18	0.81	0.94
					Grapes bunches	7	0.12	0.12	0.072	0.071	0.38	0.42
					Grapes bunches	14	0.076	0.079	0.047	0.042	0.24	0.26
					Berries	14	0.092	0.10	0.058	0.054	0.30	0.36
					Grapes bunches	21	0.077	0.081	0.042	0.040	0.24	0.33
					Grapes bunches	28	0.067	0.070	0.037	0.035	0.21	0.23
					Grapes bunches	35	0.057	0.058	0.032	0.033	0.18	0.21
					Berries	35	0.050	0.052	0.029	0.025	0.16	0.20

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of 4 spiroxamine enantiomer	
CA 6.3.1/17 (16-2193) 16-2193-04 Germany, 67281 Bissersheim Grape (Dornfelder) 2016	1 2	0.300 0.300	800 800	BBCH 83 BBCH 85	Grapes bunches	-0	0.037	0.038	0.026	0.024	0.12	0.13
					Grapes bunches	0	0.13	0.15	0.11	0.11	0.50	0.50
					Grapes bunches	7	0.074	0.076	0.048	0.049	0.25	0.30
					Grapes bunches	14	0.041	0.043	0.026	0.026	0.14	0.17
					Berries	14	0.048	0.052	0.030	0.030	0.16	0.18
					Grapes bunches	21	0.032	0.033	0.018	0.017	0.10	0.14
					Grapes bunches	28	0.028	0.032	0.016	0.015	0.092	0.14
					Grapes bunches	35	0.034	0.035	0.020	0.019	0.11	0.15
					Berries	35	0.029	0.031	0.017	0.017	0.094	0.12

1– The plot was under-dosed by 6.4%, no significant impact on residue for MRL purposes

Table CA 6.3.1/17-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/17	01480	A1 enantiomer	Grape berries	0.00271 0.0271 0.271 Overall	3 3 1 7	94; 103; 107 91; 94; 95 100 Mean: 98, RSD: 5.9
CA 6.3.1/17	01480	A1 enantiomer	Grape bunches	0.00271 0.0271 0.271 Overall	3 3 1 7	96; 97; 104 91; 92; 95 94 Mean: 96, RSD: 4.5
CA 6.3.1/17	01480	A2 enantiomer	Grape berries	0.00271 0.0271 0.271 Overall	3 3 1 7	99; 106; 112 93; 98; 104 105 Mean: 102, RSD: 6.1
CA 6.3.1/17	01480	A2 enantiomer	Grape bunches	0.00271 0.0271 0.271 Overall	3 3 1 7	94; 99; 102 92; 93; 93 93 Mean: 95, RSD: 4.0
CA 6.3.1/17	01480	B1 enantiomer	Grape berries	0.00229 0.0229 0.229 Overall	3 3 1 7	93; 101; 103 92; 93; 104 128 Mean: 101, RSD: 9.2
CA 6.3.1/17	01480	B1 enantiomer	Grape bunches	0.00229 0.0229 0.229 Overall	3 3 1 7	95; 95; 102 92; 93; 94 95 Mean: 95, RSD: 3.8
CA 6.3.1/17	01480	B2 enantiomer	Grape berries	0.00229 0.0229 0.229 Overall	3 3 1 7	93; 100; 105 88; 91; 92 98 Mean: 95, RSD: 6.2
CA 6.3.1/17	01480	B2 enantiomer	Grape bunches	0.00229 0.0229 0.229 Overall	3 3 1 7	96; 97; 102 94; 95; 96 98 Mean: 97, RSD: 2.7

Table CA 6.3.1/17-3 Procedural recovery data for the determination of total spiroxamine (via aminodiol)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/17	01089/M001	Total spiroxamine (via aminodiol)	Grape berries	0.01 0.10 1.0 Overall	3 3 1 7	82; 88; 89 85; 89; 91 86 Mean: 87, RSD: 3.5
CA 6.3.1/17	01089/M001	Total spiroxamine (via aminodiol)	Grape bunches	0.01 0.10 1.0 Overall	3 3 1 7	103; 106; 107 96; 96; 97 94 Mean: 100, RSD: 5.3

Table CA 6.3.1/17-4 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/17	Grapes	368 to 413 (four enantiomers of spiroxamine) 433 to 476 days (spiroxamine via aminodiol)

III. Conclusions

A total of four residue trials conducted in 2016 in northern Europe are available to evaluate the residues of spiroxamine (comprising of A1, A2, B1 and B2 enantiomers and the total residue as sum of the four enantiomers), and the total residue of spiroxamine (via aminodiol) in/on grapes (bunch of grapes and berry) after two applications of spiroxamine 500 EC to vines at 34/35 days pre-harvest (wine grapes PHI).

All four trials were conducted as residue decline trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in grapes ranged from 0.39 to 0.81 mg/kg, declining to between 0.079 and 0.18 mg/kg, 34/35 DALA.

The total residues of spiroxamine (as common moiety aminodiol) found 0 DALA in grapes ranged from 0.45 to 0.94 mg/kg, declining to between 0.08 and 0.21 mg/kg, 35 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in grapes ranged from 0.39 to 0.81 mg/kg, declining to between 0.079 and 0.18 mg/kg, 34/35 DALA.

The total residues of spiroxamine (as common moiety aminodiol) found 0 DALA in grapes ranged from 0.45 to 0.94 mg/kg, declining to between 0.08 and 0.21 mg/kg, 35 DALA.

Data Point:	KCA 6.3.1/18
Report Author:	[redacted]
Report Year:	2021
Report Title:	Determination of rcsidues of spiroxamine and ib rnetabolites afrer ona ortwo applicatlons of spiroxamine EC 500 in grapes {ontdoor) at 4 sites in northern Europe and 4 sites in southern Europe 2020
Report No:	S20-02176
Document No:	M-763137-01-1
Guideline(s) followed in study:	OECD 509 Adopted 2009-09-07 OECD Guideline for the testing of chemicals, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Eight trials conducted during 2020 season are available to evaluate the magnitude of spiroxamine in/on grape (bunches of grapes and berries) after either a single foliar spray or two foliar spray applications at nominally 0.6 L/ha of Spiroxamine EC 500 in northern and southern Europe.

For the trials receiving a single spray, the application was made pre development of fruits at nominally BBCH 15-19, and the subsequent grapes allowed to ripen to maturity (normal commercial harvest) without a specifically designated PHI. For the trials receiving two sprays, the designated PHI was 34/35 days (PHI for wine grapes), with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for the trials from NEU. Residues were analysed both for spiroxamine (comprising A1 enantiomer, A2 enantiomer, B1 enantiomer, B2 enantiomer, the total residue of spiroxamine as sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Four residue trials on grapes were conducted in northern Europe (northern France, Germany, Romania and Hungary) and four in southern Europe (Bulgaria, southern France, Italy and Spain) with all trials perfomed as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.1/18-1 to Table CA 6.3.1/18-3 where values considered for MRL purposes or risk assessment are underlined.

Each trial had two plots for the separate treatment designs used in this study. The plots for a single early season application were treated at a nominal product rate of 0.6 L/ha corresponding to 300 g a.s./ha between BBCH 12 and BBCH 55. The water application rates were between 182 and 617 L/ha. Mature grape berries were harvested at normal commercial harvest.

The plots for two applications at the usual critical GAP were treated at a nominal product rate of 0.6 L/ha corresponding to 300 g a.s./ha, spray intervals were 10 days with the final treatment being made at growth stage BBCH 83-89. The water application rates were between 294 and 847 L/ha. The critical GAP for the use of spiroxamine on grapes is supported in terms of rates and timings. Samples of grape berries were taken on the day of the last treatment (just after the final application and were also sampled at 13-15 and 34-36 DALA. Control samples were taken on day of the final treatment day prior to the last treatment, 13-15 and 34-36 DALA.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 495 g/L spiroxamine).

Although the trials were nominally treated at the critical GAP of 300 g a.s./ha, residues above the analytical LOQ have been scaled proportionally to a 300 g a.s./ha application rate in line with EFSA guidance [EFSA 2015 '*When proportionality approach is used, the scaling has to be applied to the entire dataset, including the trials conducted at dose rates within the ±25% tolerance rule*'] since other trials in the grapes dataset were scaled following treatment above or below the critical GAP and outside of ±25%. In practice, this scaling did not significantly impact on the reported residue results

Samples of grapes (berries) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine as the sum of the four enantiomers and also for the total residue of spiroxamine (as aminodiol).

Spiroxamine Parent Enantiomers

Residue analysis of samples of grapes for the determination of the four enantiomers of spiroxamine was conducted using the validated analytical method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol:water 3:1 (v/v) using a high-speed blender. After filtration, spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC CHIRAL Art Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. All final extracts were analysed within 2 days after the extraction was completed; nevertheless, the stability of the final extracts was demonstrated during the validation of the method 01480 for at least 12 days at 6°C.

Total Residue of Spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as aminodiol) in/on grapes was conducted using the validated method no. 01089/M001, report reference M-592369-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetone/water (2:1; v/v) using a high-speed blender. After filtration, an aliquot of the extract is heated (90°C) under acidic conditions yielding aminodiol representing all spiroxamine metabolites containing the aminodiol common moiety.

After dilution with acetonitrile and filtration, the samples were subjected to LC-MS/MS with electrospray ionization in positive ionization mode (column used SeQuant ZIC-HILIC, 150 x 2.1 mm, 5µm particle size) without further clean-up.

Each sample was extracted and analysed once or, in case a sample was extracted and analysed multiple times, the average values are reported. The samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

All final extracts were analysed within 2 days after the preparation was completed; nevertheless, the stability of the final plant extracts was demonstrated during the validation of the method 01089/M001 for at least 2 weeks at 6°C ± 3°C.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/18-6, the maximum storage time between date of deep-freezing and date of last extraction was

175 days for the four enantiomers of spiroxamine and 136 days for the analysis of spiroxamine (via aminodiol).

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.1/18-4 and Table CA 6.3.1/18-5).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg, for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via aminodiol) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All of the trials were conducted as harvest trials, with berries sampled at normal commercial harvest corresponding to PHI of 14 days and 35 days for the plots with two applications.

After a single application at nominally BBCH 15 – 19, residues of spiroxamine (sum of all enantiomers) found at normal commercial harvest in grapes were all <0.01 mg/kg (non-detectable) from four trials conducted in both northern and southern Europe. The total residues of spiroxamine (as common moiety aminodiol) found ranged from <0.01 mg/kg (non-detectable) to 0.01 mg/kg (in two trials, one each in northern and southern Europe).

After two applications with final spray at nominally BBCH 83-85, residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) at 0 DALA in grapes ranged from 0.06 to 0.72 mg/kg declining to between <0.01 mg/kg (non-detectable) to 0.09 mg/kg at 34-36 DALA. The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found ranged from 0.06 to 0.74 mg/kg at 0 DALA, declining to between 0.01 mg/kg to 0.15 mg/kg at 34-36 DALA. For the trials in southern Europe at 13-15 DALA (PHI for table grapes) residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) in grapes ranged from <0.014 to 0.17 mg/kg.

Table CA 6.3.1/18-1 Residue trials with spiroxamine 500 g/L EC in grapes – early season single application

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
Northern Europe												
CA 6.3.1/18 (S20-02176) S20-02176-01 Northern France, 45370, Cléry-Saint-André, Loiret Grape (Pino gris) 2020	1	0.288	288	BBCH 12-14	Berries	141	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	0.01
CA 6.3.1/18 (S20-02176) S20-02176-02 Germany, 69234 Dielheim, Baden-Wurttemberg Grape (Riesling) 2020	1	0.326	218	BBCH 15	Berries	131	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	<0.01
CA 6.3.1/18 (S20-02176) S20-02176-03 Romania, 317342 Masca, Arad Grape (Cabernet Sauvignon) 2020	1	0.314	314	BBCH 13-17	Berries	172	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	<0.01 (nd)

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/18 (S20-02176) S20-02176-04 Hungary, H-8272 Veszprem Grape (Rizlingszilvani) 2020	1	0.310	413	BBCH 15	Berries	141	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	<0.01 (nd)
Southern Europe												
CA 6.3.1/18 (S20-02176) S20-02176-05 Bulgaria, 4463 Lesichovo, Pazazdzhik Grape (Mavrud) 2020	1	0.308	617	BBCH 79	Berries	135	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	0.01
CA 6.3.1/18(S20-02176) S20-02176-06 southern France, 82600 Saint Sardos, Tarn et Garonne Grape (Tannat) 2020	1	0.272	182	BBCH 14-55	Berries	148	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	<0.01 (nd)
CA 6.3.1/18 (S20-02176) S20-02176-07 Italy, 40050 Bazzano, Bologna Grape (Pignoleto) 2020	1	0.303	607	BBCH 16	Berries	145	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	<0.01 (nd)

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/18 (S20-02176) S20-02176-08 Spain, 41808 Villanueva Del Ariscal, Andalucia Grape (Garrido Fino) 2020	1	0.276	368	BBCH 19	Berries	132	<0.0027 (nd)	<0.0027 (nd)	<0.0023 (nd)	<0.0023 (nd)	<0.01 (nd)	<0.01

Table CA 6.3.1/18-2 Residue trials with spiroxamine 500 g/L EC in grapes – two applications

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
Northern Europe												
CA 6.3.1/18 (S20-02176) S20-02176-01 Northern France, 45370, Cléry-Saint-André, Loiret Grape (Pino gris) 2020	1 2	0.299 0.323	598 645	BBCH 79-81 BBCH 83	Berries Berries Berries	0 14 35	0.045 0.0086 0.0079	0.043 0.0086 0.0075	0.038 0.0052 0.0046	0.037 0.0050 0.0046	0.16 0.03 0.02	0.16 0.03 0.04
CA 6.3.1/18 (S20-02176) S20-02176-02 Germany, 69234 Dielheim, Baden-Wurttemberg Grape (Riesling) 2020	1 2	0.274 0.275	365 550	BBCH79 BBCH 83	Berries Berries Berries	0 15 36	0.073 <0.0027 (nd) 0.014	0.073 <0.0027 (nd) 0.014	0.051 <0.0023 (nd) 0.0086	0.063 <0.0023 (nd) 0.0082	0.26 <0.01 (nd) 0.05	0.26 <0.01 (nd) 0.09
CA 6.3.1/18 (S20-02176) S20-02176-03 Romania, 317342 Masca, Arad Grape (Cabernet Sauvignon) 2020	1 2	0.294 0.303	294 303	BBCH 85 BBCH 85	Berries Berries Berries	0 13 35	0.095 0.028 0.0082	0.092 0.028 0.0083	0.081 0.020 0.0054	0.074 0.019 0.0053	0.34 0.09 0.03	0.27 0.10 0.03

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/18 (S20-02176) S20-02176-04 Hungary, H-8272 Veszprem Grape (Rizlingszilvani) 2020	1 2	0.282 0.308	753 822	BBCH 83 BBCH 85	Berries Berries Berries	0 14 35	0.093 0.015 0.031	0.092 0.014 0.030	0.074 0.0063 0.014	0.066 0.0061 0.014	0.33 0.04 0.09	0.32 0.10 0.15
Southern Europe												
CA 6.3.1/18 (S20-02176) S20-02176-05 Bulgaria, 4463 Lesichovo, Pazazdzhik Grape (Mavrud) 2020	1 2	0.309 0.308	824 822	BBCH 83 BBCH 85	Berries Berries Berries	0 14 34	0.21 0.039 0.021	0.21 0.037 0.021	0.16 0.023 0.014	0.15 0.025 0.015	0.74 0.12 0.07	0.76 0.20 0.11
CA 6.3.1/18 (S20-02176) S20-02176-06 southern France, 82600 Saint Sardos, Tarn et Garonne Grape (Tannat) 2020	1 2	0.302 0.299	503 499	BBCH 79-81 BBCH 83	Berries Berries Berries	0 13 34	0.20 0.053 0.022	0.20 0.052 0.021	0.15 0.030 0.011	0.17 0.029 0.011	0.73 0.16 0.07	0.70 0.22 0.15
CA 6.3.1/18 (S20-02176) S20-02176-07 Italy, 40050 Bazzano, Bologna Grape (Pignoleto) 2020	1 2	0.323 0.313	645 627	BBCH 81 BBCH 83	Berries Berries Berries	0 13 34	0.018 0.0035 <0.0027 (nd)	0.017 0.0035 <0.0027 (nd)	0.015 <0.0023 (nd) <0.0023 (nd)	0.015 <0.0023 (nd) <0.0023 (nd)	0.06 <0.01[1] <0.01 (nd)[1]	0.06 0.02 0.01

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via aminodiol) [mg/kg]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.1/18 (S20-02176) S20-02176-08 Spain, 41808 Villanueva Del Ariscal, Andalucia Grape (Garrido Fino) 2020	1 2	0.285 0.318	760 847	BBCH 75-77 BBCH 89	Berries Berries Berries	0 14 35	0.12 0.029 <0.0041	0.12 0.027 <0.0041	0.092 <0.014 <0.0023 (nd)	0.099 <0.013 <0.0023 (nd)	0.43 0.08 <0.013	0.38 0.18 0.03

1-Mean of triplicate analysis

Table CA 6.3.1/18-3 Residue trials with spiroxamine 500 g/L EC in grapes – two applications, scaled residue results

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1]					Scaled total residue of spiroxamine (via aminodiol, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[2]	
Northern Europe												
CA 6.3.1/18 (S20-02176) S20-02176-01 Northern France, 45370, Cléry-Saint-André, Loiret Grape (Pino gris) 2020	1 2	0.299 0.323	598 645	BBCH 79-81 BBCH 83	Berries Berries Berries	0 14 35	0.0418 0.0080 0.0073	0.0418 0.0080 0.0070	0.0353 0.0048 0.0046	0.0344 0.0046 0.0043	0.153 0.025 <u>0.023</u>	0.149 0.028 0.037
CA 6.3.1/18 (S20-02176) S20-02176-02 Germany, 69234 Dielheim, Baden-Wurttemberg Grape (Riesling) 2020	1 2	0.274 0.275	365 550	BBCH79 BBCH 83	Berries Berries Berries	0 15 36	0.0796 <0.0027 (nd) 0.0153	0.0796 <0.0027 (nd) 0.0153	0.0556 <0.0023 (nd) 0.0094	0.0687 <0.0023 (nd) 0.0089	0.284 <0.01 (nd) <u>0.049</u>	0.284 <0.01 (nd) 0.098
CA 6.3.1/18 (S20-02176) S20-02176-03 Romania, 317342 Masca, Arad Grape (Cabernet Sauvignon) 2020	1 2	0.294 0.303	294 303	BBCH 85 BBCH 85	Berries Berries Berries	0 13 35	0.094 0.028 0.0081	0.091 0.028 0.0082	0.080 0.020 0.0053	0.073 0.019 0.0052	0.339 0.094 <u>0.027</u>	0.267 0.099 0.030
CA 6.3.1/18 (S20-02176) S20-02176-04 Hungary, H-8272 Veszprem Grape (Rizlingszilvani) 2020	1 2	0.282 0.308	753 822	BBCH 83 BBCH 85	Berries Berries Berries	0 14 35	0.091 0.015 0.030	0.090 0.014 0.029	0.072 0.0061 0.014	0.064 0.0059 0.014	0.317 0.040 <u>0.087</u>	0.312 0.097 0.146

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1]					Scaled total residue of spiroxamine (via aminodiol, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[2]	
Southern Europe												
CA 6.3.1/18 (S20-02176) S20-02176-05 Bulgaria, 4463 Lesichovo, Pazazdzhik Grape (Mavrud) 2020	1 2	0.309 0.308	824 822	BBCH 83 BBCH 85	Berries Berries Berries	0 14 34	0.205 0.038 0.021	0.205 0.036 0.021	0.156 0.022 0.014	0.146 0.024 0.015	0.711 <u>0.121</u> <u>0.069</u>	0.740 0.195 0.107
CA 6.3.1/18 (S20-02176) S20-02176-06 southern France, 82600 Saint Sardos, Tarn et Garonne Grape (Tannat) 2020	1 2	0.302 0.299	503 499	BBCH 79-81 BBCH 83	Berries Berries Berries	0 13 34	0.201 0.053 0.022	0.201 0.052 0.021	0.151 0.030 0.011	0.171 0.029 0.011	0.722 <u>0.165</u> <u>0.065</u>	0.702 0.221 0.151
CA 6.3.1/18 (S20-02176) S20-02176-07 Italy, 40050 Bazzano, Bologna Grape (Pignoleto) 2020	1 2	0.323 0.313	645 627	BBCH 81 BBCH 83	Berries Berries Berries	0 13 34	0.017 0.0034 <0.0027 (nd)	0.016 0.0034 <0.0027 (nd)	0.014 <0.0023 (nd) <0.0023 (nd)	0.014 <0.0023 (nd) <0.0023 (nd)	0.062 <u><0.014</u> <u><0.01 (nd)</u>	0.058 0.019 0.010
CA 6.3.1/18 (S20-02176) S20-02176-08 Spain, 41808 Villanueva Del Ariscal, Andalucia Grape (Garrido Fino) 2020	1 2	0.285 0.318	760 847	BBCH 75-77 BBCH 89	Berries Berries Berries	0 14 35	0.113 0.027 0.0039	0.113 0.026 0.0039	0.087 0.013 <0.0023 (nd)	0.093 0.012 <0.0023 (nd)	0.401 <u>0.078</u> <u><0.012</u>	0.359 0.170 0.028

1 - The proportionality (scaling) concept was applied to the last single application rate
2 - Underlined value to be used to support MRL for table grapes; Double underlined value to be used to support MRL for wine grapes

Table CA 6.3.1/18-4 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/18	01480	A1 enantiomer	Grape berries	0.0027 0.027 0.27 Overall	7 7 3 17	106; 104; 105; 104; 107; 108; 95 107; 107; 106; 106; 108; 107; 100 107;102;106 Mean: 105, RSD: 3.1
CA 6.3.1/18	01480	A2 enantiomer	Grape berries	0.0027 0.027 0.27 Overall	7 7 3 17	110; 102; 107; 103;102; 108; 97 102; 105; 107; 107; 104; 105;100 108;103; 107 Mean: 105, RSD: 3.1
CA 6.3.1/18	01480	B1 enantiomer	Grape berries	0.0023 0.023 0.23 Overall	7 7 3 17	98; 100; 89; 100; 103; 95; 100 99; 103; 106; 110; 105; 103; 103 108; 90; 110 Mean: 101, RSD: 6.1
CA 6.3.1/18	01480	B2 enantiomer	Grape berries	0.0023 0.023 0.23 Overall	7 7 3 17	80; 110; 99; 100; 104; 102; 97 91; 89; 103; 98; 100; 109; 99 108; 106; 102 Mean: 100, RSD: 7.5

Table CA 6.3.1/18-5 Procedural recovery data for the determination of total spiroxamine (via aminodiol)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/18	01089/M001	Total spiroxamine (via aminodiol)	Grape berries	0.01 0.10 1.0 Overall	6 6 3 7	100; 101; 99; 105; 100; 100 97; 94; 103; 103; 104; 103 98; 99; 97 Mean: 100, RSD: 3.0

Table CA 6.3.1/18-6 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/18	Grapes	47 to 175 (four enantiomers of spiroxamine) 43 to 136 days (spiroxamine via aminodiol)

III. Conclusions

A total of eight residue trials conducted in 2020 in northern and southern Europe are available to evaluate the residues of spiroxamine (comprising of A1, A2, B1 and B2 enantiomers and the total residue as sum of the four enantiomers), and the total residue of spiroxamine (via aminodiol) in/on grapes (berries) after either one or two applications of spiroxamine 500 EC to vines. Grapevines were treated with one application at BBCH 15-19, and the grapes allowed to ripen to maturity (normal commercial harvest) or 2 sprays, applications made at BBCH 53-85 (10 days before application 2) and then at BBCH 83-85. The designated PHI was 34/35 days (PHI for wine grapes), with sampling at 14 days after the second application (SEU PHI for table grapes).

All of the trials were conducted as harvest trials, with berries sampled at normal commercial harvest corresponding to PHI of 14 days and 35 days for the plots with two applications.

After a single application at nominally BBCH 15-19, residues of spiroxamine (sum of all enantiomers) found at normal commercial harvest in grapes were all <0.01 mg/kg (non-detectable) from four trials conducted in both northern and southern Europe. The total residues of spiroxamine (as common moiety aminodiol) found ranged from <0.01 mg/kg (non-detectable) to 0.01 mg/kg (in two trials, one each in northern and southern Europe).

After two applications with final spray at nominally BBCH 83-85, residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) at 0 DALA in grapes ranged from 0.06 to 0.72 mg/kg declining to between <0.01 mg/kg (non-detectable) to 0.09 mg/kg at 34-36 DALA. The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found ranged from 0.06 to 0.74 mg/kg at 0 DALA, declining to between 0.01 mg/kg to 0.15 mg/kg at 34-36 DALA. For the trials in southern Europe at 13-15 DALA (PHI for table grapes) residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) in grapes ranged from <0.014 to 0.19 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

After a single application at nominally BBCH 15-19, residues of spiroxamine (sum of all enantiomers) found at normal commercial harvest in grapes were all <0.01 mg/kg (non-detectable) from four trials conducted in both northern and southern Europe. The total residues of spiroxamine (as common moiety aminodiol) found ranged from <0.01 mg/kg (non-detectable) to 0.01 mg/kg (in two trials, one each in northern and southern Europe). These data support the early season single application 'fall back' GAP for the use of spiroxamine on grapes and confirms that a non-significant residue situation for parent spiroxamine on grapes can be expected for this use and consumer risk would be covered under the risk-envelope scenario from the critical GAP.

After two applications with final spray at nominally BBCH 83-85, residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) at 0 DALA in grapes ranged from 0.06 to 0.72 mg/kg declining to between <0.01 mg/kg (non-detectable) to 0.09 mg/kg at 34-36 DALA. The total residues (scaled to the cGAP) of spiroxamine (as common moiety aminodiol) found ranged from 0.06 to 0.74 mg/kg

at 0 DALA, declining to between 0.01 mg/kg to 0.15 mg/kg at 34-36 DALA. For the trials in southern Europe at 13-15 DALA (PHI for table grapes) residues (scaled to the cGAP) of spiroxamine (sum of all enantiomers) in grapes ranged from <0.01 to 0.16 mg/kg.

Data Point:	KCA 6.3.1/19
Report Author:	[redacted]; [redacted]
Report Year:	2015
Report Title:	Determination of the residues of spiroxamine in/on grape after spray application of KWG 4168 EC 500 in Germany
Report No:	13-2164
Document No:	M-510227-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No: 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Two trials conducted during season 2013 are available to evaluate the magnitude of spiroxamine in/on grapes after two foliar spray applications at 0.6 L/ha of Spiroxamine 500 EC in northern Europe. The designated PHI was 35 days (PHI for wine grapes), with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for data from NEU. Residues were analysed both for spiroxamine and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Two residue trials on grapes were established in northern Europe (Germany) with both trials conducted as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/19-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a nominal product rate of 0.6 L/ha corresponding to 300 g a.s./ha. The water application rates were 714 L/ha per metre foliage height (1000 L/ha).

Spray intervals were 9 or 14 days with the final treatment being made at growth stage BBCH 83. The critical GAP for the use of spiroxamine on grapes is supported in terms of rate and timings.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 506.8 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior to and subsequent to the application) and then on several occasions, including 34 days after application (DALA, PHI for wine grapes). The PHI was one day earlier than the critical GAP (35 days) due to environmental weather conditions. This is an acceptable harvest interval and represents a worst case scenario. Control samples

were taken on day of the final treatment day prior to the last treatment, 13 and 34 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as total spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residues of spiroxamine (parent compound) and total residue of spiroxamine were analysed using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2/1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen within 24 hours of sampling at ≤-18°C. As shown in Table CA 6.3.1/19-3, the maximum storage time between date of deep-freezing and date of last extraction was 384 days for both spiroxamine (parent compound) and the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% with the exception of one result for recovery of spiroxamine as total residue (aminodiol) at 0.50 mg/kg (114%, refer to Table CA 6.3.1/19-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine (as aminodiol) in grapes was 0.05 mg/kg. Although the LOQ has been reduced to 0.01 mg/kg in subsequent studies, as all grapes data in treated samples are above the LOQ these data can be used for MRL purposes.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples

Residues of spiroxamine (parent compound) in grapes were 1.4 and 1.8 mg/kg, 0 DALA, declining to 0.25 and 0.60 mg/kg, 34 DALA in bunches and 0.24 or 0.53 mg/kg, 34 DALA in berries.

Total residues of spiroxamine (as aminodiol) in grapes were 1.5 and 2.1 mg/kg, 0 DALA, declining to 0.32 and 0.62 mg/kg, 34 DALA in bunches and 0.29 or 0.57 mg/kg, 34 DALA in berries.

Table CA 6.3.1/19-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for northern Europe (field)

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine			
	No.	kg a.s./ha	L/ha	Growth stage			Initial	Repeat[1]	Mean value[2]	Total residue of spiroxamine
CA 6.3.1/19 (13-2164) 13-2164-01 Germany, 53557 Bad Hönningen Grape (Spätburgunder) 2013	1 2	0.300 0.300	1000 1000	BBCH 81 BBCH 83	Grapes bunches	-0	0.35	0.30	0.33	0.37
					Grapes bunches	0	1.9	1.8	1.8	2.1, 2.1
					Grapes bunches	6	0.75	0.85	0.80	0.77
					Grapes bunches	13	0.80	0.89	0.84	0.81
					Berries	13	0.54	0.56	0.55	0.58
					Grapes bunches	20	0.61	0.72	0.66	0.61
					Grapes bunches	27	0.58	0.61	0.59	0.64
					Grapes bunches	34	0.58	0.62	0.60	0.62
					Berries	34	0.54	0.52	0.53	0.57
CA 6.3.1/19 (13-2164) 13-2164-02 Germany, 65366 Geisenheim Grape (Geisenheim) 2013	1 2	0.300 0.300	1000 1000	BBCH 81 BBCH 83	Grapes bunches	-0	0.10			0.10
					Grapes bunches	0	1.4			1.5
					Grapes bunches	6	0.50			0.51
					Grapes bunches	13	0.40			0.41
					Berries	13	0.23			0.32
					Grapes bunches	20	0.30			0.31
					Grapes bunches	27	0.31			0.37
					Grapes bunches	34	0.25			0.32
					Berries	34	0.24			0.29

1 – Complete reanalysis was performed in order to confirm the residue values observed
2 – Mean values of initial result and repeat were calculated based on exact values

Table CA 6.3.1/19-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/19	01089	Spiroxamine (parent compound)	Grapes (berry)	0.05 0.50 Overall	3 3 6	101; 103; *82*[1] 81; 98; *95*[1] Mean: 93, RSD: 10.2
			Grapes (bunch)	0.05 0.50 1.00 2.50 Overall	3 2 1 1 7	97; 101; 107 97; 100 105 85 Mean: 99, RSD: 7.4
CA 6.3.1/19	01089	Total residue of spiroxamine	Grapes (berry)	0.05 0.50 Overall	2 2 4	101; 104 105; 107 Mean: 104, RSD: 2.4
			Grapes (bunch)	0.05 0.50 1.00 2.50 Overall	2 1 1 1 5	98; 105 104 104 105 Mean: 107, RSD: 5.4

1-Recoveries determined during repeat analysis are shown in italics

Table CA 6.3.1/19-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/19	Grapes	258 to 384 (spiroxamine and total residue of spiroxamine)

III. Conclusions

A total of two residue trials conducted in 2013 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after two applications of spiroxamine 500 EC to vines 34 days pre-harvest (wine grapes).

Both trials were conducted as residue decline trials. Residues of spiroxamine (parent compound) in grapes were 1.4 and 1.8 mg/kg, 0 DALA, declining to 0.25 and 0.60 mg/kg, 34 DALA in bunches and 0.24 or 0.53 mg/kg, 34 DALA in berries.

Total residues of spiroxamine (as aminodiol) in grapes were 1.5 and 2.1 mg/kg, 0 DALA, declining to 0.32 and 0.62 mg/kg, 34 DALA in bunches and 0.29 or 0.57 mg/kg, 34 DALA in berries.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (parent compound) in grapes were 1.4 and 1.8 mg/kg, 0 DALA, declining to 0.25 and 0.60 mg/kg, 34 DALA in bunches and 0.24 or 0.53 mg/kg, 34 DALA in berries.

Total residues of spiroxamine (as aminodiol) in grapes were 1.5 and 2.1 mg/kg, 0 DALA, declining to 0.32 and 0.62 mg/kg, 34 DALA in bunches and 0.29 or 0.57 mg/kg, 34 DALA in berries.

Data Point:	KCA 6.3.1/20
Report Author:	[redacted]
Report Year:	2015
Report Title:	Determination of the residues of AE C656948 and spiroxamine in/on grape after high and low-volume spray application of fluopyram & spiroxamine SE 275 in southern France, Spain, Italy and Portugal
Report No:	13-2141
Document No:	M-508894-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC „h EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) „h OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial „h US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during season 2013 are available to evaluate the magnitude of spiroxamine in/on grapes after two foliar spray applications at 1.0 L/ha of fluopyram and spiroxamine SE 275 in southern Europe. The designated PHI was 13 or 14 days (PHI for table grapes) with additional sampling at 35 days (PHI for wine grapes). Residues were analysed for both spiroxamine and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residues of fluopyram were also analysed, however are not reported in this summary.

Four residue trials on grapes were conducted in southern Europe (southern France, Spain, Italy and Portugal) with all four conducted as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/20-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.0 L/ha corresponding to 200 g a.s./ha. The water application rates were 200 L/ha in France (low-volume spraying), and between 600

to 1000 L/ha in other countries. Residues above the LOQ are therefore scaled proportionally to a 300 g a.s/ha application rate in line with the critical GAP.

Spray intervals were 10 days with the final treatment being made at growth stage BBCH 79-85. The critical GAP for the use of spiroxamine on grapes is supported in terms of timings.

The trials were conducted using fluopyram and spiroxamine SE 275 formulation containing 200 g/L spiroxamine (actual content 199.0 g/L spiroxamine).

Samples of grapes (bunches of grapes) were taken on the day of the final treatment (prior to and subsequent to the application), and then on several occasions, including at 13/14 days after last application (DALA, PHI for table grapes)and 34/35 DALA (PHI for wine grapes). Control samples were taken on day of the final treatment day prior to the last treatment, and 13 or 14 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as total spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residues of spiroxamine (parent compound) and total residue of spiroxamine were analysed using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2/1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen within 24 hours of sampling at ≤-18 °C. As shown in Table CA 6.3.1/20-3, the maximum storage time between date of deep-freezing and date of last extraction was 428 days for both spiroxamine (parent compound) and the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were generally in the range of 70-110% with the exception of four results for the total residue of spiroxamine at 0.05 mg/kg (61, 62, 66, 67%). These results are not expected to influence the validity of the study as the RSD values (21.4%) was only slightly above the range of 20% (refer to Table CA 6.3.1/20-2) and these data are not used for MRL purposes. The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.08 to 0.38 mg/kg, 0 DALA, declining to 0.08 to 0.11 mg/kg, 14 DALA and 0.08 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.22 to 0.54 mg/kg, 0 DALA, declining to 0.09 to 0.21 mg/kg, 14 DALA and 0.10 to 013 mg/kg, 34 DALA.

Values originally reported as <0.05 mg/kg (LOQ) cannot be scaled to 300 g a.s./ha., meaning that data from 3 trials can be used for MRL purposes on table grapes and 1 trial for MRL purposes on wine grapes.

Table CA 6.3.1/20-1 Residue trials with fluopyram and spiroxamine SE 275 in grapes – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue[3]	Scaled residue[1,2]	Reported residue[3]	Scaled residue[1]
CA 6.3.1/20 (13-2141) 13-2141-01 Southern France, 30290 Laudun Grape (Grenache blanc) 2013	1 2	0.200 0.200	200 200	BBCH 85 BBCH 85	Grapes bunches	-0	0.052	0.08	<0.05	n.a.
					Grapes bunches	0	0.20	0.30	0.15	0.22
					Grapes bunches	3	0.066	0.01	0.058	0.09
					Grapes bunches	7	0.050	0.08	<0.05	n.a.
					Grapes bunches	14	0.055	0.083	0.060	0.09
					Grapes bunches	21	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	28	0.054	0.08	0.066	0.10
					Grapes bunches	35	<0.05	n.a.	<0.05	n.a.
CA 6.3.1/20 (13-2141) 13-2141-02 Spain, 41820 Carrión de los Cespedes Grape (Tempranillo) 2013	1 2	0.200 0.200	1000 1000	BBCH 79 BBCH 81	Grapes bunches	-0	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	0	0.056	0.084	<0.05	n.a.
					Grapes bunches	3	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	7	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	13	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	21	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	28	<0.05	n.a.	<0.05	n.a.
					Grapes bunches	35	<0.05	n.a.	<0.05	n.a.
CA 6.3.1/20 (13-2141) 13-2141-03 Italy, 76125 Trani Grape (Moscato di Trani) 2013	1 2	0.200 0.200	1000 1000	BBCH 81 BBCH 83	Grapes bunches	-0	0.075	0.11	0.12	0.18
					Grapes bunches	0	0.31	0.18	0.36	0.54
					Grapes bunches	3	0.12	0.18	0.19	0.29
					Grapes bunches	7	0.073	0.11	0.14	0.21
					Grapes bunches	14	0.062	0.093	0.14	0.21
					Grapes bunches	21	<0.05	n.a.	0.13	0.20
					Grapes bunches	28	<0.05	n.a.	0.11	0.17
					Grapes bunches	35	<0.05	n.a.	0.088	0.13

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue[3]	Scaled residue[1,2]	Reported residue[3]	Scaled residue[1]
CA 6.3.1/20 (13-2141) 13-2141-04 Portugal, 2580-041 Espiçanderia Grape (Alicante buchet) 2013	1	0.200	600	BBCH 79	Grapes bunches	-0	<0.05	n.a.	<0.05	n.a.
	2	0.200	600	BBCH 79	Grapes bunches	0	0.25	0.38	0.27	0.41
					Grapes bunches	3	0.14	0.21	0.17	0.26
					Grapes bunches	7	0.080	0.12	0.099	0.15
					Grapes bunches	14	0.072	0.11	0.088	0.13
					Grapes bunches	21	0.053	0.08	0.066	0.10
					Grapes bunches	28	0.052	0.08	0.067	0.10
					Grapes bunches	35	0.052	0.08	0.065	0.10

1 – Residues scaled proportionally to a 0.300 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - Underlined value to be used to support MRL for table grapes; Double underlined value to be used to support MRL for wine grapes
3 - Values below the LOQ (<0.05 mg/kg) not appropriate to scale up

Table CA 6.3.1/20-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/20	01089	Spiroxamine (parent compound)	Grapes (bunch)	0.05 0.50 Overall	4 7 11	80; 84; 98; 101 89; 90; 94; 100; 101; 101; 101 Mean: 94; RSD: 8.1
CA 6.3.1/20	01089	Total residue of spiroxamine	Grapes (bunch)	0.05 0.50 Overall	4 7 11	83; 83; 107; 110 61; 62; 66; 67; 81; 93; 99 Mean: 83; RSD: 21.4

Table CA 6.3.1/20-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3/1/20	Grapes	354 to 428 (spiroxamine and total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2013 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after two applications with fluopyram and spiroxamine SE 275 to vines at 13 or 14 days pre-harvest (table grapes), and 35 days pre-harvest (wine grapes).

Both trials were conducted as residue decline trials. Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.08 to 0.38 mg/kg, 0 DALA, declining to 0.08 to 0.11 mg/kg, 14 DALA and 0.08 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.22 to 0.54 mg/kg, 0 DALA, declining to 0.09 to 0.21 mg/kg, 14 DALA and 0.10 to 013 mg/kg, 34 DALA.

Values originally reported as <0.05 mg/kg (LOQ) cannot be scaled to 300 g a.s./ha., meaning that data from 3 trials can be used for MRL purposes on table grapes and 1 trial for MRL purposes on wine grapes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.08 to 0.38 mg/kg, 0 DALA, declining to 0.08 to 0.11 mg/kg, 14 DALA and 0.08 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.22 to 0.54 mg/kg, 0 DALA, declining to 0.09 to 0.21 mg/kg, 14 DALA and 0.10 to 013 mg/kg, 34 DALA.

Values originally reported as <0.05 mg/kg (LOQ) cannot be scaled to 300 g a.s./ha., meaning that data from 3 trials can be used for MRL purposes on table grapes and 1 trial for MRL purposes on wine grapes.

Data Point:	KCA 6.3.1/21
Report Author:	[redacted]; [redacted]
Report Year:	2015
Report Title:	Determination of the residues of spiroxamine in/on grapes after low-volume spraying of KWG 4168 EC 500 in the field in France (North)
Report No:	13-2163
Document No:	M-508892-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC „h EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) „h OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial „h US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Two trials conducted during season 2013 are available to evaluate the magnitude of spiroxamine in/on grapes after two foliar spray applications of Spiroxamine 500 EC in northern Europe. The designated PHI was 35 days (PHI for wine grapes) with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for data from NEU. Residues were analysed both for spiroxamine and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents

Two residue trials on grapes were conducted in northern Europe (northern France). With both trials conducted as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/21-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 0.6 L/ha corresponding to 300 g a.s./ha with water application rates at 200 L/ha (low-volume spraying). Spray intervals were 10

days with the final treatment being made at growth stage BBCH 85. The critical GAP for the use of spiroxamine on grapes is supported in terms of rates and timings.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 506.8 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior to and subsequent to the application), and then on several occasions, including at 14 days after last application (DALA, PHI for table grapes)and 35 DALA (PHI for wine grapes). Control samples were taken on day of the final treatment day prior to the last treatment, 14 and 35 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as total spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residues of spiroxamine (parent compound) and total residue of spiroxamine were analysed using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2:1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen within 24 hours of sampling at ≤-18 °C. As shown in Table CA 6.3.1/21-3, the maximum storage time between date of deep-freezing and date of last extraction was 311 days for both spiroxamine (parent compound) and the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110%, with the exception of three results. Two for total residue of spiroxamine at 0.05 mg/kg (114% spiroxamine) and at 0.1 mg/kg (114% spiroxamine), in addition to one sample for spiroxamine (parent compound) at 1.0 mg/kg (114% spiroxamine) (refer to Table CA 6.3.1/21-2). These results do not impact the validity of the data. The limit of quantification (LOQ) for both spiroxamine (parent compound) and total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Residues of spiroxamine (parent compound) in grapes were 0.32 and 0.42 mg/kg, 0 DALA, declining to 0.05 and 0.075 mg/kg 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes were 0.32 to 0.45 mg/kg, 0 DALA, declining to 0.11 and 0.083 mg/kg 35 DALA.

Table CA 6.3.1.21-1 Residue trials with Spiroxamine 500 g/L EC in grapes – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine (parent compound)	Total residues of spiroxamine
							Reported residue	Reported residue
CA 6.3.1/21 (13-2163) 13-2163-01 Northern France, 37140 Saint Nicolas de Bourgueil Grape (Cabernet Franc) 2013	1 2	0.300 0.300	200 200	BBCH 81 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Berries Bunch of grapes Bunch of grapes Bunch of grapes Berries	-0 0 7 14 14 21 28 35 35	0.072 0.42 0.11 0.12 0.082 0.075 0.074 0.098 0.075	0.079 0.48 0.13 0.15 0.085 0.089 0.078 0.074 0.11
CA 6.3.1/21 (13-2163) 13-2163-02 Northern France, 37270 Athée sur Cher Grape (Chardonnay) 2013	1 2	0.300 0.300	200 200	BBCH 81 BBCH 85	Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes Berries Bunch of grapes Bunch of grapes Bunch of grapes Berries	-0 0 7 14 14 21 28 35 35	0.053 0.32 0.17 0.11 0.059 0.089 0.061 0.050 <0.05	0.068 0.32 0.23 0.18 0.091 0.15 0.11 0.10 0.083

Table CA 6.3.1.21-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/21	01089	Spiroxamine (parent compound)	Grapes (berry)	0.05 0.50 Overall	1 1 2	95 106 Mean: 101, RSD: -
			Grapes (bunch)	0.05 0.50 1.0 Overall	3 2 1 6	102; 103; 108 101; 108 114 Mean: 106, RSD: 4.7
CA 6.3.1/21	01089	Total residue of spiroxamine	Grapes (berry)	0.05 0.50 Overall	1 1 2	108 108 Mean: 108, RSD: -
			Grapes (bunch)	0.05 0.50 1.0 Overall	3 2 1 6	98; 107; 114 103; 105 114 Mean: 107, RSD: 5.9

Table CA 6.3.1.21-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/21	Grapes	273 to 311 (spiroxamine and total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2013 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after two applications of spiroxamine EC 500 to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

The trials were conducted as residue decline trials, with bunches of grapes and berries sampled at 14 (PHI for table grapes) to 28, and 35 (PHI for wine grapes) DALA.

Residues of spiroxamine (parent compound) in grapes were 0.32 and 0.42 mg/kg, 0 DALA, declining to 0.05 and 0.075 mg/kg 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes were 0.32 to 0.45 mg/kg, 0 DALA, declining to 0.11 and 0.083 mg/kg 35 DALA.

<u>Assessment and conclusion by applicant:</u>

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (parent compound) in grapes were 0.32 and 0.42 mg/kg, 0 DALA, declining to 0.05 and 0.075 mg/kg 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes were 0.32 to 0.45 mg/kg, 0 DALA, declining to 0.11 and 0.083 mg/kg 35 DALA.

Data Point:	KCA 6.3.1/22
Report Author:	[redacted]; [redacted]
Report Year:	2015
Report Title:	Determination of the residues of spiroxamine in/on grapes after spraying and low-volume spraying of KWG 4168 EC 500 in the field in France (South), Spain, Italy and Greece
Report No:	13-2161
Document No:	M-507373-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during season 2013 are available to evaluate the magnitude of spiroxamine in/on grapes after two foliar spray applications at 0.6 L/ha of Spiroxamine 500 EC in southern Europe. The designated PHI was 14 days (PHI for table grapes) with additional sampling at 35 days (PHI for wine grapes). Residues were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Four residue trials on grapes were conducted in southern Europe (southern France, Spain, Italy and Greece) with all four conducted as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/22-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 0.6 L/ha corresponding to 300 g a.s./ha. The water application rates were between 800-100 L/ha in Spain, Italy and Greece, and 200 L/ha in France (low-volume spraying).

Spray intervals were 9 or 10 days with the final treatment being made at growth stage BBCH 79-85. The critical GAP for the use of spiroxamine on grapes is supported in terms of timings.

The trials were conducted using a 500 g/L formulation of spiroxamine (actual content 506.8 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior and subsequent to the application), and then on several occasions, including 14 days after application (DALA, PHI for table grapes) and 35 days (PHI for wine grapes). Berries were also sampled at 14 and 35 days after the last application. Control samples were taken on day of the final treatment day prior to the last treatment, 14 and 35 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as total spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residues of spiroxamine (parent compound) and total residue of spiroxamine were analysed using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2/1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen within 24 hours of sampling at ≤-18°C. As shown in Table CA 6.3.1/22-3, the maximum storage time between date of deep-freezing and date of last extraction was 358 days for both spiroxamine (parent compound) and the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/22-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples with the exception of total residue of spiroxamine (as aminodiol) for trial 13-2161-03 (Italy). It was not obvious why significant total residues of spiroxamine were detected in these control samples. The pesticide history for years 2010 and 2011 was not available for this trial site, however an application of interfering products in these years could not be excluded. As no contamination with spiroxamine itself was reported and as the results of this trial for spiroxamine are consistent with other trials the data for MRL purposes are considered to be reliable, however the use of total spiroxamine data for e.g. conversion factor calculations will not be applied for this trial.

Residues of spiroxamine (parent compound) in grapes ranged from 0.16 to 0.60 mg/kg, 0 DALA, declining to 0.076 to 0.12 mg/kg, 14 DALA and <0.05 to 0.090 mg/kg, 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes ranged from 0.18 to 0.36 mg/kg, 0 DALA, declining to 0.09 to 0.23 mg/kg, 14 DALA and 0.053 to 0.14 mg/kg, 35 DALA (data from trial 13-2161-03 not included).

Table CA 6.3.1/22-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine (parent compound)[1]	Total residues of spiroxamine[2]
CA 6.3.1/22 (13-2161) 13-2161-01 Southern France, 30290 Laudun Grape (Grenache blanc) 2013	1 2	0.300 0.300	200 200	BBCH 85 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Berries Grapes bunches Grapes bunches Grapes bunches Berries	-0 0 7 14 14 21 28 35 35	<0.05 0.16 0.079 0.076 <0.05 0.061 0.066 0.05 <0.05	<0.05 0.18 0.097 0.093 0.066 0.060 0.083 0.055 0.053
CA 6.3.1/22 (13-2161) 13-2161-02 Spain, 08784 Piera - La Fortesa Grape (Macabeo) 2013	1 2	0.300 0.300	800 800	BBCH 79 BBCH 81	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Berries Grapes bunches Grapes bunches Grapes bunches Berries	-0 0 6 14 14 21 28 35 35	0.077 0.33 0.12 0.076 0.11 0.059 <0.05 <0.05 0.056	0.11 0.39 0.19 0.17 0.19 0.063 <0.05 0.051 0.13
CA 6.3.1/22 (13-2161) 13-2161-03 Italy, 40128 Bologna Grape (Moscato) 2013	1 2	0.300 0.300	1000 1000	BBCH 79 BBCH 81	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Berries Grapes bunches Grapes bunches Grapes bunches Berries	-0 0 7 14 14 22 27 35 35	0.17 0.52 0.29 0.11 0.092 0.11 0.072 <0.05 <0.05	0.44 0.79 0.54 0.32 0.35 0.36 0.31 0.20 0.26

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine (parent compound)[1]	Total residues of spiroxamine[2]
CA 6.3.1/22 (13-2161) 13-2161-04 Greece, GR-60100 Paleo Keramidi, Katerini – Pieria - Hellas Grape (Sangiovese) 2013	1 2	0.300 0.300	1000 1000	BBCH 77 BBCH 79	Grapes bunches	-0	0.088	0.14
					Grapes bunches	0	0.60	0.67
					Grapes bunches	7	0.17	0.23
					Grapes bunches	14	0.12	0.16
					Berries	14	0.12	0.23
					Grapes bunches	21	0.093	0.16
					Grapes bunches	28	0.081	0.15
					Grapes bunches	35	0.090	0.15
					Berries	35	0.062	0.14

1 - Underlined value to be used to support MRL for table grapes; Double underlined value to be used to support MRL for wine grapes
2 - Data for trial 13-2161-03 (Italy) not reliable due to apparent contamination in control samples

Table CA 6.3.1/22-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/22	01089	Spiroxamine (parent compound)	Grapes (berry)	0.05 0.50 Overall	1 1 2	91 91 Mean: 91, RSD: -
			Grapes (bunch)	0.05 0.50 1.0 Overall	7 8 2 17	94; 100; 101; 102; 105; 108; 108 72; 77; 78; 81; 82; 92; 98; 102 100; 101 Mean: 94, RSD: 12.4
CA 6.3.1/22	01089	Total residue of spiroxamine	Grapes (berry)	0.05 0.50 Overall	1 1 2	90 108 Mean: 99, RSD: -
			Grapes (bunch)	0.05 0.50 1.0 Overall	7 8 2 17	95; 96; 97; 98; 101; 105; 106 98; 101; 101; 102; 102; 103; 109; 109 102; 103 Mean: 102, RSD: 4.0

Table CA 6.3.1/22-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/22	Grapes	256 to 358 (spiroxamine and total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2013 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after two applications of spiroxamine EC 500 to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

All four trials were conducted as residue decline trials. Residues of spiroxamine (parent compound) in grapes ranged from 0.16 to 0.60 mg/kg, 0 DALA, declining to 0.076 to 0.12 mg/kg, 14 DALA and <0.05 to 0.090 mg/kg, 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes ranged from 0.18 to 0.36 mg/kg, 0 DALA, declining to 0.09 to 0.23 mg/kg, 14 DALA and 0.053 to 0.14 mg/kg, 35 DALA (data from trial 13-2161-03 not included).

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (parent compound) in grapes ranged from 0.16 to 0.60 mg/kg, 0 DALA, declining to 0.076 to 0.12 mg/kg, 14 DALA and <0.05 to 0.090 mg/kg, 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes ranged from 0.18 to 0.36 mg/kg, 0 DALA, declining to 0.09 to 0.23 mg/kg, 14 DALA and 0.053 to 0.14 mg/kg, 35 DALA (data from trial 13-2161-03 not included).

Data Point:	KCA 6.3.1/23
Report Author:	[redacted]; [redacted]
Report Year:	2014
Report Title:	Determination of the residues of AE C656948 and spiroxamine in/on grape after high and low-volume spray application fluopyram & spiroxamine SE 275 in northern France and Germany
Report No:	13-2134
Document No:	M-506727-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07; OECD GUIDELINE FOR THE TESTING OF CHEMICALS Crop Field Trial, US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during season 2013 are available to evaluate the magnitude of spiroxamine in/on grapes after two foliar spray applications at 1.0 L/ha of fluopyram and spiroxamine SE 275 in northern Europe. The designated PHI was 35 days (PHI for wine grapes) with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for data from NEU. Residues were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residues of fluopyram were also analysed, however will not reported in this summary.

Four residue trials on grapes were conducted in northern Europe (northern France and Germany) with all four conducted as residue decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/23-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.0 L/ha corresponding to 200 g a.s./ha The water application rates were 200 L/ha in France (low-volume spraying), and 800 L/ha in Germany. Residues above the LOQ are therefore scaled proportionally to a 300 g a.s/ha application rate in line with the critical GAP.

Spray intervals were 10 days with the final treatment being made at growth stage BBCH 85. The critical GAP for the use of spiroxamine on grapes is supported in terms of timings.

The trials were conducted using fluopyram and spiroxamine SE 275 formulation containing 200 g/L spiroxamine (actual content 199.0 g/L spiroxamine).

Samples of grape bunches were taken on the day of the final treatment (prior and subsequent to the application), in addition to several points thereafter, including at 14 (PHI for table grapes) and 35 days (PHI for wine grapes). Control samples were taken on day of the final treatment day prior to the last treatment, 14 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as total spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residues of spiroxamine (parent compound) and total residue of spiroxamine were analysed using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2:1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen within 24 hours of sampling at ≤-18°C. As shown in Table CA 6.3.1/23-3, the maximum storage time between date of deep-freezing and date of last extraction was 404 days for both spiroxamine (parent compound) and the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% with the exception of one sample for the total residue of spiroxamine at 0.05 mg/kg (111% spiroxamine). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.44 to 0.65 mg/kg, 0 DALA, declining to 0.09 to 0.30 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.45 to 0.65 mg/kg, 0 DALA, declining to 0.24 to 0.27 mg/kg, 35 DALA.

Values originally reported as <0.05 mg/kg (LOQ) cannot be scaled to 300 g a.s./ha., meaning that data from 3 trials can be used for MRL purposes on wine grapes.

Table CA 6.3.1/23-1 Residue trials with fluopyram and spiroxamine SE 275 in grapes – residue results for northern Europe (field)

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue[3]	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/23 (13-2134) 13-2134-01 Northern France 37270, Athée sur Cher Grape (Chardonnay) 2013	1 2	0.200 0.200	200 200	BBCH 81 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 4 7 14 21 28 35	<0.05 0.29 0.16 0.097 0.061 0.058 <0.05 <0.05	n.a. 0.44 0.24 0.15 0.10 0.09 n.a. n.a.	0.084 0.34 0.25 0.19 0.15 0.14 0.13 0.16	0.13 0.51 0.38 0.29 0.23 0.21 0.20 0.24
CA 6.3.1/23 (13-2134) 13-2134-02 Northern France, 37140 Saint Nicolas de Bourgueil Grape (Cabernet Franc) 2013	1 2	0.200 0.200	200 200	BBCH 81 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 14 21 28 35	0.050 0.35 0.13 0.11 0.069 0.059 <0.05 0.060	0.08 0.53 0.20 0.17 0.10 0.09 n.a. <u>0.09</u>	0.096 0.43 0.22 0.18 0.12 0.14 0.12 0.16	0.14 0.65 0.33 0.27 0.18 0.21 0.18 0.24
CA 6.3.1/23 (13-2134) 13-2134-03 Germany, 76889 Steinfeld Grape (Müller Thurgau) 2013	1 2	0.200 0.200	800 800	BBCH 83 BBCH 85	Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches Grapes bunches	-0 0 3 7 14 21 28 35	0.12 0.42 0.28 0.24 0.27 0.27 0.18 0.20	0.18 0.63 0.42 0.36 0.41 0.41 0.27 <u>0.30</u>	0.088 0.30 0.21 0.16 0.23 0.28 0.22 0.18	0.13 0.45 0.32 0.24 0.35 0.42 0.33 0.27

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue[3]	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/23 (13-2134) 13-2134-04 Germany, 67281 Bisserheim Grape (Dornfelder) 2013	1 2	0.200 0.200	800 800	BBCH 83 BBCH 85	Grapes bunches	-0	0.16	0.24	0.12	0.18
					Grapes bunches	0	0.43	0.65	0.38	0.57
					Grapes bunches	3	0.24	0.36	0.24	0.36
					Grapes bunches	7	0.22	0.33	0.17	0.26
					Grapes bunches	14	0.20	0.30	0.16	0.24
					Grapes bunches	21	0.17	0.26	0.17	0.26
					Grapes bunches	28	0.18	0.27	0.17	0.26
					Grapes bunches	35	0.18	0.27	0.17	0.26

1 – Residues scaled proportionally to a 0.300 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - Underlined value to be used to support MRL for table grapes
3 - Value not appropriate to scale up as reported value below the LOQ (<0.05 mg/kg)

Table CA 6.3.1/23-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/23	01089	Spiroxamine (parent compound)	Grapes (bunch)	0.05 0.50 Overall	4 4 8	84; 87; 95; 96 86; 91; 91; 94 Mean: 91, RSD: 4.8
CA 6.3.1/23	01089	Total residue of spiroxamine	Grapes (bunch)	0.05 0.50 Overall	2 4 6	95; 111 72; 78; 100; 104 Mean: 93, RSD: 16.3

Table CA 6.3.1/23-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/23	Grapes	366 to 404 (spiroxamine and total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2013 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after two applications of with fluopyram and spiroxamine SE 275 to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

All four trials were conducted as residue decline trials. Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.44 to 0.65 mg/kg, 0 DALA, declining to 0.09 to 0.30 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.45 to 0.65 mg/kg, 0 DALA, declining to 0.24 to 0.27 mg/kg, 35 DALA.

Values originally reported as <0.05 mg/kg (LOQ) cannot be scaled to 300 g a.s./ha., meaning that data from 3 trials can be used for MRL purposes on wine grapes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.44 to 0.65 mg/kg, 0 DALA, declining to 0.09 to 0.30 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.45 to 0.65 mg/kg, 0 DALA, declining to 0.24 to 0.27 mg/kg, 35 DALA.

Values originally reported as <0.05 mg/kg (LOQ) cannot be scaled to 300 g a.s./ha., meaning that data from 3 trials can be used for MRL purposes on wine grapes.

Data Point:	KCA 6.3.1/24
Report Author:	[redacted]; [redacted]
Report Year:	2010
Report Title:	Determination of the residues of spiroxamine in/on grape after spraying and spraying, low-volume of KWG 4168 EC 500 in the field in France (South), Italy and Spain
Report No:	09-2036
Document No:	M-390949-01-1
Guideline(s) followed in study:	91/414/EEC, 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2009 are available to evaluate the magnitude of Spiroxamine in/on grapes after three foliar spray applications at 0.6 L/ha of spiroxamine 500 EC in southern Europe. The designated PHI was 14 days (PHI for table grapes) with additional sampling at 34/35 days (PHI for wine grapes). Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Four residue trials on grapes were conducted in southern Europe (southern France, Italy and Spain). Two of the trials were conducted as harvest trials and the remaining trials were conducted as residue decline trials. The trial parameters and residue results are summarised in Table CA 6.3.1/24-1 where values considered for MRL purposes or risk assessment are underlined.

Three spray applications of spiroxamine were made at a product rate of 0.6 L/ha corresponding to 300 g a.s./ha with a water application rate of 200 L/ha in France (low-volume spraying) and 500 L/ha in Italy and Spain. Spray intervals were 10 days with the final treatment being made at growth stage BBCH 81-85. It can be seen that the additional treatments in these trials, which always were made early in the season, have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes.

The critical GAP for the use of spiroxamine on grapes is therefore supported in terms of rates and timings.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 501 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior to and subsequent to the application), in addition to several points thereafter, including at 14 (PHI for table grapes) and 34/35 days (PHI for wine grapes). Berries were also sampled at 14 and 34/35 days after the last application Control samples were taken on day of the final treatment day prior to the last treatment, 14 and 35 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as total spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residues of spiroxamine (parent compound) and total residue of spiroxamine were analysed using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2/1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen after sampling at ≤18°C. As shown in Table CA 6.3.1/24-3, the maximum storage time between date of deep-freezing and date of last extraction was 343 days for both spiroxamine (parent compound) and the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/24-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Residues in fruit from treated crop ranged from <0.05 to 0.35 mg/kg for spiroxamine (parent compound), and <0.05 to 0.28 mg/kg for total residues of spiroxamine 0 to 35 DALA (days after last application).

Two of the trials were conducted as residue decline trials (trial 09-2036-01 and trial 09-2036-03) and the remaining trials (trial 09-2036-02 and trial 09-2036-04) were conducted as harvest trials. Residue bunches of grapes and berries of spiroxamine (parent compound) and total spiroxamine were sampled at 14 (PHI for table grapes) or 21 DALA, and 34 or 35 (PHI for wine grapes) DALA.

Residues of spiroxamine (parent compound) in grapes ranged from 0.19 to 0.35 mg/kg, 0 DALA, declining to 0.05 to 0.15 mg/kg, 14-21 DALA and declining to <0.05 to 0.11 mg/kg, 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes ranged from 0.14 to 0.28 mg/kg, 0 DALA, declining to between <0.05 to 0.21 mg/kg, 35 DALA.

Table CA 6.3.1/24-1 Residue trials with Spiroxamine 500 g/L EC in grapes – residue results for southern Europe (field)

Doc. No. Trial Ref Location Crop (variety) Year	Application						Reported residue (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage	Crop part	DALA (days)	Spiroxamine (parent compound)[1]	Total residues of spiroxamine
CA 6.3.1/24 (09-2036)	1	0.300	200	BBCH 83	Bunch of grapes	-0	0.08	0.07
09-2036-01	2	0.300	200	BBCH 85	Bunch of grapes	0	0.35	0.26
France, 86380 Vendeuvre du Poitou	3	0.300	200	BBCH 85	Bunch of grapes	7	0.10	0.12
Grape (Gamey)					Bunch of grapes	14	0.10	0.10
2009					Berries	14	0.12	0.13
					Bunch of grapes	21	0.15[2]	0.15
					Bunch of grapes	35	0.11	0.13
					Berries	35	0.10	0.13
CA 6.3.1/24 (09-2036)	1	0.300	200	BBCH 79	Bunch of grapes	-0	0.13	0.13
09-2036-02	2	0.300	200	BBCH 81	Bunch of grapes	0	0.25	0.24
France, 69380 Chazay d'Azergues	3	0.300	200	BBCH 85	Bunch of grapes	14	0.10	0.16
Grape (Chardonnay)					Berries	14	0.12	0.17
2009					Bunch of grapes	35	0.08	0.16
					Berries	35	0.10	0.21
CA 6.3.1/24 (09-2036)	1	0.300	500	BBCH 81	Bunch of grapes	-0	0.08	0.08
09-2036-03	2	0.300	500	BBCH 83	Bunch of grapes	0	0.28	0.28
Italy, 35020 Due Carrare (PD)	3	0.300	500	BBCH 85	Bunch of grapes	7	0.09	0.09
Grape (Raboso Piave)					Bunch of grapes	14	0.06	0.06
2009					Berries	14	0.10	0.11
					Bunch of grapes	21	0.05	0.05
					Bunch of grapes	35	<0.05	<0.05
					Berries	35	<0.05	<0.05
CA 6.3.1/24 (09-2036)	1	0.300	500	BBCH 79	Bunch of grapes	-0	<0.05	0.06
09-2036-04	2	0.300	500	BBCH 79	Bunch of grapes	0	0.19	0.14
Spain, 11405 Jerez de La Frontera	3	0.300	500	BBCH 81	Bunch of grapes	14	0.05	0.10
Grape (Palomino)					Berries	14	0.05	0.09
2009					Bunch of grapes	34	<0.05	<0.05
					Berries	34	<0.05	0.06

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine (parent compound)[1]	Total residues of spiroxamine

1 – Underlined value to be used to support MRL for table grapes; Double underlined value to be used to support MRL for wine grapes
2 – Higher value from later PHI used

Table CA 6.3.1/24-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/24	01089	Spiroxamine (parent compound)	Grapes (berries)	0.05 2.5 Overall	3 1 4	95; 81; 95 98 Mean: 92, RSD: 8.3
			Grapes (bunches)	0.05 2.5 Overall	3 3 6	76; 96; 98 99; 107; 97 Mean: 96, RSD: 10.8
CA 6.3.1/24	01089	Total residues of spiroxamine	Grapes (berries)	0.027 1.35 Overall	3 1 4	95; 95; 93 94 Mean: 94 RSD: 1.0
			Grapes (bunches)	0.027 1.35 Overall	3 2 5	86; 85; 88 89; 92 Mean: 88, RSD: 3.1

Table CA 6.3.1/24-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/24	Grapes	280 to 343 (spiroxamine and total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2009 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after application of spiroxamine 500 EC to vines at 14 days pre-harvest (table grapes) and 34/35 days pre-harvest (wine grapes).

Two trials were conducted as harvest trials and the two remaining trials as residue decline trials. Residues of spiroxamine (parent compound) in grapes ranged from 0.19 to 0.35 mg/kg, 0 DALA, declining to 0.05 to 0.15 mg/kg, 14-21 DALA and declining to <0.05 to 0.11 mg/kg, 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes ranged from 0.14 to 0.28 mg/kg, 0 DALA, declining to between <0.05 to 0.21 mg/kg, 35 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (parent compound) in grapes ranged from 0.19 to 0.35 mg/kg, 0 DALA, declining to 0.05 to 0.15 mg/kg, 14-21 DALA and declining to <0.05 to 0.11 mg/kg, 35 DALA.

Residues of total spiroxamine (as aminodiol) in grapes ranged from 0.14 to 0.28 mg/kg, 0 DALA, declining to between <0.05 to 0.21 mg/kg, 35 DALA..

Data Point:	KCA 6.3.1/01
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of KWG 4168 in/on grape after low-volume spraying and spraying of KWG 4168 (500 EC) in the field in Southern France, Italy, Spain and Greece
Report No:	RA-2650/07
Document No:	M-301988-01-1
Guideline(s) followed in study:	91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2007 are available to evaluate the magnitude of residues of spiroxamine in/on grapes after three spray applications of Spiroxamine 500 EC at 0.8 L/ha in southern Europe. The designated PHI was 14 days (PHI for table grapes) with additional sampling at 34/35 days (PHI for wine grapes). Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach determined as aminodiol and expressed as spiroxamine equivalents).

Four residue trials on grapes were conducted in southern Europe (southern France, Italy, Spain and Greece). Two trials were conducted as residue decline trials and the remaining trials were conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.1/01-1 where values considered for MRL purposes or risk assessment are underlined.

Three spray applications of Spiroxamine 500 EC were made at a product rate of 0.8 L/ha corresponding to 400 g a.s./ha. Water rates were 200 l/ha in France (low-volume spraying), and 1000 L/ha in the other countries. Spray intervals were 9-11 days, with the final treatment being made at growth stage BBCH 81-85. It can be seen that the additional treatments in these trials, which always were made early in the season, have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes. However, as an application rate of 0.400 kg a.s./ha was used, residues are therefore scaled proportionally to a 0.300 kg a.s./ha application rate in line with the critical GAP.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 501.0 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior to and subsequent to the application) in addition to several points thereafter, including at 14 (PHI for table grapes) and 35 days (PHI for wine grapes).

The samples from the trials were analysed both for spiroxamine parent compound and as the total spiroxamine residue (common moiety approach determined as aminodiol and expressed as spiroxamine equivalents). The LOQ for both analytes was 0.05 mg/kg.

Residue analysis of samples of grapes were conducted using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2/1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen after sampling at ≤18°C. As shown in Table CA 6.3.1/01-3, the maximum storage time between date of deep-freezing and date of last extraction was 259 days.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/01-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

The residues detected in the control specimens were all below the LOQ (0.05 mg/kg) with one exception of 0.17 mg/kg total residues of spiroxamine in one sample from trial R 2007 0703/7 in Spain. No explanation was given in the report for this finding, however it is not expected to have an impact on the validity of the study or for MRL purposes as spiroxamine data are used.

Two of the trials were conducted as residue decline trials and two trials were conducted as harvest trials, with grapes sampled at a 14 day PHI representing table grapes and at 35 day PHI representing wine grapes.

Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.27 to 1.13 mg/kg, 0 DALA, declining to 0.15 to 0.23 mg/kg, 13/14 DALA and declining to <0.05 to 0.11 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.14 to 0.28 mg/kg, 0 DALA, declining to between 0.07 to 0.15 mg/kg, 35 DALA.

Table CA 6.3.1/01-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/01 (RA-2650/07) R 2007 0675/8 Southern France, Morancé (Rhone-Alpes), F-69480 Grape (Gamay à jus blanc) 2007	1 2 3	0.400 0.400 0.400	200 200 200	BBCH 81 BBCH 85 BBCH 85	Grapes Grapes Grapes Grapes Grapes Grapes	-0 0 7 14 21 35	0.13 0.36 0.24 0.21 0.18 0.17	0.10 0.27 0.18 <u>0.16</u> 0.14 <u>0.13</u>	0.20 0.41 0.33 0.30 0.30 0.27	0.15 0.31 0.25 0.23 0.23 0.20
CA 6.3.1/01 (RA-2650/07) R 2007 0676/6 Italy, Andria, (BA) (Puglia), I-70031 Grape (Montepulciano) 2007	1 2 3	0.400 0.400 0.400	1000 1000 1000	BBCH 79 BBCH 81 BBCH 83	Grapes Grapes Grapes Grapes	-0 0 14 35	0.22 0.80 0.20 0.20	0.17 0.60 <u>0.15</u> <u>0.15</u>	0.33 0.90 0.33 0.31	0.25 0.68 0.25 0.23
CA 6.3.1/01 (RA-2650/07) R 2007 0703/7 Spain, La Fortesa, Barcelona, Catalonia, E-08784 Grape(Macabeo) 2007	1 2 3	0.400 0.400 0.400	1000 1000 1000	BBCH 77 BBCH 79 BBCH 81	Grapes Grapes Grapes Grapes Grapes Grapes	-0 0 7 14 21 35	0.17 0.65 0.32 0.24 0.16 0.09	0.13 0.49 0.24 <u>0.18</u> 0.12 <u>0.07</u>	0.28 0.68 0.48 0.46 0.37 0.32	0.21 0.51 0.36 0.35 0.28 0.24
CA 6.3.1/01 (RA-2650/07) R 2007 0704/5 Greece, Piperia-Aridea, Macedonia, GR-58400 Grape (Chardonnay) 2007	1 2 3	0.400 0.400 0.400	1000 1000 1000	BBCH 75 BBCH 77 BBCH 81	Grapes Grapes Grapes Grapes	-0 0 13 35	0.28 1.50 0.31 0.14	0.21 1.13 <u>0.23</u> <u>0.11</u>	0.43 1.70 0.47 0.29	0.32 1.28 0.35 0.22

1 - Residues scaled proportionally to a 0.300 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - Underlined value to be used to support MRL for table grapes; Double underlined value to be used to support MRL for wine grapes

Table CA 6.3.1/01-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/01	01089	Spiroxamine (parent compound)	Grapes	0.05 0.5 5.0 Overall	5 6 2 13	89; 86; 82; 86; 85 86; 95; 95; 93; 88; 95 99; 104 Mean: 91, RSD: 7.0
CA 6.3.1/01	01089	Total residues of spiroxamine	Grapes	0.05 0.5 5.0 Overall	5 6 2 13	93; 97; 108; 99; 109 104; 95; 100; 101; 96; 102 92; 100 Mean: 100, RSD: 5.3

Table CA 6.3.1/01-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/01	Grapes	197 to 259

III. Conclusions

A total of four residue trials conducted in 2007 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after application of Spiroxamine 500 g/L EC formulation to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

Two of the trials were conducted as residue decline trials and two trials were conducted as harvest trials.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.27 to 1.13 mg/kg, 0 DALA, declining to 0.15 to 0.23 mg/kg, 13/14 DALA and declining to <0.05 to 0.11 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.14 to 0.28 mg/kg, 0 DALA, declining to between 0.07 to 0.15 mg/kg, 35 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/11. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.27 to 1.13 mg/kg, 0 DALA, declining to 0.15 to 0.23 mg/kg, 13/14 DALA and declining to <0.05 to 0.11 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.14 to 0.28 mg/kg, 0 DALA, declining to between 0.07 to 0.15 mg/kg, 35 DALA.

Data Point:	KCA 6.3.1/02
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of KWG 4168 in/on grape after low-volume spraying and spraying of KWG 4168 (500 EC) in the field in Northern France and Germany
Report No:	RA-2649/07
Document No:	M-301984-01-1
Guideline(s) followed in study:	91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2007 are available to evaluate the magnitude of residues of spiroxamine in/on grapes after three spray applications of Spiroxamine 500 EC at 0.8 L/ha in northern Europe. The designated PHI was 35 days (PHI for wine grapes) with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for data from NEU. Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach determined as aminodiol and expressed as spiroxamine equivalents).

Four trials on grapes were conducted in northern Europe (northern France and Germany). Two trials were conducted as residue decline trials and the remaining trials were conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.1/02-1 where values considered for MRL purposes or risk assessment are underlined.

In the northern France trials, three spray applications of spiroxamine were made at a nominal product rate of 0.8 L/ha corresponding to 400 g a.s./ha. The trial in Germany was conducted at 0.8 L/ha per m crop height which, based on a vine height of 1.6 m, resulted in a final 2-dimensional application rate of 1.28 L/ha corresponding to 640 g a.s./ha. The water rates in France ranged from 150 L/ha to 200 L/ha (low-volume spraying), and 1600 L/ha in Germany. Spray intervals were 10-11 days, with the final treatment being made at growth stage BBCH 85. It can be seen that the additional treatments in these trials, which always were made early in the season, have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes. However, as an application rate of ≥0.400 kg a.s./ha was used, residues are therefore scaled proportionally to a 0.300 kg a.s/ha application rate in line with the critical GAP.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 501.0 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior to and subsequent to the application) in addition to several points thereafter, including at 35 days (PHI for wine grapes).

The samples from the trials were analysed both for spiroxamine parent compound and as the total spiroxamine residue (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. The LOQ for both analytes was 0.05 mg/kg.

Residue analysis of samples of grapes were conducted using the validated analytical method no. 01089, report reference M-304677-01-1 **(see Doc MCA Section 4)**.

Samples were extracted with 30 mL of acetone/water (2/1, v/v), and after filtration and adjustment of total volume, samples were analysed for spiroxamine by mixing an aliquot with a known amount of ISTD standard (spiroxamine d7, 0.01 mg/L) and with methanol/water (2/8, v/v) prior to determination using LC-MS/MS analysis with a C18 HPLC column.

For the determination of the total residue of spiroxamine as aminodiol, an aliquot of the same sample extract was hydrolysed to aminodiol under acid conditions by refluxing with 1M HCl. After rinsing and neutralising with ammonia solution, total spiroxamine was determined as aminodiol using LC-MS/MS analysis with a Hypercarb HPLC column.

The samples were stored deep frozen within 24 hours of sampling at ≤ -18°C. As shown in Table CA 6.3.1/02-3, the maximum storage time between date of deep-freezing and date of last extraction was 235 days.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/02-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

The residues detected in the control specimens were all below the LOQ (0.05 mg/kg).

Two of the trials were conducted as residue decline trials and two trials were conducted as harvest trials, with grapes sampled at a 35 day PHI representing wine grapes.

Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.29 to 0.80 mg/kg, 0 DALA, declining to 0.05 to 0.47 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.32 to 0.89 mg/kg, 0 DALA, declining to 0.26 to 0.66 mg/kg, 35 DALA.

Table CA 6.3.1/02-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for Northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1]	Reported residue	Scaled residue[1]
CA 6.3.1/02 (RA-2649/07) R 2007 0672/3 Northern France, Charnay les Macon (Bourgogne), F-71850 Grape (Pinot noir) 2007	1 2 3	0.400 0.400 0.400	200 200 200	BBCH 81 BBCH 85 BBCH 85	Grapes Grapes Grapes Grapes Grapes Grapes	-0 0 7 14 20 35	0.19 0.38 0.28 0.21 0.23 0.15	0.14 0.29 0.21 0.16 0.17 0.11	0.34 0.43 0.41 0.36 0.42 0.40	0.26 0.32 0.31 0.27 0.32 0.30
CA 6.3.1/02 (RA-2649/07) R 2007 0673/1 Northern France, Saint Nicolas de Bourgueil (Centre), F-37140 Grape (Cabernet franc) 2007	1 2 3	0.400 0.400 0.400	150 150 150	BBCH 81 BBCH 85 BBCH 85	Grapes Grapes Grapes Grapes	-0 0 14 35	0.10 0.84 0.22 0.12	0.08 0.63 0.17 0.09	0.24 0.69 0.36 0.34	0.18 0.52 0.27 0.26
CA 6.3.1/02 (RA-2649/07) R 2007 0701/0 Northern France, Athée sur Cher (Centre), F-37270 Grape (Gamey) 2007	1 2 3	0.400 0.400 0.376	200 200 188	BBCH 81 BBCH 85 BBCH 85	Grapes Grapes Grapes Grapes Grapes Grapes	-0 0 7 14 21 35	0.08 0.49 0.12 0.10 0.07 0.06	0.06 0.39 0.10 0.08 0.06 0.05	0.23 0.55 0.24 0.26 0.26 0.29	0.18 0.44 0.19 0.21 0.21 0.23
CA 6.3.1/02 (RA-2649/07) R 2007 0702/9 Germany, Diedesfeld (Rheinland-Pfalz), D-67434 Grape (Dornfelder) 2007	1 2 3	0.640 0.640 0.640	1600 1600 1600	BBCH 85 BBCH 85 BBCH 85	Grapes Grapes Grapes Grapes	-0 0 14 35	0.78 1.70 1.10 1.00	0.37 0.80 0.52 0.47	1.00 1.90 1.40 1.40	0.47 0.89 0.66 0.66

1 – Residues scaled proportionally to a 0.300 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503

Table CA 6.3.1/02-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/02	01089	Spiroxamine (parent compound)	Grapes	0.05 0.5 5.0 Overall	5 6 2 13	89; 86; 82; 86; 85 86; 95; 95; 93; 88; 95 99; 104 Mean: 91; RSD: 7.0
CA 6.3.1/02	01089	Total residues of spiroxamine	Grapes	0.05 0.5 5.0 Overall	5 6 2 13	93; 97; 108; 99; 109 104; 95; 100; 101; 90; 102 92; 100 Mean: 100, RSD: 5.3

Table CA 6.3.1/02-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/02	Grapes	190 to 235

III. Conclusions

A total of four residue trials conducted in 2007 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after application of Spiroxamine 500 g/L EC formulation to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

Two of the trials were conducted as residue decline trials and two trials were conducted as harvest trials. Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.29 to 0.80 mg/kg, 0 DALA, declining to 0.05 to 0.47 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.32 to 0.89 mg/kg, 0 DALA, declining to 0.26 to 0.66 mg/kg, 35 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/06. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grapes ranged from 0.29 to 0.80 mg/kg, 0 DALA, declining to 0.05 to 0.47 mg/kg, 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes ranged from 0.32 to 0.89 mg/kg, 0 DALA, declining to 0.26 to 0.66 mg/kg, 35 DALA.

Data Point:	KCA 6.3.1/03
Report Author:	[redacted]
Report Year:	2001
Report Title:	Determination of residues of Spiroxamine in/on grapes after spray application of KWG 4168 300 CS and KWG 4168 500 EC to grape vines in the field in Italy and Spain
Report No:	RA-2142/00
Document No:	M-083248-01-1
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Two trials conducted during growing season 2000 are available to evaluate the magnitude of residues of spiroxamine in/on grape bunches and berries after three spray applications of either Spiroxamine 500 EC or Spiroxamine 300 CS to grapevines in Spain and Italy. The designated PHI was 14 days (PHI for table grapes) and 35 days (PHI for wine grapes). Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents).

All trials were conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.1/03-1 where values considered for MRL purposes or risk assessment are underlined.

In trials R 2000 0194/0 and R 2000 0195/9, spiroxamine in the form of an emulsifiable concentrate containing 500 g/L a.s., was sprayed three times with a product rate of 0.8 L/ha and a spray volume of 1000 L/ha, corresponding to a product concentration of 0.08%. The rate of a.s. spiroxamine was 0.400 kg/ha. The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 485.9 g/L spiroxamine).

In trials R 2000 0193/2 and R 2000 0225/4 conducted for comparison purposes, spiroxamine, in the form of a capsule suspension containing 300 g/L a.s. spiroxamine, was applied three times to grapes with a product rate of 1.3 L/ha and a spray volume of 1000 L water/ha, corresponding to a product concentration of 0.13%. The rate of a.s. spiroxamine was 0.390 kg/ha. The trials were conducted using a 300 g/L CS formulation of spiroxamine (actual content 294.5 g/L spiroxamine). The 300 CS trials data are summarised here but are not used for MRL purposes in this renewal dossier.

It can be seen that the additional treatments in these trials have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes. However, as an application rate of 0.400 kg a.s./ha was used, residues are therefore scaled proportionally to a 0.300 kg a.s/ha application rate in line with the critical GAP.

Samples of grapes (bunches) were taken on day 0, 14 (PHI for tables grapes) and 33-35 (PHI for wine grapes) after the last application. Samples of grape berries were taken at day 14 and 33-35 after the last application.

According to the study protocol, the first treatment had to be performed at day 34 before harvest and at a growth stage of ca. BBCH 55. However, since it was impossible to combine these two requirements,

in all trials the first application was conducted at later growth stages (BBCH 81-85). The remaining applications were conducted at intervals of 9 or 10 days, with the last treatment being performed 14 days prior to harvest (PHI for table grapes).

The samples from the trials were analysed both for spiroxamine parent compound and as the total spiroxamine residue (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residue of spiroxamine parent were conducted using the validated analytical method no. 00506, report reference M-020658-01-1 **(see Doc MCA Section 4)** and the total residues of spiroxamine were analysed using analytical method no. 00407, report reference M-019430-01-1 **(see Doc MCA Section 4)**.

The LOQ for both analytes was 0.05 mg/kg.

Spiroxamine

For the analysis of spiroxamine, the samples were extracted with acetone/water (3/1, v/v). After filtration and rinsing an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on an RP-18 column using two different eluent systems; a water/methanol mixture and after column drying, an n-hexane/ethyl acetate mixture. Any spiroxamine was eluted from the column with ammonia in methanol. The residues were determined by EI-GC/MS in the single-ion-monitoring mode.

Total spiroxamine (common moiety method)

For the analysis of total residues of spiroxamine, the samples were extracted with acetone/water (3.5/1, v/v). After filtration and rinsing with the aid of Celite, an aliquot of the extract was taken, acidified with 1M HCL and refluxed. Spiroxamine and metabolites with the N-ethyl-N-propyl-1,2-dihydroxy-3-aminopropane structure was hydrolysed to a common moiety (aminodiol). The residues were concentrated to the aqueous remainder and co-extractives were removed by partition with dichloromethane followed by ethyl acetate. The remaining aqueous layer was cleaned up by chromatography separation on a polystyrenedivinylbenzene column. The residues were determined after silylation with GC/MS in the single-ion-monitoring mode.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/03-3, the maximum storage time between date of deep-freezing and date of last extraction of spiroxamine parent compound was 266 days and spiroxamine total residues was 284 days.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/03-2) with the exception of one result of 116% for spiroxamine. The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

The trials were conducted as harvest trials with bunches of grapes and berries sampled at 14 day PHI representing table grapes and 35 day PHI representing wine grapes

Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Following treatment with Spiroxamine 500 EC, scaled residues of spiroxamine (parent compound) in grapes were 0.34 and 0.42 mg/kg, 0 DALA, declining to 0.10 and 0.14 mg/kg, 14 DALA and <0.05 and 0.07 mg/kg, 33/35 DALA. Residues in berries were slightly lower than those reported in bunches.

Following treatment with Spiroxamine 500 EC, scaled residues of total spiroxamine (as aminodiol) in grapes were not determined 0 DALA but were reported as 0.12 and 0.28 mg/kg, 14 DALA and 0.11 and 0.20 mg/kg, 33/35 DALA. Residues in berries and grapes were similar.

Table CA 6.3.1/03-1 Residue trials with spiroxamine 500 g/L EC and 300 g/L CS in grapes – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha[3]	L/ha	Growth stage			Reported residue	Scaled residue[1]	Reported residue	Scaled residue[1]
CA 6.3.1/03 (RA-2142/00)	1	0.400	1000	BBCH 85	Grapes bunches	0	0.556	0.42	-	-
R 2000 0194/0	2	0.400	1000	BBCH 87	Grapes bunches	14	0.186	<u>0.14</u>	0.16	0.12
Italy, Trinitapoli, (Barletta-Andria), I-71049	3	0.400 [500 EC]	1000	BBCH 87	Grapes bunches	35	0.098	<u>0.07</u>	0.12	0.09
Grape (Montepulciano)					Berries	14	0.159	0.12	0.15	0.11
2000					Berries	35	0.067	0.05	0.13	0.10
CA 6.3.1/03 (RA-2142/00)	1	0.390	1000	BBCH 85	Grapes bunches	0	0.393	0.30	-	-
R 2000 0193/2	2	0.390	1000	BBCH 87	Grapes bunches	14	0.125	0.10	0.195	0.15
Italy, Trinitapoli, (Barletta-Andria), I-71049	3	0.390 [300 CS]	1000	BBCH 87	Grapes bunches	35	0.073	0.06	0.160	0.12
Grape (Montepulciano)					Berries	14	0.179	0.14	0.188	0.14
2000					Berries	35	<0.05	<0.05	0.078	0.06
CA 6.3.1/03 (RA-2142/00)	1	0.400	1000	BBCH 81	Grapes bunches	0	0.427	0.34	-	-
R 2000 0195/9	2	0.400	1000	BBCH 83	Grapes bunches	14	0.130	<u>0.10</u>	0.345	0.28
Spain, La Fortesa (Barcelona), E 08784	3	0.374 [500 EC]	934	BBCH 85	Grapes bunches	33	<0.05	<0.05	0.205	0.16
Grape (Xarelo)					Berries	14	0.110	0.09	0.272	0.22
2000					Berries	33	0.056	<u><0.05</u>	0.255	0.20
CA 6.3.1/03 (RA-2142/00)	1	0.390	1000	BBCH 81	Grapes bunches	0	0.196	0.15	-	-
R 2000 0225/4	2	0.390	1000	BBCH 83	Grapes bunches	14	0.087[2]	0.07	0.179	0.14
Spain, La Fortesa (Barcelona), E 08784	3	0.390 [300 CS]	1000	BBCH 85	Grapes bunches	33	<0.05	<0.05	0.116	0.09
Grape (Xarelo)					Berries	14	0.063	0.05	0.160	0.12
2000					Berries	33	0.050	<0.05	0.085	0.07

1 – Residues scaled proportionally to a 0.300 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 – <u>Underlined</u> value to be used to support MRL for table grapes; <u><u>Double underlined</u></u> value to be used to support MRL for wine grapes
3 – Only data from trials using Spiroxamine 500 EC are considered for MRL purposes

Table CA 6.3.1/03-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/03	00506	Spiroxamine (parent compound)	Grapes bunches/berries	0.05 0.5 Overall	3 5 8	96, 89, 94 80, 83, 80, 90, 116 Mean: 91, RSD: 12.9
CA 6.3.1/03	00407	Total residues of spiroxamine	Grapes bunches/berries	0.05 0.5 Overall	3 4 7	93, 100, 103 106, 107, 104, 86 Mean: 100, RSD: 7.7

Table CA 6.3.1/03-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/03	Grapes	212 to 266 (spiroxamine parent) 234 to 284 (spiroxamine total residues)

III. Conclusions

Two residue trials conducted in 2000 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in/on grape bunches and berries after application of spiroxamine 500 g/L EC formulation to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

The trials were conducted as harvest trials with bunch of grapes and berries sampled at 14 day PHI for table grapes and 35 day PHI for wine grapes. Following treatment with Spiroxamine 500 EC, scaled residues of spiroxamine (parent compound) in grapes were 0.34 and 0.42 mg/kg, 0 DALA, declining to 0.10 and 0.14 mg/kg, 14 DALA and <0.05 and 0.07 mg/kg, 33/35 DALA. Residues in berries were slightly lower than those reported in bunches.

Following treatment with Spiroxamine 500 EC, scaled residues of total spiroxamine (as aminodiol) in grapes were not determined 0 DALA but were reported as 0.12 and 0.28 mg/kg, 14 DALA and 0.11 and 0.20 mg/kg, 33/35 DALA. Residues in berries and grapes were similar.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/10. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Following treatment with Spiroxamine 500 EC, scaled residues of spiroxamine (parent compound) in grapes were 0.34 and 0.42 mg/kg, 0 DALA, declining to 0.10 and 0.14 mg/kg, 14 DALA and <0.05 and 0.07 mg/kg, 33/35 DALA. Residues in berries were slightly lower than those reported in bunches.

Following treatment with Spiroxamine 500 EC, scaled residues of total spiroxamine (as aminodiol) in grapes were not determined 0 DALA but were reported as 0.12 and 0.28 mg/kg, 14 DALA and 0.11 and 0.20 mg/kg, 33/35 DALA. Residues in berries and grapes were similar.

Data Point:	KCA 6.3.1/04
Report Author:	[redacted]; [redacted]
Report Year:	2000
Report Title:	Determination of residues of KWG 4168 & Quinoxyfen 480 SE (A.S.: spiroxamine, Quinoxyfen) in/on grape in the field following spray application in France and Germany
Report No:	RA-2169/98
Document No:	M-024140-01-1
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Six trials conducted during growing season 1998 are available to evaluate the magnitude of residues of spiroxamine in/on grapes after either four or six foliar spray applications of spiroxamine and quinoxyfen 480 SE at rates between 0.2 and 0.8 L/ha in northern Europe. The designated PHI was 35 days (PHI for wine grapes). Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residues of quinoxyfen were also analysed, however will not reported in this summary.

Three independent trials on grapes were conducted in northern Europe (Germany and northern France). The additional three trials were performed at the same trial site/location in Germany. Therefore, despite using different varieties, the replicates cannot be considered independent and the most critical residue value between these four trials (in Germany) is used for MRL purposes. All trials were conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.1/04-1 where values considered for MRL purposes or risk assessment are underlined.

Four spray applications of spiroxamine were made in Germany at a product application rate of 0.8 L/ha corresponding to 320 g a.s./ha with water application rates of 1600 L/ha.

Six spray applications of spiroxamine were made in France at product application rates between 0.2 to 0.4 L/ha corresponding to 80 to 160 g a.s./ha, respectively, with water application rates of 400 to 800 L/ha. Depending on plant height, the product was sprayed four times post-blossom with rates of 0.45 to 0.55 L/ha and spray volumes of 900 to 1100 L/ha, corresponding to 180 to 220 g/ha spiroxamine.

Spray intervals were 13-15 days, with the final treatment being made at growth stage BBCH 81.

It can be seen that the additional treatments in these trials, which always were made early in the season, have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes. However, as application rates of below and above the critical GAP were used, residues are therefore scaled proportionally to a 0.300 kg a.s./ha application rate in line with the critical GAP.

The trials were conducted using a spiroxamine and quinoxyfen 480 SE formulation containing 400 g/L of spiroxamine (actual content 398.9 g/L spiroxamine).

Samples of grapes (bunches) were taken on day 0 and day 35 (PHI for wine grapes), after the last application. At day 35, grape bunches were taken and divided into subsamples in order to obtain berry

samples. Control samples were taken on the day of final treatment day (prior to the last treatment) and 35 days after the last application (DALA).

The samples from the trials were analysed both for spiroxamine parent compound and as the total spiroxamine residue (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residue of spiroxamine parent were conducted using the validated analytical method no. 00506, report reference M-020658-01-1 **(see Doc MCA Section 4)** and the total residues of spiroxamine were analysed using analytical method no. 00407, report reference M-019430-01-1 **(see Doc MCA Section 4)**. The LOQ for both analytes was 0.05 mg/kg.

Spiroxamine

For the analysis of spiroxamine, the samples were extracted with acetone/water (3/1, v/v). After filtration and rinsing an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on an RP-18 column using two different eluent systems; a water/methanol mixture and after column drying, an n-hexane/ethyl acetate mixture. Any spiroxamine was eluted from the column with ammonia in methanol. The residues were determined by EI-GC/MS in the single-ion-monitoring mode.

Total spiroxamine (common moiety method)

For the analysis of total residues of spiroxamine, the samples were extracted with acetone/water (3.5/1, v/v). After filtration and rinsing with the aid of Celite, an aliquot of the extract was taken, acidified with 1M HCL and refluxed. Spiroxamine and metabolites with the N-ethyl-N-propyl-1,2-dihydroxy-3-aminopropane structure was hydrolysed to a common moiety (aminodiol). The residues were concentrated to the aqueous remainder and co-extractives were removed by partition with dichloromethane followed by ethyl acetate. The remaining aqueous layer was cleaned up by chromatography separation on a polystyrene/divinylbenzene column. The residues were determined after silylation with GC/MS in the single-ion-monitoring mode.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/04-3, the maximum storage time between date of deep-freezing and date of last extraction was for a maximum of 360 days for spiroxamine parent and 334 days for total spiroxamine residues.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/04-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

The residues detected in the control samples were all below the LOQ (0.05 mg/kg).

Scaled residues of spiroxamine (parent compound) in grapes were between 0.34 and 0.71 mg/kg from all trials, 0 DALA. For the three independent trials, residues had decline to between 0.07 and 0.19 mg/kg 35 DALA. Residues in berries were slightly lower than those reported in bunches.

Scaled residues of total spiroxamine (as aminodiol) in grapes were not determined 0 DALA but were reported between 0.12 and 0.38 mg/kg 35 DALA from the three independent trials. Residues in berries and grapes were similar, tending to be slightly lower in berries.

Table CA 6.3.1/04-1 Residue trials with spiroxamine and quinoxyfen 480 SE in grapes – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/04 (RA-2169/98) 816531 Germany (D-55234 Albig) Grape (Portugeiser) 1998	1 2 3 4	0.320 0.320 0.320 0.320	1600 1600 1600 1600	BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grapes bunches Grapes bunches Berries	0 35 35	0.362 0.097 0.067	0.34 0.09 0.06	- 0.205 0.162	- 0.19 0.15
CA 6.3.1/04 (RA-2169/98) 816558 Germany (D-55234 Albig) Grape (Mueller-Thurgau) 1998	1 2 3 4	0.320 0.320 0.320 0.320	1600 1600 1600 1600	BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grapes bunches Grapes bunches Berries	0 35 35	0.688 0.199 0.174	0.65 0.19 0.16	- 0.474 0.406	- 0.44 0.38
CA 6.3.1/04 (RA-2169/98) 816566 Germany (D-55234 Albig) Grape (Portugieser) 1998	1 2 3 4 5 6	0.080 0.160 0.320 0.320 0.320 0.320	400 800 1600 1600 1600 1600	BBCH 55-57 BBCH 61 BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grapes bunches Grapes bunches Berries	0 35 35	0.403 0.127 0.083	0.38 0.12 0.08	- 0.270 0.219	- 0.25 0.21
CA 6.3.1/04 (RA-2169/98) 816574 Germany (D-55234 Albig) Grape (Mueller-Thurgau) 1998	1 2 3 4 5 6	0.080 0.160 0.320 0.320 0.320 0.320	400 800 1600 1600 1600 1600	BBCH 55-57 BBCH 61 BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grapes bunches Grapes bunches Berries	0 35 35	0.752 0.168 0.135	0.71 0.16 0.13	- 0.372 0.401	- 0.35 0.38
CA 6.3.1/04 (RA-2169/98) 816582 France (F-37420 Beaumont en Veron) Grape (Cabernet Franc) 1998	1 2 3 4 5 6	0.080 0.160 0.220 0.220 0.220 0.220	400 800 1100 1100 1100 1100	BBCH 55 BBCH 65 BBCH 75 BBCH 77 BBCH 79 BBCH 81	Grapes bunches Grapes bunches Berries	0 35 35	0.239 0.050 <0.050	0.33 0.07 n.a.[3]	- 0.123 0.110	- 0.17 0.15

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine (parent compound)		Total residues of spiroxamine	
							Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/04 (RA-2169/98)	1	0.080	400	BBCH 55	Grapes bunches	0	0.155	0.23	-	-
816590	2	0.160	800	BBCH 61	Grapes bunches	35	0.057	0.09	0.098	0.15
France (F-37210 Rochecorbon “La	3	0.180	900	BBCH 75						
Valiniere")	4	0.200	1000	BBCH 79	Berries	35	0.050	0.08	0.081	0.12
Grape (Chenin)	5	0.180	900	BBCH 79						
1998	6	0.200	1000	BBCH 81						

1 - Residues scaled proportionally to a 0.300 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503. Scaling factors were derived from the last single application rate and not from the seasonal application rate.
2 – Underlined value to be used to support MRL for wine grapes
3 – Value not appropriate to scale up as reported value below the LOQ (<0.05 mg/kg).

Table CA 6.3.1/04-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/04	00506	Spiroxamine (parent compound)	Grapes	0.05 0.5 Overall	3 7 12	99; 102; 73 101; 99; 91; 81; 77; 106; 94; 95; 94 Mean: 93, RSD: 11.2
CA 6.3.1/04	00407	Total residues of spiroxamine	Grapes	0.05 0.5 Overall	4 5 9	98; 93; 102; 109 99; 106; 108; 103; 107 Mean: 103, RSD: 5.2

Table CA 6.3.1/04-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/04	Grapes	309 to 360 (spiroxamine parent) 317 to 334 (spiroxamine total residues)

III. Conclusions

A total of three independent residue harvest trials conducted in 1998 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after application of spiroxamine and quinoxyfen 480 SE formulation to vines at 35 days pre-harvest (wine grapes).

All three trials were conducted as harvest trials, with grape bunches and berries sampled at day 35 (PHI for wine grapes), after the last application. At day 35, grape bunches were taken and divided into subsamples in order to obtain berry samples. Scaled residues of spiroxamine (parent compound) in grapes were between 0.34 and 0.71 mg/kg from all trials, 0 DALA. For the three independent trials, residues had decline to between 0.07 and 0.19 mg/kg 35 DALA. Residues in berries were slightly lower than those reported in bunches.

Scaled residues of total spiroxamine (as aminodiol) in grapes were not determined 0 DALA but were reported between 0.12 and 0.38 mg/kg 35 DALA from the three independent trials. Residues in berries and grapes were similar, tending to be slightly lower in berries.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/04. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grapes were between 0.34 and 0.71 mg/kg from all trials, 0 DALA. For the three independent trials, residues had decline to between 0.07 and 0.19 mg/kg 35 DALA. Residues in berries were slightly lower than those reported in bunches.

Scaled residues of total spiroxamine (as aminodiol) in grapes were not determined 0 DALA but were reported between 0.12 and 0.38 mg/kg 35 DALA from the three independent trials. Residues in berries and grapes were similar, tending to be slightly lower in berries.

Data Point:	KCA 6.3.1/05
Report Author:	[redacted]
Report Year:	1999
Report Title:	Determination of residues of KWG 4168 500 EC (A.S. spiroxamine) in on grape following spray application in France and Germany
Report No:	RA-2760/98
Document No:	M-015466-01-2
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing 1998 season are available to evaluate the magnitude of residues of spiroxamine in/on bunches of grapes and berries after six foliar spray applications of spiroxamine 500 EC in northern Europe. The designated PHI was 35 days (PHI for wine grapes). Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents.

Two independent trials on grapes were conducted in northern Europe (Germany and northern France). The additional two trials were performed at the same trial site locations in each country. Therefore, despite using different varieties, the replicates cannot be considered independent and the most critical residue values between these two trials (in Germany and France) are used for MRL purposes. All trials were conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.1/05-1 where values considered for MRL purposes or risk assessment are underlined.

In the northern France trials, six applications of Spiroxamine 500 EC were made at a product rate of 0.3 L/ha and a spray volume of 600 L/ha for the 1st and 2nd application and with a rate of 0.6 L/ha and a spray volume of 120 L/ha for the 3rd to 6th application, corresponding to 150 g a.s./ha for the 1st and 2nd application and 300 g a.s./ha for the 3rd to 6th applications. In the German trials, six spray applications of Spiroxamine 500 EC were made at the rate of 0.3 to 0.318 L/ha with a spray volume of 600 to 637 L/ha for the 1st and 2nd applications and 0.75 L/ha with a spray volume of 1500 L/ha for the

3rd to 6th applications corresponding to 150 to 159 g a.s./ha for the 1st and 2nd application and 375 g a.s./ha for the 3rd to 6th application. The applications were performed at growth stages BBCH 52 to 81 at intervals of 12 to 23 days with the last application performed 35 days before harvest.

It can be seen that the additional treatments in these trials, which always were made early in the season, have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes. However, as application rates of below and above the critical GAP were used, residues are therefore scaled proportionally to a 300 g a.s/ha application rate in line with the critical GAP.

The trials were conducted using a 500 g/L EC formulation of spiroxamine (actual content 510.5 g/L spiroxamine).

Samples of mature grape bunches (including controls) were taken at harvest, 35 days after last application (DALA, PHI for wine grapes).

The samples from the trials were analysed both for spiroxamine parent compound and as the total spiroxamine residue (common moiety approach determined as aminodiol and expressed as spiroxamine equivalents). Residue of spiroxamine parent were conducted using the validated analytical method no. 00506, report reference M-020658-01-1 (**see Doc MCA Section 4**) and the total residues of spiroxamine were analysed using validated method no. 00407, report reference M-019430-01-1 (**see Doc MCA Section 4**). The LOQ for both analytes was 0.05 mg/kg.

Spiroxamine

For the analysis of spiroxamine, the samples were extracted with acetone/water (3/1, v/v). After filtration and rinsing an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on an RP-18 column using two different eluent systems; a water/methanol mixture and after column drying, an n-hexane/ethyl acetate mixture. Any spiroxamine was eluted from the column with ammonia in methanol. The residues were determined by EI-GC/MS in the single-ion-monitoring mode.

Total spiroxamine (common moiety method)

For the analysis of total residues of spiroxamine, the samples were extracted with acetone/water (3.5/1, v/v). After filtration and rinsing with the aid of Celite, an aliquot of the extract was taken, acidified with 1M HCL and refluxed. Spiroxamine and metabolites with the N-ethyl-N-propyl-1,2-dihydroxy-3-aminopropane structure was hydrolysed to a common moiety (aminodiol). The residues were concentrated to the aqueous remainder and co-extractives were removed by partition with dichloromethane followed by ethyl acetate. The remaining aqueous layer was cleaned up by chromatography separation on a polystyrenedivinylbenzene column. The residues were determined after silylation with GC/MS in the single-ion-monitoring mode.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/05-3, the maximum storage time between date of deep-freezing and date of last extraction of spiroxamine parent compound was 149 days and spiroxamine total residues was 166 days.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/05-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

The residues detected in the control samples were all below the LOQ (0.05 mg/kg).

Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Scaled residues of spiroxamine (parent compound) in grapes were between 0.10 and 0.21 mg/kg from all trials, 35 DALA. For the two independent trials, residues were 0.12 and 0.21 mg/kg 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes were between 0.24 and 0.79 mg/kg from all trials, 35 DALA. For the two independent trials, residues were 0.79 and 0.39 mg/kg 35 DALA.

Residues in berries and grapes were similar, the highest value is used for MRL purposes.

Table CA 6.3.1/05-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1]	Reported residue	Scaled residue[1]
CA 6.3.1/05 (RA-2160/98) 811432 France, Uchizy, Bourgogne, F-71700 Grape (Pinot noir) 1998	1 2 3 4 5 6	0.150 0.150 0.300 0.300 0.300 0.300	600 600 120 120 120 120	BBCH 53 BBCH 56 BBCH 70 BBCH 74 BBCH 78 BBCH 79	Grape bunches Berries	35 35	0.10 0.10	n.a.	0.52 0.56	n.a.
CA 6.3.1/05 (RA-2160/98) 816329 France, Uchizy, Bourgogne, F-71700 Grape (Chardonnay) 1998	1 2 3 4 5 6	0.150 0.150 0.300 0.300 0.300 0.300	600 600 120 120 120 120	BBCH 52 BBCH 56 BBCH 71 BBCH 75 BBCH 77 BBCH 79	Grape bunches Berries	35 35	0.12 0.07	n.a.	0.79 0.52	n.a.
CA 6.3.1/05 (RA-2160/98) 816337 Germany, Rhineland-Palatinate, Albig, D-55234 Grape (Portugieser) 1998	1 2 3 4 5 6	0.150 0.150 0.375 0.375 0.375 0.375	600 600 1500 1500 1500 1500	BBCH 55-57 BBCH 61 BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grape bunches Berries	35 35	0.17 0.18	0.14 0.14	0.294 0.381	0.24 0.30
CA 6.3.1/05 (RA-2160/98) 816345 Germany, Rhineland-Palatinate, Albig, D-55234 Grape (Muller-Thurgau) 1998	1 2 3 4 5 6	0.150 0.159 0.375 0.375 0.375 0.375	600 637 1500 1500 1500 1500	BBCH 55 BBCH 61 BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grape bunches Berries	35 35	0.22 0.26	0.18 0.21	0.476 0.483	0.38 0.39

1 – Residues scaled proportionally to a 0.300 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503. Scaling factors were derived from the last single application rate and not from the seasonal application rate

Table CA 6.3.1/05-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/05	00506	Spiroxamine (parent compound)	Grapes	0.05 0.5 Overall	4 4 8	86, 71, 95, 99 84, 80, 98, 92 Mean: 88, RSD: 11.0
CA 6.3.1/05	00407	Total residues of spiroxamine	Grapes	0.05 0.5 Overall	4 4 8	97, 107, 96, 108 100, 98, 95, 99 Mean: 100, RSD: 4.9

Table CA 6.3.1/05-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/05	Grapes	149 to 166 (spiroxamine parent) 138 to 155 (spiroxamine total residues)

III. Conclusions

A total of two residue trials conducted in 1998 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after application of spiroxamine 500 g/L EC formulation to vines at 35 days pre-harvest (wine grapes).

The trials were conducted as harvest trials, with bunch of grapes and berries sampled at 35 day PHI for wine grapes. Scaled residues of spiroxamine (parent compound) in grapes were between 0.10 and 0.21 mg/kg from all trials, 35 DALA. For the two independent trials, residues were 0.12 and 0.21 mg/kg 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes were between 0.24 and 0.79 mg/kg from all trials, 35 DALA. For the two independent trials, residues were 0.79 and 0.39 mg/kg 35 DALA.

Residues in berries and grapes were similar, the highest value is used for MRL purposes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/05. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grapes were between 0.10 and 0.21 mg/kg from all trials, 35 DALA. For the two independent trials, residues were 0.12 and 0.21 mg/kg 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes were between 0.24 and 0.79 mg/kg from all trials, 35 DALA. For the two independent trials, residues were 0.79 and 0.39 mg/kg 35 DALA.

Residues in berries and grapes were similar, the highest value is used for MRL purposes.

Data Point:	KCA 6.3.1/06
Report Author:	[redacted]
Report Year:	1998
Report Title:	Determination of residues of KWG 4168 & HWG 1608 (500 EC) on grape in the field in Federal Republic of Germany and France
Report No:	RA-2168/97
Document No:	M-010796-01-1
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2015)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Six trials conducted during growing 1997 season are available to evaluate the magnitude of spiroxamine in/on grapes after four or six foliar spray applications of spiroxamine and tebuconazole 500 EC at rates between 0.2 and 1.27 L/ha in northern Europe. The designated PHI was 35 days (DHI for wine grapes) with sampling at 14 days PHI included (SEU table grapes PHI) but not considered for MRL purposes for data from NEU. Samples were analysed for both spiroxamine parent compound and as the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residues of tebuconazole were also analysed, however will not reported in this summary.

Two independent trials on grapes were conducted in northern Europe (Germany and northern France). The additional four trials were performed at the same trial site locations in Germany or France. Therefore, despite using different varieties, the replicates cannot be considered independent and the most critical residue value between these four trials in Germany and two trials in France are used for MRL purposes. All trials were conducted as harvest trials.

The trial parameters and residue results are summarised in Table CA 6.3.1/06-1 where values considered for MRL purposes or risk assessment are underlined.

Four or six spray applications of spiroxamine were made at an application concentration of 0.05% corresponding to between 80 to 320 g a.s./ha with a water rate of 400 to 1600 L/ha in Germany and 80 L/ha in France (low-volume spraying). For two trials in Germany (706590 and 707570) higher spray volumes of around 2500 L/ha were used due to a miscalculation, resulting in higher application rates of 490 to 520 g a.s./ha. The generally lower rates for initial applications were to early growth stages before fruit development and are not considered to have impacted on the magnitude of residue in mature grapes, therefore these trials can be considered as supporting the GAP.

It can be seen that the additional treatments in these trials, which always were made early in the season, have no significant effect on terminal residue levels taken after the final application. For this reason, all relevant residue trials summarised here are considered valid to support the representative use (unless noted otherwise) and are evaluated as part of a single data set for MRL purposes. However, as application rates of below and above the critical GAP were used, residues are therefore scaled proportionally to a 0.300 kg a.s/ha application rate in line with the critical GAP.

The trials were conducted using a 500 g/L EC co-formulation of spiroxamine and tebuconazole containing 400 g/L of spiroxamine (actual content 394.0 g/L spiroxamine).

Samples of grapes (bunches) were taken on the day of the final treatment (prior to and subsequent to the application) and at 35 days (PHI for wine grapes). Berries were also sampled 35 days after the last application (DALA). Control samples were taken on day of the final treatment, prior to the last treatment and 35 days after the last application.

The samples from the trials were analysed both for spiroxamine parent compound and as the total spiroxamine residue (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. Residue of spiroxamine parent were conducted using the validated analytical method no. 00506, report reference M-020658-01-1 **(see Doc MCA Section 4)** and the total residues of spiroxamine were analysed using analytical method no. 00407, report reference M-019430-01-1 **(see Doc MCA Section 4)**. The LOQ for both analytes was 0.05 mg/kg.

Spiroxamine

For the analysis of spiroxamine, the samples were extracted with acetone/water (3/1; v/v). After filtration and rinsing an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on an RP-18 column using two different eluent systems; a water/methanol mixture and after column drying, an n-hexane/ethyl acetate mixture. Any spiroxamine was eluted from the column with ammonia in methanol. The residues were determined by EI-GC/MS in the single-ion-monitoring mode.

Total spiroxamine (common moiety method)

For the analysis of total residues of spiroxamine, the samples were extracted with acetone/water (3.5/1, v/v). After filtration and rinsing with the aid of Celite, an aliquot of the extract was taken, acidified with 1M HCL and refluxed. Spiroxamine and metabolites with the N-ethyl-N-propyl-1,2-dihydroxy-3-aminopropane structure was hydrolysed to a common moiety (aminodiol). The residues were concentrated to the aqueous remainder and co-extractives were removed by partition with dichloromethane followed by ethyl acetate. The remaining aqueous layer was cleaned up by chromatography separation on a polystyrenedivinylbenzene column. The residues were determined after silylation with GC/MS in the single-ion-monitoring mode.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.1/06-3, the maximum storage time between date of deep-freezing and date of last extraction was 157 days for spiroxamine (parent compound) and 122 days for total spiroxamine.

This storage period is covered by the available storage stability data for high acid content commodities which demonstrate stability of spiroxamine for up to 720 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The recoveries from fortified samples analysed concurrently with treated samples were in the range of 70-110% (refer to Table CA 6.3.1/06-2). The limit of quantification (LOQ) for both spiroxamine (parent compound) and residues of total spiroxamine in grapes was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Scaled residues of spiroxamine (parent compound) in grapes were between 0.13 and 1.89 mg/kg, 0 DALA. For the two independent trials, residues had declined to 0.15 and 0.38 mg/kg 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes were between 0.18 and 1.06 mg/kg, 35 DALA. For the two independent trials, residues were 0.42 and 1.06 mg/kg 35 DALA.

Residues in berries and grapes were similar, the highest value is used for MRL purposes.

Table CA 6.3.1/06-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/06 (RA-2168/97)	1	0.080	400	BBCH 53	Bunch of grapes	-0	0.370	0.35	-	-
706582	2	0.160	800	BBCH 61-63	Bunch of grapes	0	0.882	0.83	-	-
Germany (Albig), D-55234	3	0.320	1600	BBCH 73-75	Bunch of grapes	14	0.409	0.38	0.502	0.47
Grape (Portugieser)	4	0.320	1600	BBCH 75	Bunch of grapes	35	0.332	0.31	0.486	0.46
1997	5	0.320	1600	BBCH 78						
	6	0.320	1600	BBCH 80	Berries	14	0.325	0.30	0.459	0.43
					Berries	35	0.190	0.18	0.415	0.39
CA 6.3.1/06 (RA-2168/97)	1	0.510	2528	BBCH 73-75	Bunch of grapes	-0	0.278	0.17	-	-
706590[3]	2	0.500	2518.4	BBCH 75	Bunch of grapes	0	0.813	0.50	-	-
Germany (Albig), D-55234	3	0.490	2464	BBCH 78	Bunch of grapes	14	0.367	0.22	0.581	0.36
Grape (Portugieser)	4	0.490	2433.6	BBCH 80	Bunch of grapes	35	0.200	0.12	0.355	0.22
1997										
					Berries	14	0.259	0.16	0.524	0.32
					Berries	35	0.154	0.09	0.407	0.25
CA 6.3.1/06 (RA-2168/97)	1	0.160	80	BBCH 75	Bunch of grapes	-0	<0.05	n.a.[4]	-	-
706604	2	0.160	80	BBCH 77	Bunch of grapes	0	0.067	0.13	-	-
France (Brinay), F-18120	3	0.160	80	BBCH 81	Bunch of grapes	14	<0.05	n.a.[4]	0.089	0.17
Grape (Sauvignon)	4	0.160	80	BBCH 83	Bunch of grapes	35	<0.05	n.a.[4]	0.096	0.18
1997										
					Berries	14	0.058	0.11	0.123	0.23
					Berries	35	<0.05	n.a.[4]	0.053	0.10
CA 6.3.1/06 (RA-2168/97)	1	0.160	80	BBCH 75	Bunch of grapes	--0	0.052	0.10	-	-
706612	2	0.150	75.25	BBCH 77	Bunch of grapes	0	0.206	0.39	-	-
France (Brinay), F-18120	3	0.160	80	BBCH 81	Bunch of grapes	14	0.077	0.14	0.213	0.40
Grape (Pinot Meunier)	4	0.160	80	BBCH 83	Bunch of grapes	35	0.073	0.14	0.226	0.42
1997										
					Berries	14	0.084	0.16	0.298	0.56
					Berries	35	0.079	0.15	0.189	0.35

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residues of spiroxamine	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.1/06 (RA-2168/97) 707570[3] Germany (Albig), D-55234 Grape (Müller Thurgau) 1997	1	0.080	400	BBCH 53	Bunch of grapes	-0	0.421	0.26	-	-
	2	0.160	800	BBCH 61-63	Bunch of grapes	0	0.776	0.48	-	-
	3	0.500	2477	BBCH 73-75	Bunch of grapes	14	0.388	0.24	0.846	0.52
	4	0.521	2533	BBCH 75	Bunch of grapes	35	0.263	0.16	0.770	0.47
	5	0.500	2523.4	BBCH 78						
	6	0.490	2429	BBCH 78-80	Berries	14	0.360	0.22	0.925	0.57
					Berries	35	0.195	0.12	0.731	0.45
CA 6.3.1/06 (RA-2168/97) 707589 Germany (Albig), D-55234 Grape (Müller Thurgau) 1997	1	0.160	80	BBCH 73-75	Bunch of grapes	-0	0.359	0.67	-	-
	2	0.150	75.25	BBCH 75	Bunch of grapes	0	1.010	1.89	-	-
	3	0.160	80	BBCH 78	Bunch of grapes	14	0.386	0.73	0.916	1.72
	4	0.160	80	BBCH 78-80	Bunch of grapes	35	0.204	<u>0.38</u>	0.567	1.06
					Berries	14	0.246	0.46	0.800	1.50
					Berries	35	0.184	0.35	0.631	1.18

1 – Residues scaled proportionally to a 0.300 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503 Scaling factors were derived from the last single application rate and not from the seasonal application rate
2 – <u>Underlined</u> value used to support MRL for wine grapes
3 – The applications were overdosed for about 52-58% because of a calculation error
4 – Value not appropriate to scale up as reported value below the LOQ (<0.05)

Table CA 6.3.1/06-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residues of spiroxamine in grapes

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.1/06	00506	Spiroxamine (parent compound)	Grapes	0.05 0.5 Overall	6 6 12	90; 98; 106, 99; 82; 88 95; 86; 98, 95; 77; 85 Mean: 92, RSD: 9.1
CA 6.3.1/06	00407	Total residues of spiroxamine	Grapes	0.5 Overall	7 7	84; 84; 95; 92; 96; 92 95 Mean: 91, RSD: 5.6

Table CA 6.3.1/06-3 Storage of grape samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.1/06	Grapes	112to 157 (spiroxamine parent) 112 to 122 (spiroxamine total residues)

III. Conclusions

A total of two residue trials conducted in 1997 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in grapes after application of spiroxamine and tebuconazole 500 EC to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

The trials were conducted as harvest trials, with bunches of grapes and berries samples at 35 DALA (PHI for wine grapes). Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Scaled residues of spiroxamine (parent compound) in grapes were between 0.13 and 1.89 mg/kg, 0 DALA. For the two independent trials, residues had declined to 0.15 and 0.38 mg/kg 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes were between 0.18 and 1.06 mg/kg, 35 DALA. For the two independent trials, residues were 0.42 and 1.06 mg/kg 35 DALA.

Residues in berries and grapes were similar, the highest value is used for MRL purposes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/03. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (parent compound) and total spiroxamine were scaled to match the critical GAP. The proportionality concept was applied per trial using the last single application rate.

Scaled residues of spiroxamine (parent compound) in grapes were between 0.13 and 1.89 mg/kg, 0 DALA. For the two independent trials, residues had declined to 0.15 and 0.38 mg/kg 35 DALA.

Scaled residues of total spiroxamine (as aminodiol) in grapes were between 0.18 and 1.06 mg/kg, 35 DALA. For the two independent trials, residues were 0.42 and 1.06 mg/kg 35 DALA.

Residues in berries and grapes were similar, the highest value is used for MRL purposes.

Residues of total spiroxamine ranged from 0.40 to 1.72 mg/kg, for bunch of grapes and between 0.56 to 1.50 mg/kg in berry samples, 14 DALA and for wine grapes between 0.56 to 1.50 mg/kg for bunch of grapes and between 0.35 to 1.18 mg/kg for berry samples, 35 DALA.

Data Point:	KCA 6.3.1/07
Report Author:	[redacted]
Report Year:	1996
Report Title:	Determination of residues of KWG 4168 500 EC on grape and tablegrape in France, Portugal, Spain and Italy
Report No:	RA-2022/94
Document No:	M-010822-01-1
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts IA and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Five trials conducted during growing season 1994 are available to evaluate the magnitude of residues of spiroxamine in/on grapes after three foliar applications of Spiroxamine 500 EC in southern Europe. The designated PHI was 14 days (PHI for table grapes), with additional sampling at various intervals including 35 days (PHI for wine grapes). Samples were analysed only for the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grapes of total spiroxamine equivalents (as aminodiol) as the contribution from spiroxamine alone (CF = x0.5 was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.3.1 'Derivation of conversion factors'). Although currently available data can be used to update and derive a similar conversion factor,

including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for grapes where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Total spiroxamine residues in grapes ranged from 0.07 to 1.76 mg/kg, 0 to 36 DALA (days after last application) in the five trials.

Five residues trials conducted as residue decline trials of total spiroxamine had grape bunches and berries sampled at 14 (PHI for table grapes) or 21 DALA, with grape bunches further sampled at 35 (PHI for wine grapes) or 36 DALA. Total residues of spiroxamine in grape bunches ranged from 0.33 to 1.76 mg/kg, 0 DALA, declining to a range of 0.11 to 0.44 mg/kg, 14 or 21 DALA, and between 0.07 to 0.44 mg/kg, 35 or 36 DALA. In berry samples, residues ranged from 0.47 to 0.52 mg/kg, 14 or 21 DALA.

Table CA 6.3.1/07-1 Residue trials with spiroxamine 500 EC in grapes – residue results for Southern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**	**Active substance**	:	**spiroxamine**
Country	: **Germany**	**Crop/Crop Group**	:	**Vines**
Content of active substance (g/kg or g/L)	: **500 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	: **500 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	: **Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	: **Bayer AG**	**Residues determined as**	:	**ammodiol**
		Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2022/94 40311/3 0311-94 Portugal 2580 Alenquer 1994	Grape Seminario	1) 1983 2) 01.05.1994 - 15.05.1994 3) 01.09.1994	SRU SRU SRU	0.4250 0.4000 0.4000	531 500 500	0.08000 0.08000 0.08000	26.07.1994/0 09.08.1994/14 23.08.1994/14	91	segment of a bunch of grapes must wine, mash fermentation wine, must fermentation	0.43 1.1 0.59 0.41 0.28 0.55 0.32 0.30 0.39	0** 0 7 14 21 35< 35< 35< 35<<	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**
Country : **Germany**
Content of active substance (g/kg or g/L) : **500 g/L**
Formulation (e.g. WP) : **500 EC**
Commercial product (name) : **Spiroxamine EC 500**
Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**
Crop/Crop Group : **Vines**
Page : **1**
Indoor/outdoor : **Outdoor**
Other a.s. in formulation (common name and content) :
Residues determined as : **aminodiol**
Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2022/94 40426/8 0426-94 Italy 48010 Filetto 1994	Grape Trebbiano	1) 1979 2) 05.06.1994 - 12.06.1994 3) 30.09.1994	SPI SPI SPI	0.4000 0.4000 0.4000	1000 1000 1000	0.04000 0.04000 0.04000	25.07.1994/0 08.08.1994/14 22.08.1994/14	81	segment of a bunch of grapes	0.29 0.56 0.47 0.29 0.30 0.28	0** 0 7 14 21 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2022/94 40197/8 0197-94 France, south 30290 Laudun 1994	Grape Grenache	1) 1988 3) 08.09.1994	SRU SRU SRU	0.4000 0.4000 0.4000	100 100 100	0.40000 0.40000 0.40000	06.07.1994/0 20.07.1994/14 03.08.1994/14	81	segment of a bunch of grapes must wine	0.13 0.46 0.21 0.15 0.15 0.09 0.05 0.11	0** 0 7 14 21 36 36 36	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**
Country : **Germany**
Content of active substance (g/kg or g/L) : **500 g/L**
Formulation (e.g. WP) : **500 EC**
Commercial product (name) : **Spiroxamine EC 500**
Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**
Crop/Crop Group : **Vines**
Page : **1**
Indoor/outdoor : **Outdoor**
Other a.s. in formulation (common name and content) :
Residues determined as : **aminodiol**
Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2022/94 40388/1 0388-94 Spain 43720 Sant Marcal 1994	Grape Xarelo	1) 1986 2) 21.05.1994 - 10.06.1994 3) 21.08.1994	SPI SPI SPI	0.1690 0.2250 0.2250	450 600 600	0.03750 0.03750 0.03750	30.06.1994/0 13.07.1994/13 27.07.1994/14	83	segment of a bunch of grapes	0.27 1.3 0.65 0.25 0.31 0.33	0** 0 7 14 21 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**
Country : **Germany**
Content of active substance (g/kg or g/L) : **500 g/L**
Formulation (e.g. WP) : **500 EC**
Commercial product (name) : **Spiroxamine EC 500**
Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**
Crop/Crop Group : **Vines**
Page : **2**
Indoor/outdoor : **Outdoor**
Other a.s. in formulation (common name and content) :
Residues determined as : **aminodiol**
Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2022/94 40427/6 0427-94 Portugal 2630 Arruda dos Vinhos 1994	Table grape Cardinal	1) 1986 2) 26.05.1994 - 01.06.1994 3) 26.07.1994	SRU SRU SRU	0.3305 0.3035 0.4190	412.9 379.5 524	0.08000 0.08000 0.08000	09.06.1994/0 23.06.1994/16 05.07.1994/12	85	berry, table grape	0.45 2.1 0.67 0.69 0.69 0.73	0** 0 3 7 14< 21	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spraying, dusting etc, overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last

(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of five residue trials conducted in 1994 in southern Europe are available to evaluate the residues of total spiroxamine in grapes after application of spiroxamine 500 g/L EC formulation to wines at 14 days pre-harvest (table grapes) and 35/36 days pre-harvest (wine grapes).

All five trials were conducted as residue decline trials. Total residues of spiroxamine in grape bunches ranged from 0.33 to 1.76 mg/kg, 0 DALA, and declined to a range of 0.11 to 0.44 mg/kg, 14 or 21 DALA, and to between 0.07 to 0.44 mg/kg, 35 or 36 DALA. In berry samples, residues ranged from 0.47 to 0.52 mg/kg, 14 or 21 DALA.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/07. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of total spiroxamine in grapes bunches ranged from 0.11 to 0.44 mg/kg, 14 or 21 DALA, and between 0.07 to 0.44 mg/kg, 35 or 36 DALA after three applications of 500 g/L spiroxamine. In berry samples, residues ranged from 0.47 to 0.52 mg/kg, 14 or 21 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.1/08
Report Author:	[redacted]
Report Year:	1996
Report Title:	Determination of residues of KWG 4168 (500 EC) and KWG 4168 (800 EC) in/on grape in the Federal Republic of Germany
Report No:	RA-2125/95
Document No:	M-010818-01-1
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Eight trials conducted during growing season 1995 in Germany are available to evaluate the magnitude of spiroxamine in/on grapes after three foliar applications of Spiroxamine 500 EC or Spiroxamine 800 EC in northern Europe. The designated PHI was 14 days (PHI for table grapes), with additional sampling at various intervals including 35 days (PHI for wine grapes). Samples were analysed for the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grapes of total spiroxamine equivalents (as

aminodiol) as the contribution from spiroxamine alone (CF = x0.5 was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.3.1 'Derivation of conversion factors'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for grapes where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes, but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Total spiroxamine residues in grape segments from treated crop ranged from 0.22 to 0.86 mg/kg, 0 to 42 DALA (days after last application) in the three acceptable trials.

Three acceptable residues trials conducted as residue decline trials of total spiroxamine had residue grape segments sampled at 14 (PHI for table grapes), 28, 35 (PHI for wine grapes) and 42 DALA. Total residues of spiroxamine ranged from 0.79 to 0.86 mg/kg, 0 DALA, declining to a range of 0.27 to 0.60 mg/kg, 14 or 28 DALA, and between 0.30 and 0.53 mg/kg, 35 or 42 DALA.

Table CA 6.3.1/08-1 Residue trials with spiroxamine 500 g/L or 800 g/L EC in grapes – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**	**Active substance**	:	**spiroxamine**
Country	: **Germany**	**Crop/Crop Group**	:	**Vines**
Content of active substance (g/kg or g/L)	: **800 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	: **800 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	: **KWG 4168 EC 800**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	: **Bayer AG**	**Residues determined as**	:	**ammodiol**
		Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2125/95 50239/1 0239-95 Germany 55234 Albig 1995	Grape Müller-Thurgau	1) 1984 2) 27.06.1995 - 05.07.1995 3) 20.09.1995	SPI SPI SPI	0.3376 0.3840 0.5120	1056 1200 1600	0.03200 0.03200 0.03200	06.07.1995/0 17.07.1995/11 16.08.1995/30	81	segment of a bunch of grapes	0.45 0.96 0.50 0.40 0.32 0.33	0** 0 14 28 35< 42	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **500 g/L**

Formulation (e.g. WP) : **500 EC**

Commercial product (name) : **KWG 4168 EC 500**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1- B**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2125/95 50084/4 0084-95 Germany 55234 Albig 1995	Grape Müller-Thurgau	1) 1984 2) 27.06.1995 - 05.07.1995 3) 20.09.1995	SPI SPI SPI	0.3250 0.3900 0.5490	1000 1200 1689	0.03250 0.03250 0.03250	06.07.1995/0 17.07.1995/11 16.08.1995/396	81	segment of a bunch of grapes	0.74 1.4 0.82 0.49 0.54 0.47	0** 0 14 28 35< 42	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2125/95 50092/5 0092-95 Germany 55234 Albig 1995	Grape Müller-Thurgau	1) 1984 2) 27.06.1995 - 05.07.1995 3) 20.09.1995	SPI SPI SPI SPI	0.1650 0.2750 0.3300 0.4400	600 1000 1200 1600	0.02750 0.02750 0.02750 0.02750	26.06.1995/0 07.07.1995/11 17.07.1995/10 16.08.1995/30	81	segment of a bunch of grapes	0.58 1.0 0.60 0.39 0.42 0.41	0** 0 14 28 35< 42	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2125/95 50090/9 0090-95 Germany 67487 Maikammer 1995	Grape Spätburgunder	1) 1985 2) 21.06.1995 - 02.07.1995 3) 25.09.1995	SPI SPI SPI	0.3250 0.3900 0.5200	1000 1200 1600	0.03250 0.03250 0.03250	07.07.1995/0 21.07.1995/14 21.08.1995/31	81	segment of a bunch of grapes must	0.39 1.4 0.80 0.57 0.59 0.56 0.31	0** 0 14 28 35< 42 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **500 g/L**

Formulation (e.g. WP) : **500 EC**

Commercial product (name) : **KWG 4168 EC 500**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1- B**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) (f)	DALT/ PHI (days)	Remarks
									wine at bottling	0.34	35<<	
RA-2125/95 50091/7 0091-95 Germany 79112 Waltershofen 1995	Grape Müller-Thurgau	1) 1975 2) 24.06.1995 - 03.07.1995 3) 26.09.1995	SPI SPI SPI	0.3245 0.3900 0.5195	333 400 533	0.09750 0.09750 0.09750	04.07.1995/0 13.07.1995/13 22.08.1995/36	81	segment of a bunch of grapes	1.3 5.3 2.6 2.0 2.4 2.4	0** 0 14 28 35< 42	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2125/95 50240/5 0240-95 Germany 55234 Albig 1995	Grape Portugieser	2) 27.06.1995 - 05.07.1995 3) 20.09.1995	SRU SRU SRU	0.2985 0.4285 0.5730	305.9 439.3 587.9	0.09750 0.09750 0.09750	06.07.1995/0 17.07.1995/11 16.08.1995/30	81	segment of a bunch of grapes	0.76 1.7 0.68 0.63 0.52 0.50	0** 0 14 28 35< 42	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **800 g/L**

Formulation (e.g. WP) : **800 EC**

Commercial product (name) : **KWG 4168 EC 800**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1- C**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2125/95 50451/3 0451-95 Germany 79112 Waltershofen 1995	Grape Spätburgunder	1) 1975 2) 23.06.1995 - 02.07.1995 3) 10.09.1995	SPI SPI SPI	0.3200 0.3840 0.5120	1000 1200 1600	0.03200 0.03200 0.03200	04.07.1995/0 17.07.1995/13 05.09.1995/50	85	segment of a bunch of grapes	0.47 1.5 1.0 0.82 0.81 0.91	0** 0 14 28 35< 42	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2125/95 50241/3 0241-95 Germany 67487 Maikammer 1995	Grape Spätburgunder	1) 1985 2) 21.06.1995 - 02.07.1995 3) 25.09.1995	SPI SPI SPI	0.3200 0.3840 0.5120	1000 1200 1600	0.03200 0.03200 0.03200	07.07.1995/0 22.07.1995/14 21.08.1995/31	81	segment of a bunch of grapes must wine at bottling	0.32 1.4 0.73 0.66 0.60 0.56 0.22 0.28	0** 0 14 28 35< 42 35< 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

(a) According to Codex (or other e.g. EU Classification Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...

(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of three residue trials conducted in 1995 in northern Europe are available to evaluate the residues of total spiroxamine in grapes after application of either spiroxamine 500 g/L or 800 g/L EC formulation to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

All three trials were conducted as residue decline trials, with grape bunches sampled at 14 (PHI for table grapes), 28, 35 (PHI for wine grapes) or 42 DALA. Total residues of spiroxamine ranged from 0.79 to 0.86 mg/kg, 0 DALA, declining to a range of 0.27 to 0.60 mg/kg, 14 or 28 DALA, and between 0.30 and 0.53 mg/kg, 35 or 42 DALA.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/02. The study is considered compliant with OECD Guideline 509 - Crop Field Trials, September 2009.

Residues of total spiroxamine in table grapes ranged from 0.27 to 0.60 mg/kg, 14 or 28 DALA and wine grapes and between 0.30 to 0.53 mg/kg, 35 or 42 DALA (scaled residues) after three applications of either 500 g/L or 800 g/L spiroxamine EC.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.1/09
Report Author:	[redacted]
Report Year:	1996
Report Title:	Determination of residues of KWG 4168 (500 EC) on grape in the Federal Republic of Germany
Report No:	RA-2047/94
Document No:	M-010814-01-1
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Five trials conducted during growing season 1994 are available to evaluate the magnitude of spiroxamine in/on grapes after three or four foliar treatments of Spiroxamine 500 EC in northern Europe. The designated PHI was 35 days (PHI for wine grapes). Residues were analysed for the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grapes of total spiroxamine equivalents (as aminodiol) as the contribution from spiroxamine alone (CF = x0.5 was used by the previous RMS; refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.3.1 'Derivation of conversion factors'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for grapes where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Total spiroxamine residues in grape segments from treated crop ranged from 0.31 mg/kg to 0.82 mg/kg, 0 to 42 DALA (days after last application) in the acceptable trials.

The acceptable residue trial was conducted as a residue decline trial of total spiroxamine had residues grape segments sampled at 14 (PHI for table grapes), 28, 35 (PHI for wine grapes) and 42 DALA. Total residues of spiroxamine ranged from 0.74 to 0.82 mg/kg, 0 DALA, declining to a range of 0.37 to 0.58 mg/kg, 14 or 28 DALA, and between 0.31 and 0.37 mg/kg, 35 or 42 DALA.

Table CA 6.3.1/09-1 Residue trials with spiroxamine 500 g/L EC in grapes – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	**: Bayer AG**	**Active substance**	**: spiroxamine**
Country	**: Germany**	**Crop/Crop Group**	**: Vines**
Content of active substance (g/kg or g/L)	**: 500 g/L**	**Page**	**: 1**
Formulation (e.g. WP)	**: 500 EC**	**Indoor/outdoor**	**: Outdoor**
Commercial product (name)	**: Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	**:**
Producer of commercial product	**: Bayer AG**	**Residues determined as**	**: aminodiol**
		Residues calculated as	**: spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s)/ Application interval/ or no of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2047/94 40445/4 0445-94 Germany 55234 Albig 1994	Grape Portugieser	1) 1988 2) 12.06.1994 - 27.06.1994 3) 20.09.1994	SRU SRU SRU	0.3245 0.3900 0.5195	333 400 533	0.09750 0.09750 0.09750	30.08.1994/0 11.07.1994/11 16.08.1994/36	81	segment of a bunch of grapes	0.51 1.1 0.49 0.44 0.47 0.46	0** 0 14 28 35< 42	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg
RA-2047/94 40560/4 0560-94 Germany 67487 Maikammer 1994	Grape Spätburgunder	1) 1985 2) 17.06.1994 - 27.06.1994 3) 21.09.1994	SPI SPI SPI SPI	0.1500 0.2500 0.3000 0.4000	600 1000 1200 1600	0.02500 0.02500 0.02500 0.02500	24.05.1994/0 29.06.1994/36 15.07.1994/16 17.08.1994/33	81	segment of a bunch of grapes must	0.51 1.1 0.46 0.40 0.46 0.41 0.26	-1 0 14 28 35< 42 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	**Active substance**	:	**spiroxamine**
Country	:	**Germany**	**Crop/Crop Group**	:	**Vines**
Content of active substance (g/kg or g/L)	:	**500 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	:	**500 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	:	**Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	:	**Bayer AG**	**Residues determined as**	:	**aminodiol**
			Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hl						
									wine at bottling wine at first taste test	0.26 0.25	35<< 35<<<	

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**		**Active substance**	:	**spiroxamine**
Country	: **Germany**		**Crop/Crop Group**	:	**Vines**
Content of active substance (g/kg or g/L)	: **500 g/L**		**Page**	:	**1**
Formulation (e.g. WP)	: **500 EC**		**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	: **Spiroxamine EC 500**		**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	: **Bayer AG**		**Residues determined as**	:	**aminodiol**
			Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2047/94 40444/6 0444-94 Germany 67489 Kirrweiler 1994	Grape Müller Thurgau	1) 1982 2) 17.06.1994 - 27.06.1994 3) 21.09.1994	SPI SPI SPI	0.3250 0.3900 0.5200	1000 1200 1600	0.03250 0.03250 0.03250	29.06.1994/0 15.07.1994/16 17.08.1994/33	81	segment of a bunch of grapes must wine at bottling wine at first taste test	0.62 1.4 0.93 0.67 0.59 0.62 0.22 0.23 0.21	-1 0 14 28 35< 42 35< 35<< 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**	**Active substance**	:	**spiroxamine**
Country	: **Germany**	**Crop/Crop Group**	:	**Vines**
Content of active substance (g/kg or g/L)	: **500 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	: **500 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	: **Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	: **Bayer AG**	**Residues determined as**	:	**aminodiol**
		Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s) Application interval/ or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2047/94 40446/2 0446-94 Germany 55234 Albig 1994	Grape Müller-Thurgau	1) 1988 2) 17.06.1994 - 27.06.1994 3) 20.09.1994	SRU SRU SRU	0.5345 0.3900 0.5195	333 400 533	0.09250 0.09750 0.09750	30.06.1994/0 11.07.1994/11 16.08.1994/36	81	segment of a bunch of grapes	0.54 0.70 0.50 0.44 0.35 0.41	0** 0 14 28 35< 42	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg
RA-2047/94 40443/8 0443-94 Germany 67487 St. Martin 1994	Grape Spätburgunder	1) 1985 2) 17.06.1994 - 27.06.1994 3) 21.09.1994	SPI SPI SPI	0.3250 0.3900 0.5200	1000 1200 1600	0.03250 0.03250 0.03250	29.06.1994/0 15.07.1994/16 17.08.1994/33	81	segment of a bunch of grapes must wine at bottling	0.51 1.5 0.56 0.60 0.54 0.53 0.38 0.38	-1 0 14 28 35< 42 35< 35<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	
Active substance	:	**spiroxamine**	
Crop/Crop Group	:	**Vines**	
Page	:	**1**	
Indoor/outdoor	:	**Outdoor**	
Other a.s. in formulation (common name and content)	:		
Residues determined as	:	**aminodiol**	
Residues calculated as	:	**spiroxamine**	

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) Application interval/ or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hl						
									wine at first taste test	0.31	35<<	

(a) According to Codex (or other e.g. EU) Classification Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(j) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A residue trial conducted in 1994 in northern Europe is available to evaluate the residues of total spiroxamine in grapes after application of spiroxamine 500 g/L EC formulation to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

The trial was conducted as a residue decline trial, with grape segments sampled at 14 (PHI for table grapes) or 28 DALA, and 35 (PHI for wine grapes) or 42 DALA. Total residues of spiroxamine ranged from 0.74 to 0.82 mg/kg, 0 DALA (days after last application), declining to a range of 0.37 to 0.58 mg/kg, 14 or 28 DALA, and between 0.31 and 0.37 mg/kg, 35 or 42 DALA.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/01. The study is considered compliant with OECD Guideline 509 "Crop Field Trials", September 2009.

Residues of total spiroxamine in table grapes ranged from of 0.37 to 0.58 mg/kg, 14 or 28 DALA, and between 0.31 and 0.37 mg/kg for wine grapes, 35 or 42 DALA after three applications of 500 g/L spiroxamine.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.1/10
Report Author:	[redacted]
Report Year:	1996
Report Title:	Determination of residues of KWG 4168 (500 EC) and KWG 4168 (800 EC) in/on grape in France, Portugal, Greece and Italy
Report No:	RA-2126/95
Document No:	M-010811-01-1
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Seven trials conducted at five sites during growing season 1995 are available to evaluate the magnitude of spiroxamine in/on table grapes after three foliar spray applications of Spiroxamine 500 EC or Spiroxamine 800 EC, in southern Europe. The designated PHI was 14 days (PHI for table grapes), with additional sampling at various intervals including 35 days (PHI for wine grapes). Samples were analysed for the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grapes of total spiroxamine equivalents (as aminodiol) as the contribution from spiroxamine alone (CF = x0.5 was used by the previous RMS; refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.3.1 'Derivation of conversion factors'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for grapes where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Total spiroxamine residues in grape bunches and berries from treated crop ranged from 0.13 to 1.43 mg/kg, 0 to 42 DALA (days after last application) in the five acceptable trials.

Five acceptable residue trials conducted as residue decline trials, with grape bunches sampled at 14 (PHI for table grapes), 21, 35 (PHI for wine grapes) and 42 DALA. Additional samples of berries were also taken at 14 DALA. Total residues of spiroxamine ranged from 0.47 to 1.43 mg/kg, 0 DALA, declining to a range of 0.13 to 0.95 mg/kg, 14 or 21 DALA, and between <0.05 to 0.32 mg/kg, 35 or 42 DALA in grape bunches samples. For berry samples, residues ranged from 0.12 to 0.82 mg/kg, 14 DALA.

Table CA 6.3.1/10-1 Residue trials with spiroxamine 500 or 800 g/L EC in grapes – residue results for southern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**	**Active substance**	:	**spiroxamine**
Country	: **Germany**	**Crop/Crop Group**	:	**Vines**
Content of active substance (g/kg or g/L)	: **500 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	: **500 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	: **KWG 4168 EC 500**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	: **Bayer AG**	**Residues determined as**	:	**ammodiol**
		Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2126/95 50080/1 0080-95 Portugal 2580 Ribafria Alenquer 1995	Grape Seminario	1) 1993 2) 02.05.1995 - 09.05.1995 3) 13.09.1995	SRU SRU SRU	0.4000 0.4000 0.4000	500 500 500	0.08000 0.08000 0.08000	25.07.1995/0 08.08.1995/14 22.08.1995/14	85	segment of a bunch of grapes berry, table grape wine must	0.41 1.9 0.72 1.3 1.0 1.1 0.26 0.22	0** 0 7 14 21 14 21 21	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg (h) 0.02 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **500 g/L**

Formulation (e.g. WP) : **500 EC**

Commercial product (name) : **KWG 4168 EC 500**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2126/95 50083/6 0083-95 Italy 58100 Vetulonia 1995	Grape San Giovesa	1) 1975 2) 25.05.1995 - 05.06.1995 3) 01.08.1995	SPI SPI SPI	0.4000 0.4000 0.4000	1000 1000 1000	0.04000 0.04000 0.04000	25.07.1995/0 08.08.1995/14 22.08.1995/14	85	segment of a bunch of grapes berry, table grape	0.38 0.91 0.75 0.59 0.59 0.22 0.26 0.69	0** 0 7 14 21 35 42 14	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2126/95 50077/1 0077-95 France, south 30290 Laudun 1995	Grape Syrah	1) 1984 3) 13.09.1995	SRU SRU SRU	0.4000 0.4000 0.4000	100 100 100	0.40000 0.40000 0.40000	12.07.1995/0 26.07.1995/14 09.08.1995/14	77	segment of a bunch of grapes berry, table grape	0.22 0.62 0.32 0.17 0.42 0.17 0.28 0.16	0** 0 7 14 21 35 42 14	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **500 g/L**

Formulation (e.g. WP) : **500 EC**

Commercial product (name) : **KWG 4168 EC 500**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) (g)	DALT/ PHI (days) (f)	Remarks
									wine juice	0.12 0.12	35 35	(h) 0.02 mg/kg
RA-2126/95 50082/8 0082-95 Greece 20200 Diminio 1995	Grape Sultanina	2) 15.05.1995 - 30.08.1995 3) 15.09.1995	SPI SPI SPI	0.1690 0.4500 0.4500	450 600 600	0.03750 0.07500 0.07500	11.07.1995/0 24.07.1995/13 02.08.1995/14	81	segment of a bunch of grapes berry, table grape raisin	0.26 0.84 0.48 0.35 0.36 0.27 0.33 0.30 1.2	0** 0 7 14 21 35 42 14 14	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **800 g/L**

Formulation (e.g. WP) : **800 EC**

Commercial product (name) : **KWG 4168 EC 800**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1- B**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2126/95 50662/1 0662-95 Italy 70031 Andria 1995	Grape San Giovese	1) 1974 2) 10.05.1995 - 02.06.1995 3) 13.09.1995	SPI SPI SPI	0.4000 0.4000 0.4000	1000 1000 1000	0.04000 0.04000 0.04000	25.07.1995/0 26.07.1995/14 09.08.1995/14	77	segment of a bunch of grapes berry, table grape	0.21 0.93 0.23 0.21 0.17 0.17 0.26 0.18	0** 0 7 14 21 35 42 14	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg
RA-2126/95 50235/9 0235-95 Italy 58100 Vetulonia 1995	Grape San Giovese	1) 1975 2) 25.05.1995 - 05.06.1995 3) 01.08.1995	SPI SPI SPI	0.4000 0.4000 0.4000	1000 1000 1000	0.04000 0.04000 0.04000	25.07.1995/0 08.08.1995/14 22.08.1995/14	85	segment of a bunch of grapes berry, table grape	0.41 0.92 0.69 0.62 0.50 0.43 0.40 0.57	0** 0 7 14 21 35 42 14	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**
Country : **Germany**
Content of active substance (g/kg or g/L) : **800 g/L**
Formulation (e.g. WP) : **800 EC**
Commercial product (name) : **KWG 4168 EC 800**
Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**
Crop/Crop Group : **Vines**
Page : **1- B**
Indoor/outdoor : **Outdoor**
Other a.s. in formulation (common name and content) :
Residues determined as : **aminodiol**
Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2126/95 50234/0 0234-95 France, south 30290 Laudun 1995	Grape Syrah	1) 1984 3) 13.09.1995	SPU SRU SRU	0.4000 0.4000 0.4000	100 100 100	0.40000 0.40000 0.40000	19.07.1995/0 26.07.1995/14 09.08.1995/14	77	segment of a bunch of grapes berry, table grape	0.19 0.70 0.29 0.35 0.09 0.07 <0.05 0.19	0** 0 7 14 21 35 42 14	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc, overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last

(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of five residue trials conducted in 1995 in southern Europe are available to evaluate the residues of total spiroxamine in grapes after application of either spiroxamine 500 g/L EC or 800 g/L EC formulation to vines at 14 days pre-harvest (table grapes) and 35 days pre-harvest (wine grapes).

All five of the trials were conducted as residue decline trials. The total residues of spiroxamine ranged from 0.13 to 1.43 mg/kg, 0 DALA (days after last application), declining between 0.13 to 1.00 mg/kg, 14 or 21 DALA and between <0.05 to 0.32 mg/kg, 35 or 42 DALA in grape segment samples. For berry samples, residues ranged from 0.12 to 0.82 mg/kg, 14 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/08. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of total spiroxamine in table grapes ranged from 0.13 to 1.43 mg/kg, in grape segments, 14 or 21 DALA and berry samples between 0.12 to 0.82 mg/kg, 14 DALA. Wine grapes were between <0.05 to 0.32mg/kg in grape segments, 35 or 42 DALA after three applications of either 500 g/L or 800 g/L spiroxamine.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	(KCA 6.3.1/11)
Report Author:	[redacted]; [redacted]
Report Year:	1997
Report Title:	Determination of residues of KWG 4168 in/on tablegrape following spray application with different formulations (500 EC, 800 EC) in Italy, Portugal and France
Report No:	RA-2071/96
Document No:	M-010796-01-2
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Five trials conducted at three sites during growing season 1996 are available to evaluate the magnitude of spiroxamine in/on table grapes after three foliar spray treatments of Spiroxamine 500 EC and Spiroxamine 800 EC in southern Europe. The designated PHI was 14 days (valid for table grapes), with additional sampling at various intervals. Residues were analysed for the total residue of spiroxamine (common moiety approach) determined as aminodiol and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue

approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grapes of total spiroxamine equivalents (as aminodiol) as the contribution from spiroxamine alone (CF = x0.5 was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.3.1 'Derivation of conversion factors'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for grapes where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment of MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Total spiroxamine residues in berries from treated crop ranged from 0.04 to 0.67 mg/kg, 0 to 21 DALA (days after last application) in the three acceptable trials.

Three acceptable residues trials conducted as residue decline trials of total spiroxamine had residues berries samples at 14 days PHI for table grapes and again at 21 DALA. Total residues of spiroxamine ranged from 0.08 to 0.26 mg/kg, 0 DALA, declining to a range of 0.04 to 0.67 mg/kg, 14 or 21 DALA.

Table CA 6.3.1/11-1 Residue trials with spiroxamine 500 g/L EC or 800 g/L EC in grapes – residue results for southern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	**: Bayer AG**	**Active substance**	**:**	**spiroxamine**
Country	**: Germany**	**Crop/Crop Group**	**:**	**Vines**
Content of active substance (g/kg or g/L)	**: 800 g/L**	**Page**	**:**	**1**
Formulation (e.g. WP)	**: 800 EC**	**Indoor/outdoor**	**:**	**Outdoor**
Commercial product (name)	**: KWG 4168 EC 800**	**Other a.s. in formulation (common name and content)**	**:**	
Producer of commercial product	**: Bayer AG**	**Residues determined as**	**:**	**ammodiol**
		Residues calculated as	**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2071/96 60250/7 0250-96 Portugal P-2090 Alpiarca 1996	Table grape D. Maria	1) 1988 2) 01.05.1996 - 31.05.1996 3) 01.08.1996	SRU SRU SRU	0.4000 0.4000 0.4000	500 500 500	0.08000 0.08000 0.08000	04.06.1996/0 18.06.1996/14 03.07.1996/15	75	berry, table grape	0.35 0.89 0.39 0.32 0.24	0** 0 7 14< 21	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg
RA-2071/96 60247/7 0247-96 Italy I-70052 Bisceglie 1996	Table grape Blush	1) 1991 2) 05.06.1996 - 15.06.1996 3) 28.08.1996	SPI SPI SPI	0.4000 0.4000 0.4000	1000 1000 1000	0.04000 0.04000 0.04000	17.07.1996/0 31.07.1996/14 14.08.1996/14	81	berry, table grape	0.14 0.87 0.72 0.31 0.64	0** 0 7 14< 21	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **500 g/L**

Formulation (e.g. WP) : **500 EC**

Commercial product (name) : **KWG 4168 EC 500**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1- B**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) (a)	DALT/ PHI (days) (f)	Remarks
RA-2071/96 60248/5 0248-96 France, south F-13670 St. Andiol 1996	Table grape Muscat de Hambourg	1) 1952 3) 16.09.1996	SRU SRU SRU	0.4000 0.4000 0.4000	100 100 100	0.40000 0.40000 0.40000	05.08.1996/0 19.08.1996/14 02.09.1996/14	87	berry, table grape	0.10 0.15 0.06 0.05 <0.05	0** 0 7 14< 21	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg
RA-2071/96 60249/3 0249-96 Portugal P-2090 Alpiarca 1996	Table grape D. Maria	1) 1988 2) 01.05.1996 - 31.05.1996 3) 01.08.1996	SRU SRU SRU	0.4000 0.4000 0.4000	500 500 500	0.08000 0.08000 0.08000	04.06.1996/0 18.06.1996/14 03.07.1996/15	75	berry, table grape	0.31 0.90 0.44 0.31 0.20	0** 0 7 14< 21	(c) SRU:Spraying, low-volume (g) 00407 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**

Country : **Germany**

Content of active substance (g/kg or g/L) : **500 g/L**

Formulation (e.g. WP) : **500 EC**

Commercial product (name) : **KWG 4168 EC 500**

Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**

Crop/Crop Group : **Vines**

Page : **1- B**

Indoor/outdoor : **Outdoor**

Other a.s. in formulation (common name and content) :

Residues determined as : **aminodiol**

Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval/ or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2071/96 60246/9 0246-96 Italy I-70052 Bisceglie 1996	Table grape Blush	1) 1991 2) 05.06.1996 - 15.06.1996 3) 28.08.1996	SPI SPI SPI	0.4000 0.4000 0.4000	1000 1000 1000	0.04000 0.04000 0.04000	07.07.1996/0 31.07.1996/14 14.08.1996/14	81	berry, table grape raisin	<0.05 2.0 1.5 0.58 0.67 4.2	0** 0 7 14< 21 14<<	(c) SPI:Spraying (g) 00407 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy.

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of three residue decline trials conducted in 1996 in southern Europe are available to evaluate the residues of total spiroxamine in table grapes after application of either spiroxamine 500 g/L EC or 800 g/L EC formulation to vines at 14 days pre-harvest (table grapes).

All three trials were conducted as residue decline trials, with berries sampled at 14 or 21 day PHI for table grapes. The total residues of spiroxamine ranged from 0.08 to 0.26 mg/kg, 0 DALA (days after last application) and declined to a range of 0.04 to 0.67 mg/kg, 14 or 21 DALA.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.3/09. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of total spiroxamine in table grapes ranged from 0.04 to 0.67 mg/kg, 14 or 21 DALA after three applications of either 500 g/L or 800 g/L spiroxamine.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Overview on grapes residue trials

Residue trials conducted after the previous EU approval evaluation from 2016 onwards employed residue methods measuring both spiroxamine itself with a fully chiral method and total spiroxamine by means of a common moiety approach. Residues of spiroxamine diastereomers A and B and their enantiomers A1: A2, B1: B2 were measured and reported as mg/kg both individually and as a sum.

The residues data submitted does include trials conducted at the nominal individual application rate of 300 g a.s./ha but to provide a complete dataset from the large number of existing trials conducted at higher or lower application individual rates (from 200 to 400 g a.s./ha), all such trials are included. As these rates are outside of the usually applied ±25% rule, the accepted approach of proportionality or scaling has been used to adjust any residues reported above the analytical LOQ to the nominal rate.

Trials on grapes previously evaluated but considered not appropriate for MRL purposes are provided in this submission for information but are not relied upon. These trials are where:

- The use pattern did not fit the cGAP and residues could not be scaled
- The data were only for total residues of spiroxamine (via the aminodiol common moiety)
- The analytical LOQ was 0.05 mg/kg and therefore data could not be scaled or used reliably in MRL calculations

Table grapes:

A total of 31 residue trials conducted between 2000 and 2020 are available to evaluate the residues of spiroxamine (scaled spiroxamine as a sum of total isomers) in or on table grapes after application of spiroxamine formulations to vines nominally 14 days pre-harvest, in support of the critical GAP. All trials were performed in the southern European climatic zone. Reported spiroxamine residues in table grapes for the 14 days PHI ranged from <0.01 to 0.27 mg/kg.

Table grapes are a major crop in southern Europe and as such require eight residue trials per climatic zone when treated with products after formation of the edible commodity and where residues are above

the LOQ. Therefore the data set for table grapes satisfies the data requirements outlined in Regulation (EU) 283/2013 of 01 March 2013 and is appropriate for MRL purposes.

Data from these trials are also available for total residues of spiroxamine (via the aminodiol common moiety) and for the 14 days PHI ranged from 0.02 to 0.39 mg/kg. These values, where appropriate, are used to determine conversion factors for use in consumer risk assessment.

Wine grapes:

A total of 49 residue trials conducted between 1997 and 2020 are available to evaluate the residues of spiroxamine (scaled spiroxamine as a sum of total isomers) in or on wine grapes after application of spiroxamine formulations to vines nominally 35 days pre-harvest in support of the critical GAP. Of these, 26 trials were performed in the northern European climatic zone and 23 trials were performed in the southern European climatic zone. Reported spiroxamine residues in wine grapes for the 35 days PHI ranged from 0.02 to 0.60 mg/kg for the northern zone and from <0.01 to 0.15 mg/kg for the southern zone. The worst case data set for MRL purposes was the northern zone.

Wine grapes are a major crop in Europe and as such requires eight residue trials per climatic zone when treated with products after formation of the edible commodity and where residues are above the LOQ. Therefore the data set for wine grapes satisfies the data requirements outlined in Regulation (EU) 283/2013 of 01 March 2013 and is appropriate for MRL purposes.

Data from these trials are also available for total residues of spiroxamine (via the aminodiol common moiety) and for the 35 days PHI ranged from 0.03 to 1.18 mg/kg for the northern zone and from 0.03 to 0.54 mg/kg for the southern zone. These values, where appropriate, are used to determine conversion factors for use in consumer risk assessment.

Grapes – fall back GAP

After a single application at nominally BBCH 15-19, residues of spiroxamine (sum of all enantiomers) found at normal commercial harvest in grapes were all <0.01 mg/kg (non-detectable) from four trials conducted in both northern and southern Europe. The total residues of spiroxamine (as common moiety aminodiol) found ranged from <0.01 mg/kg (non-detectable) to 0.01 mg/kg (in two trials, one each in northern and southern Europe). These data support the early season single application 'fall back' GAP for the use of spiroxamine on grapes and confirms that a non-significant residue situation for parent spiroxamine on grapes can be expected for this use and consumer risk would be covered under the risk-envelope scenario from the critical GAP.

For procedural reasons studies listed in the Table CA 6.3.1-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.

Table CA 6.3.1-1: Studies previously submitted and not relied upon for the risk assessment

Data Point	Document No.	Date	Title
KCA 6.3.1/12	M-304376-01-1	2008	Statistical evaluation of a metric response: Total residues of spiroxamine in grapes
KCA 6.3.1/13	M-304379-01-1	2008	Statistical evaluation of a metric response: Spiroxamine (parent) residues in grapes

CA 6.3.2 Barley

Spiroxamine is supported for application to barley according to the critical GAP detailed in Table CA 6.3-1, involving up to 2 applications at 375 g a.s./ha.

Residue trials conducted after the previous EU approval evaluation from 2016 onwards employed residue methods measuring both spiroxamine itself with a fully chiral method and total spiroxamine by means of a common moiety approach. Residues of spiroxamine diastereomers A and B and their enantiomers A1: A2, B1 : B2 were measured and reported as mg/kg both individually and as a sum. The summaries in this section present these data but do not include enantiomer or A / B diastereomer ratios. The ratios are discussed and presented under Section CA 6.7 in order to consider the impact of isomer ratios on consumer risk assessment.

The residues data presented here does include trials conducted at the nominal individual application rate of 375 g a.s./ha but to provide a complete dataset from the existing trials conducted at higher or lower application individual rates (from 150 to 750 g a.s./ha) and supporting the GAP for timing or analytical LOQ, all such trials are included. As these rates are outside of the usually applied ±25% rule, the accepted approach of proportionality or scaling has been used to adjust any residues reported above the analytical LOQ to the nominal rate.

The proportionality approach for scaling of residues with examples is described in detail within various guidance documents, notably the following:

- ENV/JM/MONO(2011)50/REV1, 07 September 2016, OECD Guidance Document on Crop Field Trials, Second Edition, Series on Pesticides - No. 66, Series on Testing & Assessment - No. 164
- EFSA Supporting publication 2018:EN-1503, 06 November 2018, Recommendations on the use of the proportionality approach in the framework of risk assessment for pesticide residues
- EFSA September 2015, Residues trials and MRL calculations - Proposals for a harmonised approach for the selection of the trials and data used for the estimation of MRL, STMR and HR

The representative use GAP considered in this dossier is for barley with two applications at 375 g a.s./ha BBCH 30-61 for both NEU and SEU residue zones.

For the trials presented here on barley, spiroxamine (total isomers) data in plant, grain and straw have been scaled using the actual reported (nominal) last application rate in comparison to the critical GAP rate. The alternative approach of using the seasonal loading or the average seasonal rate to take account of trials with two or three applications, compared to the critical GAP rate would be expected to give almost identical data and therefore the selected approach is considered to be appropriate.

Finally, the scaled data includes those trials conducted at the nominal GAP rate and therefore within ±25% of the GAP. This is compliant with the cited above guidance and the following text from OECD 2016 paragraph 32 justifies this approach. In reality the scaled data used for MRL purposes from trials conducted at the nominal rate is approximately equal to the reported values but the scaling of these data (either slightly higher or slightly lower) is consistent with OECD and EFSA guidance:

OECD 2016

32. All data points under consideration, i.e. data points corresponding to application rates within/outside the acceptable range of ± 25% of the nominal application rate, should be adjusted to the nominal (1x) application rate to prevent issues of bias.

An example calculation from the barley lower rate data set is shown here.

Trial reference: CA 6.3.2/18, M-638944-01-1, 17-2075-01

Final application rate: 150 g a.s./ha

Critical GAP final application rate: 375 g a.s./ha

Reported barley plant residue 0 DALA: 3.00 mg/kg spiroxamine (total isomers)

Scaling factor: 375 / 150 = 2.5

Scaled residue: 3.00 x 2.5 = 7.50 mg/kg spiroxamine (total isomers)

Data Point:	KCA 6.3.2/13
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, the Netherlands, the United Kingdom and Belgium
Report No:	16-2045
Document No:	M-618660-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four residue trials conducted during growing season 2016 are available to evaluate the magnitude of residues of spiroxamine in/on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 in northern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in northern Europe (Germany, The Netherlands, United Kingdom and Belgium) with all four conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/13-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha, corresponding to 375 g a.s./ha at a water application rate of 200 to 400 L/ha. Spray intervals were 14 to 21 days, with the final treatment being made at growth stage BBCH 59 to 61. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using a spiroxamine and prothioconazole 460 EC formulation containing 300 g/L spiroxamine (actual content 296.2 g/L spiroxamine).

Samples of green material were taken on the day of the final application, and between 41 to 57 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last treatment and 41 to 57 DALA.

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/13-4, the maximum storage time between date of deep-freezing and date of last extraction was 540 days for spiroxamine enantiomers and 595 days for the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/13-2 and Table CA 6.3.2/13-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (as 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as harvest trials, with straw and grain sampled between 41 and 57 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 4.90 to 5.40 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.025 mg/kg with corresponding residues in straw ranging from 0.23 to 0.62 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 5.90 to 12.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.086 mg/kg with corresponding residues in straw ranging from 1.60 to 3.00 mg/kg.

Table CA 6.3.2/13-1 Residue trials with 300 g/L spiroxamine EC in barley – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/13 (16-2045) 16-2045-01 Germany, D- 04451 Borsdorf Winter barley (Meridian) 2016	1 2	0.375 0.375	300 300	BBCH 47 BBCH 61	Green material Grain Straw	0 42 42	1.40 0.006 0.17	1.40 0.008 0.17	1.10 0.006 0.140	1.10 0.004 0.13	5.00 <u>0.025</u> 0.62	5.90 0.086 3.00
CA 6.3.2/13 (16-2045) 16-2045-02 The Netherlands, N-1437 EG Rozenburg Winter barley (Cassiopée Winter brew barley) 2016	1 2	0.375 0.375	300 300	BBCH 32 BBCH 59	Green material Grain Straw	0 48 48	1.40 <0.00271 0.13	1.30 <0.00271 0.13	1.10 <0.00229 0.098	1.10 <0.00229 0.097	4.90 <u><0.010</u> 0.45	6.00 0.019 2.00
CA 6.3.2/13 (16-2045) 16-2045-03 United Kingdom, GB-CB22 5EU Little Shelford, Cambridge Spring barley (Odyssey Typical UK Malting) 2016	1 2	0.375 0.375	300 300	BBCH 32 BBCH 61	Green material Grain Straw	0 57 57	2.60 <0.00271 0.065	2.60 <0.00271 0.071	2.20 <0.00229 0.051	2.10 <0.00229 0.047	9.40 <u><0.010</u> 0.23	12.00 <0.010 1.90
CA 6.3.2/13 (16-2045) 16-2045-04 Belgium, B- 6211 Mellet Spring barley (Hilford) 2016	1 2	0.375 0.375	300 300	BBCH 33 BBCH 59	Green material Grain Straw	0 41 41	2.40 0.003 0.079	2.40 0.003 0.082	1.90 <0.00247 0.060	1.90 <0.00229 0.058	8.50 <u>0.011</u> 0.28	9.90 0.023 1.90

1 – <u>Underlined</u> value to be used to support MRL for grain

Table CA 6.3.2/13-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/13	01480	A1 enantiomer	Barley green material	0.0027 0.027 0.27 1.1 2.7 Overall	3 3 1 1 1 9	92; 94; 105 87; 88; 89 98 94 80 Mean: 92, RSD: 7.8
CA 6.3.2/13	01480	A1 enantiomer	Barley grain	0.0027 0.027 0.27 Overall	3 3 1 7	92; 94; 111 92; 93; 96 111 Mean: 99, RSD: 8.7
CA 6.3.2/13	01480	A1 enantiomer	Barley straw	0.0027 0.027 0.27 Overall	3 3 1 7	91; 92; 94 91; 92; 93 93 Mean: 92, RSD: 1.2
CA 6.3.2/13	01480	A2 enantiomer	Barley green material	0.0027 0.027 0.27 1.1 2.7 Overall	3 3 1 1 1 9	91; 91; 100 87; 88; 88 98 93 80 Mean: 91, RSD: 6.6
CA 6.3.2/13	01480	A2 enantiomer	Barley grain	0.0027 0.027 0.27 Overall	3 3 1 7	92; 93; 103 91; 92; 95 112 Mean: 97, RSD: 8.1
CA 6.3.2/13	01480	A2 enantiomer	Barley straw	0.0027 0.027 0.27 Overall	3 3 1 7	87; 91; 93 90; 92; 93 90 Mean: 91, RSD: 2.3
CA 6.3.2/13	01480	B1 enantiomer	Barley green material	0.0023 0.023 0.23 0.9 2.3 Overall	3 3 1 1 1 9	102; 112; 113 101; 101; 102 99 98 83 Mean: 101, RSD: 8.6
CA 6.3.2/13	01480	B1 enantiomer	Barley grain	0.0023 0.023 0.23 Overall	3 3 1 7	100; 104; 117 95; 98; 101 112 Mean: 104, RSD: 7.6
CA 6.3.2/13	01480	B1 enantiomer	Barley straw	0.0023 0.023 0.23 Overall	3 3 1 7	91; 92; 96 91; 92; 93 95 Mean: 93, RSD: 2.1
CA 6.3.2/13	01480	B2 enantiomer	Barley green material	0.0023 0.023 0.23 0.9 2.3 Overall	3 3 1 1 1 9	94; 95; 102 86; 92; 94 99 97 82 Mean: 93, RSD: 6.6
CA 6.3.2/13	01480	B2 enantiomer	Barley grain	0.0023 0.023	3 3	93; 94; 103 91; 92; 95

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
				0.23 Overall	1 7	111 Mean: 97, RSD: 7.6
CA 6.3.2/13	01480	B2 enantiomer	Barley straw	0.0023 0.023 0.23 Overall	3 3 1 7	90; 91; 93 91; 93; 94 94 Mean: 92, RSD: 1.7

Table CA 6.3.2/13-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/13	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.10 9.9 Overall	3 3 1 7	105 (144); 102 (167); 102 (167) 89 (93); 98 (102); 101 (105)[1] 92 Mean: 101, RSD: 11.0
CA 6.3.2/13	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.10 Overall	3 3 6	97; 97; 106 83; 86; 86 Mean: 93, RSD: 9.2
CA 6.3.2/13	00312/M002	Total residue of spiroxamine	Barley straw	0.01 0.10 4.9 Overall	3 3 1 7	93 (158); 102 (167); 102 (167)[1] 77 (84); 85 (92); 89 (96)[1] 102 Mean: 93, RSD: 10.6

1 - These recoveries were background corrected since the control sample used for spiking were found to contain (apparent) residues with differing levels. The uncorrected recoveries are shown in brackets.

Table CA 6.3.2/13-4 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/13	Barley	450 to 540 (parent spiroxamine enantiomers) 476 and 595 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2016 in northern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on barley BBCH 32 to 61.

All four trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 4.90 to 5.40 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.025 mg/kg with corresponding residues in straw ranging from 0.23 to 0.62 mg/kg.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 5.90 to 12.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.086 mg/kg with corresponding residues in straw ranging from 1.60 to 3.00 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 4.90 to 5.40 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.025 mg/kg with corresponding residues in straw ranging from 0.23 to 0.62 mg/kg.

The total residues (scaled to the cGAP) of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 5.90 to 12.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.086 mg/kg with corresponding residues in straw ranging from 1.60 to 3.00 mg/kg.

Data Point:	KCA 6.3.2/14
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Amendment no. 01 to final report: Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on barley after spray application of bixafen & spiroxamine & trifloxystrobin EC 325 in southern France and Portugal
Report No:	17-2145
Document No:	M-630826-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Two residue trials conducted during growing season 2017 to evaluate the magnitude of residues of spiroxamine in/on barley (green material, grain and straw) after two applications of spiroxamine, bixafen and trifloxystrobin EC 325 in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of bixafen and trifloxystrobin were also analysed, however are not reported in this summary.

Two residue trials on barley were conducted in southern Europe (southern France and Portugal) with both conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/14-1 and Table CA 6.3.2/14-2.

Two spray applications of spiroxamine were made at a product rate of 1.00 L/ha, corresponding to 150 g a.s./ha spiroxamine at a water application rate of 300 or 350 L/ha. Spray intervals were 13 to 21 days with the final treatment being made at growth stage BBCH 61. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using bixafen and spiroxamine and trifloxystrobin EC 325 formulation containing 150 g/L spiroxamine (actual content 148.1 g/L spiroxamine).

Samples of green material were taken on the day of the final application, and 33 or 48 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last application and 33 or 48 DALA.

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers were determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/14-5, the maximum storage time between date of deep-freezing and date of last extraction was 202 days for spiroxamine enantiomers and 366 days for the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/14-3 and Table CA 6.3.2/14-4).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

Both trials were conducted as harvest trials, with straw and grain sampled 33 or 48 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) were 6.50 and 7.00 mg/kg. At maturity, residues in grain were 0.028 mg/kg in one trial and as reported data were below the LOQ for the other trial the values could not be scaled. Corresponding residues in straw were 0.23 and 0.33 mg/kg.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) were 7.25 and 10.28 mg/kg. At maturity, residues in grain were 0.04 and 0.10 mg/kg with corresponding residues in straw both at 4.00 mg/kg.

Table CA 6.3.2/14-1 Residue trials spiroxamine, trifloxystrobin and bixafen EC 325 in barley – reported residue results for southern Europe

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.2/14 (17-2145) 17-2145-01 Southern France, F- 30000 Nîmes Spring barley 2017	1 2	0.150 0.150	300 300	BBCH 30 BBCH 61	Green material Grain Straw	0 33 33	0.76 <0.00271 0.027	0.75 <0.00271 0.026	0.64 <0.00229 0.020	0.63 <0.00229 0.019	2.80 <0.01 0.093	2.90 0.017 1.60
CA 6.3.2/14 (17-2145) 17-2145-02 Portugal, P- 2070-330 Ereira Spring barley 2017	1 2	0.150 0.150	350 350	BBCH 52 BBCH 61	Green material Grain Straw	0 48 48	0.71 0.003 0.039	0.69 0.003 0.037	0.61 <0.00229 0.029	0.58 <0.00229 0.023	2.60 0.011 0.13	4.10 0.038 1.60

Table CA 6.3.2/14-2 Residue trials spiroxamine, trifloxystrobin and bixafen EC 325 in barley – scaled residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,2]					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[3]	
CA 6.3.2/14 (17-2145) 17-2145-01 Southern France, F- 30000 Nîmes Spring barley (Calcule) 2017	1 2	0.150 0.150	300 300	BBCH 30 BBCH 61	Green material Grain Straw	0 33 33	1.900 n.a. 0.070	1.875 n.a. 0.065	1.60 n.a. 0.050	1.575 n.a. 0.048	7.00 n.a. 0.233	7.25 0.043 4.00

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[2]					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[3]	
CA 6.3.2/14 (17-2145) 17-2145-02 Portugal, P- 2070-330 Ereira Spring barley (Pewter) 2017	1 2	0.150 0.150	350 350	BBCH 52 BBCH 61	Green material Grain Straw	0 48 48	1.775 0.008 0.098	1.725 0.008 0.093	1.525 n.a. 0.073	1.45 n.a. 0.063	6.50 0.028 0.325	10.25 0.095 4.00

1 – Residues scaled proportionally to a 0.375 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 – n.a. denotes not appropriate to scale up as reported value below the LOQ

Table CA 6.3.2/14-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/14	01480	A1 enantiomer	Barley green material	0.00271 0.0271 5.4 Overall	3 3 1 7	97; 98; 98 91; 94; 94 105 Mean: 97, RSD: 4.6
CA 6.3.2/14	01480	A1 enantiomer)	Barley grain	0.00271 0.0271 Overall	3 3 6	91; 92; 93 94; 96; 96 Mean: 94, RSD: 2.2
CA 6.3.2/14	01480	A1 enantiomer	Barley straw	0.00271 0.0271 0.271 Overall	3 3 1 7	104; 108; 112 94; 96; 97 93 Mean: 101, RSD: 7.4
CA 6.3.2/14	01480	A2 enantiomer	Barley green material	0.00271 0.0271 5.4 Overall	3 3 1 7	94; 94; 95 92; 92; 93 106 Mean: 95, RSD: 5.2
CA 6.3.2/14	01480	A2 enantiomer	Barley grain	0.00271 0.0271 Overall	3 3 6	89; 91; 91 93; 95; 96 Mean: 93, RSD: 2.9
CA 6.3.2/14	01480	A2 enantiomer	Barley straw	0.00271 0.0271 0.271 Overall	3 3 1 7	100; 101; 102 93; 94; 95 92 Mean: 97, RSD: 4.3
CA 6.3.2/14	01480	B1 enantiomer	Barley green material	0.00229 0.0229 4.6 Overall	3 3 1 7	97; 108; 111 101; 102; 103 108 Mean: 104, RSD: 5.5
CA 6.3.2/14	01480	B1 enantiomer	Barley grain	0.00229 0.0229 Overall	3 3 6	94; 96; 99 100; 101; 104 Mean: 99, RSD: 3.6
CA 6.3.2/14	01480	B1 enantiomer	Barley straw	0.00229 0.0229 0.229 Overall	3 3 1 7	105; 111; 115 95; 97; 104 98 Mean: 104, RSD: 7.2
CA 6.3.2/14	01480	B2 enantiomer	Barley green material	0.00229 0.0229 4.6 Overall	3 3 1 7	101; 103; 106 100; 100; 100 108 Mean: 103, RSD: 3.2
CA 6.3.2/14	01480	B2 enantiomer	Barley grain	0.00229 0.0229 Overall	3 3 6	91; 93; 94 94; 98; 98 Mean: 95, RSD: 3.0
CA 6.3.2/14	01480	B2 enantiomer	Barley straw	0.00229 0.0229 0.229 Overall	3 3 1 7	101; 102; 105 95; 97; 98 95 Mean: 99, RSD: 3.8

Table CA 6.3.2/14-4 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/14	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.10 5.1 Overall	4 3 1 8	94 (129); 97 (132); 108 (149); 118 (139)[1] 84 (88); 91 (95); 92 (96)[1] 89 Mean: 97, RSD: 11.5
CA 6.3.2/14	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.10 Overall	3 3 6	80 (92); 82 (210); 113 (227) 76 (77); 77 (78); 82 (83)[1] Mean: 80, RSD: 3.6
CA 6.3.2/14	00312/M002	Total residue of spiroxamine	Barley straw	0.01 0.10 1.0 2.0 Overall	3 3 1 1 8	91 (205); 96 (210); 113 (227)[1] 84 (95); 89 (100); 90 (101)[1] 94 (95)[1] 110[1] Mean: 96, RSD: 10.7

1 - These recoveries were background-corrected by the inherent amount of residues in the corresponding control samples. The uncorrected values are shown in brackets.

Table CA 6.3.2/14-5 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/14	Barley	141 to 202 (parent spiroxamine enantiomers) 294 and 366 (total residue of spiroxamine)

III. Conclusions

A total of two residue trials conducted in 2016 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on barley (green material, grain and straw) after two applications of bixafen, spiroxamine and trifloxystrobin EC 325 on barley BBCH 30-61.

Both trials were conducted as harvest trials. Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) were 6.50 and 7.00 mg/kg. At maturity, residues in grain 0.028 mg/kg in one trial and as reported data were below the LOQ for the other trial the values could not be scaled. Corresponding residues in straw were 0.23 and 0.33 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) were 7.25 and 10.25 mg/kg. At maturity, residues in grain were 0.04 and 0.10 mg/kg with corresponding residues in straw both at 4.00 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) were 6.50 and 7.00 mg/kg. At maturity, residues in grain 0.028 mg/kg in one trial and as reported data were below the LOQ for the other trial the values could not be scaled. Corresponding residues in straw were 0.23 and 0.33 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) were 7.25 and 10.25 mg/kg. At maturity, residues in grain were 0.04 and 0.10 mg/kg with corresponding residues in straw both at 4.00 mg/kg.

Data Point:	KCA 6.3.2/15
Report Author:	[redacted]
Report Year:	2018
Report Title:	Determination of the residues of prothioconazole and spiroxamine in/on barley after spraying of JAU 6476 & KWG 4168 EC 460 in the field in France (South), Spain, Italy and Portugal
Report No:	16-2162
Document No:	M-631606-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2016 are available to evaluate the magnitude of residues of spiroxamine in/on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in southern Europe (southern France, Spain, Italy and Portugal) with all four conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/15-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha, corresponding to 375 g a.s./ha and at a water application rate of 300 L/ha. Spray intervals were 20 to 21 days with the final treatment being made at growth stage BBCH 51-59. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 296.2 g/L spiroxamine).

Samples of green material were taken on the day of the final application, and between 61 to 74 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final application prior to the last application and 61 to 74 DALA

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617914-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2.15-4, the maximum storage time between date of deep-freezing and date of last extraction was 566 days before the analysis of the parent spiroxamine enantiomers, and 656 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/15-2 and Table CA 6.3.2/15-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as harvest trials, with straw and grain sampled between 61 and 74 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 7.30 to 14.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.011 mg/kg with corresponding residues in straw ranging from 0.10 to 0.51 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 9.10 to 13.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.018 mg/kg with corresponding residues in straw ranging from 1.40 to 4.60 mg/kg.

Table CA 6.3.2/15-1 Residue trials with spiroxamine and prothioconazole 460 EC in barley – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/15 (16-2162) 16-2162-01 Southern France, F-86200 Ceaux en Loudun Barley (Limpid) 2016	1 2	0.375 0.375	300 300	BBCH 32 BBCH 58	Green material Grain Straw	0 66 66	2.00 <0.00271 0.085	2.00 <0.00271 0.10	1.60 <0.00229 0.076	1.60 <0.00229 0.075	7.30 <u>0.01</u> 0.340	9.10 0.012 2.80
CA 6.3.2/15 (16-2162) 16-2162-02 Spain, E-41600 Arahal Barley (Odissey) 2016	1 2	0.375 0.375	300 300	BBCH 32 BBCH 53	Green material Grain Straw	0 64 64	3.90 <0.00271 0.160	3.90 <0.00271 0.150	3.20 <0.00229 0.110	3.20 <0.00229 0.099	14.0 <u>0.01</u> 0.510	15.00 <0.01 4.60
CA 6.3.2/15 (16-2162) 16-2162-03 Italy, I-01016 Tarquinia (VT) Barley (Mercur) 2016	1 2	0.375 0.375	300 300	BBCH 32 BBCH 59	Green material Grain Straw	0 61 61	2.40 0.003 0.032	2.40 0.003 0.030	1.90 0.00229 0.022	1.90 0.002 0.020	8.50 <u>0.011</u> 0.104	11.00 0.018 1.60
CA 6.3.2/15 (16-2162) 16-2162-04 Portugal, P-2000-210 Santarem Barley (Pewter) 2016	1 2	0.375 0.375	300 300	BBCH 34 BBCH 51	Green material Grain Straw	0 74 74	2.40 <0.00271 0.055	2.30 <0.00271 0.056	2.00 <0.00229 0.041	2.00 <0.00229 0.041	8.80 <u><0.010</u> 0.190	9.40 <0.01 1.40

1 – <u>Underlined</u> value to be used to support MRL for grain

Table CA 6.3.2/15-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/15	01480	A1 enantiomer	Barley green material	0.00271 0.0271 0.271 13.5 Overall	3 3 1 1 8	91; 96; 100 91; 92; 92 97 98 Mean: 95, RSD: 3.7
CA 6.3.2/15	01480	A1 enantiomer	Barley grain	0.00271 0.0271 0.271 Overall	3 3 1 7	97; 101; 105 92; 93; 93 99 Mean: 97, RSD: 5.0
CA 6.3.2/15	01480	A1 enantiomer	Barley straw	0.00271 0.0271 0.271 Overall	3 3 1 7	97; 103; 115 93; 93; 93 102 Mean: 99, RSD: 8.1
CA 6.3.2/15	01480	A2 enantiomer	Barley green material	0.00271 0.0271 0.271 13.5 Overall	3 3 1 1 8	91; 98; 101 90; 91; 92 97 99 Mean: 95, RSD: 4.6
CA 6.3.2/15	01480	A2 enantiomer	Barley grain	0.00271 0.0271 0.271 Overall	3 3 1 7	94; 99; 100 91; 91; 91 99 Mean: 95, RSD: 4.4
CA 6.3.2/15	01480	A2 enantiomer	Barley straw	0.00271 0.0271 0.271 Overall	3 3 1 7	97; 99; 118 92; 93; 95 103 Mean: 100, RSD: 9.0
CA 6.3.2/15	01480	B1 enantiomer	Barley green material	0.00229 0.0229 0.229 11.5 Overall	3 3 1 1 8	90; 96; 104 92; 92; 93 99 99 Mean: 96, RSD: 5.0
CA 6.3.2/15	01480	B1 enantiomer	Barley grain	0.00229 0.0229 0.229 Overall	3 3 1 7	95; 100; 102 92; 93; 95 103 Mean: 97, RSD: 4.6
CA 6.3.2/15	01480	B1 enantiomer	Barley straw	0.00229 0.0229 0.229 Overall	3 3 1 7	95; 97; 106 93; 93; 95 105 Mean: 98, RSD: 5.6
CA 6.3.2/15	01480	B2 enantiomer	Barley green material	0.00229 0.0229 0.229 11.5 Overall	3 3 1 1 8	90; 95 98 89; 89; 89 95 99 Mean: 93, RSD: 4.6
CA 6.3.2/15	01480	B2 enantiomer	Barley grain	0.00229 0.0229 0.229 Overall	3 3 1 7	95; 99; 102 88; 90; 91 100 Mean: 95, RSD: 5.8
CA 6.3.2/15	01480	B2 enantiomer	Barley straw	0.00229 0.0229	3 3	101; 103; 119 91; 91; 91

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
				0.229 Overall	1 7	101 Mean: 100, RSD: 10.2

Table CA 6.3.2/15-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/15	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.10 20 Overall	3 3 1 7	94; 97; 105 97; 104; 105 103 Mean: 101, RSD: 4.5
CA 6.3.2/15	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.10 Overall	3 3 6	81 (117); 93 (146); 120 (173) 92; 94; 104 Mean: 97, RSD: 13.7
CA 6.3.2/15	00312/M002	Total residue of spiroxamine	Barley straw	0.01 0.10 1.0 40 Overall	3 3 1 1 8	109 (217); 112 (220); 118 (226) 92 (102); 95 (105); 105 (115)[1] 103 84 Mean: 102, RSD: 11.0

1 - These recoveries were background-corrected since the control sample used for spiking were found to contain (apparent) residues with differing levels. The uncorrected recoveries are shown in brackets.

Table CA 6.3.2/15-4 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/15	Barley	479 to 566 (parent spiroxamine enantiomers) 570 to 656 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2016 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on barley BBCH 32-59.

All four of the trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 7.30 to 14.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.011 mg/kg with corresponding residues in straw ranging from 0.10 to 0.51 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 9.10 to 15.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.018 mg/kg with corresponding residues in straw ranging from 1.40 to 4.60 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 7.30 to 14.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.011 mg/kg with corresponding residues in straw ranging from 0.10 to 0.51 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 9.10 to 15.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.018 mg/kg with corresponding residues in straw ranging from 1.40 to 4.60 mg/kg.

Data Point:	KCA 6.3.2/16
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the residues of prothioconazole and spiroxamine in/on barley after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Italy, Spain and Portugal
Report No:	17-2159
Document No:	M-656996-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2017 are available to evaluate the magnitude of residues of spiroxamine in/on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in southern Europe (southern France, Spain, Italy and Portugal) with all four conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/16-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha, corresponding to 375 g a.s./ha at a water application rate of 300 to 350 L/ha. Spray intervals were 13 to 21 days with the final treatment being made at growth stage BBCH 61. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 294.2 g/L spiroxamine).

Samples of green material were taken on the day of the final application and between 28 to 48 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final application prior to the last application and 28 to 48 DALA.

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617914-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/16-4, the maximum storage time between date of deep-freezing and date of last extraction was 457 days before the analysis of the parent spiroxamine enantiomers, and 551 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/16-2 and Table CA 6.3.2/16-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the LOQ were detected in the control samples for spiroxamine (sum of enantiomers). For total spiroxamine (via 4-t-butylcyclohexanone), no residues above the LOQ were detected in the control samples of grain (all trials), however in green material 1 trial (17-2159-04) reported an apparent residue of 0.013 mg/kg total spiroxamine and in straw 3 trials (17-2159-01, 17-2159-03 and 17-2159-04) reported apparent residues of 0.018, 0.012 and 0.024 mg/kg total spiroxamine, respectively. The reason for the presence of 4-t-butylcyclohexanone total residues in these control samples of green material or straw could not be identified; however, considering the low levels seen and their relative concentrations when compared to the residues in treated samples (<1%), they did not appreciably affect the study results. In addition to this, the reported levels of both spiroxamine and prothioconazole in grain from trial 17-2159-02 and also from 17-2159-03 (especially prothioconazole for this trial) were attributed in the report to suspected contamination of harvested grain with non-typical amounts of straw material, probably due to insufficient cleaning of the sample grain specimens. Due to these concerns the data from these two trials are not considered for MRL purposes.

All four trials were conducted as harvest trials, with straw and grain sampled between 28 to 48 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 5.20 to 9.10 mg/kg. At maturity, residues in grain ranged from 0.01 to 0.14 mg/kg with corresponding residues in straw ranging from 0.32 to 0.42 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 7.60 to 14.00 mg/kg. At maturity, residues in grain ranged from 0.07 to 0.63 mg/kg with corresponding residues in straw ranging from 4.70 to 6.50 mg/kg.

Table CA 6.3.2/16-1 Residue trials with spiroxamine and prothioconazole 460 EC in barley – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/16 (17-2159) 17-2159-01 Southern France, F-30000 Nîmes Spring barley (Calcule) 2017	1 2	0.375 0.375	300 300	BBCH 30 BBCH 61	Green material Grain Straw	0 33 33	1.40 0.0027 0.090	1.40 0.0023 0.094	1.20 0.0023 0.068	1.10 0.0023 0.066	5.20 <u>0.010</u> 0.320	7.60 0.070 6.50
CA 6.3.2/16 (17-2159) 17-2159-02 Italy, I-95041 Caltagirone Winter barley (Asso) 2017	1 2	0.375 0.375	300 300	BBCH 54 BBCH 61	Green material Grain Straw	0 28 28	2.40 0.036 0.120	2.40 0.036 0.120	2.00 0.036 0.091	2.00 0.036 0.086	8.90 <u>0.144</u> 0.420	13.00 0.630 4.70
CA 6.3.2/16 (17-2159) 17-2159-03 Spain, E- 11140 Conil de la Frontera Winter barley (Pandora) 2017	1 2	0.375 0.375	350 350	BBCH 53 BBCH 61	Green material Grain Straw	0 41 41	2.60 0.0078 0.110	2.50 0.0081 0.110	2.10 0.0070 0.078	2.00 0.0064 0.075	9.10 0.029 0.380	14.00 0.350 5.10
CA 6.3.2/16 (17-2159) 17-2159-04 Portugal, P-2070-057 Cartaxo Winter barley (Pewter) 2017	1 2	0.375 0.375	350 350	BBCH 51 BBCH 61	Green material Grain Straw	0 48 48	2.00 0.0091 0.110	2.00 0.011 0.110	1.60 0.010 0.077	1.50 0.080 0.076	7.10 <u>0.038</u> 0.370	11.00 0.130 5.30

1 – Underlined value to be used to support MRL for grain
2 - Suspected contamination of harvested grain with non-typical amounts of straw material

Table CA 6.3.2/16-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/16	01480	A1 enantiomer	Barley green material	0.00271 0.0271 7.2 Overall	3 3 1 7	107; 108; 109 109; 109; 112 90 Mean: 106, RSD: 6.9
CA 6.3.2/16	01480	A1 enantiomer	Barley grain	0.00271 0.0271 0.05 Overall	3 3 1 7	99; 99; 100 110; 110; 110 104 Mean: 105, RSD: 5.1
CA 6.3.2/16	01480	A1 enantiomer	Barley straw	0.00271 0.0271 0.80 Overall	3 3 1 7	103; 105; 110 105; 107; 111 90 Mean: 104, RSD: 6.7
CA 6.3.2/16	01480	A2 enantiomer	Barley green material	0.00271 0.0271 7.2 Overall	3 3 1 7	97; 98; 99 108; 109; 110 103 Mean: 103, RSD: 5.4
CA 6.3.2/16	01480	A2 enantiomer	Barley grain	0.00271 0.0271 0.05 Overall	3 3 1 7	103; 103; 104 107; 107; 107 89 Mean: 103, RSD: 6.2
CA 6.3.2/16	01480	A2 enantiomer	Barley straw	0.00271 0.0271 0.80 Overall	3 3 1 7	100; 101; 106 107; 108; 113 89 Mean: 103, RSD: 7.5
CA 6.3.2/16	01480	B1 enantiomer	Barley green material	0.00229 0.0229 6.1 Overall	3 3 1 7	106; 108; 108 108; 110; 111 89 Mean: 106, RSD: 7.1
CA 6.3.2/16	01480	B1 enantiomer	Barley grain	0.00229 0.0229 0.05 Overall	3 3 1 7	98; 98; 100 106; 107; 109 102 Mean: 103, RSD: 4.4
CA 6.3.2/16	01480	B1 enantiomer	Barley straw	0.00229 0.0229 0.70 Overall	3 3 1 7	102; 102; 102 104; 107; 114 89 Mean: 103, RSD: 7.3
CA 6.3.2/16	01480	B2 enantiomer	Barley green material	0.00229 0.0229 6.1 Overall	3 3 1 7	100; 100; 103 106; 106; 108 91 Mean: 102, RSD: 5.6
CA 6.3.2/16	01480	B2 enantiomer	Barley grain	0.00229 0.0229 0.05 Overall	3 3 1 7	95; 97; 98 102; 103; 105 107 Mean: 101, RSD: 4.4
CA 6.3.2/16	01480	B2 enantiomer	Barley straw	0.00229 0.0229 0.70 Overall	3 3 1 7	96; 98; 101 101; 104; 104 88 Mean: 99, RSD: 5.7

Table CA 6.3.2/16-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/16	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.10 20 Overall	3 3 1 7	95 (158); 96 (159); 98 (161)[1] 100 (106); 101 (107); 108 (114)[1] 101 Mean: 101, RSD: 6.6
CA 6.3.2/16	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.10 1.0 Overall	3 3 3 9	91; 97; 105 102; 107; 110 99; 108; 110 Mean: 103, RSD: 6.3
CA 6.3.2/16	00312/M002	Total residue of spiroxamine	Barley straw	0.01 0.10 15 Overall	3 3 3 8	86 (179); 90 (183); 105 (198)[1] 93 (102); 100 (109); 105 (114)[1] 101; 108; 108 Mean: 100, RSD: 8.1

1 - These recoveries were background-corrected by the inherent amount of residues in corresponding control samples. The uncorrected recoveries are shown in brackets.

Table CA 6.3.2/16-4 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/16	Barley	396 to 457 (parent spiroxamine enantiomers) 490 to 551 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2017 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on barley BBCH 30-61.

All four trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 5.20 to 9.10 mg/kg. At maturity, residues in grain ranged from 0.01 to 0.14 mg/kg with corresponding residues in straw ranging from 0.32 to 0.42 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 7.60 to 14.00 mg/kg. At maturity, residues in grain ranged from 0.07 to 0.63 mg/kg with corresponding residues in straw ranging from 4.70 to 6.50 mg/kg.

The reported levels of both spiroxamine and prothioconazole in grain from trial 17-2159-02 and also from 17-2159-03 (especially prothioconazole for this trial) were attributed in the report to suspected contamination of harvested grain with non-typical amounts of straw material, probably due to insufficient cleaning of the sample grain specimens. Due to these concerns the data from these two trials are not considered for MRL purposes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 5.20 to 9.10 mg/kg. At maturity, residues in grain ranged from 0.01 to 0.14 mg/kg with corresponding residues in straw ranging from 0.32 to 0.42 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 7.60 to 14.00 mg/kg. At maturity, residues in grain ranged from 0.07 to 0.63 mg/kg with corresponding residues in straw ranging from 4.70 to 6.50 mg/kg.

Data Point:	KCA 6.3.2/17
Report Author:	[redacted]; [redacted]
Report Year:	2019
Report Title:	Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Hungary, the United Kingdom and northern France
Report No:	17-2016
Document No:	M-663690-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2017 are available to evaluate the magnitude of residues of spiroxamine in/on winter and spring barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 in northern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in northern Europe (Hungary, the United Kingdom, and northern France) with all four conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/17-1 and Table CA 6.3.2/17-2 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a nominal product rate of 1.25 L/ha, corresponding to 375 a.s. g/ha at a water application rate of 200 to 300 L/ha. For the trial conducted in the United Kingdom (17-2016-02), the second application was overdosed by 6.8% due to walking too slowly over the treated plot area, resulting in a product rate of 1.34 L/ha corresponding to 401 a.s. g/ha. This was not expected to impact the study as it was in the acceptable range for the study parameters. However, residues for this trial are scaled proportionally to a 0.375 kg a.s/ha application rate in line with the critical GAP and in compliance with the guidance on use of scaling / proportionality. Spray intervals were 21 days with the final treatment being made at growth stage BBCH 61. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 294.2 g/L spiroxamine).

Samples of green material were taken on the day of the final application and between 17 to 47 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final application prior to the last application and 17 to 47 DALA.

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/17-5, the maximum storage time between date of deep-freezing and date of last extraction was 429 days before the analysis of the parent spiroxamine enantiomers, and 516 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/17-3 and Table CA 6.3.2/17-4).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the LOQ were detected in the control samples for spiroxamine (sum of enantiomers). For total spiroxamine (via 4-t-butylcyclohexanone), no residues above the LOQ were detected in the control samples of grain (all trials), however in green material 2 trials (17-2016-01 and 17-2016-03) reported an apparent residue of 0.013 mg/kg total spiroxamine and in straw 3 trials (17-216-01, 17-2159-03 and 17-2159-04) reported apparent residues of 0.018, 0.012 and 0.024 mg/kg total spiroxamine, respectively. The reason for the presence of 4-t-butylcyclohexanone total residues in these control samples of green material or straw could not be identified; however, considering the low levels seen and their relative concentrations when compared to the residues in treated samples (<1%), they did not appreciably affect the study results. The residue levels determined in treated samples from these trials were considered to be valid.

All four trials were conducted as harvest trials, with straw and grain sampled between 17 to 47 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 5.30 to 10.3 mg/kg, the highest residue was from the UK trial and is therefore scaled to the nominal GAP. At maturity, residues in grain ranged from 0.024 to 0.040 mg/kg (again highest residue is scaled) with corresponding residues in straw ranging from 0.22 to 0.44 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 7.80 to 14.0 mg/kg, the highest residue was from the UK trial and is therefore scaled to the nominal GAP. At maturity, residues in grain ranged from 0.077 to 0.20 mg/kg (again highest residue is scaled) with corresponding residues in straw ranging from 3.30 to 5.60 mg/kg (lowest residue was scaled).

Table CA 6.3.2/17-1 Residue trials with spiroxamine and prothioconazole 460 EC in barley – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/17 (17-2016) 17-2016-01 Hungary, H- 9700 Szombathely Winter barley (GK Stramm) 2017	1 2	0.375 0.375	300 300	BBCH 55 BBCH 59	Green material Grain Straw	0 30 30	1.50 0.0066 0.13	1.40 0.0062 0.13	1.20 0.0056 0.096	1.20 0.0051 0.091	5.30 <u>0.024</u> 0.44	7.80 0.077 4.10
CA 6.3.2/17 (17-2016) 17-2016-02 United Kingdom, GB-IP312NG Pakenham Spring barley (CONCERTO) 2017	1 2	0.375 0.401	200 214	BBCH 43 BBCH 59	Green material Grain Straw	0 47 47	3.20 0.012 0.076	3.20 0.012 0.081	2.50 0.010 0.064	2.50 0.010 0.061	11.0 0.043 0.29	15.0 0.21 3.50
CA 6.3.2/17 (17-2016) 17-2016-03 Hungary, H- 9144 Kony Spring barley (GK Habzo) 2017	1 2	0.375 0.375	350 350	BBCH 49 BBCH 58	Green material Grain Straw	0 15 17	1.60 0.0071 0.10	1.60 0.0076 0.10	1.30 0.0065 0.078	1.20 0.0057 0.076	5.60 <u>0.027</u> 0.360	8.30 0.13 4.70
CA 6.3.2/17 (17-2016) 17-2016-04 Northern France, F-71570 La Chapelle de Guinchay Spring barley (Sebastian) 2017	1 2	0.375 0.375	350 350	BBCH 30 BBCH 61	Green material Grain Straw	0 42 42	2.10 0.0070 0.065	2.00 0.073 0.062	1.70 0.080 0.044	1.60 0.068 0.044	7.50 <u>0.029</u> 0.22	9.40 0.15 5.60

1 – <u>Underlined</u> value to be used to support MRL for grain

Table CA 6.3.2/17-2 Residue trials with spiroxamine and prothioconazole 460 EC in barley –scaled residue results for northern Europe

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1,2]	
CA 6.3.2/17 (17-2016) 17-2016-02 United Kingdom, GB-IP312NG Pakenham Spring barley 2017	1 2	0.375 0.401	200 214	BBCH 33 BBCH 59	Green material Grain Straw	0 47 47	3.0 0.011 0.071	3.0 0.011 0.076	2.34 0.0094 0.060	2.34 0.0094 0.057	10.30 <u>0.040</u> 0.27	14.0 0.20 3.30

1 – Residues scaled proportionally to a 0.375 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503.
2 – <u>Underlined</u> value to be used to support MRL for grain

Table CA 6.3.2/17-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/17	01480	A1 enantiomer	Barley green material	0.00271 0.0271 5.4 Overall	3 3 1 7	97 (121); 88 (122); 91 (125)[1] 101 (104); 101 (104); 104 (107) 95 Mean: 95, RSD: 7.2
CA 6.3.2/17	01480	A1 enantiomer	Barley grain	0.00271 0.0271 Overall	3 3 6	94; 94; 95 104; 105; 105 Mean: 100, RSD: 5.7
CA 6.3.2/17	01480	A1 enantiomer	Barley straw	0.00271 0.0271 0.80 Overall	3 3 1 7	105; 106; 109 104; 106; 106 87 Mean: 103, RSD: 7.1
CA 6.3.2/17	01480	A2 enantiomer	Barley green material	0.00271 0.0271 5.4 Overall	3 3 1 7	86 (122); 87 (123); 90 (126)[1] 97 (101); 103 (107); 103 (107)[1] 97 Mean: 95, RSD: 7.5
CA 6.3.2/17	01480	A2 enantiomer	Barley grain	0.00271 0.0271 Overall	3 3 6	90; 92; 92 101; 103; 106 Mean: 97, RSD: 7.0
CA 6.3.2/17	01480	A2 enantiomer	Barley straw	0.00271 0.0271 0.80 Overall	3 3 1 7	103; 105; 107 102; 102; 104 85 Mean: 101, RSD: 7.3
CA 6.3.2/17	01480	B1 enantiomer	Barley green material	0.00229 0.0229 4.6 Overall	3 3 1 7	86 (124); 86 (124); 91 (129)[1] 104 (108); 104 (108); 104 (108)[1] 95 Mean: 96, RSD: 8.7
CA 6.3.2/17	01480	B1 enantiomer	Barley grain	0.00229 0.0229 Overall	3 3 6	89; 91; 92 101; 105 106 Mean: 97, RSD: 7.8
CA 6.3.2/17	01480	B1 enantiomer	Barley straw	0.00229 0.0229 0.70 Overall	3 3 1 7	109; 109; 110 105; 106; 107 87 Mean: 105, RSD: 7.7
CA 6.3.2/17	01480	B2 enantiomer	Barley green material	0.00229 0.0229 4.6 Overall	3 3 1 7	89 (122); 90 (123); 90 (123)[1] 101 (104); 102 (105); 104 (107)[1] 92 Mean: 95, RSD: 6.9
CA 6.3.2/17	01480	B2 enantiomer	Barley grain	0.00229 0.0229 Overall	3 3 7	87; 88; 91 100; 100; 104 Mean: 95, RSD: 7.6
CA 6.3.2/17	01480	B2 enantiomer	Barley straw	0.00229 0.0229	3 3	106; 111; 111 100; 100; 102

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
				0.70 Overall	1 7	87 Mean: 102, RSD: 8.1

1 - These recoveries were background-corrected by the inherent amount of residues in corresponding control samples. The uncorrected recoveries are shown in brackets

Table CA 6.3.2/17-4 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/17	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.10 20 Overall	3 3 3 9	93 (132); 101 (140); 102 (141)[1] 86 (90); 98 (102); 98 (102)[1] 91; 95; 99 Mean: 96, RSD: 5.4
CA 6.3.2/17	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.10 1.0 Overall	3 3 3 9	96 (112); 98 (124); 100 (126)[1] 103 (106); 104 (107); 112 (115)[1] 99; 101; 104 Mean: 101, RSD: 6.9
CA 6.3.2/17	00312/M002	Total residue of spiroxamine	Barley straw	0.01 0.10 15 Overall	3 3 3 9	87 (137); 89 (139); 93 (143)[1] 93 (98); 93 (98); 95 (100)[1] 86 89; 93 Mean: 91, RSD: 3.5

1 - These recoveries were background-corrected by the inherent amount of residues in corresponding control samples. The uncorrected recoveries are shown in brackets

Table CA 6.3.2/17-5 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/17	Barley	367 to 429 (parent spiroxamine enantiomers) 435 to 516 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2017 in northern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on barley (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 400 on barley BBCH 33-61.

All four trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 5.30 to 10.3 mg/kg, the highest residue was from the UK trial and is therefore scaled to the nominal GAP. At maturity, residues in grain ranged from 0.024 to 0.040 mg/kg (again highest residue is scaled) with corresponding residues in straw ranging from 0.22 to 0.44 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 7.80 to 14.0 mg/kg, the highest residue was from the UK trial and is therefore scaled to the nominal GAP. At maturity, residues in grain ranged from 0.077 to 0.20 mg/kg (again highest residue is scaled) with corresponding residues in straw ranging from 3.30 to 5.60 mg/kg (lowest residue was scaled).

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 5.30 to 10.3 mg/kg, the highest residue was from the UK trial and is therefore scaled to the nominal GAP. At maturity, residues in grain ranged from 0.024 to 0.040 mg/kg (again highest residue is scaled) with corresponding residues in straw ranging from 0.22 to 0.44 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 7.80 to 14.0 mg/kg, the highest residue was from the UK trial and is therefore scaled to the nominal GAP. At maturity, residues in grain ranged from 0.077 to 0.20 mg/kg (again highest residue is scaled) with corresponding residues in straw ranging from 3.30 to 5.60 mg/kg (lowest residue was scaled).

Data Point:	KCA 6.3.2/18
Report Author:	[redacted]
Report Year:	2018
Report Title:	Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on spring barley and winter barley after spray application of bixafen & spiroxamine & trifloxystrobin EC 325 in Hungary and northern France
Report No:	17-2075
Document No:	M-638944-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500: Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Two residue trials conducted during growing season 2017 to evaluate the magnitude of residues of spiroxamine in/on barley (green material, grain and straw) after two applications of spiroxamine, bixafen and trifloxystrobin EC 325 in northern Europe. Samples were analysed for both spiroxamine

(comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of bixafen and trifloxystrobin were also analysed, however are not reported in this summary.

Two residue trials on barley were conducted in northern Europe (northern France and Hungary) with both conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/18-1 and Table CA 6.3.2/18-2.

Two spray applications of spiroxamine were made at a product rate of 1.00 L/ha, corresponding to 150 g a.s./ha spiroxamine at a water application rate of 300 or 250 L/ha. Spray intervals were 20 to 21 days with the final treatment being made at growth stage BBCH 59 to 61. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s./ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using bixafen and spiroxamine and trifloxystrobin EC 325, formulation containing 150 g/L spiroxamine (actual content 148.1 g/L spiroxamine).

Samples of green material were taken on the day of the final application, and 17 or 54 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last application and 17 or 54 DALA.

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers, A1, A2, B1 and B2, paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers were determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v)

and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/18-5, the maximum storage time between date of deep-freezing and date of last extraction was 184 days for spiroxamine enantiomers and 331 days for the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/18-3 and Table CA 6.3.2/18-4).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples, except the straw sample in trial 17-2075-01, which had 0.017 mg/kg total residue of spiroxamine (via 4-TBCH).

Both trials were conducted as harvest trials, with straw and grain sampled 17 or 54 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) were 4.25 and 7.50 mg/kg. At maturity, residues in grain were below the LOQ and could not be scaled. Corresponding residues in straw were 0.220 and 0.275 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) were 5.25 and 10.75 mg/kg. At maturity, residues in grain were 0.038 and 0.085 mg/kg with corresponding residues in straw at 1.63 and 2.25 mg/kg.

Table CA 6.3.2/18-1 Residue trials spiroxamine, trifloxystrobin and bixafen EC 325 in barley – reported residue results for northern Europe

Doc. No. Trial Ref Location Crop Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.2/18 (17-2075) 17-2075-01 Hungary, 8949 Mikekaracsonyfa Spring barley (GK Toma) 2017	1 2	0.150 0.150	300 300	BBCH 51 BBCH 59	Green material Grain Straw	0 15 17	0.83 <0.00271 0.033	0.81 <0.00271 0.033	0.68 <0.00229 0.022	0.68 <0.00229 0.022	3.00 <0.01 0.11	4.30 0.034 0.90
CA 6.3.2/18 (17-2075) 17-2075-02 Northern France, 63260 Pruns Winter barley (Campanile) 2017	1 2	0.150 0.150	250 250	BBCH 32 BBCH 61	Green material Grain Straw	0 54 54	0.46 <0.00271 0.026	0.46 <0.00271 0.026	0.38 <0.00229 0.018	0.38 <0.00229 0.018	1.70 <0.01 0.088	2.10 0.015 0.65

Table CA 6.3.2/18-2 Residue trials spiroxamine, trifloxystrobin and bixafen EC 325 in barley – scaled residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,2]					Scaled total residue of spiroxamine (via 4-TBC-H, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.2/18 (17-2075) 17-2075-01 Hungary, 8949 Mikekaracsonyfa Spring barley (GK Toma) 2017	1 2	0.150 0.150	300 300	BBCH 51 BBCH 59	Green material Grain Straw	0 17 17	2.075 n.a. 0.083	2.075 n.a. 0.083	1.700 n.a. 0.055	1.700 n.a. 0.055	7.50 n.a. 0.275	10.75 0.085 2.25
CA 6.3.2/18 (17-2075) 17-2075-02 Northern France, 63260 Pruns Winter barley (Campanile) 2017	1 2	0.150 0.150	250 250	BBCH 32 BBCH 61	Green material Grain Straw	0 54 54	1.150 n.a. 0.065	1.150 n.a. 0.065	0.950 n.a. 0.045	0.950 n.a. 0.045	4.25 n.a. 0.220	5.25 0.038 1.63

1 – Residues scaled proportionally to a 0.375 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 – n.a. denotes not appropriate to scale up as reported value below the LOQ

Table CA 6.3.2/18-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/18	01480	A1 enantiomer	Barley green material	0.00271 0.0271 0.271 5.4 Overall	3 3 1 1 8	88, 91, 92 86, 87, 88 93 92 Mean: 90, RSD: 3.0
CA 6.3.2/18	01480	A1 enantiomer)	Barley grain	0.00271 0.0271 0.271 Overall	3 3 1 7	90, 96, 97 86, 87, 92 98 Mean: 92, RSD: 5.3
CA 6.3.2/18	01480	A1 enantiomer	Barley straw	0.00271 0.0271 0.271 Overall	3 3 1 7	96, 98, 104 86, 89, 89 93 Mean: 94, RSD: 6.7
CA 6.3.2/18	01480	A2 enantiomer	Barley green material	0.00271 0.0271 0.271 5.4 Overall	3 3 1 1 8	84, 88, 89 85, 86, 87 92 93 Mean: 88, RSD: 3.6
CA 6.3.2/18	01480	A2 enantiomer	Barley grain	0.00271 0.0271 0.271 Overall	3 3 1 7	90, 93, 98 85, 85, 93 99 Mean: 92, RSD: 6.1
CA 6.3.2/18	01480	A2 enantiomer	Barley straw	0.00271 0.0271 0.271 Overall	3 3 1 7	97, 98, 100 87, 87, 89 92 Mean: 93, RSD: 5.9
CA 6.3.2/18	01480	B1 enantiomer	Barley green material	0.00229 0.0229 0.229 4.6 Overall	3 3 1 1 8	92, 93, 93 68, 70, 70 95 93 Mean: 84, RSD: 14.7
CA 6.3.2/18	01480	B1 enantiomer	Barley grain	0.00229 0.0229 0.229 Overall	3 3 1 7	72, 80, 85 69, 70, 77 97 Mean: 79, RSD: 12.7
CA 6.3.2/18	01480	B1 enantiomer	Barley straw	0.00229 0.0229 0.229 Overall	3 3 1 7	93, 89, 104 82, 85, 86 95 Mean: 92, RSD: 8.6
CA 6.3.2/18	01480	B2 enantiomer	Barley green material	0.00229 0.0229 0.229 4.6 Overall	3 3 1 1 8	68, 75, 78 99, 108, 110 94 94 Mean: 91, RSD: 17.1
CA 6.3.2/18	01480	B2 enantiomer	Barley grain	0.00229 0.0229 0.229 Overall	3 3 1 7	104, 105, 108 102, 103, 107 105 Mean: 105, RSD: 2.0
CA 6.3.2/18	01480	B2 enantiomer	Barley straw	0.00229 0.0229	3 3	95, 99, 110 91, 91, 93

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
				0.229 Overall	1 7	94 Mean: 96, RSD: 7.0

Table CA 6.3.2/18-4 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/18	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.10 5.1 Overall	3 3 1 7	84 (131); 94 (181); 108 (195) 88 (97); 91 (100); 94 (103)[1] 90 Mean: 93, RSD: 8.2
CA 6.3.2/18	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.10 Overall	3 3 6	91 (128); 94 (131); 102 (139) 78 (82); 82 (86); 86 (90) Mean: 89, RSD: 9.8
CA 6.3.2/18	00312/M002	Total residue of spiroxamine	Barley straw	0.01 0.10 1.0 Overall	3 3 1 7	77 (252); 104 (279); 107 (282)[1] 85 (103); 86 (104); 92 (110)[1] 85 (87)[1] Mean: 91, RSD: 12.0

1 - These recoveries were background-corrected by the inherent amount of residues in the corresponding control samples. The uncorrected values are shown in brackets.

Table CA 6.3.2/18-5 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/18	Barley	128 to 184 (parent spiroxamine enantiomers) 277 and 331 (total residue of spiroxamine)

III. Conclusions

A total of two residue trials conducted in 2017 in northern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on barley (green material, grain and straw) after two applications of bixafen, spiroxamine and trifloxystrobin EC 325 on barley BBCH 32-61.

Both trials were conducted as harvest trials. Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) were 4.25 and 7.50 mg/kg. At maturity, residues in grain were below the LOQ and could not be scaled. Corresponding residues in straw were 0.220 and 0.275 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) were 5.25 and 10.75 mg/kg. At maturity, residues in grain were 0.038 and 0.085 mg/kg with corresponding residues in straw at 1.63 and 2.25 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) were 4.25 and 7.50 mg/kg. At maturity, residues in grain were below the LOQ and could not be scaled. Corresponding residues in straw were 0.220 and 0.275 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) were 5.25 and 10.75 mg/kg. At maturity, residues in grain were 0.038 and 0.085 mg/kg with corresponding residues in straw at 1.63 and 2.25 mg/kg.

Data Point:	KCA 6.3.2/19
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain
Report No:	E19RP003
Document No:	M-683313-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Seven trials conducted during growing season 2019 are available to evaluate the magnitude of residues of spiroxamine in/on barley (green material, grain and straw) after two applications of spiroxamine, prothioconazole and trifloxystrobin EC 280.3 in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole and trifloxystrobin were also analysed, however are not reported in this summary.

Seven residue trials on barley were conducted in southern Europe (southern France, Spain, Italy and Greece) with all seven trials conducted as semi-decline trials. The trial parameters and residue results are summarised in Table CA 6.3.2/19-1 and Table CA 6.3.2/19-2 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a nominal product rate of 1.50 L/ha, corresponding to 160.5 g a.s./ha at a water application rate of 288 to 401 L/ha. Spray intervals were 18 to 32 days with the final treatment being made at growth stage BBCH 61. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using spiroxamine, prothioconazole and trifloxystrobin EC 280.3 formulation containing 107 g/L spiroxamine (actual content 107.4 g/L spiroxamine).

Samples of green material were taken on the day of the final application and again between 7 and 22 DALA on 1 to 3 occasions and then straw and grain were sampled between 33 to 64 DALA (PHI is growth stage dependent). Control samples were taken on the day of final application prior to the last application and 33 to 64 DALA.

Samples of barley (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-

phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/19-5, the maximum storage time between date of deep-freezing and date of last extraction was 126 to 208 days before the analysis of the parent spiroxamine enantiomers, and 143 to 235 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/19-3 and Table CA 6.3.2/19-4).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the LOQ were detected in the control samples for spiroxamine (sum of enantiomers). For total spiroxamine (via 4-t-butylcyclohexanone), no residues above the LOQ were detected in the control samples of grain (all trials), however in green material 1 trial (E19RP003-04) reported an apparent residue of 0.01 mg/kg total spiroxamine and in straw 1 trial (E19RP003-01) reported apparent residues of 0.01 mg/kg total spiroxamine. The reason for the presence of 4-t-butylcyclohexanone total residues in these control samples of green material or straw could not be identified; however, considering the low levels seen and their relative concentrations when compared to the residues in treated samples (≤0.5%), they did not affect the study results.

All seven trials were conducted as semi-decline trials, with samples of green material sampled between 7 and 22 DALA on 1 to 3 occasions and then straw and grain sampled between 33 to 64 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 4.48 to 10.95 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.048 mg/kg with corresponding residues in straw ranging from 0.060 to 0.693 mg/kg.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 4.953 to 11.058 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.159 mg/kg with corresponding residues in straw ranging from 1.331 to 4.299 mg/kg.

For the samples of barley whole plant (green material) taken between 7 and 22 DALA, scaled residues of spiroxamine (sum of all enantiomers) and total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) all declined from the reported values 0 DALA.

Table CA 6.3.2/19-1 Residue trials with spiroxamine, prothioconazole and trifloxystrobin 280.3 EC in barley – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/19 (E19RP003) E19RP003-01 Southern France, 31330 St Caprais Barley (Etincel) 2019	1 2	0.162 0.159	303 297	BBCH 39 BBCH 61	Green material Green material Grain Straw	0 17 33 33	0.56 0.086 <0.00271 0.031	0.56 0.088 <0.00271 0.034	0.46 0.066 <0.00229 0.030	0.45 0.068 <0.00229 0.028	2.0 0.34 <0.01 0.12	2.1 0.68 0.024 0.78
CA 6.3.2/19 (E19RP003) E19RP003-02 Southern France, 13103St Etienne du Gres Barley (Rafaela) 2019	1 2	0.158 0.159	295 298	BBCH 37 BBCH 61	Green material Green material Green material Green material Grain Straw	0 8 14 21 64 64	0.53 0.068 0.066 0.041 <0.00271 0.012	0.53 0.068 0.066 0.043 <0.00271 0.012	0.43 0.052 0.051 0.030 <0.00229 0.008	0.42 0.054 0.051 0.031 <0.00229 0.008	1.9 0.24 0.23 0.14 <0.01 0.040	2.3 0.65 0.75 0.48 <0.01 0.66
CA 6.3.2/19 (E19RP003) E19RP003-03 Italy, 40128 Bologna Barley (Lutece) 2019	1 2	0.166 0.169	393 401	BBCH 32 BBCH 61	Green material Green material Grain Straw	0 16 55 55	0.71 0.070 <0.00271 0.015	0.71 0.071 <0.00271 0.016	0.60 0.056 <0.00229 0.012	0.59 0.057 <0.00229 0.011	2.6 0.25 <0.01 0.054	3.5 0.50 0.037 0.60
CA 6.3.2/19 (E19RP003) E19RP003-04 Italy, 01016 Tarquinia (VT) Barley (Sunshine) 2019	1 2	0.154 0.157	288 292	BBCH 32 BBCH 61	Green material Green material Grain Straw	0 14 49 49	0.78 0.098 <0.00271 0.048	0.78 0.099 <0.00271 0.049	0.64 0.073 <0.00229 0.038	0.65 0.073 <0.00229 0.035	2.9 0.34 <0.01 0.17	3.3 0.70 0.016 1.3

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-HBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/19 (E19RP003) E19RP003-05 Greece, 58005 Agrosikia Barley (Colorado) 2019	1 2	0.164 0.157	307 293	BBCH 35 BBCH 61	Green material Green material Green material Green material Grain Straw	0 7 14 22 39 39	1.0 0.19 0.12 0.093 0.006 0.082	0.99 0.19 0.13 0.094 0.006 0.088	0.81 0.15 0.090 0.072 0.004 0.060	0.81 0.15 0.090 0.070 0.004 0.057	3.6 0.69 0.43 0.33 0.020 0.29	2.8 1.3 0.79 0.80 0.063 1.8
CA 6.3.2/19 (E19RP003) E19RP003-06 Spain, 41610 Paradas Barley (RGT planet) 2019	1 2	0.155 0.161	289 300	BBCH 37 BBCH 61	Green material Green material Green material Grain Straw	0 7 13 60 60	1.3 0.12 0.090 <0.00271 0.053	1.3 0.12 0.091 <0.00271 0.054	1.1 0.093 0.069 <0.00229 0.038	1.1 0.096 0.071 <0.00229 0.036	4.7 0.43 0.32 <0.01 0.18	4.7 0.99 0.59 0.010 1.0
CA 6.3.2/19 (E19RP003) E19RP003-07 Greece, 54100 Lagkadas Barley (Colorado) 2019	1 2	0.161 0.156	300 292	BBCH 35 BBCH 61	Green material Green material Green material Green material Grain Straw	0 7 14 22 50 50	0.95 0.15 0.090 0.046 0.003 0.008	0.95 0.15 0.091 0.048 0.003 0.008	0.76 0.11 0.066 0.032 <0.00229 0.005	0.74 0.12 0.069 0.034 <0.00229 0.005	3.4 0.53 0.32 0.16 0.011 0.025	4.6 1.4 1.0 1.0 0.066 0.68

Table CA 6.3.2/19-2 Residue trials with spiroxamine, prothioconazole and trifloxystrobin 280.3 EC in barley – scaled residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,2]					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/19 (E19RP003) E19RP003-01 Southern France, 31330 St Caprais Barley (Etincel) 2019	1 2	0.162 0.159	303 297	BBCH 39 BBCH 61	Green material Green material Grain Straw	0 17 33 33	1.321 0.203 n.a. 0.073	1.321 0.208 n.a. 0.080	1.085 0.156 n.a. 0.071	1.061 0.160 n.a. 0.066	4.717 0.731 n.a. 0.283	4.953 1.604 0.057 1.840
CA 6.3.2/19 (E19RP003) E19RP003-02 Southern France, 13103St Etienne du Gres Barley (Rafaela) 2019	1 2	0.158 0.159	295 298	BBCH 37 BBCH 61	Green material Green material Green material Green material Grain Straw	0 8 14 21 64 64	1.250 0.160 0.156 0.097 n.a. 0.028	1.250 0.160 0.156 0.099 n.a. 0.028	1.014 0.123 0.120 0.071 n.a. 0.019	0.991 0.127 0.120 0.073 n.a. 0.019	4.481 0.566 0.542 0.330 n.a. 0.094	5.425 1.533 1.769 1.132 <0.01 1.557
CA 6.3.2/19 (E19RP003) E19RP003-03 Italy, 40128 Bologna Barley (Lutece) 2019	1 2	0.166 0.169	393 401	BBCH 32 BBCH 61	Green material Green material Grain Straw	0 16 55 55	1.575 0.155 n.a. 0.033	1.575 0.158 n.a. 0.036	1.331 0.124 n.a. 0.027	1.309 0.126 n.a. 0.024	5.769 0.555 n.a. 0.120	7.766 1.109 0.082 1.331
CA 6.3.2/19 (E19RP003) E19RP003-04 Italy, 01016 Tarquinia (VT) Barley (Sunshine) 2019	1 2	0.154 0.157	288 292	BBCH 32 BBCH 61	Green material Green material Grain Straw	0 14 49 49	1.863 0.234 n.a. 0.115	1.863 0.236 n.a. 0.117	1.529 0.174 n.a. 0.091	1.553 0.174 n.a. 0.084	6.927 0.812 n.a. 0.406	7.882 1.672 0.038 3.105

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[2]					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.2/19	1	0.164	307	BBCH 35	Green material	0	2.389	2.365	1.935	1.935	8.599	6.688
(E19RP003)	2	0.157	293	BBCH 61	Green material	7	0.454	0.454	0.358	0.358	1.648	3.105
E19RP003-05					Green material	14	0.287	0.311	0.215	0.215	1.027	1.887
Greece, 58005					Green material	22	0.222	0.225	0.172	0.167	0.788	1.911
Agrosikia					Grain	39	0.014	0.014	0.010	0.010	0.048	0.150
Barley (Colorado)					Straw	39	0.196	0.210	0.143	0.136	0.693	4.299
2019												
CA 6.3.2/19	1	0.155	289	BBCH 37	Green material	0	3.028	3.028	2.562	2.562	10.947	10.947
(E19RP003)	2	0.161	300	BBCH 61	Green material	7	0.280	0.280	0.217	0.224	1.002	2.306
E19RP003-06					Green material	13	0.210	0.212	0.161	0.163	0.745	1.374
Spain, 41610 Paradas					Grain	60	n.a.	n.a.	n.a.	n.a.	n.a.	0.023
Barley (RGT planet)					Straw	60	0.123	0.126	0.089	0.084	0.419	2.329
2019												
CA 6.3.2/19	1	0.161	300	BBCH 35	Green material	0	2.284	2.284	1.827	1.779	8.173	11.058
(E19RP003)	2	0.156	292	BBCH 61	Green material	7	0.361	0.361	0.264	0.288	1.274	3.365
E19RP003-07					Green material	14	0.216	0.219	0.159	0.166	0.769	2.404
Greece, 54100					Green material	22	0.111	0.115	0.077	0.082	0.385	2.404
Lagkadas					Grain	50	0.007	0.007	n.a.	n.a.	0.026	0.159
Barley (Colorado)					Straw	50	0.019	0.019	0.012	0.012	0.060	1.635
2019												

1 – Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 – n.a. denotes not appropriate to scale up as reported value below the LOQ
3 – Underlined value to be used to support MRL for grain

Table CA 6.3.2/19-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/19	01480	A1 enantiomer	Barley green material	0.00271 0.136 2.7 Overall	3 3 3 9	82; 83; 83 98; 99; 99 105; 105; 107 Mean: 96, RSD: 10.7
CA 6.3.2/19	01480	A1 enantiomer	Barley grain	0.00271 0.136 Overall	3 3 6	83; 84; 85 98; 98; 99 Mean: 91, RSD: 8.6
CA 6.3.2/19	01480	A1 enantiomer	Barley straw	0.00271 0.136 Overall	3 3 6	70; 71; 74 93; 94; 95 Mean: 83, RSD: 14.9
CA 6.3.2/19	01480	A2 enantiomer	Barley green material	0.00271 0.136 2.7 Overall	3 3 3 9	79; 81; 82 98; 99; 99 106; 106; 109 Mean: 96.6, RSD: 11.1
CA 6.3.2/19	01480	A2 enantiomer	Barley grain	0.00271 0.136 Overall	3 3 6	88; 88; 89 96; 98; 98 Mean: 93, RSD: 5.4
CA 6.3.2/19	01480	A2 enantiomer	Barley straw	0.00271 0.136 Overall	3 3 6	72; 75; 78 92; 93; 94 Mean: 84, RSD: 12.0
CA 6.3.2/19	01480	B1 enantiomer	Barley green material	0.00229 0.115 2.3 Overall	3 3 3 9	80; 81; 81 96; 96; 97 107; 107; 110 Mean: 95, RSD: 12.5
CA 6.3.2/19	01480	B1 enantiomer	Barley grain	0.00229 0.115 Overall	3 3 6	84; 85; 86 93; 94; 97 Mean: 90, RSD: 6.1
CA 6.3.2/19	01480	B1 enantiomer	Barley straw	0.00229 0.115 Overall	3 3 6	76; 78; 80 93; 93; 94 Mean: 86, RSD: 9.9
CA 6.3.2/19	01480	B2 enantiomer	Barley green material	0.00229 0.115 2.3 Overall	3 3 3 9	80; 80; 81 97; 98; 99 106; 106; 110 Mean: 95, RSD: 12.5
CA 6.3.2/19	01480	B2 enantiomer	Barley grain	0.00229 0.115 Overall	3 3 6	78; 80; 81 95; 96; 96 Mean: 88, RSD: 10.0
CA 6.3.2/19	01480	B2 enantiomer	Barley straw	0.00229 0.115 Overall	3 3 6	70; 74; 75 91; 93; 93 Mean: 83, RSD: 13.0

Table CA 6.3.2/19-4 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/19	00312/M002	Total residue of spiroxamine	Barley green material	0.01 0.50 8.9 Overall	3 3 3 9	73 (112); 79 (118); 86 (125)[1] 85(86); 86(87); 89(90) 89; 91; 92 Mean: 85, RSD: 7.0

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/19	00312/M002	Total residue of spiroxamine	Barley grain	0.01 0.50 Overall	3 3 6	86 (102); 88 (104); 102 (118)[1] 99; 103; 107 Mean: 97.5, RSD: 8.8
CA 6.3.2/19	00312/M002	Total residue of spiroxamine	Barley straw	0.01 1.0 3.0 Overall	3 3 3 9	97; 105; 117 81; 84; 90 74; 90; 93 Mean: 92, RSD: 14.0

1 - These recoveries were background-corrected by the inherent amount of residues in corresponding control samples. The uncorrected recoveries are shown in brackets.

Table CA 6.3.2/19-5 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/19	Barley green material	158 to 208 (parent spiroxamine enantiomers) 185 to 235 (total residue of spiroxamine)
CA 6.3.2/19	Barley grain	126 to 147 (parent spiroxamine enantiomers) 142 to 164 (total residue of spiroxamine
CA 6.3.2/19	Barley straw	126 to 147 (parent spiroxamine enantiomers) 147 to 168 (total residue of spiroxamine

III. Conclusions

A total of seven residue trials conducted in 2019 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on barley (green material, grain and straw) after two applications of spiroxamine, prothioconazole and trifloxystrobin EC 280 3 on barley BBCH 32-61.

All seven trials were conducted as semi-decline trials. Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 4.48 to 10.95 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.048 mg/kg with corresponding residues in straw ranging from 0.060 to 0.693 mg/kg.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 4.953 to 11.058 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.159 mg/kg with corresponding residues in straw ranging from 1.331 to 4.299 mg/kg.

For the samples of barley whole plant (green material) taken between 7 and 22 DALA, scaled residues of spiroxamine (sum of all enantiomers) and total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) all declined from the reported values 0 DALA.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in barley whole plant (green material) ranged from 4.48 to 10.95 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.048 mg/kg with corresponding residues in straw ranging from 0.060 to 0.693 mg/kg.

The total scaled residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in barley whole plant (green material) ranged from 4.953 to 11.058 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.159 mg/kg with corresponding residues in straw ranging from 1.331 to 4.299 mg/kg.

Data Point:	KCA 6.3.2/01
Report Author:	[redacted]
Report Year:	1999
Report Title:	Determination of residues of KWG 4168 & ICIA 5504 400 SE in/on spring barley and winter wheat following spray application in Germany, Sweden and Great Britain
Report No:	RA-2002/97
Document No:	M-010782-01-1
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Four trials conducted during growing season 1997 are available to evaluate the magnitude of residues of spiroxamine in/on spring barley and winter wheat (green material, ear, straw and grain) after two applications of spiroxamine and azoxystrobin 400 SE to cereals in northern Europe. The two trials on barley are summarised here. Samples were analysed for spiroxamine and for grain only as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of azoxystrobin were also analysed, however are not reported in this summary.

Two residue trials on barley were conducted in northern Europe (Germany and United Kingdom) with both performed as semi-decline trials. The trial parameters and residue results are summarised in Table CA 6.3.2/01-1.

Two spray applications of spiroxamine were made at a product rate of 1.5 L/ha, corresponding to 400 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 17 to 22 days, with the final treatment being made at growth stage BBCH 57 to 63 which is slightly beyond the critical GAP of BBCH 61 and therefore presents a worst case. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using spiroxamine and azoxystrobin 400 SE formulation containing 267 g/L spiroxamine (actual content 267.3 g/L spiroxamine [R703796 first application only] and 262.7 g/L spiroxamine).

Samples of green material were taken on the day of the final application, with ear and straw samples taken 35 days after the last application (DALA), and mature grain and straw samples taken at 49 DALA. Control samples were taken on the day of final treatment prior to the last application and 49 DALA.

Samples of barley (grain, ear, green material and straw) were analysed for spiroxamine and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone (mature grain only).

Spiroxamine (parent compound)

The samples of green material, ear, grain and straw were analysed for spiroxamine parent compound. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00506 and its supplement 00506/E001, report reference M-020658-01-1 and M-020694-01-1, respectively (**see Doc MCA Section 4)**. The LOQ for spiroxamine was 0.05 mg/kg.

Sample were extracted with acetone/water (3/1; v/v) using a high-speed blender. After filtration and rinsing, an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on a RP 18 column with elution of spiroxamine in methanol/ammonia (999/1; v/v). Residues were determined by LC-GC/MS (DB 1701 capillary column) using external standardisation.

Total spiroxamine (common moiety approach)

The samples of green material, ear, straw and grain were analysed for the residue of total spiroxamine. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00312, report reference M-018352-02-1 (**see Doc MCA Section 4**). The LOQ for residues of total spiroxamine was 0.05 mg/kg.

Samples were extracted and simultaneously hydrolysed by refluxing with a methanol/1M hydrochloric acid (1/1; v/v) mixture. The total residue of spiroxamine itself and all metabolites containing the 4-t-butylcyclohexanone moiety, was hydrolysed yielding 4-t-butylcyclohexanone. After filtration an aliquot of the extract was diluted with water and clean-up using a RP-18 chromatography column. Any 4-t-butylcyclohexanone was eluted from the column with dichloromethane. Extracts were subject to final clean-up using a silica SPE cartridge eluting any 4-t-butylcyclohexanone with dichloromethane. After addition of n-heptane and careful removal of solvent by rotary evaporation under vacuum, the 4-t-butylcyclohexanone was determined by LC-GC/MS (Ultra 2 capillary column) using external standardisation.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/01-3, the maximum storage time between date of deep-freezing and date of last extraction was 397 days for spiroxamine parent and 311 days for total spiroxamine residues.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.2/01-2). The limit of quantification (LOQ) for spiroxamine and total residue of spiroxamine from both methods was 0.05 mg/kg.

No residues above the respective LOQ were detected in the control samples.

Both trials were conducted as semi-decline trials, with green material sampled on 0 DALA, ears and straw sampled 35 DALA and mature grain and straw sampled 49 DALA.

Scaled residues of spiroxamine in green material were 7.06 and 7.09 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 49 DALA with corresponding scaled residues in straw of 0.27 and 0.55 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Reported or scaled residues of total spiroxamine in grain 49 DALA were <0.05 mg/kg.

Table CA 6.3.2/01-1 Residue trials with spiroxamine and azoxystrobin 400 SE in barley – residue results for northern Europe (field)

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine (parent compound)		Total residue of spiroxamine (via 4-TBCH)	
							Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.2/01 (RA-2002/97) 703796 Germany, D-51399 Burscheid Spring barley (Scarlett) 1997	1 2	0.400 0.400	300 300	BBCH 32 BBCH 61	Green material Ear Straw Straw Grain	0 35 35 49 49	7.53 0.068 0.354 0.588 <0.050	7.06 0.06 0.33 0.55 <0.05	<0.05	<0.05
CA 6.3.2/01 (RA-2002/97) 703826, GB-IP31 3SH, Bury St. Edmunds Thurston Spring barley (Cooper) 1997	1 2	0.400 0.400	300 300	BBCH 32 BBCH 57-63	Green material Ear Straw Straw Grain	0 35 35 49 49	7.56 0.05 0.543 0.289 <0.050	7.09 0.05 0.51 0.27 <0.05	<0.05	<0.05

1 – Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503

Table CA 6.3.2/01-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residue of spiroxamine

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/01	00506 and 00506/E001	Spiroxamine (parent compound)	Barley green material	0.05 0.50 5.0 Overall	3 4 3 10	81; 81; 79 92; 96; 91; 87 97; 97; 100 Mean: 90, RSD: 8.5
CA 6.3.2/01	00506 and 00506/E001	Spiroxamine (parent compound)	Barley straw	0.05 0.50 5.0 Overall	3 4 3 10	92; 91; 95 104; 105; 109; 80 104; 109; 116 Mean: 101, RSD: 10.7
CA 6.3.2/01	00506 and 00506/E001	Spiroxamine (parent compound)	Barley grain/ear	0.05 0.50 Overall	2 1 3	84; 73 88 Mean: 82, RSD: 9.5
CA 6.3.2/01	00312	Total residue of spiroxamine	Barley grain	0.05 Overall	3 3	104; 93; 96 Mean: 98, RSD 5.8

Table CA 6.3.2/01-3 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/01	Barley	328 to 397 (spiroxamine parent) 306 to 311 (spiroxamine total residues)

III. Conclusions

A total of two residue trials conducted in 1997 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in/on barley green material, ear, straw and grain harvested after two applications of spiroxamine and azoxystrobin SE 400 on barley BBCH 32 to 63.

Both trials were conducted as semi-decline trials. Scaled residues of spiroxamine in green material were 7.06 and 7.09 mg/kg, 0 DALA. In mature grain, residues were <0.05 mg/kg 49 DALA with corresponding scaled residues in straw of 0.27 and 0.55 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Scaled residues of total spiroxamine in grain 49 DALA were <0.05 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/07. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine in green material were 7.06 and 7.09 mg/kg, 0 DALA. In mature grain, residues were <0.05 mg/kg 49 DALA with corresponding scaled residues in straw of 0.27 and 0.55 mg/kg. Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Scaled residues of total spiroxamine in grain 49 DALA were <0.05 mg/kg.

Data Point:	KCA 6.3.2/02
Report Author:	[redacted]
Report Year:	1997
Report Title:	Determination of residues of KWG 4168 500 EC in/on winter rye, winter wheat, spring barley, spring wheat and winter barley under actual use conditions in England, France and Germany
Report No:	RA-2079/93
Document No:	M-010690-01-2
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Part 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Eight trials conducted during growing season 1993 are available to evaluate the magnitude of residues of spiroxamine in/on winter rye, winter wheat, spring barley, spring wheat and winter barley (green material, ear, straw and grain) after two applications of Spiroxamine 500 EC at 1.5 L/ha to cereals in northern Europe, 750 g a.s./ha. The three trials on barley are summarised here. Samples were analysed for the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grain or straw of total spiroxamine equivalents (as 4-t-butylcyclohexanone) as the contribution from spiroxamine alone (CF = x0.23 for cereal grain and CF = x0.17 for straw was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.2.1 ‘Derivation of conversion factors for cereals’). Although

currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for cereals where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Three trials on barley were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 49 and 66 DALA. Total residues of spiroxamine in green material ranged from 11 to 13 mg/kg, 0 DALA, declining to between 1.6 to 4.2 mg/kg in mature straw and between <0.05 to 0.30 mg/kg in mature grain, 49 to 66 DALA.

Table CA 6.3.2/02-1 Residue trials with spiroxamine 500 EC in barley – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**	**Active substance**	:	**spiroxamine**
Country	: **Germany**	**Crop/Crop Group**	:	**Cereals**
Content of active substance (g/kg or g/L)	: **500 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	: **500 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	: **Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	: **Bayer AG**	**Residues determined as**	:	**4-t-butylcyclohexanone**
		Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2079/93 30125/6 0125-93 Germany 51399 Burscheid 1993	Barley, spring Marina	1) 19.03.1993 2) 11.06.1993 - 13.06.1993 3) 02.08.1993	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	12.05.1993/0 07.08.1993/26	Begin of flowering	green material straw ear grain	0.27 11 2.4 1.6 4.2 0.61 0.10 0.07	0** 0 35< 43 56 35<< 43 56	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**
Crop/Crop Group	:	**Cereals**
Page	:	**2**
Indoor/outdoor	:	**Outdoor**
Other a.s. in formulation (common name and content)	:	
Residues determined as	:	**4-t-butylcyclohexanone**
Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2079/93 30260/0 0260-93 France, north 27630 Molincourt 1993	Barley, winter Systel	1) 14.10.1992 2) 17.05.1993 - 28.05.1993 3) 30.06.1993	SPI SPI	0.7500 0.7500	280 280	0.26800 0.26800	20.04.1993/0 12.05.1993/2	51	green material ear grain straw	1.3 13 2.9 1.7 1.9 0.38 0.30 3.0	0** 0 14 28 35 35 49< 49<<	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**
Crop/Crop Group	:	**Cereals**
Page	:	**2**
Indoor/outdoor	:	**Outdoor**
Other a.s. in formulation (common name and content)	:	
Residues determined as	:	**4-t-butylcyclohexanone**
Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2079/93 30004/7 0004-93 United Kingdom IP 31, 3 SJ / Thurston, Bury ST. Edmunds 1993	Barley, winter Marinka	1) 13.10.1992 3) 23.07.1993	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	21.04.1993/0 18.05.1993/27	49	green material ear grain straw	0.66 12 2.4 1.5 1.5 <0.05 <0.05 <0.05 1.6	0** 0 14 28 35 28 35 66 66	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last

(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of three residue trials conducted in 1993 in northern Europe are available to evaluate the residues of total spiroxamine in/on barley green material, ear, straw and grain harvested after two application of Spiroxamine 500 EC on barley BBCH 31-61.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Three trials on barley were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 49 to 66 DALA. Total residues of spiroxamine in green material ranged from 11 to 13 mg/kg, 0 DALA, declining to between 1.6 to 4.2 mg/kg in mature straw and between <0.05 to 0.30 mg/kg in mature grain, 49 to 66 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B5 RAR (2010), IIA 6.3.2/04. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Three trials on barley were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 49 to 66 DALA. Total residues of spiroxamine in green material ranged from 11 to 13 mg/kg, 0 DALA, declining to between 1.6 to 4.2 mg/kg in mature straw and between <0.05 to 0.30 mg/kg in mature grain, 49 to 66 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.2.2/03
Report Author:	[redacted]
Report Year:	1995
Report Title:	Determination of residues of HWG 1608 & KWG 4168 383 EW in/on spring barley, spring wheat, winter rye, winter wheat and winter barley in the Federal Republic of Germany and France
Report No:	RA-2004/94
Document No:	M-010783-01-1
Guideline(s) followed in study:	None stated
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Six trials conducted during growing season 1994 are available to evaluate the magnitude of residues of spiroxamine in/on winter rye, winter wheat, spring barley, spring wheat and winter barley after two

applications of spiroxamine and tebuconazole 383 EW in northern Europe, 375 g a.s./ha for spiroxamine. The two trials on barley are summarised here. Samples were analysed for the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grain or straw of total spiroxamine equivalents (as 4-t-butylcyclohexanone) as the contribution from spiroxamine alone (CF = x0.23 for cereal grain and CF = x0.17 for straw was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.2.1 'Derivation of conversion factors for cereals'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for cereals where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control specimens with the exception of some of the samples of straw where the total apparent residue of spiroxamine was up to 0.22 mg/kg. No explanation was given in the report, however as the data are not relied on there is no impact on the validity of the study.

Both trials were conducted as residue decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 49 DALA. Total average residues of spiroxamine in green material were 4.3 and 7.2 mg/kg 0 DALA, declining to 1.2 and 5.1 mg/kg in mature straw, 0.07 and 0.11 mg/kg in mature grain, 35 or 49 DALA.

Table CA 6.3.2/03-1 Residue trials with spiroxamine and tebuconazole 383 g/L EW in barley – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	**: Bayer AG**	**Active substance**	**:**	**spiroxamine**
Country	**: Germany**	**Crop/Crop Group**	**:**	**Cereals**
Content of active substance (g/kg or g/L)	**: 250 g/L**	**Page**	**:**	**1**
Formulation (e.g. WP)	**: 383 EW**	**Indoor/outdoor**	**:**	**Outdoor**
Commercial product (name)	**: HWG 1608 & KWG 4168 EW 383**	**Other a.s. in formulation (common name and content)**	**:**	**tebuconazole 133 g/L**
Producer of commercial product	**: Bayer AG**	**Residues determined as**	**:**	**4-t-butylcyclohexanone**
		Residues calculated as	**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hl						
RA-2004/94 40015/7 0015-94 Germany 51399 Burscheid, Versuchsgut Höfchen 1994	Barley, spring Sissy	1) 20.04.1994 2) 28.06.1994 - 00.07.1994 3) 10.08.1994	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	07.08.1994/0 00.07.1994/24	69	green material straw grain	0.88 7.2 4.2 5.2 0.10 0.11	0** 0 35<< 40 35<< 40	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 35<<: c=0.21 mg/kg day 40: c=0.22 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	**: Bayer AG**	**Active substance**	**: spiroxamine**
Country	**: Germany**	**Crop/Crop Group**	**: Cereals**
Content of active substance (g/kg or g/L)	**: 250 g/L**	**Page**	**: 2**
Formulation (e.g. WP)	**: 383 EW**	**Indoor/outdoor**	**: Outdoor**
Commercial product (name)	**: HWG 1608 & KWG 4168 EW 383**	**Other a.s. in formulation (common name and content)**	**: tebuconazole 133 g/L**
Producer of commercial product	**: Bayer AG**	**Residues determined as**	**: 4-t-butylcyclohexanone**
		Residues calculated as	**: spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (l/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
RA-2004/94 40195/1 0195-94 France, north 27110 Daubeuf la Campagne 1994	Barley, winter Plaisant	1) 18.10.1993 2) 18.05.1994 - 04.06.1994 3) 06.07.1994	SPI SPI	0.3750 0.3750	280 280	0.13400 0.13400	20.04.1994/0 18.05.1994/28	61	green material ear grain straw	0.35 4.3 1.3 0.68 1.4 0.12 0.07 1.2	0** 0 14 28 35 35 49<< 49<<	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg <<: c=0.06 mg/kg

(a) According to Codex (or other e.g. EU) Classification (Glide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

III. Conclusions

A total of two residue trials conducted in 1994 in northern Europe are available to evaluate the total residues of spiroxamine in/on spring and winter barley (green material, ear, straw and grain) after two applications of spiroxamine and tebuconazole 383 EW on barley BBCH 32-69.

Both trials were conducted as residue decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 49 DALA. Total average residues of spiroxamine in green material were 4.3 and 7.2 mg/kg 0 DALA, declining to 1.2 and 5.1 mg/kg in mature straw, 0.07 and 0.11 mg/kg in mature grain, 40 or 49 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment of MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/03. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Both trials were conducted as residue decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 49 DALA. Total average residues of spiroxamine in green material were 4.3 and 7.2 mg/kg 0 DALA, declining to 1.2 and 5.1 mg/kg in mature straw, 0.07 and 0.11 mg/kg in mature grain, 40 or 49 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.2/04
Report Author:	[redacted]
Report Year:	1994
Report Title:	Determination of residues of HWG1608 & KWG 4168 383 EW in/on winter rye, winter wheat, spring barley, spring wheat and winter barley under actual use conditions in the Federal Republic of Germany and France
Report No:	RA-2077/93
Document No:	M-010788-01-1
Guideline(s) followed in study:	FVA Guideline, Residue Trials, Part 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Eight trials conducted during growing season 1993 are available to evaluate the magnitude of residues of spiroxamine in/on winter rye, winter wheat, spring barley, spring wheat and winter barley (green material, ear, straw, grain) after two applications of spiroxamine and tebuconazole 383 EW at 1.5 L/ha to cereals in northern Europe, 375 g a.s./ha for spiroxamine. The three trials on barley are summarised

here. Samples were analysed for the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grain or straw of total spiroxamine equivalents (as 4-t-butylcyclohexanone) as the contribution from spiroxamine alone (CF = x0.23 for cereal grain and CF = x0.17 for straw was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.2.1 'Derivation of conversion factors for cereals'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for cereals where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

All trials were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 56 DALA. Total average residues of spiroxamine in green material were between 4.8 and 9.1 mg/kg 0 DALA, declining to between 0.66 and 3.8 mg/kg in mature straw, 0.05 and 0.10 mg/kg in mature grain, 49 or 56 DALA.

Table CA 6.3.2/04-1 Residue trials with spiroxamine and tebuconazole 383 g/L EC in barley – residue results for Northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	*Bayer AG, Leverkusen*	**Active substance**	:	*spiroxamine*
Country	:	*Germany*	**Crop/Crop Group**	:	*Cereals*
Content of active substance (g/kg or g/l)	:	*250 g/l*	**Page**	:	*1- 4*
Formulation (e.g. WP)	:	*383 EW*	**Indoor/outdoor**	:	*Outdoor*
Commercial product (name)	:	*HWG 1608 & KWG 4168 EW 383*	**Other a.s. in formulation (common name and content)**	:	*tebuconazole 133 g/l*
Producer of commercial product	:	*BAYER AG*	**Residues determined as**	:	*4-t-Butylcyclohexanone*
			Residues calculated as	:	*spiroxamine*

1	2	3	4	5			6	7	8	9	10	11
Report No.; Study No Location incl. postal code	Commodity / Variety	Date 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	DALT/PHI (days)	Remarks
	(a)	(b)	(c)				(d)	(e)	(a)		(f)	
RA-2077/93 30133/7 0133-93 Germany Versuchsgut Höfchen, 51399 Burscheid 1993	Barley, spring Marina	1) 19.03.1993 2) 10.06.1993 - 13.06.1993 3) 02.08.1993	SPI	0.3750	300	0.125	2/07.06.1993	Beginning of flowering	*green material* *ear* *straw* *grain*	*2.7* *9.1* *0.71* *2.9* *3.8* *0.05*	*0** *0* *35<<* *35<<* *56* *56*	*(c) SPI:Spraying* *(g) 00312* *(h) 0.05 mg/kg* ** prior to last application*

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : *Bayer AG, Leverkusen*

Country : *Germany*

Content of active substance (g/kg or g/l) : *250 g/l*

Formulation (e.g. WP) : *383 EW*

Commercial product (name) : *HWG 1608 & KWG 4168 EW 383*

Producer of commercial product : *BAYER AG*

Active substance : *spiroxamine*

Crop/Crop Group : *Cereals*

Page : *1- A*

Indoor/outdoor : *Outdoor*

Other a.s. in formulation (common name and content) : *tebuconazole 133 g/l*

Residues determined as : *4-tButylcyclohexanone*

Residues calculated as : *spiroxamine*

1	2	3	4	5			6	7	8	9	10	11
Report No.; Study No Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	DALT/PHI (days)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL	treatments and last date					
	(a)	(b)	(c)				(d)	(e)	(a)		(f)	
RA-2077/93 30301/1 0301-93 France 27220 Mousseaux-Neuville 1993	Barley, winter Plaisant	1) 20.10.1992 2) 17.05.1993 - 29.05.1993 3) 30.06.1993	SPI	0.3750	280	0.1340	2/12.05.1993	Beginning of heading	*green material* *ear* *grain* *straw*	*0.71* *5.3* *1.5* *1.0* *0.89* *0.10* *0.05* *0.66*	*0** *0* *14* *28* *35* *35* *49<<* *49<<*	*(c) SPI:Spraying* *(g) 00312* *(h) 0.05 mg/kg* ** prior to last application*

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	*Bayer AG, Leverkusen*	**Active substance**	:	*spiroxamine*
Country	:	*Germany*	**Crop/Crop Group**	:	*Cereals*
Content of active substance (g/kg or g/l)	:	*250 g/l*	**Page**	:	*2- A*
Formulation (e.g. WP)	:	*383 EW*	**Indoor/outdoor**	:	*Outdoor*
Commercial product (name)	:	*HWG 1608 & KWG 4168 EW 383*	**Other a.s. in formulation (common name and content)**	:	*tebuconazole 133 g/l*
Producer of commercial product	:	*BAYER AG*	**Residues determined as**	:	*4-tButylcyclohexanone*
			Residues calculated as	:	*spiroxamine*

1	2	3	4	5			6	7	8	9	10	11
Report No.; Study No Location incl. postal code	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) of no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2077/93 30303/8 0303-93 France 27220 Garencieres 1993	Barley, winter Express	1) 07.10.1992 2) 17.05.1993 - 28.05.1993 3) 30.06.1993	SPI	0.3750	280	0.1340	2/12.05.1993	Beginning of heading	*green material*	*0.82* *4.8* *1.6* *0.98* *0.96*	*0** *0* *14* *28* *35*	*(c) SPI:Spraying* *(g) 00312* *(h) 0.05 mg/kg* ** prior to last application*
									ear	*0.19*	*35*	
									grain	*0.10*	*49<<*	
									straw	*1.3*	*49<<*	

a) According to Codex (or other e.g. EU) Classification Guide
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc., overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last treatment)
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

III. Conclusions

Three residue trials conducted in 1993 in northern Europe are available to evaluate the residues of total spiroxamine in/on barley green material, ear, straw and grain harvested after two applications of spiroxamine and tebuconazole 383 EW on barley BBCH 32 to 61.

All trials were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 56 DALA. Total average residues of spiroxamine in green material were between 4.8 and 9.1 mg/kg 0 DALA, declining to between 0.66 and 3.8 mg/kg in mature straw, 0.05 and 0.10 mg/kg in mature grain, 49 or 56 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment of MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/02. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

All trials were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 56 DALA. Total average residues of spiroxamine in green material were between 4.8 and 9.1 mg/kg 0 DALA, declining to between 0.66 and 3.8 mg/kg in mature straw, 0.05 and 0.10 mg/kg in mature grain, 49 or 56 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.2/05
Report Author:	[redacted]
Report Year:	2001
Report Title:	Determination of residues of JAU 6476-desthio & KWG 4168 on spring barley following spray application of JAU 6476 & KWG 4168 460 EC in Germany, France and Great Britain
Report No:	RA-2096/00
Document No:	M-088987-01-1
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Six trials conducted during growing season 2000 are available to evaluate the magnitude of residues of spiroxamine in/on spring barley (rest of plant, ear, straw and grain) after two applications of spiroxamine and prothioconazole 460 EC in northern Europe (4 trials) or southern Europe (2 trials).

Samples were analysed for spiroxamine. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in northern Europe (Germany and United Kingdom), and two trials in southern Europe (southern France) with all six performed as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.2/05-1 and Table CA 6.3.2/05-2.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha, corresponding to 375 g a.s./ha spiroxamine at a water application rate of 300 L/ha. Spray intervals were 15 to 24 days with the final treatment being made at growth stage BBCH 61-69 which is just beyond the critical GAP of BBCH 61. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using spiroxamine and prothioconazole 460 EC formulation containing 300 g/L spiroxamine (actual content 295.2 g/L spiroxamine).

Samples of separated green material (plant remaining) and ears were taken on the day of the final application. Additional samples of either green material (plant remaining) and ears or immature grain and straw were taken at subsequent intervals until harvest when samples of mature grain and straw were sampled, 42 to 61 days after last application (DALA).

Samples of barley (rest of plant, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 00709, report reference M-082616-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.05 mg/kg.

Samples were extracted with acetonitrile/water (4/1; v/v) using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/05-4, the maximum storage time between date of deep-freezing and date of last extraction was 437 days for total spiroxamine residues.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.2/05-3), with the exception of one result (111% for grain/ears at 0.5 mg/kg). The limit of quantification (LOQ) for spiroxamine was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

All four trials were conducted as residue decline trials, with mature grain and straw sampled between 42 to 61 DALA (PHI is growth stage dependent).

For trials in northern Europe, residues of spiroxamine found 0 DALA in barley whole plant remaining (green material) ranged from 4.50 to 7.90 mg/kg. Residues of spiroxamine found 0 DALA in barley immature ears ranged from 6.70 to 9.30 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg with corresponding straw residues ranging from 0.23 to 0.41 mg/kg.

For trials in southern Europe, residues of spiroxamine found 0 DALA in barley whole plant remaining (green material) were 3.80 and 6.00 mg/kg. Residues of spiroxamine found 0 DALA in barley immature ears were 2.90 to 3.50 mg/kg. In mature grain, both trials reported residues of <0.05 mg/kg with corresponding straw residues of 0.35 and 0.54 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.2/05-1 Residue trials with spiroxamine and prothioconazole 300 g/L EC in barley – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported Residue (mg/kg) Spiroxamine (parent compound)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.3.2/05 (RA-2096/00) R 2000 0083/9 Germany, D-51399 Burscheid Spring barley (Baronesse) 2000	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Rest of plant Rest of plant Rest of plant Ear Ear Ear Straw Straw Grain Grain (mature)	0 28 34 0 28 34 42 61 42 61	6.20 0.91 0.73 9.10 0.12 0.09 0.61 0.29 <0.05 <0.05
CA 6.3.2/05 (RA-2096/00) R 2000 0425/7 Germany, D-40789 Monheim Spring barley (Alexis) 2000	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Rest of plant Rest of plant Rest of plant Ear Ear Ear Straw Straw Grain Grain (mature)	0 28 34 0 28 34 42 55 42 55	7.30 0.93 0.61 6.70 0.16 0.06 0.59 0.25 <0.05 <0.05

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported Residue (mg/kg) Spiroxamine (parent compound)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.3.2/05 (RA-2096/00) R 2000 0426/5 Northern France, F-27700 Fresne 1'Archeveque Spring barley (Nevada) 2000	1 2	0.375 0.375	300 300	BBCH 39 BBCH 61	Rest of plant Rest of plant Rest of plant Rest of plant Ear Ear Ear Ear Straw Grain (mature)	0 28 35 42 0 28 35 42 56 56	4.50 0.62 0.50 0.27 7.30 0.05 <0.05 <0.05 0.23 <0.05
CA 6.3.2/05 (RA-2096/00) R 2000 0427/3 England, GB-IP31 3SH, Thurston, Bury St. Edmunds (EFDS) Spring barley (Optic) 2000	1 2	0.375 0.375	300 300	BBCH 37 BBCH 63	Rest of plant Rest of plant Rest of plant Rest of plant Ear Ear Ear Ear Straw Grain (mature)	0 29 35 42 0 29 35 42 57 57	7.90 1.20 0.86 0.53 9.30 0.16 0.07 0.09 0.41 <0.05

Table CA 6.3.2/05-2 Residue trials with spiroxamine and prothioconazole 300 g/L EC in barley – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported Residue (mg/kg) Spiroxamine (parent compound)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.3.2/05 (RA-2096/00) R 2000 0084/7 Southern France, F-26750 St. Paul les Romans Spring barley (Nevada) 2000	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61-69	Rest of plant Ear Straw Straw Straw Straw Grain Grain Grain Grain (mature)	0 0 28 35 45 52 28 35 45 52	6.00 3.50 0.77 0.71 0.47 0.35 <0.05 <0.05 <0.05 <0.05
CA 6.3.2/05 (RA-2096/00) R 2000 0428/1 Southern France, F-82600 Mas Grenier Spring barley (Nevada) 2000	1 2	0.375 0.375	300 300	BBCH 37 BBCH 69	Rest of plant Rest of plant Ear Ear Straw Straw Grain Grain (mature)	0 28 0 28 35 42 35 42	3.80 0.79 2.90 0.09 0.70 0.54 <0.05 <0.05

Table CA 6.3.2/05-3 Procedural recovery data for the determination of spiroxamine (parent compound)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/05	00709	Spiroxamine (parent compound)	Barley grain/ear	0.05 0.50 Overall	8 8 16	103; 105; 108; 103; 105; 87; 98; 94 106; 107; 105; 106; 109; 111; 99; 90 Mean: 102, RSD: 7.3
CA 6.3.2/05	00709	Spiroxamine (parent compound)	Barley rest of plant	0.05 0.50 5 Overall	5 7 2 14	109; 109; 109; 108; 108 104; 107; 106; 106; 106; 99; 94 85; 84 Mean: 102, RSD: 8.5
CA 6.3.2/05	00709	Spiroxamine (parent compound)	Barley straw	0.05 0.50 Overall	5 7 12	81; 95; 99; 103; 102 100; 108; 107; 107; 106; 76; 86 Mean: 98, RSD: 11.1

Table CA 6.3.2/05-4 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/05	Barley	307 to 437

III. Conclusions

A total of six residue trials conducted in 2000 in northern Europe (4 trials) or southern Europe (2 trials) are available to evaluate the residues of spiroxamine (parent compound) in/on spring barley (rest of plant, ear, straw and grain) after two applications of spiroxamine and prothioconazole 460 EC on barley BBCH 37 to 69.

For trials in northern Europe, residues of spiroxamine found 0 DALA in barley whole plant remaining (green material) ranged from 4.50 to 7.90 mg/kg. Residues of spiroxamine found 0 DALA in barley immature ears ranged from 6.70 to 9.30 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg with corresponding straw residues ranging from 0.23 to 0.41 mg/kg.

For trials in southern Europe, residues of spiroxamine found 0 DALA in barley whole plant remaining (green material) were 3.80 and 6.00 mg/kg. Residues of spiroxamine found 0 DALA in barley immature ears were 2.90 to 3.50 mg/kg. In mature grain, both trials reported residues of <0.05 mg/kg with corresponding straw residues of 0.35 and 0.54 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/08. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

For trials in northern Europe, residues of spiroxamine found 0 DALA in barley whole plant remaining (green material) ranged from 4.50 to 7.90 mg/kg. Residues of spiroxamine found 0 DALA in barley immature ears ranged from 6.70 to 9.30 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg with corresponding straw residues ranging from 0.23 to 0.41 mg/kg.

For trials in southern Europe, residues of spiroxamine found 0 DALA in barley whole plant remaining (green material) were 3.80 and 6.00 mg/kg. Residues of spiroxamine found 0 DALA in barley immature ears were 2.90 to 3.50 mg/kg. In mature grain, both trials reported residues of <0.05 mg/kg with corresponding straw residues of 0.35 and 0.54 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.2/06
Report Author:	[redacted]
Report Year:	2006
Report Title:	Determination of the residues of JAU 6476, tebuconazole and KWG 4168 in/on spring barley after spraying of JAU 6476 & HWG 1608 & KWG 4168 (450 EC) in the field in Northern France, United Kingdom, Germany and Sweden
Report No:	RA-2571/05
Document No:	M-272012-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Four trials conducted during growing season 2005 are available to evaluate the magnitude of residues of spiroxamine in/on spring barley (green material, grain and straw) after two applications of spiroxamine, prothioconazole and tebuconazole EC 450 on barley in northern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and tebuconazole were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in northern Europe (Germany, France, the United Kingdom and the Netherlands) with all four performed as semi-decline trials. The trial parameters and residue results are summarised in Table CA 6.3.2/06-1.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha, corresponding to 313 g a.s./ha spiroxamine at a water application rate of 300 L/ha. Spray intervals were 6 to 12 days, with the final treatment being made at growth stage BBCH 61. The application rates differ from the critical GAP. Residues above the LOQ are therefore scaled proportionally to a 0.375 kg a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using a 450 g/L EC formulation of spiroxamine, prothioconazole and tebuconazole containing 250 g/L spiroxamine (actual content 243.51 g/L spiroxamine).

Samples of green material were taken on the day of final application. Additional samples of green material were taken at subsequent intervals until harvest when samples of mature grain and straw were sampled, 39 to 51 days after last application (DALA).

Samples of barley (green material, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 00709, report reference M-082616-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.05 mg/kg.

Samples were extracted with acetonitrile/water (4/1, v/v) using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.2/06-3, the maximum storage time between date of deep-freezing and date of last extraction was 187 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.2/06-2). The limit of quantification (LOQ) for spiroxamine was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

All four trials were conducted as residue decline trials, with mature grain and straw sampled between 39 to 51 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found 0 DALA in green material ranged from 5.88 to 8.76 mg/kg. All residues for grain samples were reported below the LOQ (<0.05 mg/kg) and therefore are not appropriate to scale up to meet the cGAP. Scaled residues of spiroxamine at 39 to 51 DALA were between 0.11 to 0.68 mg/kg in straw samples.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.2/06-1 Residue trials with spiroxamine, prothioconazole and tebuconazole EC 450 in barley – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg) Spiroxamine (parent compound)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]
CA 6.3.2/06 (RA-2571/05) R 2005 0030/0 Northern France, St. Cyr en Arthies (Ild-de-France), F-95510 Spring barley (Carafe) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 61	Green material Green material Green material Green material Grain Straw	-0 0 28 35 39 39	1.2 6.3 0.45 0.21 <0.05 0.49	1.44 7.56 0.54 0.25 <n.a. 0.59
CA 6.3.2/06 (RA-2571/05) R 2005 0031/9 United Kingdom, Little Shelford (Cambridgeshire), GB-CB2 5EU Spring barley (Optic) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 61	Green material Green material Grain Straw	0 35 47 47	4.9 0.09 <0.05 0.12	5.88 0.11 n.a. 0.14
CA 6.3.2/06 (RA-2571/05) R 2005 0801/8 Germany, Vechta-Langförden (Niedersachsen), D-49377 Spring barley (Adonis) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 61	Green material Green material Grain Straw	0 38 49 49	7.1 0.07 <0.05 0.09	8.52 0.08 n.a. 0.11
CA 6.3.2/06 (RA-2571/05) R 2005 0802/6 Sweden, Lund (Malmö), SE-245 61 Spring barley (Pasadena) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 61	Green material Green material Grain Straw	0 35 51 51	7.3 0.29 <0.05 0.57	8.76 0.35 n.a. 0.68

1 – Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503. Scaling factors were derived from the last single application rate and not from the seasonal application rate.
2 – Values reported below the LOQ (< 0.05 mg/kg) not appropriate to scale up.

Table CA 6.3.2/06-2 Procedural recovery data for the determination of spiroxamine in barley

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/06	00709	Spiroxamine	Barley green material	0.05 0.50 5.0 10 Overall	2 1 2 1 6	99,99 89 97, 98 98 Mean: 97, RSD: 4.0
CA 6.3.2/06	00709	Spiroxamine	Barley grain	0.05 0.50 Overall	3 1 4	97, 102, 102 76 Mean: 95, RSD: 13.8
CA 6.3.2/06	00709	Spiroxamine	Barley straw	0.05 5.0 Overall	2 1 3	80, 81 117 Mean: 93, RSD: 22.7

Table CA 6.3.2/06-3 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/06	Barley	68 to 187

III. Conclusions

A total of four residue trials conducted in 2005 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on barley green material, grain and straw harvested after two spray applications with the formulation spiroxamine, prothioconazole and tebuconazole EC 450 on barley at BBCH 47 to 61.

Scaled residues of spiroxamine found 0 DALA in green material ranged from 5.88 to 8.76 mg/kg. All residues for grain samples were reported below the LOQ (<0.05 mg/kg) and therefore are not appropriate to scale up to meet the eGAP. Scaled residues of spiroxamine at 39 to 51 DALA were between 0.11 to 0.68 mg/kg in straw samples.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/09. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine found 0 DALA in green material ranged from 5.88 to 8.76 mg/kg. All residues for grain samples were reported below the LOQ (<0.05 mg/kg) and therefore are not appropriate to scale up to meet the cGAP. Scaled residues of spiroxamine at 39 to 31 DALA were between 0.11 to 0.68 mg/kg in straw samples.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.2/07
Report Author:	[redacted]
Report Year:	2006
Report Title:	Determination of the residues of JAU 6476, Tebuconazole and KWG 4168 in/on winter barley after spraying of JAU 6476 & HWG 1608 & KWG 4168 (450 EC) in the field in Southern France and Spain
Report No:	RA-2572/05
Document No:	M-272115-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Two trials conducted during growing season 2005 are available to evaluate the magnitude of residues of spiroxamine in/on winter barley (green material, grain and straw) after two applications of spiroxamine, prothioconazole and tebuconazole 450 EC on barley in southern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and tebuconazole were also analysed, however are not reported in this summary.

Two residue trials on barley were conducted in southern Europe (southern France and Spain) with both performed as semi-decline trials. The trial parameters and residue results are summarised in Table CA 6.3.2/07-1.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 313 g a.s./ha, at a water application rate of 300 L/ha. Spray intervals were 8 or 9 days, with the final treatment being made at growth stage BBCH 61. The application rates differ from the critical GAP. Residues above the LOQ are therefore scaled proportionally to a 0.375 kg a.s/ha application rate in line

with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using a 450 g/L EC formulation of spiroxamine, prothioconazole and tebuconazole containing 250 g/L spiroxamine (actual content 243.51 g/L spiroxamine).

Samples of green material were taken on the day of final application. Additional samples of green material or immature grain/straw were taken at subsequent intervals until harvest when samples of mature grain and straw were sampled, 47 or 61 days after last application (DALA).

Samples of barley (green material, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 00709, report reference M-082616-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.05 mg/kg.

Samples were extracted with acetonitrile/water (4/1; v/v) using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤-18°C. As shown in Table CA 6.3.2/07-3, the maximum storage time between date of deep-freezing and date of last extraction was 234 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.2/07-2) with the exception of one result of 117% for spiroxamine in straw at 5.0 mg/kg. The limit of quantification (LOQ) for spiroxamine was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Scaled residues of spiroxamine found 0 DALA in green material were 8.76 and 14.4 mg/kg. All residues for grain samples were reported below the LOQ (<0.05 mg/kg) and therefore are not appropriate to scale up to meet the cGAP. Scaled residues of spiroxamine at 47 to 61 DALA were 0.12 and 0.37 mg/kg in mature straw samples.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.2/07-1 Residue trials with spiroxamine, prothioconazole and tebuconazole EC 450 in barley – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg) Spiroxamine (parent compound)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]
CA 6.3.2/07 (RA-2572/05) R 2005 0468/3 Southern France, F-13150 Tarascon, (Provence-Côte D'Azur) Winter barley (Bakara) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 61	Green material Green material Green material Green material Grain Straw	-0 0 28 35 61 61	1.70 12.00 0.21 0.13 <0.05 0.10	2.04 14.40 0.25 0.16 n.a. 0.12
CA 6.3.2/07 (RA-2572/05) R 2005 0469/1 Spain, E-17468 Vilademuls (Cataluña) Winter barley (Graphic) 2005	1 2	0.3125 0.3125	300 300	BBCH 51 BBCH 59	Green material Grain Grain Straw Straw	0 35 47 35 47	7.30 <0.05 <0.05 0.32 0.31	8.76 n.a. n.a. 0.38 0.37

1 - Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503. Scaling factors were derived from the last single application rate and not from the seasonal application rate
2 - Values reported below the LOQ (<0.05 mg/kg) not appropriate to scale up

Table CA 6.3.2/07-2 Procedural recovery data for the determination of spiroxamine in barley

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/07	00709	Spiroxamine	Barley green material	0.05 0.50 5.0 10 Overall	2 1 2 1 6	99,99 89 97, 98 98 Mean: 97, RSD: 4.0
CA 6.3.2/07	00709	Spiroxamine	Barley grain	0.05 0.50 Overall	3 1 4	97, 102, 102 76 Mean: 95, RSD: 13.8
CA 6.3.2/07	00709	Spiroxamine	Barley straw	0.05 5.0 Overall	2 1 3	80, 81 117 Mean: 93, RSD: 22.7

Table CA 6.3.2/07-3 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/07	Barley	133 to 234

III. Conclusions

A total of two residue trials conducted in 2005 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on winter barley green material, grain and straw harvested after two spray applications with the formulation spiroxamine, prothioconazole and tebuconazole EC 450 on barley at BBCH 47 to 61.

Scaled residues of spiroxamine found 0 DALA in green material were 8.76 and 14.4 mg/kg. All residues for grain samples were reported below the LOQ (<0.05 mg/kg) and therefore are not appropriate to scale up to meet the cGAP. Scaled residues of spiroxamine at 47 to 61 DALA were 0.12 and 0.37 mg/kg in mature straw samples.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/10. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine found 0 DALA in green material were 8.76 and 14.4 mg/kg. All residues for grain samples were reported below the LOQ (<0.05 mg/kg) and therefore are not appropriate to scale up to meet the cGAP. Scaled residues of spiroxamine at 47 to 61 DALA were 0.12 and 0.37 mg/kg in mature straw samples.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.2/08
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of BYF 00587, JAU 6476 and KWG 4168 in/on spring barley and winter barley after spraying of BYF 00587 & JAU 6476 & KWG 4168 (400 EC) in the field in Northern France, Germany, the United Kingdom and the Netherla
Report No:	RA-2042/07
Document No:	M-298147-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/A/I/95, rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2007 are available to evaluate the magnitude of residues of spiroxamine in/on spring and winter barley (green material, ear, rest of plant, grain and straw) after two applications of spiroxamine, prothioconazole and bixafen EC 400 on barley in northern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and bixafen were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in northern Europe (northern France, Germany, United Kingdom and the Netherlands) with all four conducted as harvest trials although samples were taken at 35 days after last application for all trials even if the crop was not at commercial maturity. The trial parameters and residue results are summarised in Table CA 6.3.2/08-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.5 L/ha corresponding to 375 g a.s./ha, at a water application rate of 300 L/ha. One trial was overdosed by 6% (R 0527/1) therefore residues data for this trial are scaled proportionally to a 0.375 kg a.s/ha application rate in line with the critical GAP. Spray intervals were 8-20 days, with the final treatment being made at growth stage BBCH 61. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using a 400 g/L EC formulation of spiroxamine, prothioconazole and bixafen containing 250 g/L spiroxamine (actual content 256.6 g/L spiroxamine).

Samples of green material were taken on the day of final application (prior to and subsequent to the application). Additional samples of ear and plant remaining or immature grain/straw were taken at nominally 35 days after last application (DALA) if the crop was not commercially mature and then at full commercial maturity when samples of mature grain and straw were sampled, 55 to 96 DALA.

Samples of barley (green material, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 01013 (modified by 01013/M001), report reference M-283439-03-1 and M-297777-02-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetonitrile/water (4/1, v/v) in the presence of cysteine hydrochloride after initial soaking, using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤-18°C. As shown in Table CA 6.3.2/08-3, the maximum storage time between date of deep-freezing and date of last extraction was 207 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.2/08-2). The limit of quantification (LOQ) for spiroxamine was 0.01 mg/kg.

No residues above the LOQ (0.01 mg/kg) were detected in the control samples.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 55 to 96 DALA (PHI is growth stage dependent).

Residues of spiroxamine found 0 DALA in green material ranged from 4.6 to 8.8 mg/kg (highest value was scaled to the cGAP). Residues for mature grain samples ranged from <0.01 to 0.02 mg/kg 55 to 96 DALA, with corresponding residues in straw ranging from 0.06 to 0.47 mg/kg. The residue in grain of <0.01 mg/kg was from the trial slightly overdosed at 0.3975 kg a.s./ha and therefore cannot be scaled but as the reported residue is below the LOQ with a more critical application rate the value from this trial can be considered for MRL purposes.

Table CA 6.3.2/08-1 Residue trials with spiroxamine, prothioconazole and bixafen EC 400 in barley - residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Spiroxamine residue (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue[2]	Scaled residue[1,2]
CA 6.3.2/08 (RA-2042/07) R 2007 0448/8 Northern France, St Cyr en Arthies (Ile-de-France), F-95510 Spring barley (Heinly) 2007	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 58 58	0.50 4.6 0.10 0.56 0.36 <u>0.01</u>	n.a.
CA 6.3.2/08 (RA-2042/07) R 2007 0449/6 Germany, Swisttal (Nordrhein-Westfalen), D-53913 Winter barley (Naomi) 2007	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 55 55	0.69 4.6 0.06 0.71 0.47 <u>0.02</u>	n.a.
CA 6.3.2/08 (RA-2042/07) R 2007 0527/1 United Kingdom, Little Shelford (Cambridgeshire), CB2 5EU Spring barley (Cocktail) 2007	1 2	0.375 0.3975	300 318	BBCH 37 BBCH 61	Green material Green material Rest of plant Ear Straw Grain	-0 0 34 34 96 96	0.38 9.3 0.53 0.05 0.06 <0.01	0.36 8.77 0.50 0.05 0.06 <u><0.01</u>[3]
CA 6.3.2/08 (RA-2042/07) R 2007 0529/8 Netherlands, Zwaagdijk-Oost (Noord-Holland), NL-1681 ND Spring barley (Prestige) 2007	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Green material Green material Straw Grain Straw Grain	-0 0 35 35 71 71	0.64 6.0 0.54 0.02 0.13 <u>0.01</u>	n.a.

1 - Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - <u>Underlined</u> value to be used to support MRL for grain
3 - Trial slightly overdosed at 0.3975 kg a.s./ha and therefore cannot be scaled but as the reported residue is below the LOQ with a more critical application rate the value from this trial can be considered for MRL purposes

Table CA 6.3.2/08-2 Procedural recovery data for the determination of spiroxamine in barley

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/08	01013	Spiroxamine	Barley green material[1]	0.01 0.10 1.0 10 Overall	5 1 2 2 10	86, 85, 82, 87, 88 102 79, 81 72, 78 Mean: 84, RSD: 9.5
CA 6.3.2/08	01013	Spiroxamine	Barley grain[2]	0.01 0.10 Overall	5 5 10	93, 88, 86, 82, 84 85, 91, 89, 87, 85 Mean: 87, RSD: 3.8
CA 6.3.2/08	01013	Spiroxamine	Barley straw	0.01 0.10 1.0 Overall	5 2 2 9	93, 82, 82, 108, 102 80, 81 78, 80 Mean: 87, RSD: 12.6

1 - Recoveries for rest of plant are covered by recoveries for green material.

2 - Recoveries for ear are covered by recoveries for grain.

Table CA 6.3.2/08-3 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/08	Barley	48 to 207

III. Conclusions

A total of four residue trials conducted in 2007 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on barley green material, ear, rest of plant, grain and straw harvested after two spray applications with the formulation spiroxamine, prothioconazole and bixafen EC 400 on barley at BBCH 37 to 61.

Residues of spiroxamine found 0 DALA in green material ranged from 4.6 to 8.8 mg/kg (highest value was scaled to the cGAP). Residues for mature grain samples ranged from <0.01 to 0.02 mg/kg 55 to 96 DALA, with corresponding residues in straw ranging from 0.06 to 0.47 mg/kg. The residue in grain of <0.01 mg/kg was from the trial slightly overdosed at 0.3975 kg a.s./ha and therefore cannot be scaled but as the reported residue is below the LOQ with a more critical application rate the value from this trial can be considered for MRL purposes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/11. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine found 0 DALA in green material ranged from 4.6 to 8.8 mg/kg (highest value was scaled to the cGAP). Residues for mature grain samples ranged from <0.01 to 0.02 mg/kg, 55 to 96 DALA, with corresponding residues in straw ranging from 0.06 to 0.47 mg/kg. The residue in grain of <0.01 mg/kg was from the trial slightly overdosed at 0.3975 kg a.s./ha and therefore cannot be scaled but as the reported residue is below the LOQ with a more critical application rate the value from this trial can be considered for MRL purposes.

Data Point:	KCA 6.3.2/09
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of BYF 00587, JAU 6476 and KWG 4168 in/on winter barley and spring barley after spraying of BYF 00587 & JAU 6476 & KWG 4168 (400 EC) in the field in Southern France, Spain, Italy and Portugal
Report No:	RA-2043/07
Document No:	M-298412-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/I/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2007 are available to evaluate the magnitude of residues of spiroxamine in/on spring and winter barley (green material, ear, rest of plant, grain and straw) after two applications with the formulation spiroxamine, prothioconazole and bixafen EC 400 on barley in southern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and bixafen were also analysed, however are not reported in this summary.

Four residue trials on barley were conducted in southern Europe (southern France, Spain, Italy and Portugal) with all four conducted as harvest trials although samples were taken at 35 days after last application for all trials even if the crop was not at commercial maturity. The trial parameters and residue results are summarised in Table CA 6.3.2/09-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.5 L/ha corresponding to 375 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 8-20 days, with the final treatment being made at growth stage BBCH 61. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using a 400 g/L EC formulation of spiroxamine, prothioconazole and bixafen containing 250 g/L spiroxamine (actual content 256.6 g/L spiroxamine).

Samples of green material were taken on the day of final application (prior to and subsequent to the application). Additional samples of ear and plant remaining or immature grain/straw were taken at nominally 35 days after last application (DALA) if the crop was not commercially mature and then at full commercial maturity when samples of mature grain and straw were sampled, 35 to 58 DALA. Control samples were taken on day of the final application day prior to the last treatment and 35 to 58 DALA.

Samples of barley (green material, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 01013 (modified by 01013/M001), report reference M-283439-03-1 and M-297777-02-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetonitrile/water (4/1; v/v) in the presence of cysteine hydrochloride after initial soaking, using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤ -18°C. As shown in Table CA 6.3.2/09-3, the maximum storage time between date of deep-freezing and date of last extraction was 225 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.2/09-2). The limit of quantification (LOQ) for spiroxamine was 0.01 mg/kg.

No residues above the LOQ (0.01 mg/kg) were detected in the control samples.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 35 to 58 DALA (PHI is growth stage dependent).

Residues of spiroxamine found 0 DALA in green material ranged from 4.7 to 7.2 mg/kg. Residues for mature grain samples ranged from 0.01 to 0.02 mg/kg, 35 to 58 DALA, with corresponding residues in straw ranging from 0.07 to 0.73 mg/kg.

Table CA 6.3.2/09-1 Residue trials with spiroxamine, prothioconazole and bixafen EC 400 in barley - residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application No.	kg a.s./ha	L/ha	Growth stage	Crop part	DALA (days)	Reported residue of spiroxamine (mg/kg)[1,3]
CA 6.3.2/09 (RA-2043/07) R 2007 0451/8 Southern France, Cherves (Poitou-Charentes), F-86170 Winter barley (Esterel) 2007	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 58 58	0.63 6.0 0.06 0.85 0.44 <u>0.01</u>
CA 6.3.2/09 (RA-2043/07) R 2007 0452/6 Spain, Osuna Sevilla (Andalucia), E-41640 Winter barley (County R2) 2007	1 2	0.375 0.375	300 300	BBCH 39[1] BBCH 61	Green material Green material Straw Grain Straw Grain	-0 0 36 36 42 42	0.67 7.2 0.55 0.01 0.42 <u>0.01</u>
CA 6.3.2/09 (RA-2043/07) R 2007 0530/1 Italy, Bologna (Emilia-Romagna), I-40128 Spring barley (Tunica) 2007	1 2	0.375 0.375	300 300	BBCH 39[2] BBCH 61	Green material Green material Straw Grain Straw Grain	-0 0 39 39 55 55	0.95 6.6 0.07 0.02 0.07 <u>0.02</u>
CA 6.3.2/09 (RA-2043/07) R 2007 0532/8 Portugal, Santarém (Ribatejo e Oeste), P-2000-210 Winter barley (Scarlet) 2007	1 2	0.375 0.375	300 300	BBCH 37 BBCH 61	Green material Green material Straw Grain	-0 0 35 35	0.48 4.7 0.73 <u>0.01</u>

1 - Due to meteorological reasons and late arrival of the trial protocol it was impossible to find a crop in growth stage BBCH 37.
2 - No explanation given for application on late BBCH code
3 – Underlined value to be used to support MRL for grain

Table CA 6.3.2/09-2 Procedural recovery data for the determination of spiroxamine in barley

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/09	01013	Spiroxamine	Barley green material[1]	0.01 0.10 1.0 10 Overall	5 1 2 2 10	86, 85, 82, 87, 88 102 79, 81 72, 78 Mean: 84; RSD: 9.5
CA 6.3.2/09	01013	Spiroxamine	Barley grain[2]	0.01 0.10 Overall	5 5 10	93, 88, 86, 82, 84 85, 91, 89, 87, 85 Mean: 87, RSD: 3.8
CA 6.3.2/09	01013	Spiroxamine	Barley straw	0.01 0.10 1.0 Overall	5 2 2 9	95, 82, 82, 108, 102 80, 81 78, 80 Mean: 87, RSD: 12.8

1- Recoveries for rest of plant are covered by recoveries for green material
2- Recoveries for ear are covered by recoveries for grain

Table CA 6.3.2/09-3 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/09	Barley	69 to 225

III. Conclusions

A total of four residue trials conducted in 2007 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on barley green material, ear, rest of plant, grain and straw harvested after two spray applications with the formulation spiroxamine, prothioconazole and bixafen EC 400 on barley at BBCH 37 to 61.

Residues of spiroxamine found 0 DALA in green material ranged from 4.7 to 7.2 mg/kg. Residues for mature grain samples ranged from 0.01 to 0.02 mg/kg 35 to 58 DALA, with corresponding residues in straw ranging from 0.07 to 0.73 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), DA 6.12/12. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine found 0 DALA in green material ranged from 4.7 to 7.2 mg/kg. Residues for mature grain samples ranged from 0.01 to 0.02 mg/kg 35 to 58 DALA, with corresponding residues in straw ranging from 0.07 to 0.73 mg/kg.

Data Point:	KCA 6.3.2/10
Report Author:	[redacted]
Report Year:	1999
Report Title:	Determination of residues of KWG 4168 500 EC in/on spring and winter barley following spray application in France
Report No:	RA-2158/98
Document No:	M-010906-01-1
Guideline(s) followed in study:	EC guidance document 7029/VI/95 rev.5: Appendix B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and classified RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 1998 are available to evaluate the magnitude of residues of spiroxamine in/on winter or spring barley (straw and grain) after two applications of Spiroxamine 500 EC in southern Europe. Samples were analysed for spiroxamine and total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents.

Four residue trials on barley were conducted in southern Europe (southern France) with all four conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.2/10-1 where values considered for MRL purposes or risk assessment are underlined. Three of the barley trials were conducted in France, less than 50 km apart (estimated approximate distances varied from 16 to 24 km between trials). The trials less than 20 km, cannot be considered as independent and only two trials from these three are acceptable for assessment. Trial 816299 was in a different region of southern France.

Two spray applications of spiroxamine were made at a product rate of at 1.5, corresponding to 750 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 21 to 29 days, with the final treatment being made at growth stage BBCH 61 except for trial 810029 which exceeds the BBCH growth stage on the cGAP (BBCH 83) and therefore represents a more critical GAP and is not considered for assessment. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on barley is supported in terms of rates and timings.

The trials were conducted using a spiroxamine 500 EC formulation containing 500 g/L spiroxamine (actual content 510.5 g/L spiroxamine).

Samples of grain and straw were taken between 41 to 49 days after the last application (DALA). Control samples were taken 35 to 50 DALA.

Samples of barley (grain and straw) were analysed for spiroxamine and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine (parent compound)

The samples of grain and straw were analysed for spiroxamine parent compound. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00506 and its supplement 00506/E001, report reference M-020658-01-1 and M-020694-01-1, respectively (**see Doc MCA Section 4)**. The LOQ for spiroxamine was 0.05 mg/kg.

Sample were extracted with acetone/water (3/1; v/v) using a high-speed blender. After filtration and rinsing, an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on a RP 18 column with elution of spiroxamine in methanol/ammonia (999/1; v/v). Residues were determined by EI-GC/MS (DB 1701 capillary column) using external standardisation.

Total spiroxamine (common moiety approach)

The samples of grain and straw were analysed for the residue of total spiroxamine. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00312, report reference M-018352-02-1 (**see Doc MCA Section 4**). The LOQ for residues of total spiroxamine was 0.05 mg/kg.

Samples were extracted and simultaneously hydrolysed by refluxing with a methanol/1M hydrochloric acid (1/1; v/v) mixture. The total residue of spiroxamine itself and all metabolites containing the 4-t-butylcyclohexanone moiety, was hydrolysed yielding 4-t-butylcyclohexanone. After filtration an aliquot of the extract was diluted with water and clean-up using a RP-18 chromatography column. Any 4-t-butylcyclohexanone was eluted from the column with dichloromethane. Extracts were subject to final clean-up using a silica SPE cartridge eluting any 4-t-butylcyclohexanone with dichloromethane. After addition of n-heptane and careful removal of solvent by rotary evaporation under vacuum, the 4-t-butylcyclohexanone was determined by EI-GC/MS (Ultra 2 capillary column) using external standardisation.

The samples were stored deep frozen after sampling at ≤ -18 °C. As shown in Table CA 6.3.2/10-3, the maximum storage time between date of deep-freezing and date of last extraction was 153 days for parent spiroxamine and 125 days for total spiroxamine residues.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for parent spiroxamine and total residue of spiroxamine were between 70-110% (refer to Table CA 6.3.2/10-2). The limit of quantification (LOQ) for spiroxamine and total residue of spiroxamine from both methods was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

In mature grain, scaled residues of parent spiroxamine were <0.025 to <0.05 mg/kg 41 to 49 DALA with corresponding residues in straw of 0.261 to 0.600 mg/kg.

Scaled residues of total spiroxamine in grain 41 or 49 DALA were 0.085 to 0.134 mg/kg with corresponding residues in straw of 1.84 to 5.25 mg/kg.

As two of the French trials are not independent, only the higher grain and straw value at harvest can be relied on for MRL and risk assessment purposes.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.2/10-1 Residue trials with spiroxamine 500 EC in barley – residue results for Southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residue of spiroxamine (via 4-TBCH)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.2/10 (RA-2158/98) 810029 Southern France, F-01380 Bage-la-Ville Spring barley (Volga) 1998	1 2	0.750 0.750	300 300	BBCH 37 BBCH 58-83 [outside of cGAP]	Straw Grain	49 49	0.967 0.055	0.484 0.028	5.61 0.246	2.81 0.123
CA 6.3.2/10 (RA-2158/98) 810045 Southern France, F-01560 St-Julien-sur-Reyssouze Winter barley (Kelibia) 1998	1 2	0.750 0.750	300 300	BBCH 33 BBCH 61	Straw Grain	48 48	0.522 0.050	0.261 0.025	3.68 0.268	1.84 0.134
CA 6.3.2/10 (RA-2158/98) 816299 Southern France, F-32100 Beraut Spring barley (Nevada) 1998	1 2	0.750 0.750	300 300	BBCH 35 BBCH 61	Straw Grain	41 41	1.20 <0.05	0.600 <0.05	10.5 0.169	5.25 0.085
CA 6.3.2/10 (RA-2158/98) 816310 Southern France, F-01440 Viriat Winter barley (Vertige) 1998	1 2	0.750 0.750	300 300	BBCH 33 BBCH 61	Straw Grain	48 48	0.719 <0.05	0.360 <0.05	5.75 0.264	2.88 0.132

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residue of spiroxamine (via 4-TBCH)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]

1 - Residues above LOQ scaled proportionally to a 0.375 kg a.s/ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - Underlined value to be used to support MRL for grain

Table CA 6.3.2/10-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residue of spiroxamine

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.2/10	00506 and 00506/E001	Spiroxamine (parent compound)	Barley straw	0.05 0.50 Overall	3 3 6	88; 91; 97 78; 97; 102 Mean: 92, RSD: 9.2
CA 6.3.2/10	00506 and 00506/E001	Spiroxamine (parent compound)	Barley grain	0.05 0.50 Overall	3 3 6	89; 82; 108 91; 97; 110 Mean: 96, RSD: 11.5
CA 6.3.2/10	00312	Total residue of spiroxamine	Barley straw	0.05 0.50 Overall	3 4 7	114; 124; 121 88; 92; 105; 98 Mean: 106, RSD: 13.3
CA 6.3.2/10	00312	Total residue of spiroxamine	Barley grain	0.05 0.50 Overall	4 3 7	98; 86; 90; 103 88; 87; 94 Mean: 92, RSD 6.9

Table CA 6.3.2/10-3 Storage of barley samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.2/10	Barley	107 to 153 (spiroxamine parent) 79 to 125 (spiroxamine total residues via 4-t-butylcyclohexanone)

III. Conclusions

Four residue trials conducted in 1998 in southern Europe are available to evaluate the residues of (parent compound) and residues of total spiroxamine in/on barley straw and grain harvested after two applications of Spiroxamine 500 EC on barley BBCH 33-83.

The trials were conducted as harvest trials. Scaled residues of spiroxamine (parent compound) in grain were 0.025 to <0.05 mg/kg 41 to 49 DALA with corresponding residues in straw of 0.261 to 0.600 mg/kg.

Scaled residues of total spiroxamine in grain 41 or 49 DALA were 0.085 to 0.134 mg/kg with corresponding residues in straw of 1.84 to 5.25 mg/kg.

Reported or scaled residues of total spiroxamine in grain 41 or 49 DALA were <0.05 mg/kg.

As two of the French trials are not independent and one of these trials does not match the cGAP, only the grain and straw value at harvest from trial 810045 can be relied on for MRL and risk assessment purposes.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05

mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted as supplemental information in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/14. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (parent compound) in grain were 0.025 to <0.05 mg/kg 41 to 49 DALA with corresponding residues in straw of 0.261 to 0.600 mg/kg.

Scaled residues of total spiroxamine in grain 41 or 49 DALA were 0.085 to 0.134 mg/kg with corresponding residues in straw of 1.84 to 5.25 mg/kg.

As two of the French trials are not independent and one of these trials does not match the cGAP, only the grain and straw value at harvest from trial 810045 can be relied on for MRL and risk assessment purposes.

Data Point:	KCA 6.3.2/11
Report Author:	[redacted]
Report Year:	1992
Report Title:	HWG 1608 & KWG 4168 417 EC winter barley; Germany; BBA
Report No:	0841-90
Document No:	M-010866-01-2
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and classified DAR (1997), RAR (2010) RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

One trial conducted during growing season 1990 is available to evaluate the magnitude of residues of spiroxamine in/on winter barley (green material, ears, straw and grain) after two applications of Tebuconazole and Spiroxamine 417 EC at 1.5 l/ha to barley in northern Europe, 375 g a.s./ha. The trial on barley is summarised here. Samples were analysed for spiroxamine and tebuconazole, but only the spiroxamine residues are reported.

This previously evaluated study was not conducted to GLP and is not relied upon for assessment or MRL purposes as it does not fully match the GAP but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial on barley was conducted as a decline trial, with green material sampled at 0 to 28 DALA, and ears, straw and grain sampled between 28 to 52 DALA. Residues of spiroxamine in green material were 6.4 mg/kg, 0 DALA, declining to 0.62 to 1.1 mg/kg in mature straw and <0.05 mg/kg in mature grain, 35 to 52 DALA.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in barley grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.2/11-1 Residue trials with tebuconazole and spiroxamine 417 EC in barley – residue results for Northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Crop/Crop Group**	**Cereals**
Country		**:**	**Germany**	**Page**	**1**
Content of active substance	**(g/kg or g/L)**	**:**	**417 g/L**	**Indoor/outdoor**	**Outdoor**
Formulation	**(e.g. WP)**	**:**	**417 EC**	**Other a.s. in formulation (common name and content)**	**Tebuconazole**
Commercial product	**(name)**	**:**	**Tebuconazole and Spiroxamine 417 EC**	**Residues determined as**	**Spiroxamine**
Producer of commercial product		**:**	**Bayer AG**	**Residues calculated as**	**spiroxamine**

Active substance : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) spiroxamine	DALT/PHI (days) (f)	Remarks
PF 3742 00641/6 (0641-90) Germany 5093 Burscheid, Hofchen 1990	Barley, winter Borwina	1) 18.10.1989 2) 09.06.1990 13.06.1990 3) 16.07.1990	Spraying Spraying	0.3750 0.3750	300 300	0.12500 0.12500	15.05.1990,0 25.05.1990,10	61	green material ear straw grain	6.4 0.92 1.0 0.18 0.20 0.62 1.1 <0.05	0 14 28 28 35 35 52 52	(g) 00252 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

III. Conclusions

One residue trial conducted in 1990 in northern Europe is available to evaluate the residues of spiroxamine in/on barley green material, ears, straw and grain harvested after two applications of Tebuconazole and Spiroxamine 417 EC on barley up to BBCH 61.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial on barley was conducted as a decline trial, with green material sampled at 0 to 28 DALA, and ears, straw and grain sampled between 28 to 52 DALA. Residues of spiroxamine in green material were 6.4 mg/kg, 0 DALA, declining to 0.62 to 1.1 mg/kg in mature straw and <0.05 mg/kg in mature grain, 35 to 52 DALA.

This study it is not relied on for assessment or MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted as supplemental information in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/06. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial on barley was conducted as a decline trial, with green material sampled at 0 to 28 DALA, and ears, straw and grain sampled between 28 to 52 DALA. Residues of spiroxamine in green material were 6.4 mg/kg, 0 DALA, declining to 0.62 to 1.1 mg/kg in mature straw and <0.05 mg/kg in mature grain, 35 to 52 DALA.

This study is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.2/12
Report Author:	[redacted]
Report Year:	1999
Report Title:	Determination of residues of KWG 4168 500 EC in/on spring and winter barley following spray application in Germany and Great Britain
Report No:	RA-2157/98
Document No:	M-010652-01-2
Guideline(s) followed in study:	EC guidance document 7029/VI/95 rev.5: Appendix B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and classified RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Four trials conducted during growing season 1998 are available to evaluate the magnitude of residues of spiroxamine and total spiroxamine in/on winter or spring barley (straw and grain) after two applications of Spiroxamine 500 EC at 1.5 to 1.7 L/ha to barley in northern Europe, 750 g a.s./ha. The four trials on barley are summarised here. Samples were analysed for spiroxamine and total residue of

spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents.

This previously evaluated study is not relied upon for assessment or MRL purposes as it does not match the GAP since the second applications all exceed the BBCH growth stage on the cGAP. The data are still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Four trials on barley were conducted as harvest trials, with straw and grain sampled between 35 to 36 DALA. Residues of spiroxamine were 0.416 to 0.669 mg/kg in straw and 0.106 to 0.172 mg/kg in mature grain, 35 to 36 DALA. Residues of total spiroxamine via 4-TBCH were 2.58 to 4.40 mg/kg in straw and 0.312 to 0.923 mg/kg in mature grain, 35 to 36 DALA.

Table CA 6.3.2/12-1 Residue trials with spiroxamine 500 EC in barley – residue results for Northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Active substance**	**:**	**spiroxamine**
Country		**:**	**Germany**	**Crop/Crop Group**	**:**	**Cereals**
Content of active substance	**(g/kg or g/L)**	**:**	**500 g/L**	**Page**	**:**	**1**
Formulation	**(e.g. WP)**	**:**	**500 EC**	**Indoor/outdoor**	**:**	**Outdoor**
Commercial product	**(name)**	**:**	**Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	**:**	
Producer of commercial product		**:**	**Bayer AG**	**Residues determined as**	**:**	**Spiroxamine and spiroxamine via 4-t-butylcyclohexanone**
				Residues calculated as	**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9		10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) spiroxamine	spiroxamine (via 4-TBCH)	DALT/PHI (days) (f)	Remarks
RA-2157/98 810010 Germany D-51399 Burscheid 1998	Barley, spring Scarlett	1) 26.03.1998 2) 16.06.1998 - 20.06.1998 3) 12.08.1998	Spraying Spraying	0.7500 0.7500	300 300	0.25000 0.25000	28.05.1998/0 07.07.1998/40	79	straw grain	0.650 0.107	4.40 0.312	36 36	(g) 00312, 00506 and 00506/E001 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	**Active substance**	:	**spiroxamine**
Country	:	**Germany**	**Crop/Crop Group**	:	**Cereals**
Content of active substance (g/kg or g/L)	:	**500 g/L**	**Page**	:	**2**
Formulation (e.g. WP)	:	**500 EC**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	:	**Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	:	
Producer of commercial product	:	**Bayer AG**	**Residues determined as**	:	**Spiroxamine and spiroxamine via 4-t-butylcyclohexanone**
			Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9		10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) spiroxamine	spiroxamine (via 4-TBCH)	DALT/ PHI (days) (f)	Remarks
RA-2157/98 810037 Germany D-51399 Burscheid 1998	Barley, winter Loreley	1) 26.09.1997 2) 20.05.1998 - 24.05.1998 3) 22.07.1998	Spraying Spraying	0.7500 0.7500	300 300	0.25000 0.25000	29.05.1998/0 - 15.06.1998/47	75-79	straw grain	0.416 0.106	2.58 0.500	35 35	(g) 00312, 00506 and 00506/E001 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**
Country		**:**	**United Kingdom**
Content of active substance	**(g/kg or g/L)**	**:**	**500 g/L**
Formulation	**(e.g. WP)**	**:**	**500 EC**
Commercial product	**(name)**	**:**	**Spiroxamine EC 500**
Producer of commercial product		**:**	**Bayer AG**

Active substance		**:**	**spiroxamine**
Crop/Crop Group		**:**	**Cereals**
Page		**:**	**3**
Indoor/outdoor		**:**	**Outdoor**
Other a.s. in formulation (common name and content)		**:**	
Residues determined as		**:**	**Spiroxamine and spiroxamine via 4-t-butylcyclohexanone**
Residues calculated as		**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9		10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) spiroxamine	spiroxamine (via 4-TBCH)	DALT/PHI (days) (f)	Remarks
RA-2157/98 816280 United Kingdom GB-IP31 3SH, Bury St. Edmunds, Thurston 1998	Barley, spring Alexis	1) 24.03.1998 2) 17.06.1998 01.07.1998 3) 12.08.1998	Spraying Spraying	0.7500 0.3500	300 345	0.25000 0.21739	22.05.1998/0 08.07.1998/47	81	straw grain	0.669 0.172	3.90 0.923	35 35	(g) 00312, 00506 and 00506/E001 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**United Kingdom**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	
Active substance	:	**spiroxamine**	
Crop/Crop Group	:	**Cereals**	
Page	:	**4**	
Indoor/outdoor	:	**Outdoor**	
Other a.s. in formulation (common name and content)	:		
Residues determined as	:	**Spiroxamine and spiroxamine via 4-t-butylcyclohexanone**	
Residues calculated as	:	**spiroxamine**	

1	2	3	4	5			6	7	8	9		10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) spiroxamine	spiroxamine (via 4-TBCH)	DALT/PHI (days) (f)	Remarks
RA-2157/98 816302 United Kingdom GB-IP31 3SH, Bury St. Edmunds, Thurston 1998	Barley, winter Puffin	1) 04.11.1997 2) 18.05.1998 30.05.1998 3) 30.07.1998	Spraying Spraying	0.7500 0.7500	300 300	0.25000 0.25000	28.04.1998/0 24.06.1998/57	85	straw grain	0.635 0.127	3.99 0.901	36 36	(g) 00312, 00506 and 00506/E001 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

III. Conclusions

A total of four residue trials conducted in 1998 in northern Europe are available to evaluate the residues of spiroxamine and total spiroxamine in/on barley straw and grain harvested after two applications of Spiroxamine 500 EC on barley BBCH 32-85.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Four trials on barley were conducted as harvest trials, with straw and grain sampled between 35 to 36 DALA. Residues of spiroxamine were 0.416 to 0.669 mg/kg in straw and 0.106 to 0.172 mg/kg in mature grain, 35 to 36 DALA. Residues of total spiroxamine via 4-TBCH were 2.58 to 4.40 mg/kg in straw and 0.312 to 0.923 mg/kg in mature grain, 35 to 36 DALA.

This study it is not relied on for assessment or MRL purposes as it does not match the GAP.

Assessment and conclusion by applicant:

Study previously submitted and accepted as supplemental information in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.2/13. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Four trials on barley were conducted as harvest trials, with straw and grain sampled between 35 to 36 DALA. Residues of spiroxamine were 0.416 to 0.669 mg/kg in straw and 0.106 to 0.172 mg/kg in mature grain, 35 to 36 DALA. Residues of total spiroxamine via 4-TBCH were 2.58 to 4.40 mg/kg in straw and 0.312 to 0.923 mg/kg in mature grain, 35 to 36 DALA.

This study it is not relied on for assessment or MRL purposes as it does not match the GAP.

Overview on barley residue trials

Residue trials conducted after the previous EU approval evaluation from 2016 onwards employed residue methods measuring both spiroxamine itself with a fully chiral method and total spiroxamine by means of a common moiety approach. Residues of spiroxamine diastereomers A and B and their enantiomers A1: A2; B1 : B2 were measured and reported as mg/kg both individually and as a sum.

The residues data submitted does include trials conducted at the nominal individual application rate of 375 g a.s./ha but to provide a complete dataset from the large number of existing trials conducted at higher or lower application individual rates (from 150 to 750 g a.s./ha), all such trials are included. As these rates are outside of the usually applied ±25% rule, the accepted approach of proportionality or scaling has been used to adjust any residues reported above the analytical LOQ to the nominal rate.

Trials on barley or oats previously evaluated but considered not appropriate for MRL purposes are provided in this submission for information but are not relied upon. These trials are where:

- The use pattern did not fit the cGAP and residues could not be scaled
- The data were only for total residues of spiroxamine (via the 4-t-butylcyclohexanone common moiety)
- The analytical LOQ was 0.05 mg/kg and therefore data could not be scaled or used reliably in MRL calculations

Grain data:

A total of 25 residue trials conducted between 1998 and 2019 are available to evaluate the residues of spiroxamine (scaled spiroxamine as a sum of total isomers) in barley grain after application of spiroxamine formulations to barley crops in support of the critical GAP (latest application nominally BBCH 59). Of these, 12 trials were performed in the northern European climatic zone and 13 trials were performed in the southern European climatic zone. Reported spiroxamine residues in barley grain ranged from <0.01 to 0.04 mg/kg for the northern zone and from <0.01 to 0.048 mg/kg for the southern zone. The data sets were considered to be similar for MRL purposes.

Barley is a major crop in Europe and as such requires eight residue trials per climatic zone when treated with products after formation of the edible commodity and where residues are above the LOQ. Therefore the data set for barley grain satisfies the data requirements outlined in Regulation (EU) 283/2013 of 01 March 2013 and is appropriate for MRL purposes.

In accordance with the guidance from document SANTE/2019/12752 (November 2020) a minimum of 8 trials on barley (commodity 0500010) allows extrapolation of the data to oats (commodity 0500050).

Data from trials are also available for total residues of spiroxamine (via the t-butyl cyclohexanone common moiety) and ranged from <0.01 to 0.20 mg/kg for the northern zone and from <0.01 to 0.16 mg/kg for the southern zone. These values, where appropriate, are used to determine conversion factors for use in consumer risk assessment.

Straw data:

A total of 50 residue trials conducted between 1998 and 2019 are available to evaluate the residues of spiroxamine (scaled spiroxamine as a sum of total isomers) in barley straw after application of spiroxamine formulations to barley crops in support of the critical GAP (latest application nominally BBCH 59). Of these, 24 trials were performed in the northern European climatic zone and 26 trials were performed in the southern European climatic zone. Reported spiroxamine residues in barley straw ranged from 0.06 to 0.68 mg/kg for the northern zone and from 0.06 to 0.73 mg/kg for the southern zone.

Data from trials are also available for total residues of spiroxamine (via the t-butyl cyclohexanone common moiety) and ranged from 1.63 to 5.6 mg/kg for the northern zone and from 1.33 to 6.5 mg/kg for the southern zone. These values, where appropriate, can be used to determine conversion factors or used in dietary burden calculations.

CA 6.3.3 Wheat

Spiroxamine is supported for application to wheat according to the critical GAP detailed in Table CA 6.3-1, involving up to 2 applications at 375 g a.s./ha.

Residue trials conducted after the previous EU approval evaluation from 2016 onwards employed residue methods measuring both spiroxamine itself with a fully chiral method and total spiroxamine by means of a common moiety approach. Residues of spiroxamine diastereomers A and B and their enantiomers A1; A2; B1; B2 were measured and reported as mg/kg both individually and as a sum. The summaries in this section present these data but do not include enantiomer or A / B diastereomer ratios. The ratios are discussed and presented under Section CA 6.7.1 in order to consider the impact of isomer ratios on consumer risk assessment.

The residues data presented here does include trials conducted at the nominal individual application rate of 375 g a.s./ha but to provide a complete dataset from the existing trials conducted at higher or lower application individual rates (from 150 to 750 g a.s./ha) and supporting the GAP for timing or analytical LOQ, all such trials are included. As these rates are outside of the usually applied ±25% rule,

the accepted approach of proportionality or scaling has been used to adjust any residues reported above the analytical LOQ to the nominal rate. Refer to Section CA 6.3.2 for details of how the scaling approach was applied.

Data Point:	KCA 6.3.3/12
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Determination of the residues of prothioconazole and spiroxamine in/on spring wheat and winter wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the United Kingdom, Germany and the Netherlands
Report No:	16-2046
Document No:	M-626175-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four residue trials conducted during growing season 2016 are available to evaluate the magnitude of residues of spiroxamine in/on spring and winter wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat in northern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on wheat were conducted in northern Europe (Germany, the Netherlands and United Kingdom) with all four conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/12-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 375 g a.s./ha at a water application rate of 200 to 400 L/ha. Spray intervals were 20 to 24 days, with the final treatment being made at growth stage BBCH 65 or 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 296.2 g/L of spiroxamine).

Samples of green material were taken on the day of the final application, and between 50 to 62 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last treatment and 50 to 62 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/12-4, the maximum storage time between date of deep-freezing and date of last extraction was 518 days for spiroxamine enantiomers and 588 days for the total residue of spiroxamine.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.3/12-2 and Table CA 6.3.3/12-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as harvest trials, with straw and grain sampled between 50 and 62 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.20 to 9.80 mg/kg. At maturity, residues in grain were all <0.01 mg/kg with corresponding residues in straw ranging from 0.14 to 0.44 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 4.10 to 11.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.038 mg/kg with corresponding residues in straw ranging from 0.44 to 2.00 mg/kg.

Table CA 6.3.3/12-1 Residue trials with spiroxamine and prothioconazole 460 EC in wheat – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-OHBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.3/12 (16-2046) 16-2046-01 United Kingdom, SG8 8SS Great Chishill, Royston Winter wheat (Cougar) 2016	1 2	0.375 0.375	200 200	BBCH 43 BBCH 69	Green material Grain Straw	0 50 50	1.50 <0.00271 0.120	1.50 <0.00271 0.130	1.30 <0.00229 0.094	1.30 <0.00229 0.098	5.60 <u><0.01</u> 0.440	5.50 0.018 2.00
CA 6.3.3/12 (16-2046) 16-2046-02 Germany, 51399 Burscheid Winter wheat (Dekan) 2016	1 2	0.375 0.375	300 300	BBCH 45 BBCH 69	Green material Grain Straw	0 62 62	1.60 <0.00271 0.038	1.60 <0.00271 0.039	1.40 <0.00229 0.033	1.30 <0.00229 0.031	5.80 <u><0.01</u> 0.140	6.60 0.024 0.440
CA 6.3.3/12 (16-2046) 16-2046-03 The Netherlands, 1606 MG Venhuizen Spring wheat (Tybalt) 2016	1 2	0.375 0.375	400 400	BBCH 39 BBCH 65	Green material Grain Straw	0 54 54	1.30 <0.00271 0.043	1.20 <0.00271 0.044	0.930 <0.00229 0.033	0.970 <0.00229 0.034	4.20 <u><0.01</u> 0.150	4.10 <0.01 0.670
CA 6.3.3/12 (16-2046) 16-2046-04 Germany, 59609 Anröchte-Berge Spring wheat (Thasos) 2016	1 2	0.375 0.375	300 300	BBCH 39 BBCH 69	Green material Grain Straw	0 53 53	2.70 <0.00271 0.079	2.70 <0.00271 0.086	2.20 <0.00229 0.067	2.30 <0.00229 0.070	9.80 <u><0.01</u> 0.300	11.0 0.038 2.00

1 – <u>Underlined</u> value to be used to support MRL for grain

Table CA 6.3.3/12-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/12	01480	A1 enantiomer	Wheat green material	0.00271 0.0271 0.271 5.4 Overall	3 3 1 1 8	98; 101; 102 90; 91; 91 91 90 Mean: 94, RSD: 5.5
CA 6.3.3/12	01480	A1 enantiomer	Wheat grain	0.00271 0.0271 0.271 Overall	3 3 1 7	87; 87; 91 89; 89; 90 95 Mean: 90, RSD: 3.1
CA 6.3.3/12	01480	A1 enantiomer	Wheat straw	0.00271 0.0271 0.271 Overall	3 3 1 7	98; 99; 106 90; 91; 92 98 Mean: 96, RSD: 5.9
CA 6.3.3/12	01480	A2 enantiomer	Wheat green material	0.00271 0.0271 0.271 5.4 Overall	3 3 1 1 8	96; 98; 98 90; 91; 91 92 90 Mean: 93, RSD: 3.7
CA 6.3.3/12	01480	A2 enantiomer	Wheat grain	0.00271 0.0271 0.271 Overall	3 3 1 7	86; 88; 88 89; 89; 91 96 Mean: 90, RSD: 3.6
CA 6.3.3/12	01480	A2 enantiomer	Wheat straw	0.00271 0.0271 0.271 Overall	3 3 1 7	92; 96; 104 89; 91; 91 96 Mean: 94, RSD: 5.4
CA 6.3.3/12	01480	B1 enantiomer	Wheat green material	0.00229 0.0229 0.229 4.6 Overall	3 3 1 1 8	95; 99; 103 90; 91; 92 91 92 Mean: 94, RSD: 4.9
CA 6.3.3/12	01480	B1 enantiomer	Wheat grain	0.00229 0.0229 0.229 Overall	3 3 1 7	88; 88; 91 88; 90; 91 98 Mean: 91, RSD: 3.9
CA 6.3.3/12	01480	B1 enantiomer	Wheat straw	0.00229 0.0229 0.229 Overall	3 3 1 7	92; 98; 102 91; 91; 92 101 Mean: 95, RSD: 5.1
CA 6.3.3/12	01480	B2 enantiomer	Wheat green material	0.00229 0.0229 0.229 4.6 Overall	3 3 1 1 8	97; 99; 101 89; 92; 93 92 91 Mean: 94, RSD: 4.5
CA 6.3.3/12	01480	B2 enantiomer	Wheat grain	0.00229 0.0229 0.229 Overall	3 3 1 7	84; 85; 88 90; 92; 92 97 Mean: 90, RSD: 5.0
CA 6.3.3/12	01480	B2 enantiomer	Wheat straw	0.00229 0.0229	3 3	93; 93; 102 88; 90; 91

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
				0.229 Overall	1 7	97 Mean: 93, RSD: 6.1

Table CA 6.3.3/12-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/12	00312/M002	Total residue of spiroxamine	Wheat green material	0.01 0.10 9.9 Overall	3 3 1 7	95 (166); 98 (169); 105 (176)[1] 81 (88); 94 (98); 100 (107)[1] 81 Mean: 101, RSD: 11.0
CA 6.3.3/12	00312/M002	Total residue of spiroxamine	Wheat grain	0.01 0.10 Overall	3 3 6	96 (124); 116 (144); 119 (147)[1] 98 (101); 101 (104); 114 (117)[1] Mean: 107, RSD: 9.4
CA 6.3.3/12	00312/M002	Total residue of spiroxamine	Wheat straw	0.01 0.10 1.0 2.0 Overall	3 3 1 1 8	100 (199); 104 (203); 105 (204)[1] 84 (94); 91 (101); 95 (105)[1] 103 94 Mean: 97, RSD: 7.6

1 - These recoveries were background-corrected since the control sample used for spiking were found to contain (apparent) residues with differing levels. The uncorrected recoveries are shown in brackets.

Table CA 6.3.3/12-4 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/12	Wheat	456 to 518 (parent spiroxamine enantiomers) 530 and 588 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2016 in northern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat BBCH 37 to 69.

All four trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.20 to 9.80 mg/kg. At maturity, residues in grain were all <0.01 mg/kg with corresponding residues in straw ranging from 0.14 to 0.44 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 4.10 to 11.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.038 mg/kg with corresponding residues in straw ranging from 0.44 to 2.00 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.20 to 9.80 mg/kg. At maturity, residues in grain were all <0.01 mg/kg with corresponding residues in straw ranging from 0.14 to 0.44 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 4.10 to 11.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.038 mg/kg with corresponding residues in straw ranging from 0.44 to 2.00 mg/kg.

Data Point:	KCA 6.3.3/13
Report Author:	[redacted]
Report Year:	2018
Report Title:	Determination of the residues of prothioconazole and spiroxamine in on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Italy and Greece
Report No:	16-2163
Document No:	M-627049-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2016 are available to evaluate the magnitude of residues of spiroxamine, in/on spring and winter wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on wheat were conducted in southern Europe (southern France, Spain, Italy and Greece) with all four trials conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/13-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 375 g a.s./ha at a water application rate of 300 to 400 L/ha. One exception was the trial in Spain (16-2163-02) where the first application was slightly increased at 1.31 L/ha (394 g a.s./ha), this was considered to have no impact on the residues following the second application which was applied at the intended rate. Spray intervals were 21 or 22 days, with the final treatment being made at growth stage BBCH 65 or 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 296.2 g/L of spiroxamine).

Samples of green material were taken on the day of the final application, and between 42 to 50 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last treatment and 42 to 50 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/13-4, the maximum storage time between date of deep-freezing and date of last extraction was 554 days before the analysis of the parent spiroxamine enantiomers, and 678 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.3/13-2 and Table CA 6.3.3/13-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as harvest trials, with straw and grain sampled between 42 and 50 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.30 to 11.0 mg/kg. At maturity, residues in grain were all <0.01 mg/kg with corresponding residues in straw ranging from 0.55 to 1.50 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 5.90 to 13.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.012 mg/kg with corresponding residues in straw ranging from 2.5 to 9.0 mg/kg.

Table CA 6.3.3/13-1 Residue trials with spiroxamine and prothioconazole 460 EC in wheat – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomer[1]	
CA 6.3.3/13 (16-2163) 16-2163-01 Southern France, 31620 Gargas Wheat (Tiepolo) 2016	1 2	0.375 0.375	300 300	BBCH 43 BBCH 69	Green material Grain Straw	0 50 50	1.20 <0.00271 0.150	1.20 <0.00271 0.160	0.97 <0.00229 0.120	1.00 <0.00229 0.120	4.30 <u><0.01</u> 0.550	5.90 <0.01 2.50
CA 6.3.3/13 (16-2163) 16-2163-02 Spain, 41310 Brenes Wheat (Artur Nick) 2016	1 2	0.394[2] 0.375	300 300	BBCH 47 BBCH 69	Green material Grain Straw	0 46 46	1.60 <0.00271 0.210	1.60 <0.00271 0.210	1.3 <0.00229 0.160	1.30 <0.00229 0.160	5.90 <u><0.01</u> 0.740	8.30 0.012 3.80
CA 6.3.3/13 (16-2163) 16-2163-03 Italy, 94100 C. da San Benedetto; Enna (EN) Wheat (Avvento) 2016	1 2	0.375 0.375	400 400	BBCH 51 BBCH 65	Green material Grain Straw	0 43 43	1.20 <0.00271 0.440	1.20 <0.00271 0.440	0.970 <0.00229 0.310	1.10 <0.00229 0.320	4.50 <u><0.01</u> 1.50	8.50 <0.01 9.00
CA 6.3.3/13 (16-2163) 16-2163-04 Greece, 611 00 Kristoni Kilkis Wheat (Zanzibar) 2016	1 2	0.375 0.375	300 300	BBCH 49 BBCH 65	Green material Grain Straw	0 42 42	3.10 <0.00271 0.300	3.10 <0.00271 0.310	2.50 <0.00229 0.210	2.50 <0.00229 0.200	11.0 <u><0.01</u> 1.00	13.0 <0.01 6.70

1 – Underlined value to be used to support MRL for grain
2 – Application slightly overdosed, no impact

Table CA 6.3.3/13-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/13	01480	A1 enantiomer	Wheat green material	0.00271 0.0271 0.54 14 Overall	3 3 1 1 8	96; 99; 116 92; 92; 95 98 91 Mean: 97, RSD: 8.3
CA 6.3.3/13	01480	A1 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	92; 99; 102 94; 95; 95 Mean: 96, RSD: 3.8
CA 6.3.3/13	01480	A1 enantiomer	Wheat straw	0.00271 0.0271 0.54 Overall	3 3 1 7	96; 96; 98 91; 91; 93 85 Mean: 93, RSD: 4,7
CA 6.3.3/13	01480	A2 enantiomer	Wheat green material	0.00271 0.0271 0.54 14 Overall	3 3 1 1 8	93; 97; 112 93; 94; 95 96 91 Mean: 96, RSD: 6,8
CA 6.3.3/13	01480	A2 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	86; 94; 102 93; 94; 96 Mean: 94, RSD: 4.9
CA 6.3.3/13	01480	A2 enantiomer	Wheat straw	0.00271 0.0271 0.54 Overall	3 3 1 7	92; 96; 102 92; 93; 94 84 Mean: 93, RSD: 5.8
CA 6.3.3/13	01480	B1 enantiomer	Wheat green material	0.00229 0.0229 0.46 12 Overall	3 3 1 1 8	98; 101; 108 94; 94; 94 101 90 Mean: 98, RSD: 5.9
CA 6.3.3/13	01480	B1 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	94; 96; 101 96; 99; 103 Mean: 98, RSD: 3.5
CA 6.3.3/13	01480	B1 enantiomer	Wheat straw	0.00229 0.0229 0.46 Overall	3 3 1 7	96; 97; 98 92; 96; 96 83 Mean: 94, RSD: 5.5
CA 6.3.3/13	01480	B2 enantiomer	Wheat green material	0.00229 0.0229 0.46 12 Overall	3 3 1 1 8	102; 102; 112 92; 94; 97 98 94 Mean: 99, RSD: 6.5
CA 6.3.3/13	01480	B2 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	94; 96; 104 93; 93; 95 Mean: 96, RSD: 4.3
CA 6.3.3/13	01480	B2 enantiomer	Wheat straw	0.00229 0.0229 0.46 Overall	3 3 1 7	90; 94; 100 90; 91; 92 87 Mean: 92, RSD: 4.5

Table CA 6.3.3/13-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/13	00312/M002	Total residue of spiroxamine	Wheat green material	0.01 0.10 15 Overall	4 3 1 8	80 (88); 82 (90); 100 (119); 108 (127)[1] 89 (91); 94 (96); 96 (98)[1] 78 Mean: 91, RSD: 11.6
CA 6.3.3/13	00312/M002	Total residue of spiroxamine	Wheat grain	0.01 0.10 Overall	3 3 6	98; 108; 114 82; 84; 85 Mean: 95, RSD: 14.3
CA 6.3.3/13	00312/M002	Total residue of spiroxamine	Wheat straw	0.01 0.10 9.9 Overall	4 3 1 8	88 (104); 99 (115); 102 (128); 111 (137)[1] 85 (88); 88 (91); 96 (99)[1] 89 Mean: 95, RSD: 9.4

1 – Background corrected for apparent residues found in the control sample measured as 4-t-butylcyclohexanone. The values in brackets are the recovery values before correction.

Table CA 6.3.3/13-4 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/13	Wheat	484 to 554 (parent spiroxamine enantiomers) 587 and 678 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2016 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 160 on wheat BBCH 43 to 69.

All four trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.30 to 11.0 mg/kg. At maturity, residues in grain were all <0.01 mg/kg with corresponding residues in straw ranging from 0.55 to 1.50 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 5.90 to 13.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.012 mg/kg with corresponding residues in straw ranging from 2.5 to 9.0 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.30 to 11.0 mg/kg. At maturity, residues in grain were all <0.01 mg/kg with corresponding residues in straw ranging from 0.55 to 1.50 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 5.90 to 13.0 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.012 mg/kg with corresponding residues in straw ranging from 2.5 to 9.0 mg/kg.

Data Point:	KCA 6.3.3/14
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on wheat after spray application of bixafen & spiroxamine & trifloxystrobin EC 325 in Spain and Italy
Report No:	17-2144
Document No:	M-633801-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Two trials conducted during growing season 2017 are available to evaluate the magnitude of residues of spiroxamine in/on wheat (green material, grain and straw) after two applications of spiroxamine, bixafen and trifloxystrobin EC 325 in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of bixafen and trifloxystrobin were also analysed, however are not reported in this summary.

Two residue trials on wheat were conducted in southern Europe (Spain and Italy) with both trials conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/14-1 and Table CA 6.3.3/14-2.

Two spray applications of spiroxamine were made at a product rate of 1.0 L/ha corresponding to 150 g a.s./ha at a water application rate of 300 or 400 L/ha. Spray intervals were 21 days with the final

treatment being made at growth stage BBCH 69. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a bixafen and spiroxamine and trifloxystrobin EC 325 formulation containing 150 g/L spiroxamine (actual content 148.1 g/L of spiroxamine).

Samples of green material were taken on the day of the final application and 42 or 56 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last application and 42 or 56 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/14-5 the maximum storage time between date of deep-freezing and date of last extraction was 221 days before the analysis of the parent spiroxamine enantiomers, and 380 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.3/14-3 and Table CA 6.3.3/14-4).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

Both trials were conducted as harvest trials, with straw and grain sampled 42 or 56 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) were 5.00 and 15.75 mg/kg. At maturity, residues in grain were reported as below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 0.16 and 0.75 mg/kg.

The scaled total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) were 5.75 and 12.50 mg/kg. At maturity, residues in grain were again below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 2.50 and 4.25 mg/kg.

Table CA 6.3.3/14-1 Residue trials with spiroxamine, trifloxystrobin and bixafen 325 EC in wheat – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.3/14 (17-2144) 17-2144-01 Spain, 41610 Paradas Wheat (Amilcar) 2017	1 2	0.150 0.150	300 300	BBCH 49 BBCH 69	Green material Grain Straw	0 56 56	1.70 <0.00271 0.018	1.70 <0.00271 0.018	1.40 <0.00229 0.014	1.40 <0.00229 0.015	6.30 <0.01 0.065	5.00 <0.01 1.00
CA 6.3.3/14 (17-2144) 17-2144-02 Italy, 95045 Misterbianco Wheat (Core) 2017	1 2	0.150 0.150	400 400	BBCH 45 BBCH 69	Green material Grain Straw	0 42 42	0.55 <0.00271 0.085	0.52 <0.00271 0.087	0.46 <0.00229 0.065	0.44 <0.00229 0.061	2.00 <0.01 0.30	2.30 <0.01 1.70

Table CA 6.3.3/14-2 Residue trials spiroxamine, trifloxystrobin and bixafen EC 325 in wheat – scaled residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,2]					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of 4 spiroxamine enantiomers	
CA 6.3.3/14 (17-2144) 17-2144-01 Spain, 41610 Paradas Wheat (Amilcar) 2017	1 2	0.150 0.150	300 300	BBCH 49 BBCH 69	Green material Grain Straw	0 56 56	3.50 n.a. 0.03	3.50 n.a. 0.05	3.50 n.a. 0.04	3.50 n.a. 0.04	13.75 n.a. 0.16	12.50 n.a. 2.50
CA 6.3.3/14 (17-2144) 17-2144-02 Italy, 95045 Misterbianco Wheat (Core) 2017	1 2	0.150 0.150	400 400	BBCH 45 BBCH 69	Green material Grain Straw	0 42 42	1.38 n.a. 0.21	1.50 n.a. 0.22	1.15 n.a. 0.16	1.10 n.a. 0.15	5.00 n.a. 0.75	5.75 n.a. 4.25

1 – Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 – Values reported below the LOQ not appropriate to scale up

Table CA 6.3.3/14-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/14	01480	A1 enantiomer	Wheat green material	0.00271 0.0271 1.4 5.4 Overall	3 3 1 1 8	97; 99; 99 92; 94; 95 111 96 Mean: 98, RSD: 5.9
CA 6.3.3/14	01480	A1 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	90; 90; 90 92; 93; 94 Mean: 92, RSD: 1.9
CA 6.3.3/14	01480	A1 enantiomer	Wheat straw	0.00271 0.0271 0.271 Overall	3 3 1 7	93; 95; 97 90; 93; 93 85 Mean: 92, RSD: 4.2
CA 6.3.3/14	01480	A2 enantiomer	Wheat green material	0.00271 0.0271 1.4 5.4 Overall	3 3 1 1 8	97; 101; 104 93; 93; 95 109 95 Mean: 98, RSD: 5.9
CA 6.3.3/14	01480	A2 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	87; 89; 91 94; 95; 95 Mean: 92, RSD: 3.7
CA 6.3.3/14	01480	A2 enantiomer	Wheat straw	0.00271 0.0271 0.271 Overall	3 3 1 7	91; 94; 95 91; 92; 93 85 Mean: 92, RSD: 3.6
CA 6.3.3/14	01480	B1 enantiomer	Wheat green material	0.00229 0.0229 1.2 4.6 Overall	3 3 1 1 8	99; 100; 101 95; 97; 97 110 97 Mean: 99.5, RSD: 4.7
CA 6.3.3/14	01480	B1 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	91; 95; 90 94; 96; 95 Mean: 94, RSD: 2.6
CA 6.3.3/14	01480	B1 enantiomer	Wheat straw	0.00229 0.0229 0.229 Overall	3 3 1 7	103; 97; 99 93; 95; 93 88 Mean: 95, RSD: 5.1
CA 6.3.3/14	01480	B2 enantiomer	Wheat green material	0.00229 0.0229 1.2 4.6 Overall	3 3 1 1 8	98; 98; 99 91; 94; 95 112 98 Mean: 98, RSD: 6.3
CA 6.3.3/14	01480	B2 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	92; 92; 92 95; 95; 96 Mean: 94, RSD: 2.0
CA 6.3.3/14	01480	B2 enantiomer	Wheat straw	0.00229 0.0229 0.229 Overall	3 3 1 7	95; 96; 96 91; 93; 93 85 Mean: 93, RSD: 4.2

Table CA 6.3.3/14-4 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/14	00312/M002	Total residue of spiroxamine	Wheat green material	0.01 0.10 1.0 9.9 Overall	3 3 1 1 8	85 (204); 91 (210); 103 (222)[1] 87 (99); 88 (100); 90 (102)[1] 89 (90)[1] 84 Mean: 90, RSD: 6.6
CA 6.3.3/14	00312/M002	Total residue of spiroxamine	Wheat grain	0.01 0.10 Overall	3 3 6	95; 96; 101 87; 95; 96 Mean: 95, RSD: 4.8
CA 6.3.3/14	00312/M002	Total residue of spiroxamine	Wheat straw	0.01 0.10 1.0 5.1 Overall	3 3 1 1 8	85 (246); 111 (272); 117 (278)[1] 80 (96); 86 (102); 99 (115)[1] 96 (98) 90 Mean: 96, RSD: 13.6

1 – Background corrected for apparent residues found in the control sample measured as 4-t-butylcyclohexanone. The values in brackets are the recovery values before the correction.

Table CA 6.3.3/14-5 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/14	Wheat	161 to 221 (parent spiroxamine enantiomers) 313 and 380 (total residue of spiroxamine)

III. Conclusions

A total of two residue trials conducted in 2017 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on wheat (green material, grain and straw) after two applications of bixafen and spiroxamine and trifloxystrobin EC 325, BBCH 45-69.

Both trials were conducted as harvest trials. Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) were 5.00 and 15.75 mg/kg. At maturity, residues in grain were reported as below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 0.16 and 0.75 mg/kg.

The scaled total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) were 5.75 and 12.50 mg/kg. At maturity, residues in grain were again below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 2.50 and 4.25 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) were 5.00 and 15.75 mg/kg. At maturity, residues in grain were reported as below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 0.16 and 0.75 mg/kg.

The scaled total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) were 5.75 and 12.50 mg/kg. At maturity, residues in grain were again below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 2.50 and 4.25 mg/kg.

Data Point:	KCA 6.3.3/15
Report Author:	[redacted]
Report Year:	2018
Report Title:	Determination of the residues of trifloxystrobin, BYE 00587 and spiroxamine in/on winter wheat after spray application of bixafen & spiroxamine & trifloxystrobin EC 325 in The United Kingdom and Germany
Report No:	17-2030
Document No:	M-634111-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Two trials conducted during growing season 2017 are available to evaluate the magnitude of residues of spiroxamine, in/on wheat (green material, grain and straw) after two applications of spiroxamine, bixafen and trifloxystrobin EC 325 in northern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of bixafen and trifloxystrobin were also analysed, however are not reported in this summary.

Two residue trials on wheat were conducted in northern Europe (United Kingdom and Germany) with both trials conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/15-1 and Table CA 6.3.3/15-2.

Two spray applications of spiroxamine were made at a product rate of 1.0 L/ha corresponding to 150 g a.s./ha at a water application rate of 200 or 300 L/ha. Spray intervals were 18 or 21 days with the final treatment being made at growth stage BBCH 69. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a bixafen and spiroxamine and trifloxystrobin EC 325 formulation containing 150 g/L spiroxamine (actual content 148.1 g/L of spiroxamine).

Samples of green material were taken on the day of the final application, and 47 or 50 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last application and 47 or 50 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617614-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/15-5, the maximum storage time between date of deep-freezing and date of last extraction was

152 days before the analysis of the parent spiroxamine enantiomers, and 306 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.3/15-3 and Table CA 6.3.3/15-4).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

Both trials were conducted as harvest trials, with straw and grain sampled 42 or 56 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) were 4.50 and 5.75 mg/kg. At maturity, residues in grain were reported as below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 0.53 and 0.50 mg/kg.

The scaled total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) were 5.25 and 5.50 mg/kg. At maturity, residues in grain were again below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 4.50 and 3.00 mg/kg.

Table CA 6.3.3/15-1 Residue trials with spiroxamine, trifloxystrobin and bixafen325 EC in wheat – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.3.3/15 (17-2030) 17-2030-01 United Kingdom, GB-SG8 8SS Great Chishill Royston Winter wheat (KWS Santiago 2017	1 2	0.150 0.150	200 200	BBCH 51 BBCH 69	Green material Grain Straw	0 50 50	0.50 <0.00271 0.058	0.48 <0.00271 0.059	0.42 <0.00229 0.045	0.41 <0.00229 0.046	1.80 <0.01 0.21	2.10 0.011 1.80
CA 6.3.3/15 (17-2030) 17-2030-02 Germany, D-78073 Bad Dürrheim OT Unterbaldingen Winter wheat (Akteur) 2017	1 2	0.150 0.150	300 300	BBCH 37 BBCH 69	Green material Grain Straw	0 47 47	0.62 <0.00271 0.053	0.60 <0.00271 0.055	0.53 <0.00229 0.045	0.51 <0.00229 0.045	2.30 <0.01 0.20	2.20 <0.01 1.20

Table CA 6.3.3/15-2 Residue trials spiroxamine, trifloxystrobin and bixafen EC 325 in wheat – scaled residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Scaled residue spiroxamine enantiomers (mg/kg)[1,2]					Scaled total residue of spiroxamine (via 4-TBCH, mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of 4 spiroxamine enantiomers	
CA 6.3.3/15 (17-2030) 17-2030-01 United Kingdom, GB-SG8 8SS Great Chishill Royston Winter wheat (KWS Santiago) 2017	1 2	0.150 0.150	200 200	BBCH 51 BBCH 69	Green material Grain Straw	0 50 50	1.25 n.a. 0.15	1.20 n.a. 0.15	1.95 n.a. 0.11	1.03 n.a. 0.12	4.50 n.a. 0.53	5.25 n.a. 4.50
CA 6.3.3/15 (17-2030) 17-2030-02 Germany, D-78073 Bad Dürrheim OT Unterbaldingen Winter wheat (Akteur) 2017	1 2	0.150 0.150	300 300	BBCH 37 BBCH 69	Green material Grain Straw	0 47 47	1.55 n.a. 0.13	1.50 n.a. 0.14	1.33 n.a. 0.11	1.28 n.a. 0.11	5.75 n.a. 0.50	5.50 n.a. 3.00

1 – Residues scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - Values reported below the LOQ not appropriate to scale up

Table CA 6.3.3/15-3 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/15	01480	A1 enantiomer	Wheat green material	0.00271 0.0271 5.4 Overall	3 3 1 7	91; 93; 95 94; 95; 97 97 Mean: 95, RSD: 2.3
CA 6.3.3/15	01480	A1 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	89; 91; 91 89; 94; 94 Mean: 91, RSD: 2.5
CA 6.3.3/15	01480	A1 enantiomer	Wheat straw	0.00271 0.0271 0.271 Overall	3 3 1 7	96; 96; 96 94; 97; 99 96 Mean: 96, RSD: 1.6
CA 6.3.3/15	01480	A2 enantiomer	Wheat green material	0.00271 0.0271 5.4 Overall	3 3 1 7	90; 91; 92 94; 94; 97 97 Mean: 94, RSD: 2.9
CA 6.3.3/15	01480	A2 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	86; 86; 91 90; 93; 93 Mean: 90, RSD: 3.5
CA 6.3.3/15	01480	A2 enantiomer	Wheat straw	0.00271 0.0271 0.271 Overall	3 3 1 7	89; 91; 92 93; 96; 98 96 Mean: 94, RSD: 3.4
CA 6.3.3/15	01480	B1 enantiomer	Wheat green material	0.00229 0.0229 4.6 Overall	3 3 1 7	93; 93; 95 94; 94; 98 99 Mean: 95, RSD: 2.5
CA 6.3.3/15	01480	B1 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	91; 92; 92 90; 95; 96 Mean: 93, RSD: 2.5
CA 6.3.3/15	01480	B1 enantiomer	Wheat straw	0.00229 0.0229 0.229 Overall	3 3 1 7	94; 97; 97 96; 97; 99 95 Mean: 96, RSD: 1.7
CA 6.3.3/15	01480	B2 enantiomer	Wheat green material	0.00229 0.0229 4.6 Overall	3 3 1 7	92; 93; 96 93; 94; 96 100 Mean: 95, RSD: 2.9
CA 6.3.3/15	01480	B2 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	88; 90; 93 92; 94; 94 Mean: 92, RSD: 2.6
CA 6.3.3/15	01480	B2 enantiomer	Wheat straw	0.00229 0.0229 0.229 Overall	3 3 1 7	96; 96; 99 94; 97; 100 97 Mean: 97, RSD: 2.1

Table CA 6.3.3/15-4 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/15	00312/M002	Total residue of spiroxamine	Wheat green material	0.01 0.10 5.0 Overall	4 3 1 8	98 (113); 106 (145); 112 (151); 112 (127)[1] 85 (87); 86 (88); 88 (90)[1] 83 Mean: 96, RSD: 12.8
CA 6.3.3/15	00312/M002	Total residue of spiroxamine	Wheat grain	0.01 0.10 Overall	3 3 6	81 (124); 84 (127); 85 (128)[1] 82 (86); 83 (87); 84 (88)[1] Mean: 83, RSD: 1.8
CA 6.3.3/15	00312/M002	Total residue of spiroxamine	Wheat straw	0.01 0.10 1.0 Overall	3 3 1 7	73 (202); 99 (228); 104 (233)[1] 76 (89); 79 (92); 85 (98)[1] 83 (84)[1] Mean: 86, RSD: 13.7

1 – Background corrected for apparent residues found in the control sample measured as 4-t-butylcyclohexanone. The values in brackets are the recovery values before the correction.

Table CA 6.3.3/15-5 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/15	Wheat	98 to 152 (parent spiroxamine enantiomers) 240 and 306 (total residue of spiroxamine)

III. Conclusions

A total of two residue trials conducted in 2017 in northern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in on wheat (green material, grain and straw) after two applications of bixafen and spiroxamine and trifloxystrobin EC 325, BBCH 37-69.

Both trials were conducted as harvest trials. Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) were 4.50 and 5.75 mg/kg. At maturity, residues in grain were reported as below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 0.53 and 0.50 mg/kg.

The scaled total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) were 5.25 and 5.50 mg/kg. At maturity, residues in grain were again below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 4.50 and 3.00 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) were 4.50 and 5.75 mg/kg. At maturity, residues in grain were reported as below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 0.53 and 0.50 mg/kg.

The scaled total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) were 5.25 and 5.50 mg/kg. At maturity, residues in grain were again below the LOQ for both trials, therefore the values could not be scaled up to the critical GAP. Corresponding scaled residues in straw were 4.50 and 3.00 mg/kg.

Data Point:	KCA 6.3.3/16
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, northern France and the Netherlands
Report No:	17-2015
Document No:	M-659920-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2017 are available to evaluate the magnitude of residues of spiroxamine in/on spring and winter wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat in northern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2, with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on wheat were conducted in northern Europe (Germany, northern France and the Netherlands) with all four trials conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/16-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 375 g a.s./ha at a water application rate of 300 to 400 L/ha. Spray intervals were 21 days, with the final treatment being made at growth stage BBCH 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 294.2 g/L of spiroxamine).

Samples of green material were taken on the day of the final application, and between 26 to 61 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last treatment and 26 to 61 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617914-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/16-4, the maximum storage time between date of deep-freezing and date of last extraction was 441 days before the analysis of the parent spiroxamine enantiomers, and 517 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.3/16-2 and Table CA 6.3.3/16-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg and for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers. The limit of quantification (LOQ) for total spiroxamine (via 4-t-butylcyclohexanone) was 0.01 mg/kg.

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as harvest trials, with straw and grain sampled between 26 and 61 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.10 to 5.70 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.022 mg/kg with corresponding residues in straw ranging from 0.43 to 0.93 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 6.10 to 7.40 mg/kg. At maturity, residues in grain ranged from 0.011 to 0.024 mg/kg with corresponding residues in straw ranging from 2.20 to 4.50 mg/kg.

Table CA 6.3.3/16-1 Residue trials with spiroxamine and prothioconazole 460 EC in wheat – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-FBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.3/16 (17-2015) 17-2015-01 Germany, D-51399 Burscheid Winter wheat (Potential) 2017	1 2	0.375 0.375	300 300	BBCH 45 BBCH 69	Green material Grain Straw	0 61 61	1.60 <0.00271 0.21	1.50 <0.00271 0.21	1.30 <0.00229 0.17	1.30 <0.00229 0.17	5.70 <u>0.01</u> 0.77	7.10 0.014 3.80
CA 6.3.3/16 (17-2015) 17-2015-02 Germany, D-78166 Donaueschingen OT Asen Spring wheat (KWS Chamsin) 2017	1 2	0.375 0.375	300 300	BBCH 51 BBCH 69	Green material Grain Straw	0 26 26	1.20 0.006 0.25	1.20 0.006 0.26	0.97 0.005 0.21	0.96 0.005 0.21	4.30 <u>0.022</u> 0.93	6.10 0.036 2.20
CA 6.3.3/16 (17-2015) 17-2015-03 Northern France, F- 37310 Chambourg sur Indre Winter wheat (Venezio) 2017	1 2	0.375 0.375	300 300	BBCH 45 BBCH 69	Green material Grain Straw	0 43 43	1.30 <0.00271 0.20	1.30 <0.00271 0.20	1.10 <0.00229 0.16	1.10 <0.00229 0.17	4.80 <u><0.01</u> 0.72	7.20 0.011 4.50
CA 6.3.3/16 (17-2015) 17-2015-04 The Netherlands, N-1681 ND Zwaagdijk Spring wheat (Tybalt) 2017	1 2	0.375 0.375	400 400	BBCH 47 BBCH 69	Green material Grain Straw	0 39 39	1.20 0.003 0.12	1.10 0.003 0.12	0.91 0.00234[2] 0.094	0.89 0.00229[2] 0.093	4.10 <u>0.011</u> 0.43	7.40 0.022 1.00

1 – <u>Underlined</u> value to be used to support MRL for grain
2 – Values are presented with 3 significant digits to clarify that they are at or above LOQ

Table CA 6.3.3/16-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/16	01480	A1 enantiomer	Wheat green material	0.00271 0.0271 5.4 Overall	3 3 1 7	87 (100); 89 (102); 89 (102)[1] 104 (105); 104 (105); 105 (106)[1] 87 Mean: 95, RSD: 9.2
CA 6.3.3/16	01480	A1 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	91; 91; 91 102; 102; 104 Mean: 97, RSD: 6.6
CA 6.3.3/16	01480	A1 enantiomer	Wheat straw	0.00271 0.0271 0.80 Overall	3 3 1 7	94; 96; 97 102; 103; 104 85 Mean: 97, RSD: 6.8
CA 6.3.3/16	01480	A2 enantiomer	Wheat green material	0.00271 0.0271 5.4 Overall	3 3 1 7	83 (95); 85 (97); 89 (101)[1] 101 (102); 102 (103); 105 (106)[1] 86 Mean: 93, RSD: 10.0
CA 6.3.3/16	01480	A2 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	91; 91; 92 100; 103; 106 Mean: 97, RSD: 6.9
CA 6.3.3/16	01480	A2 enantiomer	Wheat straw	0.00271 0.0271 0.80 Overall	3 3 1 7	97; 97; 97 98; 99; 102 86 Mean: 94, RSD: 6.6
CA 6.3.3/16	01480	B1 enantiomer	Wheat green material	0.00229 0.0229 4.6 Overall	3 3 1 7	85 (100); 90 (105); 93 (108)[1] 100 (102); 101 (103); 102 (104)[1] 85 Mean: 94, RSD: 4.9
CA 6.3.3/16	01480	B1 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	89; 91; 92 103; 103; 103 Mean: 97, RSD: 7.0
CA 6.3.3/16	01480	B1 enantiomer	Wheat straw	0.00229 0.0229 0.70 Overall	3 3 1 7	93; 98; 99 101; 103; 104 85 Mean: 98, RSD: 6.8
CA 6.3.3/16	01480	B2 enantiomer	Wheat green material	0.00229 0.0229 4.6 Overall	3 3 1 8	85 (101); 86 (102); 90 (106)[1] 100 (102); 101 (103); 102 (104)[1] 85 Mean: 93, RSD: 8.6
CA 6.3.3/16	01480	B2 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	87; 89; 90 102; 103; 104 Mean: 96, RSD: 8.3
CA 6.3.3/16	01480	B2 enantiomer	Wheat straw	0.00229 0.0229 0.229 Overall	3 3 1 7	97; 97; 104 99; 102; 102 87 Mean: 98, RSD: 5.7

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)

1 - These recoveries were background-corrected by the inherent amount of residues in corresponding control samples. The uncorrected recoveries are shown in brackets

Table CA 6.3.3/16-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/16	00312/M002	Total residue of spiroxamine	Wheat green material	0.01 0.10 20 Overall	3 3 1 9	86 (119); 91 (124); 96 (129) 83 (86); 89 (92); 89 (92)[1] 81; 91; 99 Mean: 90, RSD: 5.4
CA 6.3.3/16	00312/M002	Total residue of spiroxamine	Wheat grain	0.01 0.10 Overall	3 3 7	96; 98; (132); 102 (136); 104 83; 94; 94 Mean: 96, RSD: 6.8
CA 6.3.3/16	00312/M002	Total residue of spiroxamine	Wheat straw	0.01 0.10 15 Overall	3 3 1 9	91 (235); 92 (128); 103 (139)[1] 83 (89); 87 (91); 96 (100)[1] 83; 87; 89 Mean: 90, RSD: 6.8

1 - These recoveries were background-corrected since the control sample used for spiking were found to contain (apparent) residues with differing levels. The uncorrected recoveries are shown in brackets.

Table CA 6.3.3/16-4 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/16	Wheat	358 to 441 (parent spiroxamine enantiomers) 423 and 517 (total residue of spiroxamine)

III. Conclusions

A total of four residue trials conducted in 2017 in northern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butylcyclohexanone) in/on wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat BBCH 45-69.

All four trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.10 to 5.70 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.022 mg/kg with corresponding residues in straw ranging from 0.43 to 0.93 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 6.10 to 7.40 mg/kg. At maturity, residues in grain ranged from 0.011 to 0.024 mg/kg with corresponding residues in straw ranging from 2.20 to 4.50 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.10 to 5.70 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.022 mg/kg with corresponding residues in straw ranging from 0.43 to 0.93 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 6.10 to 7.40 mg/kg. At maturity, residues in grain ranged from 0.011 to 0.024 mg/kg with corresponding residues in straw ranging from 2.20 to 4.50 mg/kg.

Data Point:	KCA 6.3.3/17
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the residues of prothioconazole and spiroxamine in on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal
Report No:	17-2158
Document No:	M-660074-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Five trials conducted during growing season 2017 are available to evaluate the magnitude of residues of spiroxamine in/on wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat in southern Europe. Samples were analysed for both spiroxamine (comprising the enantiomers A1, A2, B1, B2 with total spiroxamine as the sum of the four enantiomers) and as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of prothioconazole were also analysed, however are not reported in this summary.

Five residue trials on wheat were conducted in southern Europe (southern France, Spain, Greece, Italy and Portugal) with all five trials conducted as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/17-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 375 g a.s./ha at a water application rate of 300 or 400 L/ha. Spray intervals were 17 or 21 days, with the final treatment being made at growth stage BBCH 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine and prothioconazole EC 460 formulation containing 300 g/L spiroxamine (actual content 294.2 g/L of spiroxamine).

Samples of green material were taken on the day of the final application, and between 33 to 66 days after the last application (DALA) for grain and straw. Control samples were taken on the day of final treatment prior to the last treatment and 33 to 66 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine comprising the A1, A2, B1 and B2 enantiomers, the total residue of parent spiroxamine was reported as the sum of the four enantiomers and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone.

Spiroxamine parent enantiomers

Residue analysis of samples of plant material, grain and straw for the determination of the four enantiomers of spiroxamine was conducted using the validated method no. 01480, report reference M-576210-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) as the sum of the four enantiomers was 0.01 mg/kg.

Spiroxamine consists of four enantiomers - A1, A2, B1 and B2 - paired in two diastereomers A and B. The LOQ per enantiomer, based on the composition of the reference material, was established at 0.00271 mg/kg for enantiomers A1 and A2 and at 0.00229 mg/kg for enantiomers B1 and B2.

Samples were extracted with methanol/water (3/1, v/v) using a high-speed blender. After filtration, and addition of ISTD (spiroxamine d7) spiroxamine enantiomers are determined with chiral reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - YMC ChiralArt Amylose-SA, 150 x 3 mm, 3 µm particle size) without any further clean-up step.

Total residue of spiroxamine

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in/on cereal matrices was conducted using the validated method no. 00312/M002, report reference M-617914-01-1 **(see Doc MCA Section 4)**. The limit of quantification (LOQ) for the total residue of spiroxamine was 0.01 mg/kg.

Samples were extracted with methanol/water (3/1; v/v) using a high-speed blender, straw samples were extracted twice. After filtration with the aid of Celite, an aliquot of the extract is heated by reflux under acidic conditions yielding 4-t-butylcyclohexanone, representing spiroxamine and all metabolites containing the 4-t-butylcyclohexanone common moiety. The cooled extracts were partitioned into dichloromethane and taken to near dryness using N,N-dimethylformamide (DMF, 1 mL) to avoid losses. A known amount of ISTD (4-t-butylcyclohexanone d9) was added along with 2,4-dinitrophenylhydrazine (DNPH) solution in sulphuric acid/methanol (1/4, v/v) and then at room temperature any 4-t-butylcyclohexanone including the d9-ISTD was derivatised to the corresponding hydrazine. The reaction mixture was stopped and stabilised by addition of water/methanol (8/2, v/v) and ammonia acetate solution (3 mol/L). The filtered final extracts were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode (column used - Supelco Ascentis Express C18, 100 x 2.1 mm, 2.7 µm particle size).

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/17-4, the maximum storage time between date of deep-freezing and date of last extraction was 496 days before the analysis of the parent spiroxamine enantiomers, and 590 days before the analysis of the total residue of spiroxamine (via 4-t-butylcyclohexanone).

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine were between 70 to 110% (refer to Table CA 6.3.3/17-2 and Table CA 6.3.3/17-3).

The limits of quantification (LOQ) for the A1 and A2 enantiomer were 0.00271 mg/kg, for the B1 and B2 enantiomer 0.00229 mg/kg. The LOQ was therefore 0.01 mg/kg as the sum of the 4 enantiomers, and 0.01 mg/kg for total residue of spiroxamine (via 4-t-butylcyclohexanone).

No residues above the respective LOQ were detected in the control samples.

All four of the trials were conducted as harvest trials, with straw and grain sampled between 33 and 57 DALA (PHI is growth stage dependent).

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.20 to 7.80 mg/kg. At maturity, all residues in grain were <0.01 mg/kg with corresponding residues in straw ranging from 0.27 to 1.40 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 7.30 to 11.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.021 mg/kg with corresponding residues in straw ranging from 2.20 to 7.60 mg/kg.

Table CA 6.3.3/17-1 Residue trials with spiroxamine and prothioconazole 460 EC in wheat – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers[1]	
CA 6.3.3/17 (17-2158) 17-2158-01 Southern France, F-13103 St Etienne du Gres Wheat (Arkeos) 2017	1 2	0.375 0.375	400 400	BBCH 51 BBCH 69	Green material Grain Straw	0 56 56	1.40 <0.00271 0.084	1.40 <0.00271 0.078	1.20 <0.00229 0.056	1.10 <0.00229 0.061	5.10 <u>0.01</u> 0.27	8.40 0.021 2.20
CA 6.3.3/17 (17-2158) 17-2158-02 Spain, E-41610 Paradas Wheat (Amilcar) 2017	1 2	0.375 0.375	300 300	BBCH 49 BBCH 69	Green material Grain Straw	0 57 57	2.20 <0.00271 0.082	2.30 <0.00271 0.083	1.70 <0.00229 0.061	1.70 <0.00229 0.062	7.80 <u>0.01</u> 0.29	11.0 0.017 3.70
CA 6.3.3/17 (17-2158) 17-2158-03 Greece, G-59031 Meliki, Imathias Wheat (Atchilleas) 2017	1 2	0.375 0.375	400 400	BBCH 39 BBCH 69	Green material Grain Straw	0 33 33	1.70 <0.00271 0.11	1.70 <0.00271 0.11	1.30 <0.00229 0.086	1.30 <0.00229 0.084	6.00 <u>0.01</u> 0.39	9.20 0.010 7.20
CA 6.3.3/17 (17-2158) 17-2158-04 Italy, I-95045 Misterbianco Wheat (Core) 2017	1 2	0.375 0.375	400 400	BBCH 45 BBCH 69	Green material Grain Straw	0 42 42	1.10 <0.00271 0.38	1.10 <0.00271 0.38	0.95 <0.00229 0.30	0.94 <0.00229 0.30	4.20 <u>0.01</u> 1.40	7.30 <0.01 7.60
CA 6.3.3/17 (17-2158) 17-2158-05 Portugal, P-2000-336 Achete-Santarém Wheat (Valbona) 2017	1 2	0.375 0.375	300 300	BBCH 51 BBCH 69	Green material Grain Straw	0 66 66	1.90 <0.00271 0.20	1.80 <0.00271 0.20	1.50 <0.00229 0.14	1.50 <0.00229 0.13	1.50 <u>0.01</u> 0.13	9.90 0.011 4.40

1 – Underlined value to be used to support MRL for grain

Table CA 6.3.3/17-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/17	01480	A1 enantiomer	Wheat green material	0.00271 0.0271 5.4 Overall	3 3 1 7	90 (100); 92 (102); 94 (104)[1] 103 (104); 104 (105); 106 (107)[1] 87 Mean: 96, RSD: 8.6
CA 6.3.3/17	01480	A1 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	92; 93; 94 103; 105; 105 Mean: 99, RSD: 6.4
CA 6.3.3/17	01480	A1 enantiomer	Wheat straw	0.00271 0.0271 0.80 Overall	3 3 1 7	100; 104; 105 105; 105; 108 84 Mean: 102, RSD: 8.9
CA 6.3.3/17	01480	A2 enantiomer	Wheat green material	0.00271 0.0271 5.4 Overall	3 3 1 7	94 (105); 97 (108); 98 (109)[1] 104 (105); 105 (106); 108 (109)[1] 86 Mean: 99, RSD: 7.6
CA 6.3.3/17	01480	A2 enantiomer	Wheat grain	0.00271 0.0271 Overall	3 3 6	93; 93; 96 10; 105; 110 Mean: 100, RSD: 7.0
CA 6.3.3/17	01480	A2 enantiomer	Wheat straw	0.00271 0.0271 0.80 Overall	3 3 1 7	103; 105; 111 106; 106; 107 85 Mean: 103, RSD: 8.2
CA 6.3.3/17	01480	B1 enantiomer	Wheat green material	0.00229 0.0229 4.6 Overall	3 3 1 7	91 (103); 92 (104); 94 (106)[1] 102 (103); 103 (104); 103 (104)[1] 85 Mean: 96, RSD: 7.4
CA 6.3.3/17	01480	B1 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	91; 93; 93 103; 104; 106 Mean: 98, RSD: 6.8
CA 6.3.3/17	01480	B1 enantiomer	Wheat straw	0.00229 0.0229 0.70 Overall	3 3 1 7	100; 106; 108 107; 108; 108 85 Mean: 103, RSD: 7.9
CA 6.3.3/17	01480	B2 enantiomer	Wheat green material	0.00229 0.0229 4.6 Overall	3 3 1 7	92 (102); 93 (103); 94 (104)[1] 99 (100); 101 (102); 102 (103)[1] 85 Mean: 95, RSD: 6.9
CA 6.3.3/17	01480	B2 enantiomer	Wheat grain	0.00229 0.0229 Overall	3 3 6	92; 93; 94 101; 103; 104 Mean: 98, RSD: 5.5
CA 6.3.3/17	01480	B2 enantiomer	Wheat straw	0.00229 0.0229	3 3	98; 100; 104 103; 104; 105

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
				0.229 Overall	1 7	85 Mean: 100, RSD: 7.0

1 - These recoveries were background-corrected by the inherent amount of residues in corresponding control samples. The uncorrected recoveries are shown in brackets

Table CA 6.3.3/17-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/17	00312/M002	Total residue of spiroxamine	Wheat green material	0.01 0.10 20 Overall	3 3 1 9	106 (149); 107 (150); 113 (156)[1] 100 (104); 106 (110); 106 (110)[1] 102; 106; 114 Mean: 107, RSD: 4.2
CA 6.3.3/17	00312/M002	Total residue of spiroxamine	Wheat grain	0.01 0.10 1.0 Overall	3 3 1 9	92 (132); 93 (133); 97 (137)[1] 90 (94); 102 (106); 112 (116)[1] 93; 97; 102 Mean: 98, RSD: 7.0
CA 6.3.3/17	00312/M002	Total residue of spiroxamine	Wheat straw	0.01 0.10 15 Overall	3 3 1 9	86 (193); 100 (207); 114 (221)[1] 85 (96); 90 (101); 93 (104)[1] 89; 91; 106 Mean: 95, RSD: 10.3

1 - These recoveries were background-corrected since the control sample used for spiking were found to contain (apparent) residues with differing levels. The uncorrected recoveries are shown in brackets.

Table CA 6.3.3/17-4 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/17	Wheat	358 to 441 (parent spiroxamine enantiomers) 423 and 517 (total residue of spiroxamine)

III. Conclusions

A total of five residue trials conducted in 2017 in southern Europe are available to evaluate the residues of parent spiroxamine enantiomers (A1, A2, B1 and B2) and total residue of spiroxamine (via 4-t-butyl cyclohexanone) in/on wheat (green material, grain and straw) after two applications of spiroxamine and prothioconazole EC 460 on wheat BBCH 39-69.

All five trials were conducted as harvest trials. Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.20 to 7.80 mg/kg. At maturity, all residues in grain were <0.01 mg/kg with corresponding residues in straw ranging from 0.27 to 1.40 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 7.30 to 11.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.021 mg/kg with corresponding residues in straw ranging from 2.20 to 7.60 mg/kg.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study not previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Residues of spiroxamine (sum of all enantiomers) found 0 DALA in wheat whole plant (green material) ranged from 4.20 to 7.80 mg/kg. At maturity, all residues in grain were <0.01 mg/kg with corresponding residues in straw ranging from 0.27 to 1.40 mg/kg.

The total residues of spiroxamine (as common moiety 4-t-butylcyclohexanone) found 0 DALA in wheat whole plant (green material) ranged from 7.30 to 11.00 mg/kg. At maturity, residues in grain ranged from <0.01 to 0.021 mg/kg with corresponding residues in straw ranging from 2.20 to 7.60 mg/kg.

Data Point:	KCA 6.3.3/01
Report Author:	[redacted]
Report Year:	1999
Report Title:	Determination of residues of KWG 4168 & ICI A 5504 400 SE in/on spring barley and winter wheat following spray application in Germany, Sweden and Great Britain
Report No:	RA-2002/97
Document No:	M-010782-01-1
Guideline(s) followed in study:	EC guidance working document 7029/VI/95 rev. 5 (1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Four trials conducted during growing season 1997 are available to evaluate the magnitude of residues of spiroxamine in/on spring barley and winter wheat (green material, ear, straw and grain) after two applications of spiroxamine and azoxystrobin SE 400 to cereals in northern Europe. The two trials on wheat are summarised here. Samples were analysed for spiroxamine and for grain only as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. Residues of azoxystrobin were also analysed, however are not reported in this summary.

Two residue trials on wheat were conducted in northern Europe (Sweden) with both performed as semi-decline trials. The trial parameters and residue results are summarised in Table CA 6.3.3/01-1. Both wheat trials were conducted in Sweden near Malmo, considerably less than 50 km apart (approximately <10 km), in addition to using the same wheat variety (Ritmo). The replicates, therefore, cannot be considered independent and only one residue value between these two trials is selected.

Two spray applications of spiroxamine were made at a product rate of 1.5 L/ha, corresponding to 400 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 24 to 28 days, with the final treatment being made at growth stage BBCH 69. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a 400 SE formulation of spiroxamine and azoxystrobin (actual content 262.7 g/L spiroxamine).

Samples of green material were taken on the day of the final application, with ear and straw samples taken 35 days after the last application (DALA), and mature grain and straw samples taken at 43 or 49 DALA. Control samples were taken on the day of final treatment prior to the last application and 43 or 49 DALA.

Samples of wheat (grain, ear, green material and straw) were analysed for spiroxamine and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone (mature grain only).

Spiroxamine (parent compound)

The samples of green material, ear, grain and straw were analysed for spiroxamine parent compound. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00506 and its supplement 00506/E001, report reference M-020658-01-1 and M-020694-01-1, respectively (**see Doc MCA Section 4**). The LOQ for spiroxamine was 0.05 mg/kg.

Sample were extracted with acetone/water (3/1; v/v) using a high-speed blender. After filtration and rinsing, an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on a RP 18 column with elution of spiroxamine in methanol/ammonia (999/1; v/v). Residues were determined by EI-GC/MS (DB 1701 capillary column) using external standardisation.

Total spiroxamine (common moiety approach)

The samples of green material, ear, straw and grain were analysed for the residue of total spiroxamine. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00312, report reference M-018352-02-1 (**see Doc MCA Section 4**). The LOQ for residues of total spiroxamine was 0.05 mg/kg.

Samples were extracted and simultaneously hydrolysed by refluxing with a methanol/1M hydrochloric acid (1/1; v/v) mixture. The total residue of spiroxamine itself and all metabolites containing the 4-t-butylcyclohexanone moiety, was hydrolysed yielding 4-t-butylcyclohexanone. After filtration an aliquot of the extract was diluted with water and clean-up using a RP-18 chromatography column. Any 4-t-butylcyclohexanone was eluted from the column with dichloromethane. Extracts were subject to final clean-up using a silica SPE cartridge eluting any 4-t-butylcyclohexanone with dichloromethane. After addition of n-heptane and careful removal of solvent by rotary evaporation under vacuum, the 4-t-butylcyclohexanone was determined by EI-GC/MS (Ultra 2 capillary column) using external standardisation.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/01-3, the maximum storage time between date of deep-freezing and date of last extraction was 411 days for spiroxamine parent and 300 days for total spiroxamine residues.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The mean procedural recoveries for parent spiroxamine and total residue of spiroxamine were between 70 to 110% excluding five results in wheat straw samples at 0.05 mg/kg (66, 62, 61, 65 and 65 % spiroxamine). No explanation was given in the report, however it is unlikely to effect the validity of the results (refer to Table CA 6.3.3/01-2). The limit of quantification (LOQ) for spiroxamine and total residue of spiroxamine from both methods was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Scaled residues of spiroxamine in green material were 5.55 and 77.33 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 43 or 45 DALA with corresponding residues in straw of 0.39 and 0.76 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Reported or scaled residues of total spiroxamine in grain 43 or 45 DALA were <0.05 mg/kg.

As the two trials are not independent, only the higher straw value at harvest can be relied on for assessment purposes.

Table CA 6.3.3/01-1 Residue trials with spiroxamine and azoxystrobin 400 g/L SE in wheat – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residue of spiroxamine (via 4-TBCH)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]	Reported residue	Scaled residue[1]
CA 6.3.3/01 (RA-2002/97) 703818 Sweden, S-24593 Staffanstorp St. Uppakra Winter wheat (Ritmo) 1997	1 2	0.400 0.400	300 300	BBCH 37 BBCH 69	Green material Ear Straw Straw Grain	0 35 35 45 45	5.87 0.339 0.810 0.493 <0.05	5.55 0.32 0.76 0.39 <0.05	<0.05	<0.05
CA 6.3.3/01 (RA-2002/97) 703834 Sweden, S-24591 Staffanstorp, Kornheddinge Winter wheat (Ritmo) 1997	1 2	0.400 0.400	300 300	BBCH 37 BBCH 69	Green material Ear Straw Straw Grain	0 35 35 43 43	7.82 0.269 0.992 0.809 <0.05	7.33 0.25 0.93 0.76 <0.05	<0.05	<0.05

1 - Residues above LOQ scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503

Table CA 6.3.3/01-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residue of spiroxamine

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/01	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat green material	0.05 0.50 5.0 Overall	3 5 3 11	85; 89; 86 82; 77; 90; 86; 85 92; 94; 86 Mean: 87, RSD: 5.4
CA 6.3.3/01	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat straw	0.05 0.50 5.0 Overall	3 5 3 11	80; 74; 79 66; 70; 71; 62; 61 65; 65; 88 Mean: 71, RSD: 12.0
CA 6.3.3/01	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat grain/ear	0.05 0.50 Overall	3 2 5	78; 86; 92 92; 106 Mean: 91, RSD: 11.3
CA 6.3.3/01	00312	Total residue of spiroxamine	Wheat grain	0.05 Overall	5 5	118; 103; 92; 89; 92 Mean: 99, RSD 12.1

Table CA 6.3.3/01-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/01	Wheat	298 to 411 (spiroxamine parent) 298 to 300 (spiroxamine total residues via 4-t-butylcyclohexanone)

III. Conclusions

Two residues trial conducted in 1997 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in/on wheat green material, ear, straw and grain harvested after two applications of spiroxamine and azoxystrobin SE 400 on wheat BBCH 37 to 69.

The trials was conducted as semi-decline trials. Scaled residues of spiroxamine in green material were 5.55 and 77.33 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 43 or 45 DALA with corresponding residues in straw of 0.39 and 0.76 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Reported or scaled residues of total spiroxamine in grain 43 or 45 DALA were <0.05 mg/kg.

As the two trials are not independent, only the higher straw value at harvest can be relied on for assessment purposes.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/06. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine in green material were 5.55 and 77.33 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 43 or 45 DALA with corresponding residues in straw of 0.39 and 0.76 mg/kg.

Reported or scaled residues of total spiroxamine in grain 43 or 45 DALA were <0.05 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

As the two trials are not independent, only the higher straw value at harvest can be relied on for assessment purposes.

Data Point:	KCA 6.3.3/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	Determination of residues of KWG 4168 500 EC in/on spring barley, spring wheat, winter rye, winter wheat and winter barley in the Federal Republic of Germany, Great Britain and France
Report No:	RA-2003/94
Document No:	M-010687-01-1
Guideline(s) followed in study:	None stated
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Twelve trials conducted during growing season 1994 are available to evaluate the magnitude of residues of spiroxamine in/on winter rye, winter wheat, spring barley, spring wheat and winter barley (green material, ear, straw and grain) after two applications of spiroxamine 500 EC at 1.5 L/ha to cereals in northern Europe, 750 g a.s./ha. The seven trials on wheat or rye are summarised here. Samples were analysed for the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grain or straw of total spiroxamine equivalents (as 4-t-butylcyclohexanone) as the contribution from spiroxamine alone (CF = x0.23 for cereal grain and CF = x0.17 for straw was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.2.1 'Derivation of conversion factors for cereals'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for cereals where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

Generally, no residues above the LOQ (0.05 mg/kg) were reported in control samples. In a few samples of green matter and straw, residues slightly above the limit of determination (up to 0.13 mg/kg) could be detected. This was reported to be likely due to spray drift during the application.

Residues of total spiroxamine residues in wheat and rye from treated crop ranged from <0.05 to 5.95 mg/kg, 0 to 57 DALA (days after last application) in the seven trials.

One trial was performed as a harvest trial, and the remaining six as decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 28 to 57 DALA. Total residues of spiroxamine in green material ranged from 5.4 to 12 mg/kg, 0 DALA, declining to between 2.6 to 12 mg/kg in mature straw and between <0.05 to 0.07 mg/kg in mature grain, 42 to 57 DALA.

Table CA 6.3.3/02-1 Residue trials with spiroxamine 500 g/L EC in rye and wheat – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Active substance**	**: spiroxamine**
Country		**:**	**Germany**	**Crop/Crop Group**	**: Cereals**
Content of active substance	**(g/kg or g/L)**	**:**	**500 g/L**	**Page**	**: 3**
Formulation	**(e.g. WP)**	**:**	**500 EC**	**Indoor/outdoor**	**: Outdoor**
Commercial product	**(name)**	**:**	**Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	**:**
Producer of commercial product		**:**	**Bayer AG**	**Residues determined as**	**: 4-t-butylcyclohexanone**
				Residues calculated as	**: spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) (f)	DALT/PHI (days)	Remarks
RA-2003/94 40013/0 0013-94 Germany 51399 Burscheid, Versuchsgut Höfchen 1994	Rye, winter Gambit	1) 20.10.1993 2) 28.05.1994 - 11.06.1994 3) 15.08.1994	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	07.05.1994/0 16.06.1994/40	31	green material ear grain straw	0.56 12 3.2 0.71 <0.05 <0.05 4.2 5.1	0** 0 33 33 53 57 53 57	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer AG**
Country : **Germany**
Content of active substance (g/kg or g/L) : **500 g/L**
Formulation (e.g. WP) : **500 EC**
Commercial product (name) : **Spiroxamine EC 500**
Producer of commercial product : **Bayer AG**

Active substance : **spiroxamine**
Crop/Crop Group : **Cereals**
Page : **4**
Indoor/outdoor : **Outdoor**
Other a.s. in formulation (common name and content) :
Residues determined as : **4-t-butylcyclohexanone**
Residues calculated as : **spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2003/94 40012/2 0012-94 Germany 40789 Monheim, Versuchsgut Laacherhof 1994	Wheat, spring Nandu	1) 30.03.1994 2) 27.06.1994 - 04.07.1994 3) 15.08.1994	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	27.05.1994/0 04.07.1994/38	69	green material straw grain 	0.21 11 7.4 4.7 5.7 0.05 0.09 0.07	0** 0 35< 42 46 35<< 42 46	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 35<<: c=0.07 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**
Crop/Crop Group	:	**Cereals**
Page	:	**5**
Indoor/outdoor	:	**Outdoor**
Other a.s. in formulation (common name and content)	:	
Residues determined as	:	**4-t-butylcyclohexanone**
Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2003/94 40094/7 0094-94 United Kingdom GB- CV 35 9 EF Wellesbourne, HRI Warwickshire 1994	Wheat, winter Haven	1) 02.11.1993 3) 16.08.1994	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	29.04.1994/0 23.06.1994/5	70	green material ear straw grain	0.25 6.6 3.6 1.1 1.4 6.7 5.7 5.2 0.05	0** 0 14 28 35 28 35 54 54	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 0: c=0.05 mg/kg day 28: c=0.10 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**	
Crop/Crop Group	:	**Cereals**	
Page	:	**5**	
Indoor/outdoor	:	**Outdoor**	
Other a.s. in formulation (common name and content)	:		
Residues determined as	:	**4-t-butylcyclohexanone**	
Residues calculated as	:	**spiroxamine**	

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2003/94 40093/9 0093-94 United Kingdom IP 31 3 SJ Thurston, Bury St. Edmunds, Suffolk 1994	Wheat, winter Hereward	1) 02.11.1993 3) 15.08.1994	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	01.05.1994/0 24.06.1994/54	69	green material ear straw grain	<0.05 8.2 2.5 0.89 0.73 4.9 4.1 3.3 0.06	0** 0 14 28 35 28 35 52 52	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 52: c=0.06 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**
Crop/Crop Group	:	**Cereals**
Page	:	**5**
Indoor/outdoor	:	**Outdoor**
Other a.s. in formulation (common name and content)	:	
Residues determined as	:	**4-t-butylcyclohexanone**
Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2003/94 40190/0 0190-94 France, north 27400 Quatremare 1994	Wheat, winter Forbi	1) 17.10.1993 2) 06.06.1994 - 21.06.1994 3) 13.08.1994	SPI SPI	0.7500 0.7500	280 280	0.26800 0.26800	26.04.1994/0 21.06.1994/58	69	green material ear straw grain	0.08 5.4 2.1 0.50 0.57 0.40 3.2 3.7 5.5 2.6 <0.05	0** 0 14 28 35 42 28 35 42 53 53	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Active substance**	**: spiroxamine**
Country		**:**	**Germany**	**Crop/Crop Group**	**: Cereals**
Content of active substance	**(g/kg or g/L)**	**:**	**500 g/L**	**Page**	**: 5**
Formulation	**(e.g. WP)**	**:**	**500 EC**	**Indoor/outdoor**	**: Outdoor**
Commercial product	**(name)**	**:**	**Spiroxamine EC 500**	**Other a.s. in formulation (common name and content)**	**:**
Producer of commercial product		**:**	**Bayer AG**	**Residues determined as**	**: 4-t-butylcyclohexanone**
				Residues calculated as	**: spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2003/94 40189/7 0189-94 France, north 27110 Marbeuf 1994	Wheat, winter Arche	1) 02.11.1993 2) 06.06.1994 - 21.06.1994 3) 12.08.1994	SPI SPI	0.7500 0.7500	280 280	0.26800 0.26800	26.04.1994/0 21.06.1994/56	69	green material ear straw grain	<0.05 6.9 2.8 0.61 0.71 0.66 3.1 3.7 4.2 3.0 <0.05	0** 0 14 28 35 42 28 35 42 52 52	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 0: c=0.07 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**500 g/L**	
Formulation (e.g. WP)	:	**500 EC**	
Commercial product (name)	:	**Spiroxamine EC 500**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**
Crop/Crop Group	:	**Cereals**
Page	:	**5**
Indoor/outdoor	:	**Outdoor**
Other a.s. in formulation (common name and content)	:	
Residues determined as	:	**4-t-butylcyclohexanone**
Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2003/94 40014/9 0014-94 Germany 40789 Monheim, Versuchsgut Laacherhof 1994	Wheat, winter Konsul	1) 26.10.1993 2) 17.06.1994 - 24.06.1994 3) 09.08.1994	SPI SPI	0.7500 0.7500	300 300	0.25000 0.25000	27.05.1994/0 28.06.1994/2	71	green material straw grain	0.66 8.8 10 12 0.05 <0.05	0** 0 35< 42 35<< 42	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 35<<: c=0.06 mg/kg day 42: c=0.11 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall/broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last

(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

Seven residue trials conducted in 1995 in northern Europe are available to evaluate the residues of total spiroxamine in/on wheat and rye green material, ear, straw and grain harvested after two applications of spiroxamine 500 EC on wheat and rye, BBCH 30 to 71.

One trial was performed as a harvest trial, and the remaining six as decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 28 to 57 DALA. Total residues of spiroxamine in green material ranged from 5.4 to 12 mg/kg, 0 DALA, declining to between 2.6 to 12 mg/kg in mature straw and between <0.05 to 0.07 mg/kg in mature grain, 42 to 57 DALA

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/04. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

One trial was performed as a harvest trial, and the remaining six as decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 28 to 57 DALA. Total residues of spiroxamine in green material ranged from 5.4 to 12 mg/kg, 0 DALA, declining to between 2.6 to 12 mg/kg in mature straw and between <0.05 to 0.07 mg/kg in mature grain, 42 to 57 DALA

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.3/03
Report Author:	[redacted]
Report Year:	1995
Report Title:	Determination of residues of HWG 1608 & KWG 4168 383 EW in/on spring barley, spring wheat, winter rye, winter wheat and winter barley in the Federal Republic of Germany and France
Report No:	RA-2004/94
Document No:	M-010783-01-1
Guideline(s) followed in study:	None stated
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Six trials conducted during growing season 1994 are available to evaluate the magnitude of residues of spiroxamine in/on winter rye, winter wheat, spring barley, spring wheat and winter barley after two applications of spiroxamine and tebuconazole 383 EW at 1.5 L/ha in northern Europe, 375 g a.s./ha for spiroxamine. The four trials on wheat are summarised here. Samples were analysed for the total residue

of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grain or straw of total spiroxamine equivalents (as 4-t-butylcyclohexanone) as the contribution from spiroxamine alone (CF = x0.23 for cereal grain and CF = x0.17 for straw was used by the previous RMS, refer to the RAR Vol 3 B.7 Final version August 2017; Section B.7.6.1.2.1 'Derivation of conversion factors for cereals'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for cereals where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control specimens with the exception of some of the samples of straw where the total apparent residue of spiroxamine was up to 0.11 mg/kg. No explanation was given in the report however as the data are not relied on there is no impact on the validity of the study.

All trials were conducted as semi-decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 28 and 52 DALA. Total average residues of spiroxamine in green material ranged from 3.2 and 6.6 mg/kg 0 DALA, declining to between 1.6 and 7.6 mg/kg in mature straw and all trials <0.05 mg/kg in mature grain, 40 to 52 49 DALA.

Table CA 6.3.3/03-1 Residue trials with spiroxamine and tebuconazole 383 g/L EW in wheat and rye – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Active substance**	**spiroxamine**
Country		**:**	**Germany**	**Crop/Crop Group**	**Cereals**
Content of active substance	**(g/kg or g/L)**	**:**	**250 g/L**	**Page**	**3**
Formulation	**(e.g. WP)**	**:**	**383 EW**	**Indoor/outdoor**	**Outdoor**
Commercial product	**(name)**	**:**	**HWG 1608 & KWG 4168 EW 383**	**Other a.s. in formulation (common name and content)**	**tebuconazole 133 g/L**
Producer of commercial product		**:**	**Bayer AG**	**Residues determined as**	**4-t-butylcyclohexanone**
				Residues calculated as	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hl						
RA-2004/94 40017/3 0017-94 Germany 51399 Burscheid, Versuchsgut Höfchen 1994	Rye, winter Gambit	1) 20.10.1993 2) 28.05.1994 3) 11.06.1994 *) 29.07.1994	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	07.05.1994/0 29.06.1994/43	71	green material ear grain straw	0.44 5.4 2.0 0.52 <0.05 2.5	0** 0 33 33 40 40	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**250 g/L**	
Formulation (e.g. WP)	:	**383 EW**	
Commercial product (name)	:	**HWG 1608 & KWG 4168 EW 383**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**	
Crop/Crop Group	:	**Cereals**	
Page	:	**4**	
Indoor/outdoor	:	**Outdoor**	
Other a.s. in formulation (common name and content)	:	**tebuconazole 133 g/L**	
Residues determined as	:	**4-t-butylcyclohexanone**	
Residues calculated as	:	**spiroxamine**	

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2004/94 40016/5 0016-94 Germany 40789 Monheim, Versuchsgut Laacherhof 1994	Wheat, spring Nandu	1) 30.03.1994 2) 27.06.1994 - 04.07.1994 3) 15.08.1994	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	27.05.1994/0 04.07.1994/38	69	green material straw grain	0.11 6.6 5.4 4.0 <0.05 <0.05	0** 0 35<< 42 35<< 42	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Active substance**	**:**	**spiroxamine**
Country		**:**	**Germany**	**Crop/Crop Group**	**:**	**Cereals**
Content of active substance	**(g/kg or g/L)**	**:**	**250 g/L**	**Page**	**:**	**5**
Formulation	**(e.g. WP)**	**:**	**383 EW**	**Indoor/outdoor**	**:**	**Outdoor**
Commercial product	**(name)**	**:**	**HWG 1608 & KWG 4168 EW 383**	**Other a.s. in formulation (common name and content)**	**:**	**tebuconazole 133 g/L**
Producer of commercial product		**:**	**Bayer AG**	**Residues determined as**	**:**	**4-t-butylcyclohexanone**
				Residues calculated as	**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2004/94 40194/3 0194-94 France, north 27110 Marbeuf 1994	Wheat, winter Fortal	1) 24.10.1993 2) 06.06.1994 - 21.06.1994 3) 12.08.1994	SPI SPI	0.3750 0.3750	280 280	0.13400 0.13400	26.04.1994/0 21.06.1994/56	69	green material ear straw grain	0.32 3.8 1.2 0.34 0.54 2.0 2.4 1.6 <0.05	0** 0 14 28 35 28 35 52 52	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 35: c=0.11 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	**Active substance**	:	**spiroxamine**
Country	:	**Germany**	**Crop/Crop Group**	:	**Cereals**
Content of active substance (g/kg or g/L)	:	**250 g/L**	**Page**	:	**5**
Formulation (e.g. WP)	:	**383 EW**	**Indoor/outdoor**	:	**Outdoor**
Commercial product (name)	:	**HWG 1608 & KWG 4168 EW 383**	**Other a.s. in formulation (common name and content)**	:	**tebuconazole 133 g/L**
Producer of commercial product	:	**Bayer AG**	**Residues determined as**	:	**4-t-butylcyclohexanone**
			Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2004/94 40018/1 0018-94 Germany 40789 Monheim, Versuchsgut Laacherhof 1994	Wheat, winter Konsul	1) 26.10.1993 2) 17.06.1994 - 24.06.1994 3) 09.08.1994	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	27.05.1994/0 28.06.1994/2	71	green material straw grain	0.56 3.2 7.5 7.6 <0.05 <0.05	0** 0 35<< 42 35<< 42	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg day 35<<: c=0.06 mg/kg day 42: c=0.09 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of four residue trials conducted in 1994 in northern Europe are available to evaluate the total residues of spiroxamine in/on winter wheat (green material, ear, straw and grain) after two applications of spiroxamine and tebuconazole 383 EW on wheat and rye at BBCH 32-71.

All trials were conducted as semi-decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 28 and 52 DALA. Total average residues of spiroxamine in green material ranged from 3.2 and 6.6 mg/kg 0 DALA, declining to between 1.6 and 7.6 mg/kg in mature straw and all trials <0.05 mg/kg in mature grain, 40 to 52 49 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment of MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/02. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

All trials were conducted as semi-decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 28 and 52 DALA. Total average residues of spiroxamine in green material ranged from 3.2 and 6.6 mg/kg 0 DALA, declining to between 1.6 and 7.6 mg/kg in mature straw and all trials <0.05 mg/kg in mature grain, 40 to 52 49 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.3/04
Report Author:	[redacted]
Report Year:	1994
Report Title:	Determination of residues of HWG1608 & KWG 4168 383 EW in/on winter rye, winter wheat, spring barley, spring wheat and winter barley under actual use conditions in the Federal Republic of Germany and France
Report No:	RA-2077/93
Document No:	M-010788-01-1
Guideline(s) followed in study:	FVA Guideline, Residue Trials, Part 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Eight trials conducted during growing season 1993 are available to evaluate the magnitude of residues of spiroxamine in/on winter rye, winter wheat, spring barley, spring wheat and winter barley (green material, ear, straw, grain) after two applications of spiroxamine and tebuconazole 383 EW at 1.5 L/ha to cereals in northern Europe, 375 g a.s./ha for spiroxamine. The five trials on wheat are summarised

here. Samples were analysed for the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. At this time for development of spiroxamine, the proposed residue definition for monitoring was a total residue approach and therefore the methods developed subsequently to measure and report spiroxamine as parent compound were not available.

In previous EU evaluations, these residue trials were considered for MRL purposes by deriving and applying a conversion factor to express reported residues in grain or straw of total spiroxamine equivalents (as 4-t-butylcyclohexanone) as the contribution from spiroxamine alone (CF = x0.23 for cereal grain and CF = x0.17 for straw was used by the previous RMS, refer to the RAR Vol.3 B.7 Final version August 2017; Section B.7.6.1.2.1 'Derivation of conversion factors for cereals'). Although currently available data can be used to update and derive a similar conversion factor, including the also required factor for conversion of residue definition for monitoring (RD-Mo) to residue definition for risk assessment (RD-RA), the large number of residue trials available for cereals where spiroxamine is measured directly combined with the fact that due to the inherent variability of residue trials data, applying a fixed CF to the total spiroxamine data is not an accurate approach and is no longer necessary in order to meet data requirements.

Therefore this previously evaluated study is not relied upon for assessment or MRL purposes but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

All trials were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 56 DALA. Total average residues of spiroxamine in green material were between 4.1 and 9.1 mg/kg 0 DALA, declining to between 1.3 and 4.4 mg/kg in mature straw, with all trials <0.05 mg/kg in mature grain, 51 to 63 DALA.

Table CA 6.3.3/04-1 Residue trials with spiroxamine and tebuconazole 383 g/L EC in rye and wheat – residue results for northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**	**Crop/Crop Group**	**:**	**Cereals**
Country		**:**	**Germany**	**Page**	**:**	**1**
Content of active substance	**(g/kg or g/L)**	**:**	**250 g/L**	**Indoor/outdoor**	**:**	**Outdoor**
Formulation	**(e.g. WP)**	**:**	**383 EW**	**Other a.s. in formulation (common name and content)**	**:**	**tebuconazole 133 g/L**
Commercial product	**(name)**	**:**	**HWG 1608 & KWG 4168 EW 383**	**Residues determined as**	**:**	**4-t-butylcyclohexanone**
Producer of commercial product		**:**	**Bayer AG**	**Residues calculated as**	**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2077/93 30044/6 0044-93 Germany Versuchsgut Höfchen, 51399 Burscheid 1993	Rye, winter Amando	1) 29.09.1992 2) 24.05.1993 3) 29.05.1993 3) 26.07.1993	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	04.05.1993/0 24.05.1993/20	Begin of flowering	green material ear grain straw	1.4 6.1 2.4 0.46 <0.05 2.4	0** 0 35< 35<< 63 63	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting and address)	**(name**	**:**	**Bayer AG**
Country		**:**	**Germany**
Content of active substance	**(g/kg or g/L)**	**:**	**250 g/L**
Formulation	**(e.g. WP)**	**:**	**383 EW**
Commercial product	**(name)**	**:**	**HWG 1608 & KWG 4168 EW 383**
Producer of commercial product		**:**	**Bayer AG**

Active substance		**:**	**spiroxamine**
Crop/Crop Group		**:**	**Cereals**
Page		**:**	**2**
Indoor/outdoor		**:**	**Outdoor**
Other a.s. in formulation (common name and content)		**:**	**tebuconazole 133 g/L**
Residues determined as		**:**	**4-t-butylcyclohexanone**
Residues calculated as		**:**	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2077/93 30135/3 0135-93 Germany 40789 Monheim, Laacherhof 1993	Wheat, spring Hanno	1) 12.03.1993 2) 08.06.1993 - 14.06.1993 3) 29.07.1993	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	25.05.1993/0 08.06.1993/1	Begin of flowering	green material ear straw grain	3.2 9.1 0.50 5.4 4.4 <0.05	0** 0 35<< 35<< 51 51	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**250 g/L**	
Formulation (e.g. WP)	:	**383 EW**	
Commercial product (name)	:	**HWG 1608 & KWG 4168 EW 383**	
Producer of commercial product	:	**Bayer AG**	
Active substance	:	**spiroxamine**	
Crop/Crop Group	:	**Cereals**	
Page	:	**3**	
Indoor/outdoor	:	**Outdoor**	
Other a.s. in formulation (common name and content)	:	**tebuconazole 133 g/L**	
Residues determined as	:	**4-t-butylcyclohexanone**	
Residues calculated as	:	**spiroxamine**	

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) 	DALT/PHI (days) (f)	Remarks
RA-2077/93 30300/3 0300-93 France, north 27220 St. Andre/Eure 1993	Wheat, winter Thesee	1) 11.10.1992 2) 02.06.1993 - 19.06.1993 3) 04.08.1993	SPI SPI	0.3750 0.3750	280 280	0.13400 0.13400	28.09.1993/0 09.06.1993/2	65	green material ear straw grain	0.56 4.4 1.2 0.89 0.21 1.3 1.3 <0.05	0** 0 14 28 35 35 56 56	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg
RA-2077/93 30134/5 0134-93 Germany 40789 Monheim, Laacherhof 1993	Wheat, winter Greif	1) 14.10.1992 2) 02.06.1993 3) 07.06.1993 3) 28.07.1993	SPI SPI	0.3750 0.3750	300 300	0.12500 0.12500	17.05.1993/0 02.06.1993/16	Begin of flowering	green material ear straw grain	1.4 6.8 0.26 2.7 2.1 <0.05	0** 0 35< 35< 56 56	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	:	**Bayer AG**	
Country	:	**Germany**	
Content of active substance (g/kg or g/L)	:	**250 g/L**	
Formulation (e.g. WP)	:	**383 EW**	
Commercial product (name)	:	**HWG 1608 & KWG 4168 EW 383**	
Producer of commercial product	:	**Bayer AG**	

Active substance	:	**spiroxamine**	
Crop/Crop Group	:	**Cereals**	
Page	:	**3**	
Indoor/outdoor	:	**Outdoor**	
Other a.s. in formulation (common name and content)	:	**tebuconazole 133 g/L**	
Residues determined as	:	**4-t-butylcyclohexanone**	
Residues calculated as	:	**spiroxamine**	

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment(s) Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/PHI (days) (f)	Remarks
RA-2077/93 30080/2 0080-93 France, north 27220 Mousseaux-Neuville 1993	Wheat, winter Soisson	1) 26.10.1992 2) 02.06.1993 - 19.06.1993 3) 04.08.1993	SPI SPI	0.3750 0.3750	280 280	0.13400 0.13400	28.09.1993/0 09.06.1993/2	65	green material ear straw grain	0.62 4.1 1.2 1.0 0.12 1.4 1.4 <0.05	0** 0 14 28 35 35 56 56	(c) SPI:Spraying (g) 00312 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EU) Classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

III. Conclusions

A total of five residue trials conducted in 1993 in northern Europe are available to evaluate the residues of total spiroxamine in/on wheat or rye green material, ear, straw and grain harvested after two applications of spiroxamine and tebuconazole 383 EW on wheat BBCH 32 to 65.

All trials were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 56 DALA. Total average residues of spiroxamine in green material were between 4.1 and 9.1 mg/kg 0 DALA, declining to between 1.3 and 4.4 mg/kg in mature straw, with all trials <0.05 mg/kg in mature grain, 51 to 63 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment of MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU Spiroxamine Annex B7 RAR (2010) IIA 6.3.1/01. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

All trials were conducted as semi-decline or decline trials, with green material sampled at 0 DALA and ears, straw and grain sampled between 35 and 56 DALA. Total average residues of spiroxamine in green material were between 4.1 and 9.1 mg/kg 0 DALA, declining to between 1.3 and 4.4 mg/kg in mature straw, with all trials <0.05 mg/kg in mature grain, 51 to 63 DALA.

As spiroxamine (parent) residues were not determined in this study it is not relied on for assessment or MRL purposes.

Data Point:	KCA 6.3.3/05
Report Author:	[redacted]
Report Year:	2001
Report Title:	Determination of residues of JAU 6476-desthio & KWG 4168 on spring wheat following spray application of JAU 6476 & KWG 4168 460 EC in Great Britain, France, Germany and Italy
Report No:	RA-2092/00
Document No:	M-087669-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of 15 July, 1991, Annex II, part A, point 6 and Annex III, part A, point 8 Residues in or on Treated Products, Food and Feed
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Six trials conducted during growing season 2000 are available to evaluate the magnitude of residues of spiroxamine in/on spring wheat (rest of plant, ear, straw and grain) after two applications of

prothioconazole and spiroxamine 460 EC to wheat in northern Europe (4 trials) or southern Europe (2 trials). Samples were analysed for spiroxamine. Residues of prothioconazole were also analysed, however are not reported in this summary.

Four residue trials on wheat were conducted in northern Europe (United Kingdom, Germany and northern France), in addition to two trial in southern Europe (Italy and southern France) with all six performed as decline trials. The trial parameters and residue results are summarised in Table CA 6.3.3/05-1.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha, corresponding to 375 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 13 to 51 days, with the final treatment being made at growth stage BBCH 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using spiroxamine and prothioconazole 460 EC formulation containing 300 g/L spiroxamine (actual content 295.2 g/L spiroxamine).

Samples of separated green material (plant remaining) and ears were taken on the day of the final application. Additional samples of either green material (plant remaining) and ears or immature grain and straw were taken at subsequent intervals until harvest when samples of mature grain and straw were sampled, 42 to 58 days after last application (DALA).

Samples of wheat (rest of plant, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 00709, report reference M-082616-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.05 mg/kg.

Samples were extracted with acetonitrile/water (4/1; v/v) using a high-speed blender. After filtration with the aid of Celite, dilution and addition of IS-ID (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/05-3, the maximum storage time between date of deep-freezing and date of last extraction was 437 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.3/05-2) with the exception of one result of 119% for straw at 0.5 mg/kg and three results of 112, 114, 117% for ear/grain at 0.5 mg/kg. No explanations are given in the report, however these recoveries >110% have no effect on the acceptance of the results. The limit of quantification (LOQ) for spiroxamine was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

All six trials were conducted as residue decline trials, with mature grain and straw sampled between 42 to 58 DALA (PHI is growth stage dependent).

For trials in northern Europe, residues of spiroxamine found 0 DALA in wheat whole plant remaining (green material) ranged from 5.20 to 11.0 mg/kg. Residues of spiroxamine found 0 DALA in wheat immature ears ranged from 2.90 to 6.50 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg with corresponding straw residues ranging from 0.27 to 0.70 mg/kg.

For trials in southern Europe, residues of spiroxamine found 0 DALA in wheat whole plant remaining (green material) were 6.7 and 8.1 mg/kg. In mature grain, the reported residues were both <0.05 mg/kg with corresponding straw residues of 0.36 and 1.30 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically <0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.3/05-1 Residue trials with prothioconazole and spiroxamine 460 EC in wheat – residue results for northern and southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application No.	kg a.s./ha	L/ha	Growth stage	Crop part	DALA (days)	Reported residue of spiroxamine (mg/kg)[1]
Northern Europe							
CA 6.3.3/05 (RA-2092/00) R 2000 0081/2 Great Britain, GB-IP31 3SH, Thurston, Bury St. Edmunds (EFDS) Spring wheat (Chablis) 2000	1 2	0.375	300	BBCH 32 BBCH 69	Rest of plant Rest of plant Rest of plant Ear Ear Ear Straw Straw Grain Grain	0 28 35 0 28 35 42 56 42 56	11.00 1.30 0.95 2.90 0.18 0.13 0.69 0.32 <0.05 <0.05
CA 6.3.3/05 (RA-2092/00) R 2000 0430/3 Germany, D-51399 Burscheid Spring wheat (Lavett) 2000	1 2	0.375	300	BBCH 32 BBCH 69	Rest of plant Rest of plant Rest of plant Rest of plant Ear Ear Ear Ear Straw Grain	0 28 35 42 0 28 35 42 58 58	6.80 1.00 0.97 0.71 6.20 0.42 0.20 0.12 0.31 <0.05

Doc. No. Trial Ref Location Crop (variety) Year	Application No.	 kg a.s./ha	 L/ha	 Growth stage	Crop part	DALA (days)	Reported residue of spiroxamine (mg/kg)[1]
CA 6.3.3/05 (RA-2092/00) R 2000 0431/1 Germany, D-40789 Monheim Spring wheat (Lavett) 2000	1 2	0.375 0.375	300 300	BBCH 32 BBCH 69	Rest of plant Rest of plant Rest of plant Ear Ear Ear Straw Straw Grain Grain	0 28 35 0 28 35 41 49 41 49	10.00 1.90 0.57 6.50 0.32 <0.17 1.20 0.70 <0.05 <0.05
CA 6.3.3/05 (RA-2092/00) R 2000 0433/8 northern France, F-27700 Fresne 1 ' Archeveque Spring wheat (Furio) 2000	1 2	0.375 0.375	300 300	BBCH 32 BBCH 69	Rest of plant Rest of plant Ear Ear Straw Straw Grain Grain	0 28 0 28 35 42 35 42	5.20 0.53 3.80 0.25 0.40 0.27 <0.05 <0.05
Southern Europe							
CA 6.3.3/05 (RA-2092/00) R 2000 0082/0 southern France, F-82600 Mas Grenier Spring wheat (Furio) 2000	1 2	0.375 0.375	300 300	BBCH 32 BBCH 69	Rest of plant Rest of plant Ear Ear Straw Straw Straw Grain Grain Grain	0 28 0 28 35 42 49 35 42 49	6.70 1.80 3.60 0.14 2.00 1.30 1.30 <0.05 <0.05 <0.05

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Reported residue of spiroxamine (mg/kg)[1]
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.3.3/05 (RA-2092/00) R 2000 0434/6 Italy, 1-48100 Ravenna Spring wheat (Centauro) 2000	1 2	0.375 0.375	300 300	BBCH 32 BBCH 69	Forage Rest of plant Rest of plant Ear Ear Straw Straw Grain Grain	0 28 35 28 35 42 49 42 49	8.10 0.66 0.43 0.11 0.14 0.34 0.36 <0.05 <0.05

1 – Underlined value to be used to support MRL in grain

Table CA 6.3.3/05-2 Procedural recovery data for the determination of spiroxamine (parent compound)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/05	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat grain/ear	0.05 0.50 Overall	7 8 15	94; 99; 101; 103; 103; 120; 101 100; 108; 109; 109; 112; 114; 103; 117 Mean: 106, RSD: 6.9
CA 6.3.3/05	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat rest of plant/foliage	0.05 0.50 5.0 Overall	5 7 2 14	107; 108; 110; 106; 107 101; 104; 107; 105; 108; 106; 104 105; 104 Mean: 106, RSD: 2.1
CA 6.3.3/05	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat straw	0.05 0.50 Overall	5 8 13	97; 99; 103; 106; 103 97; 98; 100; 100; 98; 119; 98; 110 Mean: 102, RSD: 6.2

Table CA 6.3.3/05-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/05	Wheat	233 to 437 days

III. Conclusions

A total of six residue trials conducted in 2000 in northern and southern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on wheat green material, ear, straw and grain harvested after two applications of prothioconazole and spiroxamine 460 EC at BBCH 32-69.

All six trials were conducted as residue decline trials, with mature grain and straw sampled between 42 to 58 DALA (PHI is growth stage dependent).

For trials in northern Europe, residues of spiroxamine found 0 DALA in wheat whole plant remaining (green material) ranged from 5.20 to 11.0 mg/kg. Residues of spiroxamine found 0 DALA in wheat immature ears ranged from 2.90 to 6.50 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg with corresponding straw residues ranging from 0.27 to 0.70 mg/kg.

For trials in southern Europe, residues of spiroxamine found 0 DALA in wheat whole plant remaining (green material) were 6.7 and 8.1 mg/kg. In mature grain, the reported residues were both <0.05 mg/kg with corresponding straw residues of 0.36 and 1.30 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/07. The study is considered compliant with OECD Guideline 509 Crop Field Trials, September 2009.

All six trials were conducted as residue decline trials, with mature grain and straw sampled between 42 to 58 DALA (PHI is growth stage dependent).

For trials in northern Europe, residues of spiroxamine found 0 DALA in wheat whole plant remaining (green material) ranged from 5.20 to 11.0 mg/kg. Residues of spiroxamine found 0 DALA in wheat immature ears ranged from 2.90 to 6.50 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg with corresponding straw residues ranging from 0.27 to 0.70 mg/kg.

For trials in southern Europe, residues of spiroxamine found 0 DALA in wheat whole plant remaining (green material) were 6.7 and 8.1 mg/kg. In mature grain, the reported residues were both <0.05 mg/kg with corresponding straw residues of 0.36 and 1.30 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.3/06
Report Author:	[redacted]
Report Year:	2006
Report Title:	Determination of the residues of JAU 6476, tebuconazole and KWG 4168 in/on winter wheat after spraying of JAU 6476 & HWG 1608 & KWG 4168 (450 EC) in the field in Northern France, Germany, United Kingdom and Sweden
Report No.:	RA-2573/05
Document No:	M-271973-01-1
Guideline(s) followed in study:	91/414/EEC
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Four trials conducted during growing season 2005 are available to evaluate the magnitude of residues of spiroxamine in/on winter wheat (green material, grain and straw) after two applications of spiroxamine, prothioconazole and tebuconazole 450 EC to wheat in northern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and tebuconazole were also analysed, however are not reported in this summary.

Four residue trials on wheat were conducted in northern Europe (northern France, Germany, United Kingdom and Sweden) with one of the trials performed as a decline trial and three trials performed as harvest trials. The trial parameters and residue results are summarised in Table CA 6.3.3/06-1.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 312.5 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 15 to 25 days, with the final treatment being made at growth stage BBCH 69. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine, prothioconazole and tebuconazole 450 EC formulation containing 250 g/L spiroxamine (actual content 243.51 g/L spiroxamine).

Samples of green material were taken on the day of the final application. Additional samples of green material were taken at subsequent intervals until harvest for the decline trial. For all trials samples of mature grain and straw were sampled, 42 to 54 days after last application (DALA).

Samples of wheat (rest of plant, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 00709, report reference M-082616-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.05 mg/kg.

Samples were extracted with acetonitrile/water (4/1; v/v) using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/06-3, the maximum storage time between date of deep-freezing and date of last extraction was 182 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.3/06-2). The limit of quantification (LOQ) for spiroxamine was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial was conducted as a residue decline trial with all other trials conducted as harvest trials. Mature grain and straw were sampled between 42 to 54 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found DALA in wheat green material ranged from 5.16 to 7.92 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg and are therefore not appropriate to scale up to meet the cGAP. Corresponding straw scaled residues ranged from 0.56 to 1.3 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.3/06-1 Residue trials with spiroxamine, prothioconazole and tebuconazole 450 EC in wheat – residue results for northern Europe (field)

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue of spiroxamine (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]
CA 6.3.3/06 (RA-2573/05) R 2005 0470/5 Northern France, F-95710 Chaussy (Ile-de-France) Winter wheat (PR-22) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 69	Green material Green material Green material Green material Grain Straw	0 0 28 35 42 42	0.69 4.60 0.46 0.31 <0.05 0.47	0.83 5.52 0.55 0.37 n.a. 0.56
CA 6.3.3/06 (RA-2573/05) R 2005 0471/3 Germany, D-51377 Leverkusen (Nordrhein-Westfalen) Winter wheat (Batis) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 69	Green material Green material Grain Straw	0 35 52 52	6.60 0.36 <0.05 0.69	7.92 0.43 n.a. 0.83
CA 6.3.3/06 (RA-2573/05) R 2005 0803/4 United Kingdom, GB-SG8 8SS Royston (Hertfordshire) Winter wheat (Einstein) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 69	Green material Green material Grain Straw	0 35 50 50	4.30 0.79 <0.05 1.10	5.16 0.95 n.a. 1.32
CA 6.3.3/06 (RA-2573/05) R 2005 0804/2 Sweden, SE-225 92 Lund (Malmö) Winter wheat (Ritmo) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 69	Green material Green material Grain Straw	0 35 54 54	5.90 0.78 <0.05 1.10	7.08 0.94 n.a. 1.32

1 – Residues above LOQ scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503
2 - Values <LOQ not appropriate to scale up

Table CA 6.3.3/06-2 Procedural recovery data for the determination of spiroxamine (parent compound)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/06	00709	Spiroxamine (parent compound)	Winter wheat (green material)	0.05 0.50 5.0 Overall	3 1 1 5	91; 93; 94 99 99 Mean: 95, RSD: 3.8
CA 6.3.3/06	00709	Spiroxamine (parent compound)	Winter wheat (grain)	0.05 0.50 Overall	3 2 5	93; 96; 98 96; 98 Mean: 95, RSD: 2.3
CA 6.3.3/06	00709	Spiroxamine (parent compound)	Winter wheat (straw)	0.05 0.50 5.0 Overall	2 1 1 4	104; 97 84 94 Mean: 95, RSD: 8.8

Table CA 6.3.3/06-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/06	Wheat	72 to 182 days

III. Conclusions

A total of four residue trials conducted in 2005 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on wheat (green material, straw and grain) harvested after two applications of spiroxamine, prothioconazole and tebuconazole 450 EC at BBCH 47 to 69.

One trial was conducted as a residue decline trial with all other trials conducted as harvest trials. Mature grain and straw were sampled between 42 to 54 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found 0 DALA in wheat green material ranged from 5.16 to 7.92 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg and are therefore not appropriate to scale up to meet the cGAP. Corresponding straw scaled residues ranged from 0.56 to 1.3 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/08. The study is considered compliant with OECD Guideline 509 Crop Field Trials, September 2009.

One trial was conducted as a residue decline trial with all other trials conducted as harvest trials. Mature grain and straw were sampled between 42 to 54 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found 0 DALA in wheat green material ranged from 5.16 to 7.92 mg/kg. In mature grain, all trials reported residues of <0.05 mg/kg and are therefore not appropriate to scale up to meet the cGAP. Corresponding straw scaled residues ranged from 0.56 to 1.3 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.3/07
Report Author:	[redacted]; [redacted]
Report Year:	2006
Report Title:	Determination of the residues of JAU 6476, tebuconazole and KWG 4168 in/on winter wheat after spraying of JAU 6476 & HWG 1608 & KWG 4168 (450 EC) in the field in Southern France and Greece
Report No:	RA-2574/05
Document No:	M-271989-02-1
Guideline(s) followed in study:	91/414/EEC
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Two trials conducted during growing season 2005 are available to evaluate the magnitude of residues of spiroxamine in/on winter wheat (green material, grain and straw) after two applications of spiroxamine, prothioconazole and tebuconazole 450 EC to wheat in southern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and tebuconazole were also analysed, however are not reported in this summary.

Two residue trials on wheat were conducted in southern Europe (southern France and Greece) with one performed as a decline trial and one performed as a semi-decline trial. The trial parameters and residue results are summarised in Table CA 6.3.3/07-1.

Two spray applications of spiroxamine were made at a product rate of 1.25 L/ha corresponding to 312.5 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 18 or 28 days, with the final treatment being made at growth stage BBCH 69. Residues above the analytical LOQ have been scaled

proportionally to a 375 g a.s/ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine, prothioconazole and tebuconazole 450 EC formulation containing 250 g/L spiroxamine (actual content 243.51 g/L spiroxamine).

Samples of green material were taken on the day of the final application. Additional samples of green material were taken at subsequent intervals until harvest for the decline trial. For both trials samples of mature grain and straw were sampled, 42 or 51 days after last application (DALA).

Samples of wheat (rest of plant, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 00709, report reference M-082616-01-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.05 mg/kg.

Samples were extracted with acetonitrile/water (4/1, v/v) using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤ -18 °C. As shown in Table CA 6.3.3/07-3, the maximum storage time between date of deep-freezing and date of last extraction was 219 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.3/07-2). The limit of quantification (LOQ) for spiroxamine was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial was conducted as a residue decline trial with the other trial conducted as a semi-decline trial. Mature grain and straw were sampled between 42 or 51 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found 0 DALA in wheat green material were 4.68 and 10.92 mg/kg. In mature grain, both trials reported residues of <0.05 mg/kg and are therefore not appropriate to scale up to meet the cGAP. Corresponding straw scaled residues were 0.37 and 0.85 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.3/07-1 Residue trials with spiroxamine, prothioconazole and tebuconazole 450 EC in wheat – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue of spiroxamine (mg/kg)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1,2]
CA 6.3.3/07 (RA-2574/05) R 2005 0472/1 Southern France, F-30290 Laudun (Languedoc-Roussillon) Winter wheat (Florence Aurore) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 69	Green material Green material Green material Green material Grain Straw	-0 0 28 35 51 51	1.40 9.10 1.30 1.20 <0.05 0.71	1.68 10.92 1.56 1.44 n.a. 0.85
CA 6.3.3/07 (RA-2574/05) R 2005 0474/8 Greece, GR-59031 Meliki (Northern Greece - Macedonia) Winter wheat (Aracena) 2005	1 2	0.3125 0.3125	300 300	BBCH 47 BBCH 69	Green material Green material Grain Straw	0 35 42 42	3.90 0.25 <0.05 0.31	4.68 0.30 n.a. 0.37

1 – Residues above LOQ scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA supporting publication 2018:EN-1503
2 - Values <LOQ not appropriate to scale up

Table CA 6.3.3/07-2 Procedural recovery data for the determination of spiroxamine (parent compound)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/07	00709	Spiroxamine (parent compound)	Winter wheat (green material)	0.05 0.50 5.0 Overall	3 1 1 5	91; 93; 94 99 99 Mean: 95, RSD: 3.8
CA 6.3.3/07	00709	Spiroxamine (parent compound)	Winter wheat (grain)	0.05 0.50 Overall	3 2 5	93; 96; 98 96; 98 Mean: 95, RSD: 2.3
CA 6.3.3/07	00709	Spiroxamine (parent compound)	Winter wheat (straw)	0.05 0.50 5.0 Overall	2 1 1 4	104; 97 84 94 Mean: 95, RSD: 8.8

Table CA 6.3.3/07-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/07	Wheat	118 to 219 days

III. Conclusions

A total of two residue trials conducted in 2005 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on wheat (green material, straw and grain) harvested after two applications of spiroxamine, prothioconazole and tebuconazole 450 EC at BBCH 47 to 69.

One trial was conducted as a residue decline trial with the other trial conducted as a semi-decline trial. Mature grain and straw were sampled between 42 or 51 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found 0 DALA in wheat green material were 4.68 and 10.92 mg/kg. In mature grain, both trials reported residues of <0.05 mg/kg and are therefore not appropriate to scale up to meet the cGAP. Corresponding straw scaled residues were 0.37 and 0.85 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/09. The study is considered compliant with OECD Guideline 509 Crop Field Trials, September 2009.

One trial was conducted as a residue decline trial with the other trial conducted as a semi-decline trial. Mature grain and straw were sampled between 42 or 51 DALA (PHI is growth stage dependent).

Scaled residues of spiroxamine found 0 DALA in wheat green material were 4.68 and 10.92 mg/kg. In mature grain, both trials reported residues of <0.05 mg/kg and are therefore not appropriate to scale up to meet the cGAP. Corresponding straw scaled residues were 0.37 and 0.85 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that residues in wheat grain are typically ≤0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.3/08
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of BYF 00587, JAU 6476 and KWG 4168 in/on winter wheat and spring wheat after spraying of BYF 00587 & JAU 6476 & KWG 4168 (400 EC) in the field in Northern France, the Netherlands, the United Kingdom and German
Report No:	RA-2040/07
Document No:	M-298182-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted. RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2007 are available to evaluate the magnitude of residues of spiroxamine in/on spring and winter wheat (green material, ear, rest of plant, grain and straw) after two applications of spiroxamine, prothioconazole and bixafen EC 400 on wheat in northern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and bixafen were also analysed, however are not reported in this summary

Four residue trials on wheat were conducted in northern Europe (northern France, the Netherlands, United Kingdom and Germany) with all four conducted as harvest trials although samples were taken at 35 days after last application for all trials even if the crop was not at commercial maturity. The trial

parameters and residue results are summarised in Table CA 6.3.3/08-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.5 L/ha corresponding to 375 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 13 to 22 days, with the final treatment being made at growth stage BBCH 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a 400 g/L EC formulation of spiroxamine, prothioconazole and bixafen containing 250 g/L spiroxamine (actual content 256.6 g/L spiroxamine).

Samples of green material were taken on the day of final application (prior to and subsequent to the application). Additional samples of ear and plant remaining or immature grain/straw were taken at nominally 35 days after last application (DALA) if the crop was not commercially mature and then at full commercial maturity when samples of mature grain and straw were sampled, 44 to 73 DALA.

Samples of wheat (green material, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 01013 (modified by 01013/M001), report reference M-283439-03-1 and M-297777-02-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetonitrile/water (4/1, v/v) in the presence of cysteine hydrochloride after initial soaking, using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/08-3, the maximum storage time between date of deep-freezing and date of last extraction was 196 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.3/08-2). The limit of quantification (LOQ) for spiroxamine was 0.01 mg/kg.

No residues above the LOQ (0.01 mg/kg) were detected in the control samples.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 44 to 73 DALA (PHI is growth stage dependent).

Residues of spiroxamine (parent compound) in wheat from treated crop ranged from <0.01 to 7.20 mg/kg, 0 to 73 DALA (days after last application) in the four trials.

Residues of spiroxamine found 0 DALA in green material ranged from 3.2 to 7.2 mg/kg. Residues for mature grain samples ranged from <0.01 to 0.01 mg/kg 44 to 73 DALA, with corresponding residues in straw ranging from 0.05 to 0.55 mg/kg.

Table CA 6.3.3/08-1 Residue trials with spiroxamine, prothioconazole and bixafen 400 EC in wheat – residue results for northern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application No.	kg a.s./ha	L/ha	Growth stage	Crop part	DALA (days)	Reported residue of spiroxamine (mg/kg)[1]
CA 6.3.3/08 (RA-2040/07) R 2007 0443/7 northern France, F-37120 Braslou (Centre) Winter wheat (Mendel) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 44 44	0.48 6.00 0.09 0.56 0.05 <0.01
CA 6.3.3/08 (RA-2040/07) R 2007 0444/5 The Netherlands, NL-1681 ND Zwaagdijk-Oost (Noord-Holland) Spring wheat (Baldus) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Straw Grain Straw Grain	-0 0 35 35 61 61	0.39 4.30 0.24 0.03 0.24 0.01
CA 6.3.3/08 (RA-2040/07) R 2007 0523/9 The United Kingdom, GB-IP21 4BQ Thrandeston/ Diss (Norfolk) Spring wheat (Belvoir) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 73 73	0.47 3.20 0.03 0.32 0.39 <0.01
CA 6.3.3/08 (RA-2040/07) R 2007 0524/7 Germany, D-51399 Burscheid (Nordrhein-Westfalen) Spring wheat (Thasos) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Straw Grain Straw Grain	-0 0 35 35 56 56	1.30 7.20 0.70 0.02[2] 0.55 0.01

1 - Underlined value to be used to support MRL for grain

Table CA 6.3.3/08-2 Procedural recovery data for the determination of spiroxamine (parent compound)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/08	01013 and 01013/M001	Spiroxamine (parent compound)	Winter wheat (green material)	0.01 1.0 10 Overall	5 2 2 9	98; 94; 87; 83; 84 86; 88 78; 79 Mean: 86, RSD: 7.5
CA 6.3.3/08	01013 and 01013/M001	Spiroxamine (parent compound)	Winter wheat (grain)	0.01 0.10 Overall	6 5 11	101; 94; 92; 91; 88; 108 90; 95; 90; 92; 90 Mean: 94, RSD: 6.3
CA 6.3.3/08	01013 and 01013/M001	Spiroxamine (parent compound)	Winter wheat (straw)	0.01 0.10 1.0 Overall	5 2 2 9	108; 94; 90; 73; 98 88; 86 74; 88 Mean: 89, RSD: 12.3

Table CA 6.3.3/08-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/08	Wheat	80 to 196 days

III. Conclusions

A total of four residue trials conducted in 2007 in northern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on wheat (green material, rest of plant, ear, straw and grain) harvested after two applications of spiroxamine, bixafen and prothioconazole 400 EC at BBCH 47-69.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 44 to 73 DALA (PHI is growth stage dependent).

Residues of spiroxamine (parent compound) in wheat from treated crop ranged from <0.01 to 7.20 mg/kg, 0 to 73 DALA (days after last application) in the four trials.

Residues of spiroxamine found 0 DACA in green material ranged from 3.2 to 7.2 mg/kg. Residues for mature grain samples ranged from <0.01 to 0.01 mg/kg 44 to 73 DALA, with corresponding residues in straw ranging from 0.05 to 0.55 mg/kg.

Assessment and conclusion by applicant:

Acceptable study. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/10. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 44 to 73 DALA (PHI is growth stage dependent).

Residues of spiroxamine (parent compound) in wheat from treated crop ranged from <0.01 to 7.20 mg/kg, 0 to 73 DALA (days after last application) in the four trials.

Residues of spiroxamine found 0 DALA in green material ranged from 3.2 to 7.2 mg/kg. Residues for mature grain samples ranged from <0.01 to 0.01 mg/kg 44 to 73 DALA, with corresponding residues in straw ranging from 0.05 to 0.55 mg/kg.

Data Point:	KCA 6.3.3/09
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of BYF 00587, JAU 6476 and KWG 4168 in/on winter wheat and wheat, durum after spraying of BYF 00587 & JAU 6476 & KWG 4168 (400 EC) in the field in Southern France, Italy, Spain and Greece
Report No:	RA-2041/07
Document No:	M-298650-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

Four trials conducted during growing season 2007 are available to evaluate the magnitude of residues of spiroxamine in/on spring and winter wheat (green material, ear, rest of plant, grain and straw) after two applications with the formulation spiroxamine, prothioconazole and bixafen EC 400 on wheat in southern Europe. Samples were analysed for spiroxamine. Residues of prothioconazole and bixafen were also analysed, however are not reported in this summary.

Four residue trials on wheat were conducted in southern Europe (southern France, Spain, Italy and Greece) with all four conducted as harvest trials although samples were taken at 35 days after last application for all trials even if the crop was not at commercial maturity. The trial parameters and residue results are summarised in Table CA 6.3.3/09-1 where values considered for MRL purposes or risk assessment are underlined.

Two spray applications of spiroxamine were made at a product rate of 1.5 L/ha corresponding to 375 g a.s./ha at a water application rate of 300 L/ha. Spray intervals were 8 to 26 days, with the final

treatment being made at growth stage BBCH 69. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a 400 g/L EC formulation of spiroxamine, prothioconazole and bixafen containing 250 g/L spiroxamine (actual content 256.6 g/L spiroxamine).

Samples of green material were taken on the day of final application (prior to and subsequent to the application). Additional samples of ear and plant remaining or immature grain/straw were taken at nominally 35 days after last application (DALA) if the crop was not commercially mature and then at full commercial maturity when samples of mature grain and straw were sampled 35 to 55 DALA. Control samples were taken on day of the final application day prior to the last treatment and 35 to 55 DALA.

Samples of wheat (green material, ears, grain and straw) were analysed for spiroxamine (parent compound). Residue analysis was conducted using the validated analytical method no. 01013 (modified by 01013/M001), report reference M-283439-03-1 and M-297777-02-1 (**see Doc MCA Section 4**). The limit of quantification (LOQ) for residues of spiroxamine was 0.01 mg/kg.

Samples were extracted with acetonitrile/water (4/1; v/v) in the presence of cysteine hydrochloride after initial soaking, using a high-speed blender. After filtration with the aid of Celite, dilution and addition of ISTD (spiroxamine d7) residues were determined with reversed-phase LC-MS/MS in electrospray positive ionisation mode without any further clean-up step.

The samples were stored deep frozen within 24 hours of sampling at ≤18°C. As shown in Table CA 6.3.3/09-3, the maximum storage time between date of deep-freezing and date of last extraction was 217 days.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for spiroxamine (parent compound) were between 70-110% (refer to Table CA 6.3.3/09-2). The limit of quantification (LOQ) for spiroxamine was 0.01 mg/kg.

No residues above the LOQ (0.01 mg/kg) were detected in the control samples.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 35 to 55 DALA (PHI is growth stage dependent).

Residues of spiroxamine found 0 DALA in green material ranged from 4.7 to 11.0 mg/kg. Residues for mature grain samples ranged from <0.01 to 0.01 mg/kg 35 to 55 DALA, with corresponding residues in straw ranging from 0.45 to 0.65 mg/kg.

Table CA 6.3.3/09-1 Residue trials with spiroxamine, prothioconazole and bixafen 400 EC in wheat – residue results for southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application No.	kg a.s./ha	L/ha	Growth stage	Crop part	DALA (days)	Reported residue of spiroxamine (mg/kg)[1]
CA 6.3.3/09 (RA-2041/07) R 2007 0445/3 Southern France, F-69380 Les Chères (Rhone Alpes) Winter wheat (Autan) 2007	1 2	0.375 0.375	300 300	BBCH 52 BBCH 69	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 44 44	1.10 4.80 0.12 0.85 0.45 <u><0.01</u>
CA 6.3.3/09 (RA-2041/07) R 2007 0446/1 Italy, I-70058 Spinazzola (Bari) (Puglia) Wheat, durum (Simeto) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Ear Rest of plant Straw Grain	-0 0 35 35 44 44	0.31 4.70 0.12 0.60 0.55 <u><0.01</u>
CA 6.3.3/09 (RA-2041/07) R 2007 0525/5 Spain, E-41500 Alcala de Guadaira Sevilla (Andalucia) Winter wheat (Bolido R1) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Rest of plant Ear Straw Grain	-0 0 36 36 55 55	0.54 6.60 0.95 0.11 0.65 <u><0.01</u>
CA 6.3.3/09 (RA-2041/07) R 2007 0526/3 Greece, GR-57001 Thessaloniki (Northern Greece - Macedonia) Winter wheat (Moro (durum)) 2007	1 2	0.375 0.375	300 300	BBCH 47 BBCH 69	Green material Green material Straw Grain	-0 0 35 35	2.00 11.0 0.51 <u>0.01</u>

1 – <u>Underlined</u> value to be used to support MRL for grain

Table CA 6.3.3/09-2 Procedural recovery data for the determination of spiroxamine (parent compound)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/09	01013 and 01013/M001	Spiroxamine (parent compound)	Winter wheat (green material)	0.01 1.0 10 Overall	5 2 2 9	98; 94; 87; 83; 84 86; 88 78; 79 Mean: 86, RSD: 7.5
CA 6.3.3/09	01013 and 01013/M001	Spiroxamine (parent compound)	Winter wheat (grain)	0.01 0.10 Overall	6 5 11	101; 93; 92; 91; 88; 108 90; 95; 90; 92; 90 Mean: 94, RSD: 6.3
CA 6.3.3/09	01013 and 01013/M001	Spiroxamine (parent compound)	Winter wheat (straw)	0.01 0.10 1.0 Overall	5 2 2 9	108; 94; 90; 73; 98 88; 86 74; 88 Mean: 89, RSD: 12.3

Table CA 6.3.3/09-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/09	Wheat	75 to 217 days

III. Conclusions

A total of four residue trials conducted in 2007 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) in/on wheat (green material, rest of plant, ear, straw and grain) harvested after two applications of spiroxamine, bixafen and prothioconazole 400 EC at BBCH 47 to 69.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 35 to 55 DALA (PHI is growth stage dependent).

Residues of spiroxamine found 0 DALA in green material ranged from 4.7 to 11.0 mg/kg. Residues for mature grain samples ranged from <0.01 to 0.01 mg/kg 35 to 55 DALA, with corresponding residues in straw ranging from 0.45 to 0.65 mg/kg.

Assessment and conclusion by applicant:

Acceptable study. Study previously submitted and accepted in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/11. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

All four trials were conducted as harvest trials, with mature grain and straw sampled between 35 to 55 DALA (PHI is growth stage dependent).

Residues of spiroxamine found 0 DALA in green material ranged from 4.7 to 11.0 mg/kg. Residues for mature grain samples ranged from <0.01 to 0.01 mg/kg 35 to 55 DALA, with corresponding residues in straw ranging from 0.45 to 0.65 mg/kg.

Data Point:	KCA 6.3.3/10
Report Author:	[redacted]
Report Year:	1998
Report Title:	Determination of residues of KWG 4168 500 EC in/on winter wheat following spray application in Italy and France
Report No:	RA-2018/97
Document No:	M-010746-01-1
Guideline(s) followed in study:	EC guidance document 7029/VI/95 rev.5: Appendix B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and classified RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

Four trials conducted during growing season 1997 are available to evaluate the magnitude of residues of spiroxamine in/on winter wheat (green material, ear, straw and grain) after two applications of Spiroxamine 500 EC in southern Europe. Samples were analysed for spiroxamine and for grain only as the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents.

Four residue trials on wheat were conducted in southern Europe (southern France and Italy) with all four conducted as semi-decline trials. The trial parameters and residue results are summarised in Table CA 6.3.3/10-1. Three wheat trials were conducted in France, less than 50 km apart (estimated approximate distances ~18 km). The replicates less than 20 km, cannot be considered independent and only two residue values between these three trials could be selected.

Two spray applications of spiroxamine were made at a product rate of at 1.5 to 1.58 L/ha, corresponding to nominally 750 g a.s./ha at a water application rate of 280 to 295 L/ha. Spray intervals were 32 to 35 days, with the final treatment being made at growth stage BBCH 67 to 69. Residues above the analytical LOQ have been scaled proportionally to a 375 g a.s./ha application rate in line with the critical GAP. The critical GAP for the use of spiroxamine on wheat is supported in terms of rates and timings.

The trials were conducted using a spiroxamine 500 EC formulation containing 500 g/L spiroxamine (actual content 505.5 g/L spiroxamine).

Samples of green material were taken on the day of the final application, and between 35 to 50 days after the last application (DALA) for ears, grain and straw. Control samples were taken on the day of final treatment prior to the last treatment and 35 to 50 DALA.

Samples of wheat (grain, green material and straw) were analysed for spiroxamine and also for the total residue of spiroxamine after hydrolysis to 4-t-butylcyclohexanone (mature grain only)

Spiroxamine (parent compound)

The samples of green material, ear, grain and straw were analysed for spiroxamine parent compound. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00506 and its supplement 00506/E001, report reference M-020658-01-1 and M-020694-01-1, respectively (**see Doc MCA Section 4)**. The LOQ for spiroxamine was 0.05 mg/kg.

Sample were extracted with acetone/water (3/1; v/v) using a high-speed blender. After filtration and rinsing, an aliquot of the extract was taken and concentrated to the aqueous remainder. Clean-up of the extracts was performed by solid phase extraction on a RP 18 column with elution of spiroxamine in methanol/ammonia (999/1; v/v). Residues were determined by EI-GC/MS (DB 1701 capillary column) using external standardisation.

Total spiroxamine (common moiety approach)

The samples of grain were analysed for the residue of total spiroxamine. Residue analysis of samples of plant materials were conducted using the validated analytical method no. 00312, report reference M-018352-02-1 (**see Doc MCA Section 4**). The LOQ for residues of total spiroxamine was 0.05 mg/kg.

Samples were extracted and simultaneously hydrolysed by refluxing with a methanol/1M hydrochloric acid (1/1; v/v) mixture. The total residue of spiroxamine itself and all metabolites containing the 4-t-butylcyclohexanone moiety, was hydrolysed yielding 4-t-butylcyclohexanone. After filtration an aliquot of the extract was diluted with water and clean-up using a RP-18 chromatography column. Any 4-t-butylcyclohexanone was eluted from the column with dichloromethane. Extracts were subject to final clean-up using a silica SPE cartridge eluting any 4-t-butylcyclohexanone with dichloromethane. After addition of n-heptane and careful removal of solvent by rotary evaporation under vacuum the 4-t-butylcyclohexanone was determined by EI-GC/MS (Ultra 2 capillary column) using external standardisation.

The samples were stored deep frozen after sampling at ≤18°C. As shown in Table CA 6.3.3/10-3, the maximum storage time between date of deep-freezing and date of last extraction was 427 days for parent spiroxamine and 344 days for total spiroxamine residues.

This storage period is covered by the available storage stability data for high starch content and dry commodities, which demonstrate stability of spiroxamine for up to 722 days (24 months, refer to Point CA 6.1).

II. Results and Discussion

The procedural recoveries for parent spiroxamine and total residue of spiroxamine were between 70-110% (refer to Table CA 6.3.3/10-2). The limit of quantification (LOQ) for spiroxamine and total residue of spiroxamine from both methods was 0.05 mg/kg.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

Scaled residues of spiroxamine in green material were 3.49 to 7.25 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 49 or 50 DALA with corresponding residues in straw of 0.124 to 0.399 mg/kg.

Reported or scaled residues of total spiroxamine in grain 49 or 50 DALA were <0.05 mg/kg.

As the three French trials are not independent only two of the higher straw values at harvest can be relied on for risk assessment purposes.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in wheat grain are typically <0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

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Table CA 6.3.3/10-1 Residue trials with spiroxamine 500 EC in wheat – residue results for Southern Europe

Doc. No. Trial Ref Location Crop (variety) Year	Application				Crop part	DALA (days)	Residue (mg/kg)			
							Spiroxamine (parent compound)		Total residue of spiroxamine (via 4-TBCH)	
	No.	kg a.s./ha	L/ha	Growth stage			Reported residue	Scaled residue[1]	Reported residue	Scaled residue[1]
CA 6.3.3/10 (RA-2018/97) 702935 Southern France, F-01380 Bage-la-Ville Winter wheat (Siderol) 1997	1 2	0.750 0.750	280 280	BBCH 34 BBCH 69	Green material Ear Straw Straw Grain	0 35 35 49 49	8.71 0.129 0.570 0.428 <0.05	4.36 0.065 0.285 0.214 <0.05	<0.05	<0.05
CA 6.3.3/10 (RA-2018/97) 702943 Southern France, F-01560 Lescheroux Winter wheat (Messager) 1997	1 2	0.750 0.750	280 280	BBCH 34 BBCH 67	Green material Ear Straw Straw Grain	0 36 36 50 50	7.77 0.272 0.919 0.798 <0.05	3.89 0.136 0.460 0.399 <0.05	<0.05	<0.05
CA 6.3.3/10 (RA-2018/97) 702951 Southern France, F-01340 Marsonnas Winter wheat (Tremie) 1997	1 2	0.750 0.750	280 280	BBCH 34 BBCH 69	Green material Ear Straw Straw Grain	0 35 35 49 49	14.5 0.204 0.432 0.352 <0.05	7.25 0.102 0.216 0.176 <0.05	<0.05	<0.05
CA 6.3.3/10 (RA-2018/97) 702978 Italy, 1-40052 Mondonuovo Winter wheat (Centauro) 1997	1 2	0.790 0.790	295 295	BBCH 33 BBCH 69	Green material Ear Straw Straw Grain	0 35 35 49 49	6.98 0.189 0.308 0.247 <0.05	3.49 0.095 0.154 0.124 <0.05	<0.05	<0.05

1 - Residues above LOQ scaled proportionally to a 0.375 kg a.s./ha application rate as recommended in EFSA Supporting publication 2018:EN-1503

Table CA 6.3.3/10-2 Procedural recovery data for the determination of spiroxamine (parent compound) and total residue of spiroxamine

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.3.3/10	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat green material	0.05 0.50 5.0 Overall	3 5 3 11	85; 89; 86 82; 77; 90; 86; 85 92; 94; 86 Mean: 87, RSD: 5.4
CA 6.3.3/10	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat straw	0.05 0.50 5.0 Overall	3 5 3 11	80; 74; 79 66; 70; 71; 62; 61 65; 65; 88 Mean: 71, RSD: 12.0
CA 6.3.3/10	00506 and 00506/E001	Spiroxamine (parent compound)	Wheat grain	0.05 0.50 Overall	3 2 5	78; 86; 92 92; 106 Mean: 91, RSD: 11.3
CA 6.3.3/10	00312	Total residue of spiroxamine	Wheat grain	0.05 Overall	5 5	118; 103; 92; 89; 92 Mean: 99, RSD 12.1

Table CA 6.3.3/10-3 Storage of wheat samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.3.3/10	Wheat	362 to 427 (spiroxamine parent) 336 to 344 (spiroxamine total residues via 4-t-butylcyclohexanone)

III. Conclusions

Four residues trial conducted in 1997 in southern Europe are available to evaluate the residues of spiroxamine (parent compound) and residues of total spiroxamine in/on wheat green material, ear, straw and grain harvested after two applications of spiroxamine 500 EC on wheat BBCH 33 to 69.

The trials were conducted as semi-decline trials. Scaled residues of spiroxamine (parent compound) in green material were 3.49 and 7.25 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 49 or 50 DALA with corresponding residues in straw of 0.124 and 0.399 mg/kg.

Reported or scaled residues of total spiroxamine in grain 49 or 50 DALA were <0.05 mg/kg.

As the three French trials are not independent, only two of the higher straw values at harvest can be relied on for risk assessment purposes.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in wheat grain are typically <0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted as supplemental information in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/12. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

Scaled residues of spiroxamine in green material were 3.49 and 7.25 mg/kg, 0 DALA. In mature grain, residues (reported or scaled) were <0.05 mg/kg 49 or 50 DALA with corresponding residues in straw of 0.124 and 0.399 mg/kg.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in wheat grain are typically <0.01 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Data Point:	KCA 6.3.3/11
Report Author:	[redacted]
Report Year:	1992
Report Title:	HWG 1608 & KWG 4168, 417 EC, winter wheat, Germany, BBA
Report No:	0640-90
Document No:	M-010809-01-2
Guideline(s) followed in study:	FIVA Guideline, Residue Trials, Parts IA and IB
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and classified DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

One trial conducted during growing season 1990 is available to evaluate the magnitude of residues of spiroxamine in/on winter wheat (green material, ears, straw and grain) after two applications of Tebuconazole and Spiroxamine 417 EC at 1.5 L/ha to wheat in northern Europe, 375 g a.s./ha. The trial on wheat is summarised here. Samples were analysed for spiroxamine and tebuconazole, but only the spiroxamine residues are reported.

This previously evaluated study was not conducted to GLP and is not relied upon for assessment or MRL purposes as it does not fully match the GAP but is still submitted to allow the RMS to confirm this situation. As such, a full summary is not required and the Tier 1 supervised trials residue form is provided.

II. Results and Discussion

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial on wheat was conducted as a decline trial, with green material sampled at 0 to 28 DALA, and ears, straw and grain sampled between 28 to 57 DALA. Residues of spiroxamine in green material were 4.0 mg/kg, 0 DALA, declining to 0.74 to 1.1 mg/kg in mature straw and <0.05 mg/kg in mature grain, 35 to 57 DALA.

Reported grain data below the LOQ cannot be used for MRL purposes as it represents an unrealistic and overly conservative assessment of true residues in grain. This is justified as the trials conducted more recently with an LOQ of 0.01 mg/kg show that positive residues in wheat grain are typically <0.05 mg/kg and the values from this study would have to be used at the LOQ of 0.05 mg/kg in MRL calculations which would statistically impact the MRL calculator.

Table CA 6.3.3/11-1 Residue trials with tebuconazole and spiroxamine 417 EC in wheat – residue results for Northern Europe

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer AG**	**Active substance**	:	**spiroxamine**
Country	: **Germany**	**Crop/Crop Group**	:	**Cereals**
Content of active substance (g/kg or g/L)	: **417 g/L**	**Page**	:	**1**
Formulation (e.g. WP)	: **417 EC**	**Indoor/Outdoor**	:	**Outdoor**
Commercial product (name)	: **Tebuconazole and Spiroxamine 417 EC**	**Other a.s. in formulation (common name and content)**	:	**Tebuconazole**
Producer of commercial product	: **Bayer AG**	**Residues determined as**	:	**Spiroxamine**
		Residues calculated as	:	**spiroxamine**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment kg a.s./ha	Water (L/ha)	kg a.s./hl	Dates of treatment(s); Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg) spiroxamine	DALT/PHI (days) (f)	Remarks
PF 3742 00640/8 (0640-90) Germany 5093 Burscheid, Hofchen 1990	Wheat, winter Apollo	1) 24.10.1989 2) 09.06.1990 3) 13.06.1990 4) 07.08.1990	Spraying Spraying	0.3750 0.3750	300 300	0.12500 0.12500	15.05.1990/0 11.06.1990/27	61	green material ear straw grain	4.0 1.5 0.78 0.23 0.17 1.1 0.74 <0.05	0 14 28 28 35 35 57 57	(g) 00252 (h) 0.05 mg/kg

(a) According to Codex (or other e.g. EPPO classification/Guide treatment)
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<', ** prior to last
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

Note: All entries to be filled in as appropriate. Date format dd.mm.yy

III. Conclusions

One residue trial conducted in 1990 in northern Europe is available to evaluate the residues of spiroxamine in/on wheat green material, ears, straw and grain harvested after two applications of tebuconazole and spiroxamine 417 EC on wheat up to BBCH 61.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial on wheat was conducted as a decline trial, with green material sampled at 0 to 28 DALA, and ears, straw and grain sampled between 28 to 57 DALA. Residues of spiroxamine in green material were 4.0 mg/kg, 0 DALA, declining to 0.74 to 1.1 mg/kg in mature straw and <0.05 mg/kg in mature grain, 35 to 57 DALA.

This study it is not relied on for assessment or MRL purposes.

Assessment and conclusion by applicant:

Study previously submitted and accepted as supplemental information in the EU: Spiroxamine Annex B7 RAR (2010), IIA 6.3.1/05. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009.

No residues above the LOQ (0.05 mg/kg) were detected in the control samples.

One trial on wheat was conducted as a decline trial, with green material sampled at 0 to 28 DALA, and ears, straw and grain sampled between 28 to 57 DALA. Residues of spiroxamine in green material were 4.0 mg/kg, 0 DALA, declining to 0.74 to 1.1 mg/kg in mature straw and <0.05 mg/kg in mature grain, 35 to 57 DALA.

This study it is not relied on for assessment or MRL purposes.

Overview on wheat residue trials

Residue trials conducted after the previous EU approval evaluation from 2016 onwards employed residue methods measuring both spiroxamine itself with a fully chiral method and total spiroxamine by means of a common moiety approach. Residues of spiroxamine diastereomers A and B and their enantiomers A1: A2: B1 : B2 were measured and reported as mg/kg both individually and as a sum.

The residues data submitted does include trials conducted at the nominal individual application rate of 375 g a.s./ha but to provide a complete dataset from the large number of existing trials conducted at higher or lower application individual rates (from 150 to 750 g a.s./ha), all such trials are included. As these rates are outside of the usually applied ±25% rule, the accepted approach of proportionality or scaling has been used to adjust any residues reported above the analytical LOQ to the nominal rate.

Trials on wheat or rye previously evaluated but considered not appropriate for MRL purposes are provided in this submission for information but are not relied upon. These trials are where:

- The use pattern did not fit the cGAP and residues could not be scaled
- The data were only for total residues of spiroxamine (via the 4-t-butylcyclohexanone common moiety)
- The analytical LOQ was 0.05 mg/kg and therefore data could not be scaled or used reliably in MRL calculations

Grain data:

A total of 25 residue trials conducted between 2007 and 2017 are available to evaluate the residues of spiroxamine (scaled spiroxamine as a sum of total isomers) in wheat grain after application of

spiroxamine formulations to wheat crops in support of the critical GAP (latest application nominally BBCH 59). Of these, 12 trials were performed in the northern European climatic zone and 13 trials were performed in the southern European climatic zone. Reported spiroxamine residues in wheat grain ranged from <0.01 to 0.02 mg/kg for the northern zone and from <0.01 to 0.01 mg/kg for the southern zone. The worst case data set for MRL purposes was the northern zone.

Wheat is a major crop in Europe and as such requires eight residue trials per climatic zone when treated with products after formation of the edible commodity and where residues are above the LOQ. Therefore the data set for wheat grain satisfies the data requirements outlined in Regulation (EU) 283/2013 of 01 March 2013 and is appropriate for MRL purposes.

In accordance with the guidance from document SANTE/2019/12752 (November 2020) a minimum of 8 trials on wheat (commodity 0500090) allows extrapolation of the data to rye (commodity 0500070).

Data from trials are also available for total residues of spiroxamine (via the t-butyl cyclohexanone common moiety) and ranged from <0.01 to 0.04 mg/kg for the northern zone and from <0.01 to 0.02 mg/kg for the southern zone. These values, where appropriate, are used to determine conversion factors for use in consumer risk assessment.

Straw data:

A total of 45 residue trials conducted between 2007 and 2017 are available to evaluate the residues of spiroxamine (scaled spiroxamine as a sum of total isomers) in wheat straw after application of spiroxamine formulations to wheat crops in support of the critical GAP (latest application nominally BBCH 59). Of these, 21 trials were performed in the northern European climatic zone and 24 trials were performed in the southern European climatic zone. Reported spiroxamine residues in wheat straw ranged from 0.05 to 1.32 mg/kg for the northern zone and from 0.12 to 1.50 mg/kg for the southern zone.

Data from trials are also available for total residues of spiroxamine (via the t-butyl cyclohexanone common moiety) and ranged from 0.44 to 4.5 mg/kg for the northern zone and from 2.2 to 9.0 mg/kg for the southern zone. These values, where appropriate, can be used to determine conversion factors or used in dietary burden calculations.

CA 6.4 Feeding studies

Of the supported representative uses for spiroxamine (see CA 6.3), only small grain cereal commodities are potential contributors to livestock diet and the residue data (summarised in CA 6.3) show that residues of grain and straw do contain potentially significant residues in raw commodities relevant for livestock diet. Therefore, a dietary burden estimation is presented here for both spiroxamine alone and also for the total residue of spiroxamine (common moiety approach) determined after hydrolysis to 4-t-butylcyclohexanone (4-TBCH) and expressed as spiroxamine equivalents. This will cover the residue intake by livestock in terms of the active substance and also the total crop residue definition for consumer risk assessment.

According to the latest EFSA Guidance [Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, September 2015] the "new data requirements" (Regulation (EU) No 283/2013) are applicable for this active substance approval submission on spiroxamine. Therefore, the trigger level within Regulation (EU) No 283/2013 for livestock diet intake of 0.004 mg/kg bw/day applies.

The current EU approach is to adopt the use of HRs or STMRs as the basis for the dietary burden calculations for the different types of commodity, as proposed by JMPR, 2004 and 2007. HRs are used for those commodities which are not blended or processed, so that a representative lot of harvested RAC with a high residue (HR) could be fed to livestock. STMRs are used for those commodities which are

blended or processed, so that a representative lot of harvested RAC with a high residue (HR) will actually be mixed or diluted with other batches before being fed to livestock, which justifies use of the STMR.

Assuming no loss of residues during processing, reported residues derived from spiroxamine in relevant supported crops and any feedstuff derived from these commodities can be used to calculate maximum and median dietary burdens to assess if livestock feeding studies need to be evaluated or conducted.

In order to harmonise and facilitate the MRL setting approach, EFSA has developed an Excel dietary burden calculator (pesticides_mrl_guidelines_animal_model_2017.xlsx) based on the OECD feedstuff tables and approach detailed in the OECD guidance 73. This calculator considers only those commodities relevant for EU consideration and has been used here.

As a further revision, the dietary burden is presented without the default process factors for by-products from cereal grain (Brewer's grain, Distiller's grain, Wheat gluten meal and Wheat milled by-products). The processing factor for Brewers grain was determined in the definitive barley processing study, where an average processing factor of 0.3 was derived (refer to Table CA 6.5.3/01-2). Additionally, it can be justified to replace the default process factors for Distiller's grain, Wheat gluten meal and Wheat milled by-products with a factor of x1 for the following reasons:

a) The EU LoEP (EFSA 2010, RMS 2017) confirms that process factors for cereal grain are <1.0 due to low residues in wheat grain and based on EU processing studies for barley grain

b) Spiroxamine residues in wheat grain from use of spiroxamine in the northern zone (as a worst case data set) are very low and the median value can be expected to be 0.01 mg/kg.

Spiroxamine

The input parameters for livestock dietary burden for spiroxamine are shown in Table CA 6.4-1.

Table CA 6.4-1 Dietary burden input parameters for spiroxamine (representative uses)

Commodity	Feed matter crop group[1]	STMR (mg/kg)	Dietary burden processing factor	STMR-P (mg/kg)	HR (mg/kg)[4]	Comments
Barley straw	Forages/Fodders[2]	0.345	-	-	0.73	From worst case SEU data set
Wheat straw	Forages/Fodders[2]	0.505	-	-	1.50	From worst case SEU data set
Barley grain	Cereal Grains/Crops Seeds	0.015		-	0.04	From worst case NEU data set
Wheat grain	Cereal Grains/Crops Seeds	0.01		-	0.02	From worst case NEU data set
Brewers grain (dried)	By-products	0.015	Calculated: 0.3	0.0045	-	Calculated pf from barley processing study. Table CA 6.5.3/01-3
Distillers grain (dried)	By-products	0.01	Default: 1.0	0.01	-	
Wheat gluten (meal)	By-products	0.01	Default: 1.0	0.01	-	
Wheat (milled by-products)	By-products	0.01	Default: 1.0	0.01	-	

Commodity	Feed matter crop group[1]	STMR (mg/kg)	Dietary burden processing factor	STMR-P (mg/kg)	HR (mg/kg)[4]	Comments

1 - As given in OECD Guidance Document on Residues in Livestock, Series on Pesticides, No 73 (10/07/2013).
2 - Additional forage items for cereals such as hay and silage not relevant for EU as a use on forage crops is not proposed.

The data set used to derive the STMRs for spiroxamine in cereal straw is shown in Table CA 6.4-2.

The calculations for median and maximum dietary intake are summarised in Table CA 6.4-3 showing spiroxamine dietary burden from cereal feedstuff commodities.

Table CA 6.4-2 Data set used to derive STMRs for spiroxamine in cereal straw

Region	Commodity	Residues (mg/kg)	STMR (mg/kg	HR (mg/kg)
SEU	Wheat straw	0.12, 0.13, 0.16, 0.21, 0.27, 0.29, 0.36, 0.37, 0.39, 0.40, 0.45, 0.50, 0.51, 0.53, 2 x 0.55, 0.65, 0.74, 0.75, 0.85, 1.00, 1.30, 1.40, 1.50	**0.505**	**1.50**
NEU	Wheat straw	0.05, 0.14, 0.15, 0.24, 0.27, 0.30, 0.31, 032, 0.39, 0.43, 0.44, 0.55, 0.56, 0.70, 0.72, 0.76, 0.77, 0.83, 0.93, 2 x 1.32	0.440	1.32
EU	Wheat straw	0.05, 0.12, 0.13, 0.14, 0.15, 0.16, 0.21, 0.24, 2 x 0.27, 0.29, 0.30, 0.31, 0.32, 0.36, 0.37, 2 x 0.39, 0.40, 0.43, 0.44, 0.45, 0.50, 0.51, 0.53, 3 x 0.55, 0.56, 0.65, 0.70, 0.72, 0.74, 0.75, 0.76, 0.77, 0.83, 0.85, 0.93, 1.00, 1.30, 2 x 1.32, 1.40, 1.50	0.500	1.50
SEU	Barley straw	0.06, 0.07, 0.09, 0.10, 2 x 0.12, 0.19, 0.23, 0.26, 0.28, 0.32, 0.33, 0.34, 0.35, 0.36, 2 x 0.37, 0.41, 2 x 0.42, 0.44, 0.51, 0.54, 0.60, 0.69, 0.73	**0.345**	**0.73**
NEU	Barley straw	0.06, 0.11, 0.13, 0.14, 2 x 0.22, 2 x 0.23, 0.25, 2 x 0.27, 2 x 0.28, 0.29, 2 x 0.36, 0.41, 0.44, 0.45, 0.47, 0.55, 0.59, 0.62, 0.68	0.28	0.68
EU	Barley straw	2 x 0.06, 0.07, 0.09, 0.11, 2 x 0.12, 0.13, 0.14, 0.19, 2 x 0.22, 3 x 0.23, 0.25, 0.26, 2 x 0.27, 3 x 0.28, 0.29, 0.32, 0.33, 0.34, 0.35, 3 x 0.36, 2 x 0.37, 2 x 0.41, 2 x 0.42, 2 x 0.44, 0.45, 0.47, 0.51, 0.54, 0.55, 0.59, 0.60, 0.62, 0.68, 0.69, 0.73	0.320	0.73

Table CA 6.4-3 Calculated spiroxamine dietary burden (representative uses)

Relevant groups	Dietary burden expressed in				Most critical diet	Most critical commodity	Trigger exceeded (yes/no) [0.004 mg/kg bw]
	mg/kg bw per day		mg/kg DM				
	Median	Maximum	Median	Max			
Cattle (all diets)	0.005	0.014	0.13	0.36	Dairy cattle	Rye straw	Yes
Cattle (dairy only)	0.005	0.014	0.13	0.35	Dairy cattle	Rye straw	Yes
Sheep (all diets)	0.010	0.030	0.24	0.69	Lamb	Rye straw	Yes
Sheep (ewe only)	0.008	0.023	0.24	0.69	Ram/Ewe	Rye straw	Yes
Swine (all diets)	<0.001	<0.001	0.02	0.02	Swine (finishing)	Barley grain	No
Poultry (all diets)	0.005	0.013	0.07	0.19	Poultry layer	Wheat straw	Yes
Poultry (layer only)	0.005	0.013	0.07	0.19	Poultry layer	Wheat straw	Yes

Total residue of spiroxamine (common moiety approach)

The input parameters for livestock dietary burden for total spiroxamine (common moiety) are shown in Table CA 6.4-4.

Table CA 6.4-4 Dietary burden input parameters for total spiroxamine (representative uses) using the residue definition for risk assessment

Commodity	Feed matter crop group[1]	STMR (mg/kg)[3]	Dietary burden processing factor	STMR-P (mg/kg)	HR (mg/kg)[4]	Comments
Barley straw	Forages/Fodders[2]	2.80	-	-	6.50	From worst case SEU data set
Wheat straw	Forages/Fodders[2]	4.25	-	-	9.00	From worst case SEU data set
Barley grain	Cereal Grains/Crops Seeds	0.036	-	-	0.20	From worst case NEU data set
Wheat grain	Cereal Grains/Crops Seeds	0.020	-	-	0.038	From worst case NEU data set
Brewers grain (dried)	By-products	0.056	Calculated: 0.3	0.0168	-	Calculated pf from barley processing study. Table CA 6.5.3/01-3
Distillers grain (dried)	By-products	0.020	Default: 1.0	0.020	-	
Wheat gluten (meal)	By-products	0.020	Default: 1.0	0.020	-	
Wheat (milled by-products)	By-products	0.020	Default: 1.0	0.020	-	

1 - As given in OECD Guidance Document on Residues in Livestock, Series on Pesticides, No. 73 (10/07/2013)
2 - Additional forage items for cereals such as hay and silage not relevant for EU as a use on forage crops is not proposed

The data set used to derive the STMRs for total spiroxamine in cereal grain and straw is shown in Table CA 6.4-5.

The calculations for median and maximum dietary intake are summarised in Table CA 6.4-6 showing spiroxamine dietary burden from cereal feedstuff commodities.

Table CA 6.4-5 Data set used to derive STMRs for total spiroxamine in cereal grain and straw

Region	Commodity	Residues (mg/kg)	STMR (mg/kg	HR (mg/kg)
SEU	Wheat straw	2.20, 2 x 2.50, 3.00, 3.70, 3.80, 4.25, 4.40, 4.50, 6.70, 7.20, 7.60, 9.00	**4.25**	**9.0**
NEU	Wheat straw	0.44, 0.67, 1.00, 2.00, 2.20, 3.80, 4.50	2.00	4.5
EU	Wheat straw	0.44, 0.67, 1.00, 2.00, 2 x 2.20, 2 x 2.50, 3.00, 3.70, 2 x 3.80, 4.25, 4.40, 2 x 4.50, 6.70, 7.20, 7.60, 9.00	3.70	9.0
SEU	Wheat grain	4 x <0.01, 0.010, 0.011, 0.012, 0.017, 0.021	0.010	0.021
NEU	Wheat grain	<0.01, 0.014, 0.014, 0.018, 0.022, 0.024, 0.036, 0.038	0.020	0.038
EU	Wheat grain	5 x <0.01, 0.01, 2 x 0.011, 0.012, 0.014, 0.017, 0.018, 0.020, 0.021, 0.022, 0.024, 0.036, 0.038	**0.013**	**0.038**
SEU	Barley straw	1.33, 1.40, 1.56, 1.60, 1.64, 1.84, 2.33, 2.80, 3.11, 2 x 4.00, 4.60, 5.30, 6.50	**2.80**	**6.5**
NEU	Barley straw	1.63, 2 x 1.90, 2.00, 2.25, 3.00, 3.30, 4.10, 4.70, 5.60	2.625	5.6
EU	Barley straw	1.33, 1.40, 1.56, 1.60, 1.63, 1.64, 1.84, 2 x 1.90, 2.00, 2.25, 2.33, 2.80, 3.00, 3.11, 3.30, 2 x 4.00, 4.10, 4.30, 4.60, 4.70, 5.30, 5.60, 6.50	2.80	6.5
SEU	Barley grain	3 x <0.01, 0.012, 0.018, 0.023, 0.038, 0.043, 0.057, 0.070, 0.082, 0.095, 0.130, 0.134, 0.150, 0.159	0.050	0.159
NEU	Barley grain	<0.01, 0.015, 0.019, 0.023, 0.034, 0.077, 0.086, 0.130, 0.150, 0.200	**0.056**	**0.20**
EU	Barley grain	4 x <0.01, 0.012, 0.015, 0.018, 0.019, 2 x 0.023, 0.034, 0.043, 0.057, 0.070, 0.077, 0.082, 0.086, 0.095, 2 x 0.130, 0.134, 0.150, 0.159, 0.200	0.050	0.20

Table CA 6.4-6 Calculated total spiroxamine dietary burden (representative uses)

Relevant groups	Dietary burden expressed in				Most critical diet	Most critical commodity	Trigger exceeded (yes/no) [0.004 mg/kg bw]
	mg/kg bw per day		mg/kg DM				
	Median	Maximum	Median	Maximum			
Cattle (all diets)	0.039	0.086	1.39	2.24	Dairy cattle	Barley straw	Yes
Cattle (dairy only)	0.039	0.086	1.38	2.23	Dairy cattle	Barley straw	Yes
Sheep (all diets)	0.084	0.187	2.72	4.41	Lamb	Barley straw	Yes
Sheep (ewe only)	0.066	0.147	2.72	4.41	Ram/Ewe	Barley straw	Yes
Swine (all diets)	0.002	0.002	0.06	0.06	Swine (finishing)	Barley grain	No
Poultry (all diets)	0.037	0.074	0.54	1.08	Poultry layer	Wheat straw	Yes
Poultry (layer only)	0.037	0.074	0.54	1.08	Poultry layer	Wheat straw	Yes

Summary of livestock dietary burden

Spiroxamine and total spiroxamine

Trigger levels of 0.004 mg/kg bw/day are exceeded in all relevant livestock groups except swine. Data requirements to address livestock metabolism pathway and potential for transfer of residues into food of animal origin have been addressed, refer to data points CA 6.2.2, CA 6.2.3 and CA 6.4.

CA 6.4.1 Poultry

Data Point:	KCA 6.4.1/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	KWG 4168 -- Laying hen feeding study
Report No:	MR 640/95
Document No:	M-006154-01-1
Guideline(s) followed in study:	U.S. EPA FIFRA Guideline, Subdivision 0, No. 171-4(b)
Deviations from current test guideline:	Yes, minor deviations are noted. OECD 505 (2007) guideline recommends for poultry that sacrifice is conducted within 6 hours of the final dose. No time of euthanasia of the poultry was given in the report. As all residues in tissues and eggs were <LOQ at all feeding levels, it was not possible to provide information showing correlation between feeding level and tissue residue concentration. Thus, it was also not possible to establish a plateau residue level in eggs.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

The purpose of this study was to quantify the residues of spiroxamine in eggs and edible tissues (muscle, fat and liver) of laying hens following repeated dietary administration of spiroxamine for a minimum period of 28 days

Three groups of laying hens (*Gallus gallus domesticus,* strain Lohman White LSL) were administered spiroxamine once daily for 28 consecutive days at a rate of 0.2 (1X), 0.6 (3X) and 2.0 (10X) mg/kg in the diet. Additionally a control group was fed unspiked feed. These dose levels were equivalent to 0.19, 0.048 and 0.150 mg/kg bodyweight per day for the 1X, 3X and 10X levels, respectively. Treatments were administered orally via treated commercially available poultry feed that was prepared in weekly batches. The highest dose group represents approximately 2N the highest dietary burden based on total spiroxamine feed levels (refer to Section CA 6.4).

Hens were inspected during the study and were found to be in good health. Egg production and feed intake were considered to be normal.

Dose preparation and dosing

Commercially available poultry feed (LA 55, Hoveler feed factory) was spiked with technical grade spiroxamine. One 20 kg batch of spiked feed was prepared weekly for each treatment group (0.2, 0.6 and 2.0 mg/kg) as well as for the unspiked feed of the control group, by dissolving the appropriate amount of spiroxamine in peanut oil (DAB 9; 1% of feed) and mixed with the feed thoroughly (threefold pre-mixing). Control feed was prepared by adding peanut oil only. All feed was filled into clearly labelled containers to ensure correct allocation of feed to individual dose groups.

During the dosing period, the spiked feed was prepared weekly and stored in a freezer at ≤-18°C. Before use, the required amount of feed was thawed in a refrigerator (+4°C) and then stored for 24 hours under stable conditions. Each batch of feed was only fed for 7 days after fortification. Any remaining feed was discarded and replaced by a new batch.

Table CA 6.4.1/01-1 Dose level groups of spiroxamine to laying hens

Group no.	Treatment level	Dose level			Number of animals per group	Animal identification number
		mg a.s./kg in diet (dry matter)	Average weight (kg)	mg/kg bw/day[1]		
1	Control	0	1.53	0.00	12	1-12
2	1X	0.2	1.61	0.019	12	13-24
3	3X	0.6	1.47	0.048	12	25-36
4	10X	2.00	1.53	0.150	12	37-49[2]

1 - Mean achieved dose intake, expressed as mg/kg body weight/day, during weeks 1 to 4, calculated using the mean daily feed consumption

2 – hen number 45 died during the acclimation phase and was replaced by hen 49

The 1X dose in the is study was considered to approximately represent maximum dietary burden at the time of study conduct (0.24 mg/kg in dry feed matter). This dose is above the maximum dietary burden from the crops supported in this dossier and is appropriate to evaluate the potential for transfer into food of animal origin.

Birds were selected for use in the study on the basis of health and body weight and were randomly allocated to individual cages. All study birds were acclimatised for 7 days prior to start of dosing. Each bird was assigned a number and identified uniquely within the study by a numbered leg ring.

The birds were housed individually in constructed from galvanised steel in a building which provided suitable environmental conditions for hens. The temperature was maintained at approximately 16 – 18°C and humidity was recorded at 60 – 80%. Illumination was set for 14 hours per day. Each pen contained a concentrate feed hopper and a source of fresh drinking water (available at all times without

restriction). The hens were fed every morning. Approximately 200g of the appropriate feed was fed and any uneaten food at the end of each day was weighed and discarded.

Contamination of the hens with faeces was prevented by a wide grid cage floor.

The animals were weighed individually on arrival, on day 0, just before administration of the first and at termination (day 28).

All test birds in Groups 2, 3 and 4 received supplemented diet containing spiroxamine at the nominal concentrations shown in Table CA 6.4.1/01-1, offered continuously *ad libitum* as the only feed source, for 28 consecutive days.

Pre-mix diets were analysed using the validated analytical method 00361 (diet), report reference M-019094-02-1 (**see Doc MCA Section 4**). Data were acceptable showing that the test item was dosed correctly, with the exception of the 0.2 mg/kg group, where overdosing was noted and corrected for.

Egg collection

Eggs were collected twice daily at 11 am and 4 pm. After the first few days of acclimation, egg production was very regular. Due to the light regimen, most eggs were collected in the morning. Immediately after collection, the eggs were stored in individually labelled containers at approximately -20°C.

Animal sacrifice and sampling

After 28 days of dosing, the hens were anaesthetised by CO_2, followed by exsanguination. No treatment-related morphological or pathological findings were detected. The following tissues were collected at sacrifice: liver (whole organ without gallbladder), muscle (composite thigh, leg and breast) and fat (abdominal and subcutaneous) were taken. The remaining carcasses were disposed of. Samples were stored frozen at -20°C.

Sample analysis

Preparation of samples

All samples from each hen were stored individually deep frozen (-20°C). Just before analysis, the tissues were thawed and then tissue samples from the same subgroup of hens were combined and homogenised as was done for eggs.

Eggs

Immediately prior to analysis, eggs were taken to the analytical laboratory, where they were thawed overnight in a refrigerator (+4°C), cracked and opened. The contents of up to 3 eggs of the same sub-dose group and sampling day were pooled and homogenised. The shells were discarded. Initially, the eggs of all dose groups were analysed. As no residues were found, only the 2 mg/kg dose group was analysed for the whole dose group. Additionally some eggs of day 3 or 5 and the eggs of day 27 of all other dose groups were analysed.

Liver and fat

Samples of each of these tissue types were manually cut into small pieces with a knife, placed in a polyethylene box and mixed thoroughly.

Muscle

Muscle samples were homogenised using a mincing machine. The homogenate was placed in a polyethylene box and mixed thoroughly.

Residue analysis

Residue analysis for the determination of the total residue of spiroxamine (as derivatised 4-t-butylcyclohexanone) in samples (eggs and tissues) was conducted using the validated method no. 00392, report reference M-019285-02-1 (**see Doc MCA Section 4**).

Samples were extracted by maceration with mixtures of acetonitrile/water (tissues) or methanol/water (eggs). After centrifugation, the extract was shaken with n-heptane to remove any fatty material. After evaporation, the aqueous remainder was refluxed with a mixture of hydrochloric acid and methanol. Thus the residue of total spiroxamine (parent spiroxamine and all metabolites containing the 4-t-butylcyclohexanone moiety) was hydrolysed yielding 4-t-butylcyclohexanone. The hydrolysate was diluted with water and applied to a reverse phase SPE column. Any 4-t-butylcyclohexanone was eluted using dichloromethane. The extract was further purified by column chromatography using silica gel. The final extracts were determined by gas chromatography with a mass selective detector (MSD) in the single ion monitoring mode.

The limit of quantification (LOQ) was 0.02 mg/kg in eggs and muscle and 0.05 mg/kg in fat and liver.

II. Results and discussion

Pre-mix diet

The homogeneity and stability of spiroxamine in the spiked diet was assessed with respect to the level of concentration at all three fortification levels (0.2, 0.6 and 2.0 mg/kg). The homogeneity of spiroxamine in treated feed was confirmed at these nominal concentrations. The mean values were in the range of 90 to 96% with relative standard deviations ranging from 4.0 to 7.6%.

Concentration of spiroxamine in the feed used for feeding

Each batch used for feeding was analysed to determine the concentration of spiroxamine. The concentrations found in the batches of the 0.6 and 2.0 mg/kg dose groups fit well to the target concentrations, with concentrations between 89 and 103% of target (average 98%) for the 0.6 mg/kg feed and 83 to 125% (average 97%) for the 2.0 mg/kg feed. However, the report noted that for the lowest dose group, there was an apparent overdose situation where a concentration of 0.376 mg/kg was determined (188% of target), leading to an overall average of 125%. For the calculation of the actual consumption of spiroxamine, the results of analysis of single batches was taken.

Sample analysis

The linearity of the detector was checked prior to sample analysis and was found to be linear over the whole range tested. Procedural recoveries were conducted for spiroxamine and also the desethyl and despropyl metabolites at the 0.02 or 0.05 mg/kg level and for spiroxamine at the 2.0 mg/kg level.

The recoveries from fortified control samples analysed concurrently with dose group samples were generally acceptable and mean results for each matrix were within the range of 70 to 110% (refer to Table CA 6.4.1/01-3 and Table CA 6.4.1/01-4).

The limit of quantification (LOQ) for total spiroxamine in eggs and muscle was 0.02 mg/kg and 0.05 mg/kg in fat and liver.

No quantifiable residues were found in control samples.

Residues of total spiroxamine (expressed as spiroxamine equivalents) are presented in Table CA 6.4.1/01-2. Only the eggs from the highest dose level were analysed at regular intervals throughout the study. For the other two dose levels, only eggs on limited selected days were analysed. All residues in eggs were less than the limit of quantification at all dose levels and timepoints. No residues above the limit of quantification were detected in fat, muscle (light and dark meat) and liver at any of the dose levels.

Table CA 6.4.1/01-2 Total residues of spiroxamine in eggs and edible tissues after feeding of spiroxamine for 28 days

Dose group (mg/kg)	Total spiroxamine residue (mg/kg)[1]			
	Eggs	Fat	Muscle	Liver
Control	<0.02	<0.05	<0.02	<0.05
0.2	Not analysed	<0.05	<0.02	<0.05
0.6	<0.02	<0.05	<0.02	<0.05
2.0	<0.02	<0.05	<0.02	<0.05

1 – total spiroxamine residue includes spiroxamine and metabolites with unchanged t-butylcyclohexyl moiety, expressed as spiroxamine

Table CA 6.4.1/01-3 Procedural recovery data for the determination of spiroxamine residues in egg and tissue matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.4.1/01	00392	Whole eggs	0.02 0.20 overall	8 6 14	74 to 101 77 to 90 mean: 85 RSD: 8.1
CA 6.4.1/01	00392	Muscle	0.02 0.20 overall	3 2 5	84 to 95 92, 94 mean: 91 RSD: 6.3
CA 6.4.1/01	00392	Fat	0.05 0.50 overall	2 2 4	102, 109 91, 99 mean: 100 RSD: 7.4
CA 6.4.1/01	00392	Liver	0.05 0.50 overall	2 3 5	94, 122 87 to 104 mean: 99 RSD: 15

Table CA 6.4.1/01-4 Procedural recovery data for the determination of spiroxamine-desethyl and spiroxamine-despropyl residues in egg and tissue matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.4.1/01	00392	Whole eggs	0.02 (desethyl) 0.02 (despropyl) overall	2 2 4	73, 76 80, 94 mean: 81 RSD: 12
CA 6.4.1/01	00392	Muscle	0.02 (desethyl) 0.02 (despropyl) overall	2 2 4	88, 88 88, 89 mean: 88 RSD: 0.57
CA 6.4.1/01	00392	Fat	0.05 (desethyl) 0.05 (despropyl) overall	2 2 4	90, 91 95, 97 mean: 93 RSD: 3.5
CA 6.4.1/01	00392	Liver	0.05 (desethyl) 0.05 (despropyl) overall	2 2 4	107, 119 95, 97 mean: 105 RSD: 11

Storage stability

The stability of spiroxamine in the spiked diet formulations was tested for a period of 15 days. Storage stability was tested with feed spiked with 0.2 and 2.0 mg/kg of spiroxamine. Samples were analysed at days 0, 8 and 15. The mean values at all sampling dates were in the range of 90 to 100% of the target concentration, demonstrating that spiroxamine was stable in feed under the storage conditions.

Storage stability testing on tissue samples was not required during this study, however, tests were completed on whole eggs and demonstrated that spiroxamine was stable under conditions of frozen storage for up to 28 days.

III. Conclusions

This study measured the amount of tissue and egg yolk residues in laying hens that had been fed spiroxamine at the 1X (0.2 mg/kg), 3X (0.6 mg/kg) and 10X (2.0 mg/kg) feeding levels. Hens were dosed for 28 consecutive days. The highest dose group represents approximately 2N the highest dietary burden based on total spiroxamine feed levels (refer to Section CA 6.4). Samples were analysed for the residue of total spiroxamine (parent spiroxamine and all metabolites containing the 4-t-butylcyclohexanone moiety).

No residues above the limit of quantification were detected in eggs, fat, muscle (light and dark meat) and liver at any of the dose levels.

It is therefore concluded that it is expected there would be no significant transfer of residues resulting from intake of spiroxamine into poultry eggs and edible tissues.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.4.2/02.

No residues above the limit of quantification were detected in eggs, fat, muscle (light and dark meat) and liver at any of the dose levels.

Therefore, even though the data are not generated fully in compliance with the current and proposed residue definition for monitoring the hen metabolism data show that in all poultry edible commodities residues would be detectable if significant transfer from feed occurred. Therefore no positive MRLs above the LOQ are proposed and no new poultry feeding study is required.

CA 6.4.2 Ruminants

Data Point:	KCA 6.4.2/01
Report Author:	[redacted]
Report Year:	1996
Report Title:	KWG 4168 - Cattle feeding study
Report No:	MR-91/96
Document No:	M-006159-02-1
Guideline(s) followed in study:	U.S. EPA FIFRA Guideline, Subdivision O, No. 171-4(b)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

The purpose of this study was to quantify the residues of spiroxamine-acid (M06) in milk and edible tissues (muscle, fat, kidney and liver) of lactating ruminants following repeated dietary administration of spiroxamine for a period of 28 days

Three groups of lactating ruminants (*Bos taurus*, strain Holstein-Friesian) were administered spiroxamine once daily for 28 consecutive days at a rate of 2.0 (1X), 6.0 (3X) and 20.0 (10X) mg/kg in the diet. Additionally a control group was fed untreated feed. The weight of the cows at arrival was between 476 and 597 kg. All animals were between 3 and 4 years old and were in mid-lactation but not in calf. These dose levels were equivalent to 0.066, 0.194 and 0.600 mg/kg bodyweight for the 1X, 3X and 10X levels, respectively. Treatments were administered orally via gelatin capsules that were prepared in advance and stored for 28 days. The lowest dose group represents approximately 1N the highest dietary burden based on total spiroxamine feed levels for bovine species and approximately 0.4N the highest dietary burden for ovine (sheep) species (refer to Section CA 6.4).

Cows were inspected during acclimation and throughout the trial by a veterinarian and were found to be in good health. Milk production and feed intake were normal.

Dose preparation and dosing

Cows were dosed orally by gelatin capsule administered after the evening feed. The capsules were prepared in advance by weighing the chemical directly into the capsule. No additional filling substance was used. Capsules were sealed and stored in a glass container under deep freeze conditions (≤-20°C) until application.

Based on a daily feed consumption of 3% of the body weight and an anticipated weight of 550 – 600 kg, the cows were expected to consume 18 kg of dry feed matter daily. Therefore the amount of spiroxamine per capsule was estimated to be 36, 108 and 360 mg for the 2, 6 and 20 mg/kg dose levels respectively.

Table CA 6.4.2/01-1 Dose level groups of spiroxamine to lactating ruminants

Group no.	Treatment level	Dose level			Number of animals per group	Study animal identification number[2]
		(mg a.s./kg in diet (dry weight))	Target weight of spiroxamine (mg)	(mg/kg bw/day)[1]		
1	Control	0	0	0.00	3	1-3 (4196, 4195, 4194)
2	1X	2	36	0.066	3	4-6 (4193, 4192, 4191)
3	3X	6	108	0.194	3	7-9 (4190, 4189, 4188)
4	10X	20	360	0.600	3	10-12 (4187, 4186, 4185)

1 – based on actual dose and mean bodyweight of animals during the study conduct
2 – numbers in parentheses represent actual animal numbers

The 1X dose in the is study was considered to approximately represent maximum dietary burden at the time of study conduct (0.24 mg/kg in dry feed matter). This dose is above the maximum dietary burden from the crops supported in this dossier and is appropriate to evaluate the potential for transfer into food of animal origin.

On the day of arrival, cows were assigned study numbers by randomisation and were checked for signs of ill-health. Veterinarian checks were continued throughout the duration of the study. All study animals were acclimatised for 7 days prior to start of dosing.

The cattle were housed in individual stands (tethered housing) and handled as under normal dairy practice. Each animal had access to an individual feed trough. The feed ratio was as follows: 6 kg hay (equivalent to 5kg dry matter), 24 kg corn silage (5 kg DM), 6 kg dairy high energy concentrate (5.3 kg DM) and 0.1 kg of mineral feed. Actual feed consumption was recorded but it was noted that some corn silage was left by cattle at most feed events.

The temperature was maintained at approximately 20°C and humidity was recorded at approximately 60%. Illumination was set for 12 hours per day. Cows had access to a mineral lick and water of drinking quality *ad libitum*.

The animals were weighed individually on arrival, on day 0, just before administration of the first and at termination (day 28).

One day prior to the beginning of dosing, the capsules were analysed in an independent laboratory to confirm the spiroxamine dosing levels. Each capsule was opened and internal standard (diphenyl phthalate) was added. Dichloromethane was used to rinse the capsule. The amount of spiroxamine in each capsule was determined using the validated analytical method 2001-0034401-93, report reference M-299186-01-2 (**see Doc MCA Section 4**). Data were acceptable, showing that the test item was dosed correctly.

Capsules spiked with spiroxamine were stored in a refrigerator at 4°C for up to 28 days. The storage stability of spiroxamine in capsules under these storage conditions was determined for a period of 28 days.

Milk collection

Milk samples were taken from day 0 to day 28 of the study. To avoid contamination, milking was in the order 0, 2, 6 and 20 mg/kg dose level. The milk yields of every morning and evening milking were recorded.

From every milking (morning and evening) of each cow, the milk was mixed thoroughly and 2 samples each of 300 mL were removed and deep frozen immediately at ≤ -18°C for 1 – 4 days until transfer to the analytical laboratory. The remaining milk was discarded.

Samples were transferred to the analytical facility just before analysis. Samples from different cows and different days were not combined, but just before analysis, the respective morning and evening samples from each individual cow were pooled in the ratio of normal milk production in accordance with normal dairy practice.

Animal sacrifice and sampling

After 28 days of dosing, and straight after the morning milking, the cows were euthanised by captive bolt, followed by exsanguination. This was between 14 and 19 hours after the final dose. The process was staggered, according to the dosing schedule so that only 3 cows per day were dissected. No treatment-related morphological or pathological findings were detected.

The following tissues were collected at sacrifice: fat (mixed sample of renal and omental fat, between 664 and 1356 g), Liver (whole organ without gall bladder, between 6262 and 10408 g), kidneys (both whole organs, between 1147 and 1866 g) and muscle (mixed sample of flank, round and loin, between 938 and 1299 g). The remaining carcasses were disposed of. Samples were stored frozen at -20ºC.

Preparation of samples

All samples from each cow were stored individually deep frozen (<-18°C).

Fat and muscle

Single parts of the deep frozen tissue samples were chopped using a saw into approximately 3 cm cubes. The cubes were mixed with dry ice and homogenised in a cutter. The powdered tissues were stored in polyethylene boxes and stored deep frozen (<-18°C) until analysis.

Liver and kidney

Whole deep frozen organs were chopped using a saw into approximately 3 cm cubes. The cubes were mixed with dry ice and homogenised in a cutter. The powdered tissues were stored in polyethylene boxes and stored deep frozen (<-18°C) until analysis.

Residue analysis

Analysis of milk and tissues (LC-MS/MS method)

Samples were analysed according to method 00358 report reference M-019051-02-1 (**see Doc MCA Section 4**). The method determines the residue as spiroxamine-acid (M06). The limit of quantification was 0.01 mg/kg in milk and 0.02 mg/kg in tissues.

Milk and muscle

Spiroxamine-acid metabolite was extracted twice from the samples using methanol (milk) or acetonitrile and an acetonitrile/water mix (muscle). After centrifugation, the combined extracts were concentrated to the aqueous remainder. Acetonitrile and water were used to make up to a known volume (25 or 50 mL). Prior to analysis by HPLC, extracts were filtered. Quantitation was performed by LC-MS/MS in the multiple reaction mode.

After method validation, milk samples from the highest dose level were analysed to obtain information on both magnitude of residue and to determine when residues reached plateau. Based on this information, it was determined which samples from lower dose groups should be analysed.

Fat, liver and kidney

Spiroxamine-acid metabolite was extracted twice from the samples using acetonitrile and an acetonitrile/methanol mix (liver and kidney), the extractions were combined. The same metabolite was extracted from fat twice with a methanol/acetonitrile/cyclohexane mixture, (cyclohexane layer discarded) and the extractions combined. The combined extracted extracts were concentrated to the aqueous remainder after centrifugation. An XAD-4 column was used to purify the samples. Acetonitrile and water were used to make up to a known volume (25 or 50 mL). Prior to analysis by HPLC, extracts were filtered. Quantitation was performed by LC-MS/MS in the multiple reaction mode.

Additional analysis of milk and tissues (enforcement method)

Selected samples were also analysed using the proposed residue enforcement method (as spiroxamine-acid, M06) which was a GC/MS method in single ion monitoring mode. Samples were analysed according to method 00395 report reference M-019323-02-1 (**see Doc MCA Section 4**). The limit of quantification was 0.01 mg/kg in milk and 0.02 mg/kg in tissues.

Residues were extracted from liver, muscle and kidney with acetonitrile and acetonitrile/water. Fat was extracted with a mixture of acetonitrile, methanol and cyclohexane, and milk was extracted with methanol. After centrifugation, the supernatant was concentrated to the aqueous remainder which was then applied to a XAD-4-column. The eluate from the XAD-4-column was further purified on an RP-18-cartridge. The residues were determined with GC/MS in the single ion monitoring mode.

For quantification, the LC/MS/MS method used for sample analysis required standards in spiked control extracts. 'Bridging' data was therefore produced to validate the results with a method using a pure standard in solvent for quantification. Extracts obtained by the LC-MS/MS method were taken and further purified is the same way as the enforcement method.

Generally, samples from the highest dose group (10X) were analysed and for milk, the day 15 sample was selected. Additionally one control samples from each matrix was analysed. Validation of the enforcement method was performed by concurrent recoveries.

II. Results and discussion

Analysis of capsules

Initial analysis of 12 randomly selected capsules indicated there was a problem with achieving the theoretical weight of active substance within each capsule. These capsules were discarded and a second set prepared. Here the target weight of spiroxamine in each capsule at the three dose levels was determined to be correct with mean amounts of test item determined to be 36.1, 102.8 and 357.2 mg (target amounts 36, 108 and 360 mg) indicating that the correct amount of test item had been prepared.

The second set of capsules was used for days 0 - 27 of the 20 mg/kg (10X) group, days 1-27 of the 6 mg/kg (3X) group and days 2-27 of the 2 mg/kg (1X) group. From the mean values of the capsules fed and the feed consumption, the theoretical residue in feed was calculated.

Capsules spiked with spiroxamine were stored at 4°C for 28 days. At day 0 and at day 28, 6 capsules of each dose level were analysed. Spiroxamine was found to be stable under these conditions of storage in gelatin capsules for at least 28 days.

Sample analysis

The linearity of the detector was checked prior to sample analysis and was found to be linear over the whole range tested. Procedural recoveries were conducted for spiroxamine-acid at the 0.02 mg/kg level for all tissues and additionally and at the 0.2 and or 0.5 mg/kg levels. Procedural recoveries in milk were conducted at the 0.01, 0.1 and 0.5 mg/kg fortification levels.

The recoveries from fortified control samples analysed concurrently with dose group samples and samples for storage stability were acceptable and mean results for each matrix were within the range of 70 to 110% (refer to Table CA 6.4.2/01-5).

The limit of quantification (LOQ) for spiroxamine-acid in milk was 0.01 mg/kg and 0.02 mg/kg in edible tissues.

No quantifiable residues were found in control samples.

Residues of spiroxamine-acid in milk are presented in Table CA 6.4.2/01-2 and residues in edible tissues are presented in Table CA 6.4.2/01-3.

Milk

In milk samples from the 2 mg/kg dose group no residues above the LOQ (0.01 mg/kg) were determined. In the higher dose groups, residues reached plateau within 4 days of the first dose, with plateau levels of approximately 0.015 mg/kg seen in the 3X dose level (6 mg/kg) and 0.04 mg/kg at the 10X (20 mg/kg) level.

Fat

Detectable residues of fat were only seen in the highest (10X) dose group, with a mean residue of 0.088 mg/kg.

Muscle

Residues in muscle were low with residues above the LOQ (0.02 mg/kg) generally only seen at the 10X dose level. Average residues at this level were 0.054 mg/kg.

Kidney

Detectable residues in kidney were seen at each of the 3 dose levels, with average residues of 0.045, 0.09 and 0.22 mg/kg seen at the 1X, 3X and 10X levels respectively.

Liver

Similarly to kidney as another excretory organ, detectable residues were also seen at all three dose levels, with average residues of 0.053, 0.16 and 0.27 mg/kg seen at the 1X, 3X and 10X levels respectively.

Validation of results using the enforcement method

The residues obtained with the enforcement method confirm those obtained using the primary LC/MS/MS method. Some minor variations are noted but are due to normal sample variability. A comparison of residues of spiroxamine in tissues and milk analysed by both the primary method (LC/MS/MS) and the enforcement method are detailed in Table CA 6.4.2/01-4.

Table CA 6.4.2/01-2 **Summary of spiroxamine-acid residues in milk from a lactating ruminant after 28 daily doses of spiroxamine (at 2.0 (1X), 6.0 (3X) and 20.0 (10X) mg/kg in the diet)**

Day of treatment	Spiroxamine-acid residues in milk (mg/kg) at the different dose levels							
	Control		2 mg/kg		6 mg/kg		20 mg/kg	
	Residue	Mean	Residue	Mean	Residue	Mean	Residue	Mean
0	-	-	-	-	-	-	<0.01 <0.01 <0.01	<0.01
1	-	-	<0.01 <0.01 <0.01	<0.01	<0.01 <0.01 <0.01	<0.01	0.034 0.027 0.034	0.032
2	<0.01 <0.01 <0.01	<0.01	-	-	-	-	0.044 0.033 0.035	0.037
4	-	-	-	-	0.012 0.013 <0.01	0.01	0.053 0.037 0.041	0.044
6	-	-	<0.01 <0.01 <0.01	<0.01	-	-	0.051 0.037 0.022	0.036
8/9	<0.01	<0.01	-	-	0.012 0.013 0.015	0.014	0.046 0.035 0.030	0.037
11/12	-	-	<0.01 <0.01 <0.01	<0.01	0.010 0.013 0.013	0.012	0.044 0.037 0.031	0.037
15/16	-	-	<0.01 <0.01 <0.01	<0.01	0.014 0.016 0.015	0.015	0.052 0.033 0.017	0.034
19/20/21	-	-	<0.01 <0.01 <0.01	<0.01	0.019 0.020 0.025	0.021	0.050 0.033 0.017	0.039

Day of treatment	Spiroxamine-acid residues in milk (mg/kg) at the different dose levels							
	Control		2 mg/kg		6 mg/kg		20 mg/kg	
	Residue	Mean	Residue	Mean	Residue	Mean	Residue	Mean
23	-	-	-	-	-	-	0.037 0.034 0.040	0.037
26/27	<0.01 <0.01 <0.01	<0.01	<0.01 <0.01 <0.01	<0.01	0.015 0.016 0.014	0.015	0.054 0.037 0.040	0.044

Table CA 6.4.2/01-3 Spiroxamine-acid residues in edible tissues after daily dosing of spiroxamine for 28 days

Dose (mg/kg)	Spiroxamine-acid residue (mg/kg)			
	Fat	Kidney	Liver	muscle
Control	<0.02 <0.02 <0.02 (mean <0.02)	<0.02 <0.02 <0.02 (mean <0.02)	<0.02 <0.02 <0.02 (mean <0.02)	<0.02 <0.02 <0.02 (mean <0.02)
2.0	<0.02 <0.02 <0.02 (mean <0.02)	0.054 0.050 0.031 (mean 0.045)	0.049 0.060 0.049 (mean 0.053)	<0.02 <0.02 <0.02 (mean <0.02)
6.0	<0.02 <0.02 0.02 (mean <0.02)	0.089 0.074 0.106 (mean 0.09)	0.146 0.159 0.177 (mean 0.160)	<0.02 <0.02 0.021 (mean 0.02)
20.0	0.081 0.154 0.030 (mean 0.088)	0.309 0.169 0.169 (mean 0.220)	0.308 0.241 0.271 (mean 0.270)	0.057 0.057 0.050 (mean 0.054)

Table CA 6.4.2/01-4 Comparison of residue results from primary method (LC/MS/MS) and enforcement method (GC/MS)

Sample (replicates)	Spiroxamine-acid residues (mg/kg)									
	Fat		Kidney		Liver		Milk		Muscle	
	LC/MS/MS method (mg/kg)	Enforcement method (mg/kg)	LC/MS/MS method (mg/kg)	Enforcement method (mg/kg)	LC/MS/MS method (mg/kg)	Enforcement method (mg/kg)	LC/MS/MS method (mg/kg)	Enforcement method (mg/kg)	LC/MS/MS method (mg/kg)	Enforcement method (mg/kg)
Animal 12 A	0.029	0.019	0.178	0.135	0.266	0.260	0.053	0.053	0.051	0.040
Animal 12 B	0.030	0.019	0.160	0.135	0.275	0.300	0.050	0.056	0.049	0.042
mean	0.030	0.019	0.169	0.135	0.271	0.280	0.052	0.055	0.050	0.041
Animal 11 A	0.160	0.115	0.232	0.158	0.250	0.228	0.033	0.035	0.055	0.047
Animal 11 B	0.148	0.111	0.242	0.219	0.251	0.194	0.033	0.033	0.058	0.045
mean	0.154	0.113	0.237	0.189	0.241	0.211	0.033	0.034	0.057	0.046
Animal 10 A	0.076	0.050	0.307	0.265	0.305	0.296	0.019	0.023	0.041	0.045
Animal 10 B	0.085	0.058	0.310	0.310	0.311	0.362	0.014	0.012	0.076	0.061
Animal 10C	-	-	-	-	-	-	-	-	0.057	-
mean	0.081	0.054	0.309	0.288	0.308	0.329	0.017	0.018	0.054	0.053
Recovery %										
0.02 mg/kg	111	81	-	-	71/74	48[1]/77			99/95	85/93
0.1 mg/kg	-	-	-	-	-	-	74	95	-	-
0.2 mg/kg	-	-	80	89	-	-	-	-	-	-
0.5 mg/kg	-	-	-	-	-	-	-	-	102	119
2.0 mg/kg	-	-	-	-	103/79	81/62	-	-	-	-

1 - low recovery not explainable. Thought to be due to loss during concentration of extract to the aqueous remainder

Table CA 6.4.2/01-5 Procedural recovery data for the determination of spiroxamine-acid residues in milk and tissue matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.4.2/01	00355	Fat	0.02 0.5 overall	2 2 4	105, 111 94, 101 mean: 103 RSD: 6.9
CA 6.4.2/01	00355	Kidney	0.02 0.20 0.50 overall	1 1 2 4	125 80 80, 92 mean: 94 RSD: 23
CA 6.4.2/01	00355	Liver	0.02 0.50 overall	2 2 4	74, 71 95, 102 mean: 86 RSD: 18
CA 6.4.2/01	00355	Milk	0.01 0.1 0.5 overall	7 6 3 16	82, 88, 104, 86, 114, 83, 93 94, 88, 74, 97, 96, 82 95, 101, 100 mean: 92 RSD: 11
CA 6.4.2/01	00355	Muscle	0.02 0.50 overall	3 3 6	99, 95, 76 103, 102, 94 mean: 95 RSD: 10

Storage stability

Additional gelatin dose capsules spiked with spiroxamine were stored in a refrigerator at 4°C for up to 28 days. The storage stability of spiroxamine in capsules under these storage conditions was determined for a period of 28 days at each of the 3 treatment dose rates. The mean values at all sampling dates were in the range of 92 to 95% of the target concentration, demonstrating that spiroxamine was stable in the dose under the storage conditions.

Storage stability testing on tissue samples was conducted during this study as some samples were stored for more than 30 days before analysis. Data indicated that residues of spiroxamine carboxylic acid were stable under conditions of frozen storage in muscle, kidney and milk for between 175 and 224 days. In fat, uncorrected residue of spiroxamine carboxylic acid metabolite was just below 70% (average of 64% from 2 values) with a procedural recovery of 88%. A crude extrapolation of the results would imply that residues of spiroxamine carboxylic acid are stable in fat for approximately 200 days. Additionally, fat is an intrinsic component of meat, and frozen storage stability in muscle was fully acceptable with little or no degradation over time seen in this tissue. It can therefore be concluded that stability of spiroxamine in ruminant meat is stable under the conditions of storage.

After 222 days frozen, cubed portions of spiked liver showed degradation of spiroxamione-acid to less than 50% of the initial recovery. This was thought to be due in large part to the tissue being roughly macerated releasing degradative liver enzymes. Livers in the cattle feeding study were stored whole, and similar degradation would not be expected. However, a crude extrapolation of the results would imply that residues of spiroxamine carboxylic acid are stable in macerated liver for approximately 100 days. In other edible offal, i.e. kidney, residues of spiroxamine are shown to be stable over the duration of the study.

III. Conclusions

This study measured the amount of tissue and milk spiroxamine-acid residues in lactating ruminants that had been dosed with spiroxamine at the 1X (2 mg/kg), 3X (6 mg/kg) and 10X (20 mg/kg) feeding levels. Ruminants were dosed for 28 consecutive days. The lowest dose group represents

approximately 1N the highest dietary burden based on total spiroxamine feed levels for bovine species and approximately 0.4N the highest dietary burden for ovine (sheep) species (refer to Section CA 6.4).

In milk samples of the 2 mg/kg dose group, no residues above the LOQ (0.01 mg/kg) were determined. In the higher dose groups, residues reached plateau within 4 days of the first dose, with plateau levels of approximately 0.015 mg/kg seen in the 3X dose level (6 mg/kg) and 0.04 mg/kg at the 10X (20 mg/kg) level. Detectable residues in fat were only seen in the highest (10X) dose group, with a mean residue of 0.088 mg/kg. Residues in muscle were low, with residues above the LOQ (0.02 mg/kg) generally only seen at the 10X dose level. Average residues at this level were 0.054 mg/kg. Detectable residues in kidney were seen at each of the 3 dose levels, with average residues of 0.045, 0.09 and 0.22 mg/kg seen at the 1X, 3X and 10X levels, respectively. In liver, similarly to kidney, detectable residues were also seen at all three dose levels, with average residues of 0.053, 0.16 and 0.27 mg/kg seen at the 1X, 3X and 10X levels respectively.

The results therefore indicate that residues can transfer into edible tissues and milk following repeated dietary exposure to spiroxamine.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009), IIA 6.4.2/01.

Resides of spiroxamine-acid (M06) in fat and muscle were generally low. Higher residues seen in liver, kidney and milk indicating transfer of residue into edible tissues following dietary intake of spiroxamine.

CA 6.4.3 Pigs

For the supported representative uses, the intake from diet fed to pigs does not exceed 0.004 mg/kg bw/day (refer to Point CA 6.4) and the route of metabolism does not differ significantly in the rat when compared to the available data on ruminants. Therefore, there is no requirement to provide data from feeding studies in pigs and the ruminant data can be used for MRL setting and risk assessment purposes.

CA 6.4.4 Fish

Refer also to CA 6.2.5.

No data submitted or required to address this data point

To justify this position, an available fish dietary burden calculator [Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Dietary Burden Calculator v 2.0.3] has been used to estimate fish dietary burden from exposure to spiroxamine (as total spiroxamine).

This calculator uses STMR (and STMR-P where applicable) values for crop commodities which are potentially constituents of commercial fish diet for the EU.

Fish in aquaculture are fed based on a maximum reasonably balanced diet (MRBD) approach. For this, components for fish feed are typically characterised by fixed percentages of carbohydrate concentrate, protein concentrate and fat. The model calculates the dietary burden for an active substance with respect to two different important aquaculture species, rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*).

For the representative uses of spiroxamine, only cereal grain by-products form part of commercial fish diet. Using the STMR-P values as presented in Table CA 6.4.4-1, the estimated fish dietary burden in carp as a worst case (using the available dietary burden calculator for fish; Fraunhofer v 2.0.3) is very

low at just 0.015 mg/kg (in diet). The value for trout is given as 0.010 mg/kg (dry matter). It is proposed that for fish diet, the trigger value for considering residues in fish is set at 0.1 mg/kg in diet, therefore further data are not required as the estimated dietary burdens for carp and trout are significantly below the trigger value.

Table CA 6.4.4-1: Inputs into the fish dietary burden for total spiroxamine (representative uses)

Diet component	STMR-P (mg/kg)	STMR-P dry matter (mg/kg)
Brewers grain (dried)	0.017	0.018
Wheat (bran)	0.020	0.023
Wheat (flour)	0.020	0.023
Wheat (germ)	0.020	0.023
Wheat (middlings)	0.020	0.022
Wheat (gluten meal)	0.020	0.022
Wheat grain (extruded)	0.020	0.023

It is concluded that no additional data for transfer of residues into edible fish products are required.

CA 6.5 Effects of processing

CA 6.5.1 Nature of the residue

Data Point:	KCA 6.5.1/01
Report Author:	[redacted]
Report Year:	2012
Report Title:	Nature of the residues of [1,3-dioxolane-4-14C] spiroxamine and [cyclohexyl-1-14C] spiroxamine in processed commodities - High temperature hydrolysis
Report No:	EnSa-12-0215
Document No:	M-441801-01-1
Guideline(s) followed in study:	OECD Guideline for the Testing of Chemicals 507 Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, adopted 2007-10-16 European Parliament and Council Regulation (EC) No 1107/2009 US EPA OCSPP not applicable
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The hydrolysis of [^{14}C]spiroxamine was investigated in buffered drinking water under conditions of food processing hydrolysis (pasteurisation: 90°C at pH 4 for 20 min; baking, brewing, boiling: 100°C at pH 5 for 60 min; sterilisation: 120°C at pH 6 for 20 min).

The material balances for all tests were in the range of 90.0 to 102.4% and demonstrated that no significant radioactivity and volatile degradation products dissipated from the test system.

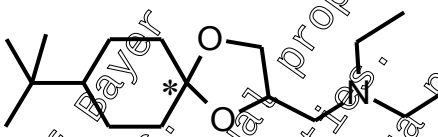
Spiroxamine (≥74.75%) was stable under conditions of food processing hydrolysis representative of sterilisation, pasteurisation, baking, brewing and boiling.

It was observed that hydrolysis rates increased under increasing acidic conditions. The main hydrolysis products were spiroxamine-aminodiol (M28) for the dioxolane label and 4-tert.-butylcyclohexanone (M15, spiroxamine-ketone) for the cyclohexyl label. All other detected compounds were found in very low amounts (≤1.2%). Most of them were impurities of the test compounds and were not further investigated.

I. Materials and methods

A. Materials

[cyclohexyl-^{14}C]spiroxamine



* Signifies position of radiolabel

Specific activity (MBq/mg)	3.60
Lot/Batch No.:	Not reported
Purity:	Radiochemical purity >97%
Storage condition:	Not reported
CAS No.:	118134-30-8

[1,3-dioxolane-^{14}C]spiroxamine

* Signifies position of radiolabel

Specific activity (MBq/mg)	4.09
Lot/Batch No.:	Not reported
Purity:	Radiochemical purity >97%
Storage condition:	Not reported
CAS No.:	118134-30-8

Test systems: Buffers

Ready for use commercial buffer concentrates were used for preparation of the buffered drinking water solutions. The buffer concentrates were delivered in sealed polymer ampoules. The buffer solutions were autoclaved (120°C, 20 min) and checked for pH values. The preparation of the buffers is presented in Table CA 6.5.1/01-1.

These buffer solutions were used for the preparation of samples for high temperature hydrolysis.

Table CA 6.5.1/01-1: Test systems

Buffer	Preparation
Buffer, pH 4	An ampoule of Tritrisol citrate buffer pH 4 was opened and poured into a volumetric flask. The flask was filled with drinking water to a final volume of 500 mL.

Buffer	Preparation
Buffer, pH 5	An ampoule of Tritrisol citrate buffer pH 5 was opened and poured into a volumetric flask. The flask was filled with drinking water to a final volume of 500 mL.
Buffer, pH 6	An ampoule of Fixanal citrate buffer pH 6 was opened and poured into a volumetric flask. The flask was filled with drinking water to a final volume of 500 mL

B. Study design

The study was conducted during the period November 2011 to November 2012 at the BCS-D-Ensa-Testing department, Bayer AG, Monheim, Germany.

Experimental conditions

A stock solution of each test compound was prepared by dissolution of the solid material in acetonitrile. The radioactivity in each stock solution was determined by LSC and amounted to approximately 63 mg/L for the dioxolane label and approximately 72 mg/L for the cyclohexyl label. The purity of the supplied radiolabelled test compounds in their stock solution was assayed by HPLC. The identity was confirmed by spectroscopic methods. The stock solutions were stored at < -18°C in a freezer.

Based on different processing operations, the study was carried out with buffered drinking water at three different pH levels and temperatures: pH 4 / 90°C, pH 5 / 100°C and pH 6 / 120°C (autoclave). The durations of the treatments were 20, 60 and 20 minutes for the three scenarios, respectively. To simulate the conditions of processing, sterilised buffered drinking water was used for the processing hydrolysis experiments. Buffered drinking water was prepared from ready to use commercial buffer concentrates. The pH value of buffers and processed samples was measured after every significant step. All pH measurements were done at room temperature.

An appropriate aliquot of each stock solution was used to give a theoretical concentration of approximately
1 mg/L in the test solution. The aliquot of the stock solution was concentrated to dryness under a gentle air stream and buffered drinking water was added to the remainder.

The pH value of all samples was measured and three aliquots of each test solution were subjected to LSC to determine the actual radioactivity in the test solution before starting the treatment. A further aliquot from each sample was taken for chromatographic analysis of the time zero purity. The test compounds were incubated in buffered drinking water at the following three representative sets of conditions to investigate the effects of hydrolysis as appropriate for the relevant processing operations.

A summary of the test conditions is summarised in Table CA 6.5.1/01-2.

Table CA 6.5.1/01-2: Test conditions

Temperature	Time of sampling	pH	Represents
90°C	0 and 20 minutes	4	Pasteurisation
100°C	0 and 60 minutes	5	Baking/brewing/boiling
120°C	0 and 20 minutes	6	Sterilisation

The tests at 90°C and 100°C were carried out using a water bath. The tests at 120°C were performed in an autoclave. The temperature was recorded in a separate vial filled with 5 mL of buffer. The vials of the test solutions were closed with a septum and crimp top and placed in a water bath or an autoclave. Samples for hydrolysis were weighed before and after hydrolysis to correct for possible losses by evaporation of water.

After termination of the test, the pH values of the samples were measured at room temperature. Three aliquots were again taken from each test solution for the determination of the radioactivity content by LSC.

Aliquots of all samples were analysed by HPLC for the detection of the hydrolysis products. Corresponding compounds in the HPLC analysis were named with the same region ID. Hydrolysis products were identified in an isolated fraction or directly in the test solution pH 4 by LC-MS and LC-MS/MS. The identified compounds were assigned in the other test solutions by comparison of the retention times

Sampling

At time zero (test start) and at test termination, the content of radioactivity was determined in the samples by liquid scintillation counting.

II. Results and discussion

Refer to Tables CA 6.5.1/01-3 and CA 6.5.1/01-4

The main portion of spiroxamine (≥ 75% TRR) was stable under all conditions of food processing. Increasing hydrolysis rates were observed under increasing acidic conditions. Normally, hydrolysis products were detected in the range of approximately 3 to 17%. As an exception, approximately 23.4% of the test compound was hydrolysed during the treatment of [cyclohexyl-1-^{14}C]spiroxamine under conditions of baking, brewing and boiling (pH 5, 100°C, for 60 min). This higher hydrolysis rate was due to the possible variance of the time periods for heating before processing and cooling after processing. Spiroxamine-aminodiol (M28) was the main hydrolysis product of the dioxolane label and was identified in an isolated HPLC fraction of test solution pH 4 by LC-MS and LC-MS/MS. The test compound and the hydrolysis product spiroxamine-ketone (spiroxamine-ketone (M15)) were directly identified in test solution pH 4 of the cyclohexyl label by LC-MS and LC-MS/MS. All other detected compounds were found in very low amounts (≤1.2%). Most of them were impurities of the test compounds and were not further investigated.

Table CA 6.5.1/01-3 Hydrolysis of [1,3-dioxolane-4-^{14}C]spiroxamine under simulated processing conditions

Hydrolysis product	% of radioactivity applied		
	pH 4 /90°C / 20 min	pH 5 /100°C / 60 min	pH 6 /120°C / 20 min
Spiroxamine (parent)	82.04	87.19	93.41
M28 (spiroxamine-aminodiol)	16.53	11.29	4.40
Unknown compound D1 peak[1]	0.27	0.20	n.d.
Unknown compound D3 peak[1]	n.d.	n.d.	0.27
Unknown compound D4 peak[1]	0.60	0.67	0.98
Unknown compound D5 peak[1]	0.55	0.65	0.94
Total	100.00	100.00	100.00

1 – Unknown compounds were also detected in the individual test solution before and after hydrolysis. These compounds were impurities of the test compound.
n.d.- Not detected

Table CA 6.5.1/01-4 Hydrolysis of [cyclohexyl-1-^{14}C]spiroxamine under simulated processing conditions

Hydrolysis product	% of radioactivity applied		
	pH 4 /90°C / 20 min	pH 5 /100°C / 60 min	pH 6 /120°C / 20 min
Spiroxamine (parent)	81.43	74.75	94.28
M15 (spiroxamine-ketone)	16.74	23.35	3.19
Unknown compound C2 peak[1]	0.33	0.48	0.51

Unknown compound C3 peak[1]	0.95	0.79	1.19
Unknown compound C5 peak[1]	0.55	0.63	0.83
Total	100.00	100.00	100.00

1 – Unknown compounds were also detected in the individual test solution before and after hydrolysis. These compounds were impurities of the test compound.

III. Conclusions

The hydrolysis of [^{14}C]spiroxamine was investigated in buffered drinking water under conditions of food processing hydrolysis (pasteurisation: 90°C at pH 4 for 20 min; baking, brewing, boiling: 100°C at pH 5 for 60 min; sterilisation: 120°C at pH 6 for 20 min).

The material balances for all tests were in the range of 90.0 to 102.4% and demonstrated that no significant radioactivity and volatile degradation products dissipated from the test system.

Spiroxamine (≥74.75%) was stable under conditions of food processing hydrolysis representative of sterilisation, pasteurisation, baking, brewing and boiling.

Increasing hydrolysis rates were observed under increasing acidic conditions. The main hydrolysis products were spiroxamine-aminodiol (M28) for the dioxolane label and 4-tert.-butylcyclohexanone (M15, spiroxamine-ketone) for the cyclohexyl label. All other detected compounds were found in very low amounts (≤1.2%). Most of them were impurities of the test compounds and were not further investigated.

The results support the proposed residue definitions. No unknown metabolites are formed under simulated hydrolysis conditions relevant for food processing.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted as supplementary data in the revised EU RAR Annex B7 (2017) IIA 6.5.1/03. The study is considered compliant with OECD Guideline 507 – Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, October 2007.

Data Point:	KCA 6.5.1/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	Hydrolysis of KWG 4168 in sterile aqueous buffer solutions
Report No:	PF4074
Document No:	M-006003-01-1
Guideline(s) followed in study:	EPA Pesticide Assessment Guidelines, Subdivision N - Chemistry: Environmental Fate §161-1 Hydrolysis Studies
Deviations from current test guideline:	Yes. Hydrolysis conditions not in accordance with OECD 507
Previous evaluation:	yes, evaluated and classified DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The hydrolysis of [^{14}C]-spiroxamine was investigated at pH 5, pH 7 and pH 9 for 30 days at 25°C in sterile aqueous buffer solutions. Solutions of aqueous buffers were prepared at the appropriate pH and dosed with [cyclohexyl-^{14}C]-spiroxamine at a nominal concentration of 0.8 mg/L.

Samples were analysed at 1, 3, 7, 14, 20, 24 and 30 days for the main experiment and 1, 7, 14, 22 and 30 days for the supplemental experiment. About 95.7% to 100% of the recovered radioactivity was unchanged parent compound. In the supplemental test at pH 9 very slow degradation of spiroxamine took place but at termination of the experiment approximately 91.0% of recovered radioactivity was due to unchanged spiroxamine. Small amounts of three metabolites were detected (max. 4.5% of applied radioactivity) and their behaviour corresponded to the reference compounds N-oxide (M03), despropyl (M02) and desethyl (M01).

The change in levels of spiroxamine was <10% at each pH test condition and spiroxamine is considered to be stable under sterile buffer conditions at 25°C.

Assessment and conclusion by applicant:

Study acceptable as supplementary data but is superseded by CA 6.5.1/01. Study previously submitted and accepted as supplementary data in the EC RAR Annex B7 (2010) IIA 6.5.1/01. The study is not considered compliant with OECD Guideline 507 – Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, October 2007.

Data Point:	KCA 6.5.1/03
Report Author:	[redacted]
Report Year:	1997
Report Title:	Hydrolysis of KWG 4168 (Spiroxamine, proposed) as a function of pH
Report No:	145000922
Document No:	M-006002-01-1
Guideline(s) followed in study:	OECD guideline for the testing of chemicals No. 111, Hydrolysis Studies
Deviations from current test guideline:	Yes. Hydrolysis conditions not in accordance with OECD 507
Previous evaluation:	yes, evaluated and classified RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The hydrolysis of non-radiolabelled spiroxamine was investigated in aqueous buffer solutions at pH 4, 7, and 9. A preliminary test was conducted with buffer solutions at pH 4, 7, and 9 at 50 °C for 8 days. In the cases of pH 7 and pH 9, no degradation was found and these parts of the test were finished. Spiroxamine was found to be stable at 50°C in buffered solutions at pH 7 and 9 corresponding to a half-life of greater than 1 year at 25 °C.

In the case of pH 4, some degradation was observed and a main test was performed measuring concentrations as a function of time. Additional measurements were performed with incubations at 30°C, and the half-lives calculated for pH 4 were extrapolated to 20°C and 25°C using the Arrhenius equation.

Due to the pH dependence of the solubility of spiroxamine in water, the experiments at pH 7 and pH 9 were performed with solutions containing 10 mg as/L, whereas for pH 4 a concentration of 0.01 mol/L was used. The two isomers of spiroxamine were separated by GLC and identified by MS. This analytical method was calibrated within the study. Checks for sterility and pH constancy were also carried out within each experiment.

The half-lives of isomer A of spiroxamine at pH 4 were approximately 1 year at 25°C and 2 years at 20°C. Isomer B was slightly instable at pH 4, with half-lives of 68 days at 25°C and 120 days at 20°C, calculated by extrapolation from the rates of hydrolysis measured at 30°C and 50°C.

Assessment and conclusion by applicant:

Study acceptable as supplementary data but is superseded by CA 6.5.1/01. Study previously submitted and accepted as supplementary data in the EU: RAR Annex B7 (2010) IIA 6.5.1/02. The study is not considered compliant with OECD Guideline 507 – Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, October 2007.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Not applicable for the representative uses on grapes and small grain cereals.

CA 6.5.3 Magnitude of residues in processed commodities

Data Point:	KCA 6.5.3/01
Report Author:	[redacted]; [redacted]
Report Year:	1995
Report Title:	Determination of residues of KWG 4168 500 EC in/on spring barley in the Federal Republic of Germany
Report No:	RA-2148/94
Document No:	M-010764-01-1
Guideline(s) followed in study:	IVA Guideline, Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

The objective of the study was to determine residue levels of spiroxamine in the raw agricultural commodity (RAC) barley and its processed fractions.

Two processing trials on spring barley were conducted in Germany with spiroxamine (500 g/L, EC) applied at a rate of 1.5 kg a.s./ha and a water application volume of 300 L/ha. Two foliar applications were made at BBCH 32-37 and BBCH 69-71.

Green material specimens were sampled at 0 days after last application (DALA, pre-spray). Mature straw and grain were sampled at 40 to 42 DALA.

The barley grain samples were processed into pearl barley, brewer's malt, malt culms, brewer's grain, brewer's yeast and beer following industrial procedures on a laboratory scale. The processing was performed at University of Hohenheim in the Institute for Food Science Technology and in Detmold in the Bundesanstalt für Getreide- und Fettforschung. A summary of the processes investigated is shown in Table CA 6.5.3/01-1. Residue values are shown in Table CA 6.5.3/01-2.

The grain samples prior to processing were shipped and stored at approximately12°C until processed. The post-processing products were shipped deep frozen with dry ice to the analytical laboratory. Samples were stored at ≤-18°C until analysis.

Table CA 6.5.3/01-1 Summary of barley processes

Process	Samples taken
Distribution on milling	Pearl barley
Preparation of alcoholic beverages (fermentation, malting, brewing and distillation)	Brewer's malt, malt culms, brewer's grain, brewer's yeast, beer

Relevant practices and standardised procedures were applied in the processing phase in order to closely simulate the common industrial processes for barley.

The grain samples were separated into two parts, one for analysis, the other part for processing. The frozen laboratory samples for analysis (green material, grain and straw) were shred with dry ice, transferred into polystyrene boxes and stored at ≤-18°C until analysis.

Processing of pearl barley: the barley grains were peeled in a laboratory peeling machine.

Processing of beer and various fractions: the barley grains were soaked for about 12 hours at approximately 14°C. Afterwards the grains were germed for 7 days at 12°C in a pilot scale malt system. Subsequently the green malt was kiln-dried for approximately 20 hours at about 85°C. The drying time depended on the air moisture and the water content of the malt. From the dried malt, the malt culms were removed by rubbing the malt over wire netting by hand.

The malt culms and the brewer's malt were transferred into polystyrene boxes and stored at ≤-18°C. After a time of rest (7 days) to regenerate the reversible inactivated enzymes in the malt, the malt was crushed in a mill into bruised malt. With the bruised malt, the mash was prepared stepwise by heating the mash up to about 70°C over a period of approximately 135 minutes. After separating the solids (brewer's grain) from the mash, the wort was cooked for one hour. The brewer's grain was transferred into polystyrene boxes and was stored at ≤-18°C. During the wort heating, hops were added after 10 and 50 minutes from the start of cooking.

After cooling the wort to 20°C, yeast was added. The fermentation was conducted at 10°C for one week. After this time the young beer was transferred into bottles and stored for a further 3 weeks at 10°C. After storage the beer was stored at ≤-18°C.

The brewer's yeast was collected from the fermentation vat, transferred into polystyrene boxes and stored at ≤-18°C.

Analytical method

Samples were analysed for total residues of spiroxamine (t-butylcyclohexanone moiety) using the validated method 00312, report reference M-018352-02-1 (**see Doc MCA Section 4**).

The residues were extracted from the plant material by refluxing with a mixture of methanol and hydrochloric acid. Through that, the total residue of spiroxamine, i.e. the active ingredient and all metabolites containing the 4-t-butylcyclohexanone moiety, was hydrolysed yielding 4-t-butylcyclohexanone. After filtration, an aliquot of the extract was taken, diluted with water and applied to a reversed phase disposable SPE column. The 4-t-butylcyclohexanone was extracted from the column with dichloromethane. This extract was further purified on a silica gel disposable SPE column. The 4-t-butylcyclohexanone was determined by gas chromatography with a mass selective detector (GC-MSD) in the single ion monitoring mode.

The limit of quantification (LOQ) of the analytical method was 0.05 mg/kg for the spiroxamine total residues for all commodities, except beer, were the LOQ was 0.02 mg/kg.

II. Results and discussion

No residues above the LOQ were found in any of the fractions derived from the untreated barley samples except for malt culms where residues were reported just above the LOQ at 0.06 mg/kg, this did not impact on the study objectives.

Following trials on barley, total spiroxamine residues in mature grain (RAC) from treated crop were 0.37 or 0.55 mg/kg, 40 to 42 DALA. The total residues in pearl barley ranged from 0.34 to 0.15 mg/kg, in brewer's malt from 0.42 to 0.25 mg/kg, in malt culms from 0.23 to 0.20 mg/kg, in brewer's grain from 0.17 to 0.12 mg/kg. In beer and brewer's yeast no residues above the limit of quantification could be measured (0.02 mg/kg and 0.05 mg/kg, respectively). The results from the processing trials are summarised in Table CA 6.5.3/01-2.

The results of the analyses showed a reduction of the total residue spiroxamine in all processing products. The transfer processing factor for pearl barley is 0.5, for brewer's malt 0.75, for malt culms

0.45, for brewer's grain 0.3, for brewer's yeast 0.1 and for beer 0.05. The transfer factors for all processing products were therefore below 1. There is no concentration of the total residues of spiroxamine during processing to beer. The results are summarised in Table CA 6.5.3./01-3 and Table CA 6.5.3/01-4 for individual processing factors and average processing factors, respectively.

Procedural recoveries for spiroxamine were performed at the LOQ (0.02 mg/kg or 0.05 mg/kg) and 10 x LOQ (0.2 mg/kg or 0.5 mg/kg) for all commodities; and additionally at 100xLOQ (5 mg/kg) for brewer's grain and grain. These are summarised in Table CA 6.5.3/01-5. The overall mean procedural recoveries of spiroxamine were 77 to 93%.

As shown in Table CA 6.5.3/01-6, the samples were stored for a maximum of 107 days (3.6 months) from sampling to analysis. These storage periods are covered by the available storage stability data for high starch content which demonstrate stability of spiroxamine for at least 24 months (refer to Point CA 6.1). Additionally, frozen stability data in beer and spent hops demonstrated that spiroxamine and total spiroxamine were stable under conditions of frozen storage for at least 147 days in both matrices also covering the storage intervals described in this study.

Table CA 6.5.3/01-2 Residue processing trials with spiroxamine 500 g/L EC in barley performed at exaggerated GAP - residue results

Doc. No. Trial Ref Location Crop / Year	Application				Commodity analysed	DAA (days)	Residue (mg/kg) Spiroxamine (total residues)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.5.3/01 (M-010764-01-1) R405175 Germany, 40789 Monheim, Versuchsgut Laacherhof Spring barley (Maresi) 1994	2	1.5 1.5	300 300	BBCH 37 BBCH 71	Barley grain (RAC)	42	0.55
					Pearl barley		0.34
					Brewer's malt		0.42
					Malt culms		0.23
					Beer		<0.02
					Brewer's grain		0.17
					Brewer's yeast		<0.05
CA 6.5.3/01 (M-010764-01-1) R407860 Germany, 51399 Burscheid, Versuchsgut Hofchen Spring barley (Sissy) 1994	2	1.5 1.5	300 300	BBCH 32 BBCH 69	Barley grain (RAC)	40	0.37
					Pearl barley		0.15
					Brewer's malt		0.25
					Malt culms		0.20
					Beer		<0.02
					Brewer's grain		0.12
					Brewer's yeast		<0.05

Table CA 6.5.3/01-3 Residue processing factors for barley

Doc. No. Trial Ref Location Crop Year	Commodity	Spiroxamine residue (total residues) (mg/kg) and process factor	
		Residue	Factor
CA 6.5.3/01 (M-010764-01-1) R405175 Germany, 40789 Monheim, Versuchsgut Laacherhof Spring barley (Maresi) 1994	Barley grain (RAC)	0.55	-
	Pearl barley	0.34	0.6
	Brewer's malt	0.42	0.8
	Malt culms	0.23	0.4
	Beer	<0.02	0.04
	Brewer's grain	0.17	0.3
	Brewer's yeast	0.05	0.1
CA 6.5.3/01 (M-010764-01-1) R407860 Germany, 51399 Burscheid, Versuchsgut Hofchen Spring barley (Sissy) 1994	Barley grain (RAC)	0.37	-
	Pearl barley	0.15	0.4
	Brewer's malt	0.25	0.7
	Malt culms	0.20	0.5
	Beer	<0.02	0.05
	Brewer's grain	0.12	0.3
	Brewer's yeast	0.05	0.1

Table CA 6.5.3/01-4 Average residue processing factors for barley

Commodity	Spiroxamine (total residues)
	Average factor
Pearl barley	0.50
Brewer's malt	0.75
Malt culms	0.45
Beer	0.05
Brewer's grain	0.3
Brewer's yeast	0.1

Table CA 6.5.3/01-5 Procedural recovery data for the determination of spiroxamine residues in barley processed matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.5.3/01	00312	Grain	0.05 0.5 5 overall	2 4 1 7	73, 75 69, 81, 76, 82 80 mean: 77 RSD: 6.2
CA 6.5.3/01	00312	Pearl barley	See grain		
CA 6.5.3/01	00312	Brewer's malt	See grain		
CA 6.5.3/01	00312	Malt culms	0.05 0.5 overall	4 3 7	111, 128, 80, 100 78, 74, 74 mean: 92 RSD: 23.1

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.5.3/01	00312	Beer	0.02 0.2 overall	4 4 8	102, 105, 85, 89 78, 80, 89, 94 mean: 90 RSD: 10.7
CA 6.5.3/01	00312	Brewer's grain	0.05 0.5 5.0 overall	4 3 2 9	85, 78, 76, 81 74, 79, 81 81, 85 mean: 80 RSD: 4.6
CA 6.5.3/01	00312	Brewer's yeast	0.05 0.5 overall	4 3 7	100, 99, 82, 90 97, 96, 88 mean: 93 RSD: 7.1

Table CA 6.5.3/01-6 Storage of barley matrices before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.5.3/01	Grain (RAC)	47 - 107
CA 6.5.3/01	Pearl barley	47 - 107
CA 6.5.3/01	Brewer's malt	47 - 107
CA 6.5.3/01	Malt culms	47 - 107
CA 6.5.3/01	Beer	47 - 107
CA 6.5.3/01	Brewer's grain	47 - 107
CA 6.5.3/01	Brewer's yeast	47 - 107

III. Conclusions

Two EU field trials were conducted to investigate the processing of spring barley into pearl barley, brewer's malt, malt culms, beer, brewer's grain and brewer's yeast. The trials involved two applications of spiroxamine at an exaggerated rate to ensure that residues of appropriate magnitude were generated for investigations on processing.

For trials on barley, total spiroxamine residues in mature grain (RAC) from treated crop were 0.37 or 0.55 mg/kg, 40 to 42 DALA.

The total residues in pearl barley ranged from 0.34 to 0.15 mg/kg, in brewer's malt from 0.42 to 0.25 mg/kg, in malt culms from 0.23 to 0.20 mg/kg, in brewer's grain from 0.17 to 0.12 mg/kg. In beer and brewer's yeast no residues above the limit of quantification could be measured (0.02 mg/kg and 0.05 mg/kg, respectively).

The results of the analyses showed a reduction of the total residue spiroxamine in all processing products. The transfer factor for pearl barley is 0.5, for brewer's malt 0.75, for malt culms 0.45, for brewer's grain 0.2, for brewer's yeast 0.1 and for beer 0.05. The transfer factors for all processing products were therefore below 1. There was no concentration of the total residues of spiroxamine during processing to beer.

Additionally, frozen stability data in beer and spent hops demonstrated that spiroxamine and total spiroxamine were stable under conditions of frozen storage for at least 147 days in both matrices also covering the storage intervals described in this study.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2010) IIA 6.5.4/01. The study is considered compliant with OECD Guideline 508: Magnitude of Pesticide Residues in Processed Commodities (October 2008).

Data Point:	KCA 6.5.3/02
Report Author:	[redacted]; [redacted]
Report Year:	1996
Report Title:	Determination of residues of KWG 4168 500 EC/ 800 EC on grape and tablegrape and in processed commodities
Report No:	RA-3125/95
Document No:	M-010769-01-1
Guideline(s) followed in study:	IVA Guideline: Residue Trials, Parts 1A and 1B
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010); RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

The objective of the study was to determine residue levels of spiroxamine in the raw agricultural commodity (RAC) grapes and its processed fractions.

Ten processing trials on grapes were conducted in Germany, France, Portugal and Greece with spiroxamine (500 g/L or 800 g/L, EC) applied at a total rate of 1.07 to 1.24 kg a.s./ha. Three or four foliar applications were made with the final application at BBCH 77-91. Water volumes ranged from 100 to 1600 L/ha.

Grapes were sampled at 21 to 35 days after last application (DALA) for processing into must, wine and juice. Grapes were sampled at 14 DALA for processing into raisin.

The grape specimens were processed into must, wine at bottling, wine at first taste test (young wine), juice and raisin following industrial procedures on a technical scale (wine and must) and laboratory scale (juice and raisin). The processing for juice and raisins was performed at the Laboratory of Food Processing at Bayer AG. The processing for wine and must was performed at VITI R&D (France), EVN (Portugal) and L. Ziegler (Germany). A summary of the processes investigated is shown in Table CA 6.5.3/02-1. Residue values are show in table CA 6.5.3/02-2.

The samples were stored at -18°C or below after sampling. The samples were shipped to the Laboratory for Trial Design and Sample logistics (Monheim). Must samples were stored in the freezer within 24 hours. Wine samples (wine at bottling) were shipped to Monheim unfrozen and divided into two groups. One group was stored in the freezer (wine at bottling) and the other (wine at first taste test) stored at 12°C for 6 months and then frozen. Samples were stored at -18°C or below until analysis.

Table CA 6.5.3/02-1 Summary of grape processes

Process	Samples taken
Preparation of alcoholic beverages	Must, wine at bottling, wine at first taste test (young wine)
Preparation of fruit juice	Juice
Dehydration	Raisin

Relevant practices and standardised procedures were applied in the processing phase in order to closely simulate the common industrial processes for grapes.

Preparation of must and wine: Red and white grapes were processed to must and wine according to slightly different vinification techniques used in France, Germany, and Portugal. The main steps during vinification are crushing, fermentation, racking, and bottling.

In Germany, the white grapes with stems were crushed and pressed. After clarification, 1 g/L Bentonit sugar was added to get an oechsle value of 88°. An aliquot of must was transferred into a bottle and stored deep frozen (-18°C or below). The remaining must was fermented with yeast for 33 days, after which the first racking took place and 40 mg/L of SO_2 was added to the young wine. After a clarification of 16 days the second racking was carried out and the wine filtered after 6 days.

In Germany, the red grapes were stemmed and mashed. The mash was heated to 80-83°C for 3 minutes, and subsequently cooled to 28°C. The mash was then pressed. After clarification, 1-2 g/L Bentonit sugar was added to get an oechsle value of 89-90°. A sample of must was removed and stored deep frozen (-18°C or below). The remaining must was fermented with yeast for 32-35 days. The first racking took place and 35-40 mg/L SO_2 was added to the young wine. The second racking took place after a clarification of either 14 days (filtration after a further 6 days) or 137 days (filtered immediately), depending on the trial.

In France, red grapes were crushed and recovered in a demijohn with the addition of potassium metabisulphite at 0.06 g/L. Dry active yeast at 0.10 g/L was added and the must fermented until the density of the must was below 1000. For one trial, after 3 days white crystallised sugar was added to the must during fermentation.

The solid part was pressed and added to the free running wine in a demijohn. Malolactic fermentation was carried out in the absence of oxygen and ambient temperature with a seeding of lactic bacteria to speed up the process. During the malolactic fermentation 0.10 g/L of potassium metabisulphite was added. After natural clarification of 14-20 days the wine was decanted and dry gelatine added. The wine was filtered, potassium metabisulphite and metatartaric acid added, then bottled.

In Portugal, the white grapes with fractional stems for mash-fermentation were crushed and 80-100 mg/L solution of sulphur added. The mash was placed into a demijohn and fermented 4-6 days. After 21-42 days the wine was decanted into another demijohn (first racking) and 30 mg/L solution of sulphur added. After 77-79 days the second racking was carried out.

After crushing the white grapes for must fermentation, mash was pressed into must and pomace. An aliquot of must was stored frozen. The remaining must was clarified at a temperature of 4° for a period of 48 hours. After clarification, 100 mg/L of solution of sulphur was added to the must. The must was decanted into a demijohn (first racking) and fermentation started. After 4-7 days or 36-38 days the fermentation was complete. After 17-34 days the wine was racked (second racking) and solution of sulphur was added to the young wine. The last racking was complete after 77-92 days.

Preparation of juice: The processing of grape juice simulated the industrial practice in a laboratory scale. After the de-pectinisation the juice was centrifuged, ultra-filtered and pasteurised at about 87°C. The grape juice was transferred into polystyrene boxes. The analytical samples were stored deep frozen (-18°C or below) until the samples were analysed.

Preparation of raisins: The preparation of raisins simulated the industrial practice in a laboratory scale. The destemmed fruit (grapes) were dried in an oven for about 18 to 19 h at a temperature of 60 to 65

°C. The water content of the raisins after drying ranged from 8 to 12%. After drying, the raisins were washed in standing water under gentle agitation. After washing, the water content of the raisins was about 19%. The raisins were transferred into polystyrene boxes and stored deep frozen (-18°C or below) until the analytical samples were analysed.

Analytical method

Residue analysis for total residues of spiroxamine (aminodiol moiety) in grape and processing samples was performed according to validated method 00407, report reference MR-738/95 (**see Doc MCA Section 4**).

The residues of spiroxamine were extracted by acetone and water and filtered. After filtration, an aliquot was acidified and refluxed and the residues were concentrated to the aqueous remainder. Co-extractives were removed by shaking with dichloromethane and ethyl acetate. The aqueous layer was cleaned by chromatography and residues determined after silylation with GC/MS in single ion mode.

The limit of quantification (LOQ) of the analytical method was 0.05 mg/kg (grapes and raisin) and 0.02 mg/kg (wine, must and juice) for spiroxamine total residues.

II. Results and discussion

No residues at or above the LOQ were found in any of the fractions derived from the untreated grape samples.

The results from the processing trials are summarised in Table CA 6.5.3/02-2. A summary of individual processing factors and average processing factors are summarised in Tables CA 6.5.3./02-3 and CA 6.5.3/02-4, respectively.

Wine: The total residues of spiroxamine in the harvested bunches of grapes at day 21-35 ranged from 0.09-1.0 mg/kg. The values in must ranged from 0.05-0.38 mg/kg, from 0.11-0.39 mg/kg in wine immediately after bottling and from 0.21-0.31 mg/kg in young wine (wine approximately 6 months after bottling). Mean transfer factors can be calculated from the residue levels as follows: 0.49 for must, 0.61 for wine after bottling, and 0.49 for young wine. As all of these transfer factors are <1, no concentration of the total residues of spiroxamine during processing to wine is to be expected.

Juice: At day 35 (designated PHI for wine grapes), total spiroxamine residues of 0.17 mg/kg were measured in bunches of grapes. The total residues of spiroxamine in juice amounted to 0.12 mg/kg. A transfer factor of 0.71 was calculated for juice. No concentration of the total residues of spiroxamine is to be expected during processing to juice, as again the transfer factor is less than 1.

Raisins: At day 14 (designated PHI for table grapes), total spiroxamine residues of 0.30 mg/kg were measured in the destemmed grapes (=berries). In raisins, the residue levels were 1.2 mg/kg. A transfer factor of 4.0 was calculated for raisins. The transfer factor is >1, thus a concentration of the total residues of spiroxamine will occur during processing to raisins

Procedural recoveries for spiroxamine were performed at the LOQ (0.02 mg/kg) for juice, at the LOQ (0.02 mg/kg or 0.05 mg/kg) and 10xLOQ (0.2 mg/kg or 0.5 mg/kg) for must, wine, raisin and grape and additionally at 0.5xLOQ (0.025 mg/kg) and 5xLOQ (0.25) for grape. These are summarised in Table CA 6.5.3/02-5. The overall mean procedural recoveries of spiroxamine were 92 to 99%.

As shown in Table CA 6.5.3/02-6, the samples were stored for a maximum of 511 days (17.0 months) from sampling to analysis. These storage periods are covered by the available storage stability data for high acid content which demonstrate stability of spiroxamine for up to 24 months (refer to Point CA 6.1). Additionally, frozen storage stability data in grapes, raisins and juice demonstrated that total spiroxamine and spiroxamine-aminodiol were stable under conditions of frozen storage (-18°C or below) for at least 18 months in all matrices also covering the storage intervals described in this study.

Table CA 6.5.3/02-2 Residue processing trials with spiroxamine 500 g/L and 800 g/L EC in grapes performed at exaggerated GAP – residue results

Doc. No. Trial Ref Location Crop / Year	Application No.	 g a.s./ha	 L/ha	 Growth stage	Commodity analysed	DAA (days)	Residue (mg/kg) Spiroxamine (total residues)
CA 6.5.3/02 (M-010769-01-1) 401978 France, 30290 Laudun Grape (Grenache) 1994	3	400 400 400	100 100 100	BBCH 71 BBCH 77 BBCH 81	Grape bunch (RAC)	36	0.09
					Wine Must		0.11 0.05
CA 6.5.3/02 (M-010769-01-1) 403113 Portugal, 2580 Alenquer Grape (Seminario) 1994	3	430 400 400	531 500 500	BBCH 85 BBCH 88 BBCH 91	Grape bunch (RAC)	35	0.55
					Must Wine mash-fermentation Wine must- fermentation		0.32 0.30 0.39
CA 6.5.3/02 (M-010769-01-1) 404438 Germany, 67487 St. Martin Red wine grape (Spätburgunder) 1994	3	320 390 520	1000 1200 1600	BBCH 72 BBCH 75 BBCH 81	Grape bunch (RAC)	35	0.54
					Must Wine at bottling Wine at first taste test (young wine)		0.38 0.38 0.34
CA 6.5.3/02 (M-010769-01-1) 404446 Germany, 67489 Kirrweiler White wine grape (Muller-Thurgau) 1994	3	320 390 520	1000 1200 1600	BBCH 72 BBCH 75 BBCH 81	Grape bunch (RAC)	35	0.59
					Must Wine at bottling Wine at first taste test (young wine)		0.22 0.23 0.21
CA 6.5.3/02 (M-010769-01-1) 405604 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1994	4	150 250 300 400	600 1000 1200 1600	BBCH 55 BBCH 72 BBCH 75 BBCH 81	Grape bunch (RAC)	35	0.46
					Must Wine at bottling Wine at first taste test (young wine)		0.26 0.26 0.25

Doc. No. Trial Ref Location Crop / Year	Application No.	g a.s./ha	L/ha	Growth stage	Commodity analysed	DAA (days)	Residue (mg/kg) Spiroxamine (total residues)
CA 6.5.3/02 (M-010769-01-1) 500909 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	3	325 390 520	1000 1200 1600	BBCH 71 BBCH 73 BBCH 81	Grape bunch (RAC) Must Wine at bottling	35	0.59 0.31 0.34
CA 6.5.3/02 (M-010769-01-1) 502413 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	3	320 384 512	1000 1200 1600	BBCH 71 BBCH 73 BBCH 81	Grape bunch (RAC) Must Wine at bottling	35	0.60 0.22 0.28
CA 6.5.3/02 (M-010769-01-1) 500771 France, 30290 Laudun Red grape (Syrah) 1995	3	400 400 400	100 100 100	BBCH 73 BBCH 75 BBCH 77	Grape bunch (RAC) Wine at bottling Juice	35	0.17 0.12 0.12
CA 6.5.3/02 (M-010769-01-1) 500801 Portugal, 2580 Ribafria White grape (Seminario) 1995	3	400 400 400	500 500 500	NR BBCH 81 BBCH 85	Grape bunch (RAC) Wine must	21	1.00 0.26 0.22
CA 6.5.3/02 (M-010769-01-1) 500828 Greece, 20200 Diminio White grape (Sultania) 1995	3	169 450 450	450 600 600	BBCH 77-79 BBCH81 BBCH 81	Grape berries (RAC) Raisin	14	0.30 1.2

Table CA 6.5.3/02-3 Residue processing factors for grapes

Doc. No. Trial Ref Location Crop Year	Commodity	Spiroxamine residue (total residues) (mg/kg) and process factor	
		Residue	Factor
CA 6.5.3/02 (M-010769-01-1) 401978 France, 30290 Laudun Grape (Grenache) 1994	Grape bunch (RAC)	0.09	-
	Wine	0.11	1.22
	Must	0.05	0.56
CA 6.5.3/02 (M-010769-01-1) 403113 Portugal, 2580 Alenquer Grape (Seminario) 1994	Grape bunch (RAC)	0.55	
	Must	0.32	0.58
	Wine mash-fermentation	0.30	0.55
	Wine must- fermentation	0.39	0.71
CA 6.5.3/02 (M-010769-01-1) 404438 Germany, 67487 St. Martin Red wine grape (Spätburgunder) 1994	Grape bunch (RAC)	0.54	-
	Must	0.38	0.70
	Wine at bottling	0.38	0.70
	Wine at first taste test (young wine)	0.31	0.57
CA 6.5.3/02 (M-010769-01-1) 404446 Germany, 67489 Kirrweiler White wine grape (Muller-Thurgau) 1994	Grape bunch (RAC)	0.59	-
	Must	0.22	0.37
	Wine at bottling	0.23	0.39
	Wine at first taste test (young wine)	0.21	0.36
CA 6.5.3/02 (M-010769-01-1) 405604 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1994	Grape bunch (RAC)	0.46	-
	Must	0.26	0.57
	Wine at bottling	0.26	0.57
	Wine at first taste test (young wine)	0.25	0.54
CA 6.5.3/02 (M-010769-01-1) 500909 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	Grape bunch (RAC)	0.59	-
	Must	0.31	0.53
	Wine at bottling	0.34	0.58
CA 6.5.3/02 (M-010769-01-1) 502413 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	Grape bunch (RAC)	0.60	-
	Must	0.22	0.37
	Wine at bottling	0.28	0.47
CA 6.5.3/02 (M-010769-01-1) 500771 France, 30290 Laudun Red grape (Syrah) 1995	Grape bunch (RAC)	0.17	-
	Wine at bottling	0.12	0.71
	Juice	0.12	0.71
CA 6.5.3/02 (M-010769-01-1) 500801 Portugal, 2580 Ribafria White grape (Seminario) 1995	Grape bunch (RAC)	1.00	-
	Wine	0.26	0.26
	must	0.22	0.22
	Grape berries (RAC)	0.30	

Doc. No. Trial Ref Location Crop Year	Commodity	Spiroxamine residue (total residues) (mg/kg) and process factor	
		Residue	Factor
CA 6.5.3/02 (M-010769-01-1) 500828 Greece, 20200 Diminio White grape (Sultania) 1995	Raisin	1.2	4.0

Table CA 6.5.3/02-4 Average residue processing factors for grapes

Commodity	Spiroxamine (total residues)
	Average factor (mean)
Raisin	4.0
Wine after bottling	0.61
Wine at first taste (young wine)	0.49
Must	0.49
Juice	0.71

Table CA 6.5.3/02-5 Procedural recovery data for the determination of spiroxamine residues in grape processed matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.5.3/02	00407	Grapes	0.025 0.05 0.25 0.5 overall	1 26 1 19 47	119 79-116 91 65-104 mean: 92 RSD: 10.0
CA 6.5.3/02	00407	Raisin	0.05 0.5 overall	4 1 5	97, 103, 98, 98 95 mean: 98 RSD: 3.0
CA 6.5.3/02	00407	Wine	0.02 0.2 overall	12 2 14	91-114 102, 102 mean: 99 RSD: 8.0
CA 6.5.3/02	00407	Must	0.02 0.2 overall	7 2 9	89-102 96, 93 mean: 97 RSD: 5.0
CA 6.5.3/02	00407	Juice	0.02 overall	5 5	91, 107, 96, 102, 93 mean: 98 RSD: 7.0

Table CA 6.5.3/02-6 Storage of grapes before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.5.3/02	Grapes (RAC)	155 - 511
CA 6.5.3/02	Raisin	155 - 511

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.5.3/02	Wine	155 - 511
CA 6.5.3/02	Must	155 - 511
CA 6.5.3/02	Juice	155 - 511

III. Conclusions

Ten EU field trials were conducted to investigate the processing of grapes into must, wine at bottling, wine at first taste test (young wine) and raisins. The trials involved three to four applications of spiroxamine at an exaggerated rate to ensure that residues of appropriate magnitude were generated for investigations on processing.

The total residues of spiroxamine in the harvested bunches of grapes for wine at day 21-35 ranged from 0.09-1.0 mg/kg. The values in must ranged from 0.05-0.38 mg/kg, from 0.11-0.39 mg/kg in wine immediately after bottling and from 0.21-0.31 mg/kg in young wine (wine approximately 6 months after bottling). Mean transfer factors can be calculated from the residue levels as follows: 0.49 for must, 0.61 for wine after bottling, and 0.49 for young wine.

At day 35 (designated PHI for wine grapes), total spiroxamine residues 0.17 mg/kg were measured in bunches of grapes for juicing. The total residues of spiroxamine in juice amounted to 0.12 mg/kg. A transfer factor of 0.71 was calculated for juice.

At day 14 (designated PHI for table grapes), total spiroxamine residues of 0.30 mg/kg were measured in the destemmed grapes. In raisin, the residue levels were 1.2 mg/kg. A transfer factor of 4.0 was calculated for raisins.

There was no concentration of the total residues of spiroxamine during processing, except for processing to raisin.

Additionally the storage periods for the study are covered by the available storage stability data for high acid content which demonstrate stability of spiroxamine for up to 24 months and frozen storage stability data in grapes, raisins and juice demonstrated that total spiroxamine and spiroxamine-aminodiol were stable under conditions of frozen storage (-18°C or below) for at least 18 months in all matrices also covering the storage intervals described in this study.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2009) IIA 6.5.4/02. The study is considered compliant with OECD Guideline 508: Magnitude of Pesticide Residues in Processed Commodities (October 2008).

Data Point:	KCA 6.5.3/03
Report Author:	[redacted]
Report Year:	1998
Report Title:	Determination of residues of KWG 4168 500 EC / 800 EC in processed commodities of grape (wine and must)
Report No:	MR-834/98
Document No:	M-010761-01-1
Guideline(s) followed in study:	7035/VI/95 rev.5
Deviations from current test guideline:	Yes RAC commodity values are estimated by using a mean conversion factor from residue trials. Storage period not covered by storage stability data
Previous evaluation:	yes, evaluated and accepted RAR (2009)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

I. Materials and methods

The purpose of the present study was to determine residues of spiroxamine according to method 00506 (parent only) and to compare the results with residues already determined in 1994/95 according to method 00407 (total residue of spiroxamine) in CA 6.5.3/02.

See CA 6.5.3/02 for the study design.

Analytical method

Residue analysis for spiroxamine (parent only) in grape and processing samples was performed according to validated method 00506, report reference MR-816/96 (**see MCA Section 4**).

The residues of spiroxamine (parent only) were extracted by acetone and water. After filtration, an aliquot was concentrated to the aqueous remainder. The extracts were cleaned up by solid phase extraction (SPE) on an RP-18 column and residues determined by GC/MS in single ion mode.

The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for must/wine.

II. Results and discussion

No residues at or above the LOQ were found in any of the fractions derived from the untreated grape samples.

The results from the processing trials are summarised in Table CA 6.5.3/03-1. A summary of average conversion factors of spiroxamine (total residues) to spiroxamine (parent only) are presented in Table CA 6.5.3/03-2. A summary of individual processing factors and average processing factors are presented in Tables CA 6.5.3/02-3 and CA 6.5.3/02-4, respectively.

The residues for must were <0.01 to 0.04 mg/kg. The residues for wine at bottling and wine at first taste test were in the range of <0.01 to 0.04 mg/kg.

The conversion factor was in the range of 0.05 to 0.17 (mean: 0.09) for must and 0.02 to 0.18 (mean: 0.07) for wine for total spiroxamine residues to parent only spiroxamine residues.

There was no concentration of residues of spiroxamine (parent only) during processing as mean transfer factors were <1 in must, wine after bottling and young wine.

Procedural recoveries for spiroxamine (parent only) were performed at the LOQ (0.01 mg/kg), 2 x LOQ (0.02 mg/kg) and 10 x LOQ (0.1 mg/kg) for must/wine. These are summarised in Table CA 6.5.3/03-5.The overall mean procedural recoveries of spiroxamine (parent only) were 99%

As shown in Table CA 6.5.3/03-6, the samples were stored for a maximum of 1517 days (50.6 months) from sampling to analysis. Although these storage periods for processed fractions are not covered by the available storage stability data for high acid content matrices including the RAC, which demonstrate frozen stability of spiroxamine for up to 24 months, there is no indication of frozen storage stability concerns in any other matrix. Therefore, this is not anticipated to have any impact on the reliability of the transfer factors in must and wine.

Table CA 6.5.3/03-1 Residue processing trials with spiroxamine 500 g/L and 800 g/L EC in grapes performed at exaggerated GAP – residue results

Doc. No. Trial Ref Location Crop / Year	Application No.	g a.s./ha	L/ha	Growth stage	Commodity analysed	DAA (days)	Residue (mg/kg) Spiroxamine (total residues)[1]	Spiroxamine (parent only)[2]	Conversion factor
CA 6.5.3/02 (M-010769-01-1) 401978 France, 30290 Laudun Grape (Grenache) 1994	3	400	100	BBCH 71	Grape bunch (RAC)	36	0.09	-	-
		400	100	BBCH 77	Wine		0.11	0.020	0.18
		400	100	BBCH 81	Must		0.05	0.005	0.10
CA 6.5.3/02 (M-010769-01-1) 403113 Portugal, 2580 Alenquer Grape (Seminario) 1994	3	430	531	BBCH 85	Grape bunch (RAC)	35	0.55	-	-
		400	500	BBCH 88	Must		0.32	0.043	0.13
		400	500	BBCH 91	Wine mash-fermentation		0.30	0.040	0.13
					Wine must- fermentation		0.39	0.036	0.09
CA 6.5.3/02 (M-010769-01-1) 404438 Germany, 67487 St. Martin Red wine grape (Spätburgunder) 1994	3	320	1000	BBCH 72	Grape bunch (RAC)	35,	0.54	-	-
		390	1200	BBCH 75	Must		0.38	0.040	0.11
		520	1600	BBCH 81	Wine at bottling		0.38	0.022	0.06
					Wine at first taste test (young wine)		0.31	0.013	0.04
CA 6.5.3/02 (M-010769-01-1) 404446 Germany, 67489 Kirrweiler White wine grape (Muller-Thurgau) 1994	3	320	1000	BBCH 72	Grape bunch (RAC)	35	0.59	-	-
		390	1200	BBCH 75	Must		0.22	0.012	0.05
		520	1600	BBCH 81	Wine at bottling		0.23	0.006	0.03
					Wine at first taste test (young wine)		0.21	0.004	0.02
CA 6.5.3/02 (M-010769-01-1) 405604 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1994	4	150	600	BBCH 55	Grape bunch (RAC)	35	0.46	-	-
		250	1000	BBCH 72	Must		0.26	0.025	0.10
		300	1200	BBCH 75	Wine at bottling		0.26	0.016	0.06
		400	1600	BBCH 81	Wine at first taste test (young wine)		0.25	0.008	0.03

Doc. No. Trial Ref Location Crop / Year	Application No.	g a.s./ha	L/ha	Growth stage	Commodity analysed	DAA (days)	Residue (mg/kg) Spiroxamine (total residues)[1]	Spiroxamine (parent only)	Conversion factor
CA 6.5.3/02 (M-010769-01-1) 500909 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	3	325 390 520	1000 1200 1600	BBCH 71 BBCH 73 BBCH 81	Grape bunch (RAC) Must Wine at bottling	35	0.59 0.31 0.34	- 0.021 0.017	- 0.07 0.05
CA 6.5.3/02 (M-010769-01-1) 502413 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	3	320 384 512	1000 1200 1600	BBCH 71 BBCH 73 BBCH 81	Grape bunch (RAC) Must Wine at bottling	35	0.60 0.22 0.28	- <0.010 0.020	- - 0.07
CA 6.5.3/02 (M-010769-01-1) 500771 France, 30290 Laudun Red grape (Syrah) 1995	3	400 400 400	100 100 100	BBCH 73 BBCH 75 BBCH 77	Grape bunch (RAC) Wine at bottling	35	0.17 0.12	- 0.011	- 0.09
CA 6.5.3/02 (M-010769-01-1) 500801 Portugal, 2580 Ribafria White grape (Seminario) 1995	3	400 400 400	500 500 500	NR BBCH 81 BBCH 85	Grape bunch (RAC) Wine must	21	1.00 0.26 0.22	- 0.013 0.037	- 0.05 0.17

1 – data from study CA 6.5.3/02

Table CA 6.5.3/03-2 Average conversion factors for total spiroxamine to parent

Commodity analysed	Conversion factor	Average conversion factor
Wine	0.02, 0.03, 0.03, 0.04, 0.05, 0.05, 0.06, 0.06, 0.07, 0.09, 0.09, 0.13, 0.18	0.07
Must	0.05, 0.07, 0.10, 0.10, 0.11, 0.13, 0.17	0.09

Table CA 6.5.3/03-3 Residue processing factors for grapes

Doc. No. Trial Ref Location Crop Year	Commodity	Spiroxamine residue (parent only) (mg/kg) and process factor	
		Residue	Factor
CA 6.5.3/02[1] (M-010769-01-1) 401978 France, 30290 Laudun Grape (Grenache) 1994	Grape bunch (RAC)[2]	0.04	-
	Wine	0.020	<1 (0.5)
	Must	0.005	<1 (0.13)
CA 6.5.3/02[1] (M-010769-01-1) 403113 Portugal, 2580 Alenquer Grape (Seminario) 1994	Grape bunch (RAC)[2]	0.25	-
	Must	0.043	<1 (0.17)
	Wine mash-fermentation	0.040	<1 (0.16)
	Wine must- fermentation	0.036	<1 (0.14)
CA 6.5.3/02[1] (M-010769-01-1) 404438 Germany, 67487 St. Martin Red wine grape (Spätburgunder) 1994	Grape bunch (RAC)[2]	0.25	-
	Must	0.040	<1 (0.16)
	Wine at bottling	0.022	<1 (0.09)
	Wine at first taste test (young wine)	0.013	<1 (0.52)
CA 6.5.3/02[1] (M-010769-01-1) 404446 Germany, 67489 Kirrweiler White wine grape (Müller-Thurgau) 1994	Grape bunch (RAC)[2]	0.27	-
	Must	0.012	<1 (0.07)
	Wine at bottling	0.006	<1 (0.02)
	Wine at first taste test (young wine)	0.004	<1 (0.01)
CA 6.5.3/02[1] (M-010769-01-1) 405604 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1994	Grape bunch (RAC)[2]	0.21	-
	Must	0.025	<1 (0.12)
	Wine at bottling	0.016	<1 (0.08)
	Wine at first taste test (young wine)	0.008	<1 (0.04)
CA 6.5.3/02[1] (M-010769-01-1) 500909 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	Grape bunch (RAC)[2]	0.27	-
	Must	0.021	<1 (0.08)
	Wine at bottling	0.017	<1 (0.06)
CA 6.5.3/02[1] (M-010769-01-1) 502413 Germany, 67487 Maikammer Red wine grape (Spätburgunder) 1995	Grape bunch (RAC)[2]	0.28	-
	Must	<0.010	<1 (<0.04)
	Wine at bottling	0.020	<1 (0.07)

Doc. No. Trial Ref Location Crop Year	Commodity	Spiroxamine residue (parent only) (mg/kg) and process factor	
		Residue	Factor[3]
CA 6.5.3/02[1] (M-010769-01-1) 500771 France, 30290 Laudun Red grape (Syrah) 1995	Grape bunch (RAC)[2]	0.08	-
	Wine at bottling	0.011	<1 (0.14)
CA 6.5.3/02[1] (M-010769-01-1) 500801 Portugal, 2580 Ribafria White grape (Seminario) 1995	Grape bunch (RAC)[2]	0.46	-
	Wine must	0.013 0.037	<1 (0.03) <1 (0.08)

1 – Trial data from study CA 6.5.3/02 and reanalysed according to method 00506
2 – Determined from total spiroxamine residue values in residue trials using a factor of x0.46
3 – All factors <1, actual value shown in parenthesis

Table CA 6.5.3/03-4 Average residue processing factors for grapes

Commodity	Spiroxamine (parent only)
	Average factor (mean)
Wine after bottling	<1
Wine at first taste (young wine)	<1
Must	<1

Table CA 6.5.3/03-5 Procedural recovery data for the determination of spiroxamine residues in grape processed matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.5.3/03	00506	Must/wine	0.01 0.02 0.10 overall	3 1 5 9	119, 112, 87 88 99, 108, 101, 64, 115 mean: 99 RSD: 17.8

Table CA 6.5.3/03-6 Storage of grapes before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.5.3/03	Wine	1493 - 1517
CA 6.5.3/03	Must Juice	1493 - 1517

III. Conclusions

Ten EU field trials were conducted to investigate the processing of grapes into must, wine at bottling, and wine at first taste test (young wine). The trials involved three to four applications of spiroxamine at an exaggerated rate to ensure that residues of appropriate magnitude were generated for investigations on processing.

The purpose of the present study was to determine residues of spiroxamine according to method 00506 (parent only) and to compare the results with residues already determined in CA 6.5.3/02 according to method 00407 (total residue of spiroxamine).

The residue data present a fairly consistent picture of a very low residue level. The residues for must were <0.01 to 0.04 mg/kg for parent only, and 0.05 to 0.38 mg/kg for spiroxamine total residue. The residues for wine at bottling and wine at first taste test were in the range of <0.01 to 0.04 mg/kg for parent only, and 0.11 to 0.39 mg/kg for spiroxamine total residue.

The conversion factor was in the range of 0.05 to 0.17 (mean: 0.09) for must and 0.02 to 0.18 (mean: 0.07) for wine.

Mean transfer factors can be calculated from the residue levels as <1 for must, wine after bottling, and young wine. There was no concentration of residues of spiroxamine (parent only) during processing.

Assessment and conclusion by applicant:

Study acceptable as supplementary data but is superseded by CA 6.5.3/02. Study previously submitted and accepted in the EU: RAR Annex B7 (2010) IIA 6.5.4/03. The study is not considered compliant with OECD Guideline 508: Magnitude of Pesticide Residues in Processed Commodities (October 2008) as RAC commodity values are estimated by using a mean conversion factor from residue trials.

The following new study is currently on-going and a report will be submitted at an agreed submission top-up time-point:

Dossier node	Draft title	Study ID	Planned submission
KCA 6.5.3	Determination of residues of spiroxamine and its metabolites after one application of JAU 6476 & KWG 4168 EC in wheat (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020, including processing from 2 sites	S20-02175	Final report Not before May 2021

CA 6.6 Residues in rotational crops

CA 6.6.1 Metabolism in rotational crops

Data Point:	KCA 6.6.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	[Cyclohexyl-1-14C] KWG 4168 residues in following crops
Report No:	PF4043
Document No:	M-006096-01-1
Guideline(s) followed in study:	US EPA §165-1 Confined accumulation studies on rotational crops, 1982
Deviations from current test guideline:	Yes. OECD 502 guideline (January 2007) requires three plant-back intervals for succeeding crops. The longest interval of 270 to 365 days to represent crops sown the following year was not conducted in this study.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A single application of ^{14}C-spiroxamine (labelled in the cyclohexyl-1-^{14}C position) was made to bare sandy loam soil in 1 m² containers at a target rate of 1.58 kg a.s./ha as an emulsifiable concentrate (EC 500) formulation. This rate is 2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Crops were planted at 30 days after application (DAA) – first rotation , and 161 DAA – second rotation . Each crop was harvested at maturity.

Seeds of wheat (*Triticum aestivum,* Kolibri), Swiss chard (*Beta vulgaris,* Lukullus) and turnips (*Brassica rapa,* Rapa) were sown into the soil at 30 and 161 DAA and crops grown to maturity. Crop samples collected were wheat straw, wheat grain, mature Swiss chard, turnip root and turnip foliage.

Soil samples were taken at 0, 30 and 161 days after application.

The total radioactive residues (TRRs) in Swiss chard, turnip roots and leaves were determined by summation of the radioactivity in organic solvent extracts and that in the residual post-extraction solids. TRRs in wheat grain and straw were determined by combustion of the solid sample. TRRs in all samples (except wheat straw) decreased between the first plant-back interval (30 days) and the second (161 days).

The TRR in wheat grain was 0.06 mg/kg (measured as mg spiroxamine equivalents per kg) at the 30 day interval and 0.05 mg/kg at the 161 day interval. Corresponding levels in wheat straw were 1.07 mg/kg and 1.27 mg/kg (30 day and 161 day intervals respectively). In Swiss chard, TRRs were 0.15 mg/kg at the 30 day interval declining to 0.07 mg/kg at the 161 day interval. Residues in turnip roots were low at both plant-back intervals.

Parent spiroxamine was the main component of the residue in Swiss chard, and turnips harvested from the 30 day plant-back interval, accounting for 29% TRR (0.13 mg/kg) in turnip leaves, 45.8% TRR (0.01 mg/kg in turnip roots) and 40.8% TRR (0.06 mg/kg) in Swiss chard. In wheat straw, the main metabolite was the spiroxamine-N-oxide (M03), accounting for 12.7% TRR (0.13 mg/kg). Desalkylated metabolites (M01, M02) were also seen in wheat straw and parent spiroxamine accounted for 6.8% TRR (0.08 mg/kg).

Parent spiroxamine was also seen in rotated crops at the 161 DAA plant-back interval, although at lower levels, accounting for between 4% TRR (0.05 mg/kg) in wheat straw and 27% TRR (<0.01 mg/kg) in turnip roots. In wheat straw, the main metabolite was the spiroxamine-N-oxide (M03), accounting for 12.1% TRR (0.15 mg/kg). In Swiss chard, the major metabolites were spiroxamine-despropyl (M02) and the spiroxamine-N-oxide (M03), each accounting for 14.2% TRR (0.01 mg/kg). In turnip leaves, the main metabolite was spiroxamine-despropyl (M02) comprising 16.7% TRR (0.02 mg/kg). Residues in turnip roots at this interval were very low, however, trace amounts of spiroxamine-desethyl, spiroxamine-despropyl and spiroxamine-N-oxide were tentatively detected.

The organic and aqueous phases of wheat straw, Swiss chard and turnip leaves were further characterised by hydrolysis with hydrochloric acid and the majority of the TRR in the organic and aqueous extract was converted to spiroxamine-ketone (M15) (25.5 – 46.5% TRR), the spiroxamine-hydroxy ketone (m16) (8.8 – 37.2% TRR) and spiroxamine-diol (M14) (3.5 - 13.2% TRR).

The nature of the residue in rotated crops when spiroxamine is applied to bare soil was essentially the same as found in the primary wheat metabolism study. Desalkylation of the parent compound occurred forming the desethyl and despropyl spiroxamine moieties. Additionally, oxidation of spiroxamine in the tertiary amine group forming the spiroxamine-N-oxide and to a minor extent in the tert.-butyl group. Hydrolysis occurred (ketone formation) which was followed by reduction of the keto group to the hydroxyl group. Conjugation at both hydroxyl positions was also observed and to a minor extent at the secondary amine group (N-formyl formation).

I. Materials and methods

A. Test material

[cyclohexyl-1-^{14}C]-spiroxamine

* Denotes radiolabel position

Specific activity (µCi/mg)	33.3 (1.23 MBq/mg)
Lot/Batch No.:	ECW 9496-A
Radiochemical purity:	99%
CAS No.:	118134-30-8

B. Study design

Soil

Soil characterisation details are presented below in Table CA 6.6.1/01-1

Table CA 6.6.1/01-1 Soil classification and physico-chemical properties

Soil Type (USDA)	pH (H_2O)	OM %	Sand %	Silt %	Clay %	Moisture holding capacity mL / 100g)	CEC mval / 100 g
Sandy loam	6.5	1.68	58.2	31.0	10.8	Not stated	10

Test system

The test systems were planted in a container (1m^2) containing a sandy loam soil (described above) and located in a greenhouse at the Institute for Metabolism Research and Residue Analysis in the crop protection centre of Bayer AG.

Crops grown following application of the test item was wheat (*Triticum aestivum*, variety 'Kolibri'), Swiss chard (*Beta vulgaris* spp, variety 'Lukullus') and turnip (*Brassica rapa*, variety 'Rapa'). These crops represent a cereal crop, a leafy crop and a root crop. The daytime temperature of the greenhouse was kept at 20°C and the night time temperature (20.00 – 06.00 h) at 16°C. Additional lighting was automatically switched on when the natural daylight intensity fell below 50 kLx. Water was automatically applied to the soil in the containers to ensure optimum conditions for growth. Plant protection and fertilisation measures were carried out as required and documented. A single container was used for this study.

For each plant back interval, the crops shared the container each taking up one third of the available area.

Plants were irrigated and fertilised as required for good crop growth.

The experimental work of the study started on December 7, 1992 and was completed on October 18, 1994.

The study was conducted at BAYER AG, Agrochemicals Division Development Department, Institute for Metabolism Research and Residue Analysis, Germany.

Experimental conditions

The uptake and metabolism of [^{14}C]-spiroxamine in rotational crops of wheat, Swiss chard and turnip was studied following a single application to bare-soil. The rate employed in this study was higher than the proposed seasonal critical GAP for the use of spiroxamine on field crops but is considered representative and appropriate for the objectives.

The dose calculations were based on an application rate of 1.58 kg a.s./ha and a plot size of 1 m^2 (i.e. 158 mg a.s./m^2). This rate is 2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use.

Preparation of radiolabelled dose

The application was conducted by spraying to bare soil in December 1992. The radiolabelled formulated active ingredient was dissolved in 80 mL of water and sprayed onto the soil by means of a modified plot sprayer. To avoid spray-drift, the plant container was enclosed by a box made of plastic sheet which was removed some hours after application. Any radioactivity adhering to the plastic sheet was rinsed off with methanol and quantified by liquid scintillation counting (LSC).

After completing the preparation of the formulated application solution an aliquot was taken for pre-application purity determination. This was repeated after application for post-application purity determination. No degradation of the radiolabelled treatment solution was observed.

The amount of test substance applied was calculated based on the total dpm dispensed, corrected for the aliquots taken for purity check and LSC and any residual activity remaining in the equipment used, including the spray bottles and plastic sheeting. The stability of the test substance was established by thin layer chromatography (TLC).

Planting and sampling of following crops

30 DAA: (first rotation)

On January 6 1993 (30 days after application, DAA), the treated soil was tilled by hand to a depth of 15 cm. The rotational crops Swiss chard, turnips and wheat were each sown in one third of the soil area. On March 22 1993, 105 DAA, Swiss chard and turnips were harvested. Wheat was harvested on May 17 1993 (161 DAA). The roots of the Swiss chard were not harvested and remained in the soil.

161 DAA: (second rotation)

Following the harvest of the first succeeding crops, the soil was mixed as per the first rotation and the second set of following crops was planted as before. Each crop was however planted in a different third of the container. The harvest date of the Swiss chard and turnips was July 21 1993 (226 DAA) and wheat, August 25 1993 (261 DAA)

After harvest, the mature wheat was separated into grain and straw. The glumes and the remainder of the ears were added to the straw fraction. The mature turnips were separated into roots and leaves. The Swiss chard was harvested as whole leaves.

Sampling of soil

Soil cores (15 cm depth x 2.5 cm diameter) were taken using a soil corer.

Immediately after application (day 0) and prior to planting of the first set of succeeding crops (30 DAA), 10 soil cores were taken randomly at each date. Prior to the planting of the second set of succeeding crops (161 DAA), 5 further soil cores were taken at random.

The soil cores from the 30 DAA sampling were used to determine the distribution of radioactivity in the soil and to characterise metabolites. The cores from the 161 DAA sampling were used to determine total residues and characterise metabolites.

Sample preparation and extraction

Plants

The plant fractions were weighed, homogenised in liquid nitrogen and stored frozen at -20°C. The total radioactive residues (TRRs) of the plant fractions (except wheat straw and grain) were determined by summation of the radioactivity in the methanol/water extracts and The post-extraction solids (PES). TRRs in wheat straw and grain were determined by combustion. Residues were expressed as mg/kg spiroxamine equivalents.

Aliquots of homogenised plant fractions were then extracted twice in methanol and once in methanol/water (1:1 v/v) using a blender. After each extraction, the solids were removed by filtration and the extracts combined. The extracts were then reduced to the aqueous remainder and partitioned with dichloromethane (3 x 100 mL). The combined DCM phases were concentrated to dryness and the residue re-dissolved in methanol (organic phase). The remaining aqueous phase was concentrated and purified using a resin column (XAD 4, Merck) which had been pre-conditioned. The aqueous phase was added to the column and then the column eluted with 250 – 700 mL distilled water followed by 250 – 500 mL methanol. The methanol eluate was then concentrated to a small volume (aqueous phase). No significant loss of radioactivity was noted using the XAD 4 column.

The solids (containing PES) from wheat straw (1st interval) were exhaustively extracted by hydrolysis with hydrochloric acid. To an aliquot of the solids (8.5 g), 200 mL HCl and 200 mL toluene were added and the mixture refluxed for 1 hour. After cooling, filtration under vacuum the two phases were separated and the aqueous phase was extracted twice with toluene (2 x 200 mL). The combined toluene phases were then reduced to a small volume.

Isolation and derivatisation of glucose from wheat grain (30 DAA samples)

The starch contained in the wheat grain (75g) was hydrolysed to glucose by adding water (300 mL) and 25% hydrochloric acid (50 mL) to the grain and the resultant suspension stirred under reflux (2 hr). The suspension was cooled and filtered and the filtrate (400 mL) added to an anion exchange column (Lewatit MP 62). The column was then eluted with water (1260 mL) and the eluate concentrated to a small syrup-like residue.

The glucosazone derivative was obtained by adding 2-ethoxyethanol (400 mL) and acetic acid (64 mL) to the dried residue and heating the solution (80°C). Phenyl hydrazine (120 mL) was then added dropwise. The solution was stirred form 1 hour (80°C) before water (1.5 L) was added. After cooling, the precipitated glucosazone was filtered and dried (yield 47.5 g). The radioactivity balance for the isolation and derivatisation of glucose from wheat grain was calculated and the yield was determined to be 66%. This value was used to calculate the actual amount of radioactivity incorporated into glucose.

The glucosazone was recrystallised by dissolving in boiling dimethylformamide/ethanol (2:1; v/v) and then adding water. The glucosazone recrystallised after cooling to room temperature. This process was repeated three times

Acid hydrolyses of plant extracts

Hydrochloric acid (1N, 500 µL) was added to an aliquot (200 µL) of either the organic or aqueous plant extracts. The solution was heated under reflux for 1 hour. After cooling (to approximately 5°C), 2 mL ethyl acetate was added. After shaking, the ethyl acetate phase was removed and the aqueous phase partitioned with ethyl acetate twice more (2 x 2 mL). The ethyl acetate phases were combined and concentrated to a small volume under nitrogen. No significant loss of radioactivity was observed during this process.

Soil

The TRRs in soil were determined by combustion of air-dried, ground soil and are expressed as mg/kg spiroxamine equivalents.

An aliquot of the soil sample (100 g) was extracted with acetonitrile (80 mL) at room temperature by shaking for 30 minutes. The suspension was centrifuged and the supernatant decanted. This was repeated twice. The acetonitrile phases were combined, concentrated and re-suspended with methanol. Residual solids were extracted with hot acetonitrile. 85g solids were refluxed with 200 mL acetonitrile for one hour. After cooling, the suspension was filtered under suction and the solids rinsed with acetonitrile. The acetonitrile phases were combined, concentrated and re-suspended with methanol.

The radioactivity in the organic phases was determined by liquid scintillation counting (LSC) and in the solids by combustion.

Combustion analysis:

Solid samples were combusted using a Harvey Ox 500 Tissue Oxidiser (Zinsser-Analytic). The resultant $^{14}CO_2$ was absorbed in scintillation cocktail (Oxysolve C-400, Zinsser) and radioactivity measured by LSC.

Liquid scintillation counting (LSC):

Liquid samples were counted in a liquid scintillation counter (Beckman LS 6000 LL, LS 6500 or LKB Rack Beta 1219) using commercial scintillation cocktails (Instant Scint-Gel, Quick-Safe A). Counts per minute (cpm) were converted to dpm using a set of commercially prepared quenched standards.

Thin layer chromatography (TLC):

The radioactive solutions were analysed by one and two dimensional TLC using silica gel plates or aluminium sheets (60F254, Merck), which were eluted with the following solvent systems:

System 1	Acetonitrile/water/ammonia (25%)	320:18:2 v/v/v
System 2	Acetonitrile/water/ammonia (25%)	80:18:2 v/v/v
System 3	Chloroform/methanol/ammonia (25%)	65:28:8 v/v/v
System 4	Dichloromethane/methanol/2-butanone	6:2:1 v/v/v
System 5	Dichloromethane/ethyl acetate/1-butanol	9:5:1 v/v/v

Radioactive components were detected using a Fuji Imaging Analyser with a software peak integration package. The reference materials used in co-chromatography were detected using iodine vapour or by spraying with vanillin/sulphuric acid and heating the plate to 120°C.

II. Results and discussion

Total radioactive residues (TRR)

TRR data for rotational crop plant matrices are summarised in Table CA 6.6.1/01-2. Summaries of extractable radioactivity in plant matrices are presented in Table CA 6.6.1/01-3 and Table CA 6.6.1/01-4. TRR data for air-dried soil samples are summarised in Table CA 6.6.1/01-5 and distribution of radioactive residues in extracted soil is shown in Table CA 6.6.1/01-6.

The TRRs in Swiss chard, turnip roots and leaves were determined by the summation of radioactivity in the methanol/water extract and The post-extraction solids (PES). TRRs in wheat straw and grain were determined by combustion. Residues are expressed as mg/kg parent spiroxamine equivalents. The TRR in the soil samples was determined by combustion of air-dried, ground soil samples and are expressed as mg/kg parent compound equivalents.

30 DAA samples (first rotation)

Wheat

The total radioactive residue (TRR) in wheat planted 30 days after application to bare soil was 1.07 mg/kg in straw and 0.06 mg/kg in grain. In straw, 36.7% TRR (0.39 mg/kg) was extractable with organic solvent, with a further 9.5% TRR (0.10 mg/kg) associated with the aqueous phase. The post-extraction solid (PES) accounted for 53.8% TRR (0.58 mg/kg). In grain, all of the available radioactivity (0.06 mg/kg) was associated with the PES.

Swiss chard

The total radioactive residue (TRR) in Swiss chard planted 30 days after application to bare soil was 0.15 mg/kg. Organo-extractable radioactivity accounted for 66% TRR (0.10 mg/kg), with a further 20.2% TRR (0.03 mg/kg) associated with the aqueous phase. The post-extraction solid (PES) accounted for 13.8% TRR (0.02 mg/kg).

Turnips

The total radioactive residue (TRR) in turnips planted 30 days after application to bare soil was 0.46 mg/kg in leaves and 0.04 mg/kg in roots. In leaves, 66.3% TRR (0.30 mg/kg) was extractable with organic solvent, with a further 24.0% TRR (0.11 mg/kg) associated with the aqueous phase. The post-extraction solid (PES) accounted for 9.7% TRR (0.05 mg/kg). In the roots, 55.2% TRR (0.02 mg/kg) was extractable with organic solvent, with a further 29.2% TRR (0.01 mg/kg) associated with the aqueous phase. The PES accounted for 15.6% TRR (<0.01 mg/kg).

161 DAA samples (second rotation)

Wheat

The total radioactive residue (TRR) in wheat planted 161 days after application to bare soil was 1.27 mg/kg in straw and 0.05 mg/kg in grain. In straw, 32.6% TRR (0.42 mg/kg) was extractable with organic solvent, with a further 17.5% TRR (0.22 mg/kg) associated with the aqueous phase. The post-extraction solid (PES) accounted for 49.9% TRR (0.63 mg/kg). In grain, a total of 3.9% and 3.3% TRR (both <0.01 mg/kg) was extractable by organic and aqueous solvent. The PES accounted for 92.8% TRR (0.05 mg/kg).

Swiss chard

The total radioactive residue (TRR) in Swiss chard planted 161 days after application to bare soil was 0.07 mg/kg. Organo-extractable radioactivity accounted for 49.3% TRR (0.07 mg/kg), with a further

31.5% TRR (0.02 mg/kg) associated with the aqueous phase. The post-extraction solid (PES) accounted for 19.2% TRR (0.01 mg/kg).

Turnips

The total radioactive residue (TRR) in turnips planted 161 days after application to bare soil was 0.13 mg/kg in leaves and 0.02 mg/kg in roots. In leaves, 49.2% TRR (0.07 mg/kg) was extractable with organic solvent, with a further 33.5% TRR (0.04 mg/kg) associated with the aqueous phase. The post-extraction solid (PES) accounted for 17.3% TRR (0.02 mg/kg). In the roots, 37.2% TRR (<0.01 mg/kg) was extractable with organic solvent, with a further 28.2% TRR (<0.01 mg/kg) associated with the aqueous phase. The PES accounted for 33.9% TRR (<0.01 mg/kg).

Total radioactive residues in soil

The residues in soil samples taken randomly from the total area declined from 1.21 mg/kg at day 0 (immediately after application) to 0.53 mg/kg after 30 days and then stayed constant until the second planting interval at 161 days, with a TRR of 0.67 mg/kg.

The day 0 soil samples was not extracted, as it was assumed that this comprised 100% parent compound. In 30 day soil, 63.2% TRR was extractable by organic solvent extraction and extraction under reflux. Under the same conditions, 55.6% TRR was extractable at the 161 day sample interval.

Table CA 6.6.1/01-2 Total radioactive residues (TRR) in rotational crops sown 30 and 161 days after application with [cyclohexyl-1-^{14}C]spiroxamine

	Wheat		Swiss chard	Turnip	
	Straw[1] (mg/kg)	Grain[1] (mg/kg)	Leaves[2] (mg/kg)	Leaves[2] (mg/kg)	Roots[2] (mg/kg)
30 DAA	1.07	0.06	0.15	0.46	0.04
161 DAA	1.27	0.05	0.07	0.13	0.02

1 - TRR values were determined by combustion analysis.
2 - TRR values were determined by summation of radioactivity in extracts and PES

Table CA 6.6.1/01-3 Distribution of TRR in extracts and solids from rotational crops sown 30 days after application with [cyclohexyl-1-^{14}C]spiroxamine

Sample	% TRR (mg/kg)				
	Wheat		Swiss chard	Turnip	
	Straw	Grain	Leaves	Leaves	Roots
Organic phase	36.7 (0.39)	-	66.0 (0.10)	66.3 (0.30)	55.2 (0.02)
Aqueous phase	9.5 (0.10)		20.2 (0.03)	24.0 (0.11)	29.2 (0.01)
PES	53.8 (0.58)	100.0 (0.06)	13.8 (0.02)	9.7 (0.05)	15.6 (<0.01)
Total	100.0 (1.07)	100.0 (0.06)	100.0 (0.15)	100.0 (0.46)	100 (0.04)

Table CA 6.6.1/01-4 Distribution of TRR in extracts and solids from rotational crops sown 161 days after application with [cyclohexyl-1-^{14}C]spiroxamine

Sample	% TRR (mg/kg)				
	Wheat		Swiss chard	Turnip	
	Straw	Grain	Leaves	Leaves	Roots
Organic phase	32.6 (0.42)	3.9 (<0.01)	49.3 (0.04)	49.2 (0.07)	37.9 (<0.01)
Aqueous phase	17.5 (0.22)	3.3 (<0.01)	31.5 (0.02)	33.5 (0.04)	28.2 (<0.01)
PES	49.9 (0.63)	92.8 (0.05)	19.2 (0.01)	17.3 (0.02)	33.9 (<0.01)

Total	100.0 (1.27)	100.0 (0.05)	100.0 (0.07)	100.0 (0.13)	100.0 (0.02)

Table CA 6.6.1/01-5 TRR in air-dried soil treated with [cyclohexyl-1-^{14}C]spiroxamine

Interval	Days after application	Bq/g	mg/kg[1]
Immediately after application	0	1494	1.21
After mixing, before planting the first set of following crops	30	659	0.53
After mixing, before planting the second set of following crops	161	825	0.67

1 – mg/kg values (expressed as spiroxamine equivalents) were calculated from the combustion value (Bq/g) and the specific radioactivity (1.23 MBq/mg)

Table CA 6.6.1/01-6 Distribution of TRR in extracts of soil after treatment with [cyclohexyl-1-^{14}C]spiroxamine

Sample	% TRR (mg/kg)	
	Day 30	Day 161
Organic phase 1	8.9 (0.05)	37.3 (0.25)
Organic phase 1	54.3 (0.29)	18.3 (0.12)
PES	36.8 (0.19)	44.4 (0.30)
Total	100 (0.53)	100 (0.67)

Characterisation

Available standard compounds for characterisation were parent test substance spiroxamine (KWG 4168) and the following potential metabolites:

KWG 4557 spiroxamine-desethyl (M01)

KWG 4669 spiroxamine-despropyl (M02)

WAK 6301 spiroxamine-N-oxide (M03)

WAK 6782 spiroxamine-N-formyl-desethyl (M04)

BNF 5567B acetyl-desethyl-spiroxamine

BNF 5567A acetyl-despropyl-spiroxamine

WAK 5868 spiroxamine-hydroxyl (M05)

WAK 5428 (spiroxamine-ketone) (M15)

BNF 5544A (spiroxamine-hydroxy ketone) (M16)

BNF 5550A (spiroxamine-cyclohexanol) (M13)

WAK6482-4 (spiroxamine-diol) (M14)

Each metabolite was identified by co-chromatography with the standard compounds.

30 DAA samples (first rotation)

The identity of the metabolites in the organic crop extracts was determined by TLC with comparison with reference materials. Qualitatively, the four crops fractions evaluated were similar. The distribution of parent spiroxamine and metabolites is shown in Table CA 6.6.1/01-7 and the distribution of residues following hydrolysis is shown in Table CA 6.6.1/01-10.

Wheat straw

The organic phase of wheat straw accounted for 36.7% TRR (0.40 mg/kg). The main metabolite was spiroxamine-N-oxide (M03), accounting for 12.7% TRR (0.13 mg/kg). Spiroxamine-N-formyl-desethyl (M04) accounted for 9.2% TRR (0.08 mg/kg). A band comprising spiroxamine-hydroxyl (M05) and spiroxamine-desethyl (M01) accounted for 4.5% TRR (0.05 mg/kg) and spiroxamine-despropyl (M02) comprised 3.5% TRR (0.04 mg/kg). Parent spiroxamine in this extract of wheat straw accounted for 6.8% TRR (0.08 mg/kg).

The aqueous phase of wheat straw accounted for 9.5% TRR (0.10 mg/kg). Water-soluble conjugated moieties formed the majority of this extract, particularly the conjugated diol accounting for 2.2% TRR (0.02 mg/kg), the glucoside of hydroxy-N-oxide (2.1% TRR, 0.01 mg/kg) and the conjugate of hydroxy ketone (1.5% TRR, 0.01 mg/kg).

The organic and aqueous phases of wheat straw were further characterised by hydrolysis with hydrochloric acid. Using this procedure, parent compound and its metabolites were converted to three common moieties; the spiroxamine-ketone (M15), the spiroxamine-hydroxy-ketone (M16) and the spiroxamine-diol (M14). The majority of the TRR in the organic and aqueous extract was converted to tert.-butylketone (spiroxamine-ketone, M15) accounting for 32.9% TRR (0.35 mg/kg).

Post-extraction solids after initial extractions accounted for 53.8% TRR (0.58 mg/kg).

Wheat grain

The TRR in wheat grain was 0.06 mg/kg and was not extracted.

However, investigation of the incorporation of radioactivity into glucose by repeated recrystallization of the glucosazone to a constant specific activity showed that approximately 43% of the total radioactivity in wheat grain was incorporated into glucose.

Swiss chard

Organo-extractable radioactivity accounted for 66% TRR (0.10 mg/kg). Parent spiroxamine accounted for 40.8% TRR (0.06 mg/kg). The main metabolite was the band comprising spiroxamine-hydroxyl and spiroxamine-desethyl accounting for 11.6% TRR (0.02 mg/kg). Spiroxamine-despropyl comprised 7.5% TRR (<0.01 mg/kg) and the spiroxamine-N-oxide, accounted for 6.1% TRR (<0.01 mg/kg).

The aqueous phase of Swiss chard accounted for 20.2% TRR (0.03 mg/kg). As with wheat straw, the same water-soluble conjugated moieties formed the majority of this extract, each however <5% TRR (<0.01 mg/kg).

The post-extraction solid (PES) accounted for 13.8% TRR (0.02 mg/kg).

The organic and aqueous phases of Swiss chard were further characterised by hydrolysis with hydrochloric acid and the majority of the TRR in the organic and aqueous extract was converted to tert.-butylketone accounting for 46.4% TRR (0.07 mg/kg) with the spiroxamine-hydroxy ketone accounting for 29.3% TRR (0.04 mg/kg) and the diol comprising 8.8% TRR (0.01 mg/kg).

Turnips

In leaves, 66.3% TRR (0.30 mg/kg) was extractable with organic solvent. Parent spiroxamine accounted for 29.2% TRR (0.13 mg/kg). The main metabolite was spiroxamine-despropyl comprising 17.7% TRR (0.08 mg/kg). The band comprising spiroxamine-hydroxyl and spiroxamine-desethyl accounted for 14.8% TRR (0.07 mg/kg).

The aqueous phase of turnip leaves accounted for 24.0% TRR (0.11 mg/kg). As with other aqueous extracts, the same water-soluble conjugated moieties formed the majority of this extract, each however <5% TRR (<0.01 mg/kg) apart from the glucoside of hydroxy-N-oxide (10.4% TRR, 0.04 mg/kg).

The post-extraction solid (PES) in turnip leaves accounted for 9.7% TRR (0.05 mg/kg).

Further characterisation of the organic and aqueous phases of turnip leaves by hydrolysis with hydrochloric acid indicated the majority of the TRR in the organic and aqueous extract was converted to tert.-butylketone accounting for 38.1% TRR (0.17 mg/kg) and the spiroxamine-hydroxy ketone accounting for 37.3% TRR (0.17 mg/kg)

In the roots, 55.2% TRR (0.02 mg/kg) was extractable with organic solvent. Parent spiroxamine accounted for 45.8% TRR (0.01 mg/kg). The main metabolite was the band comprising spiroxamine-hydroxyl and spiroxamine-desethyl (M05 and M01) accounted for 4.4% TRR (<0.01 mg/kg).

The aqueous phase of turnip roots accounted 29.2% TRR (<0.01 mg/kg). Water-soluble conjugates were tentatively identified, but each accounted for <0.01 mg/kg.

The post-extraction solid (PES) in turnip roots accounted for 15.6% TRR (<0.01 mg/kg).

161 DAA samples (second rotation)

The distribution of parent spiroxamine and metabolites is shown in Table CA 6.6.1/01-8 and the distribution of residues following hydrolysis is shown in Table CA 6.6.1/01-11.

Wheat straw

The organic phase of wheat straw accounted for 32.6% TRR (0.42 mg/kg). The main metabolite was the spiroxamine-N-oxide (M03), accounting for 12.1% TRR (0.15 mg/kg). Spiroxamine-N-formyl-desethyl (M04) accounted for 7.5% TRR (0.10 mg/kg). A band comprising spiroxamine-hydroxyl and spiroxamine-desethyl (M05 and M01) accounted for 2.4% TRR (0.03 mg/kg) and spiroxamine-despropyl (M02) comprised 2.9% TRR (0.04 mg/kg). Parent spiroxamine in this extract of wheat straw accounted for 4.0% TRR (0.05 mg/kg).

The aqueous phase of wheat straw accounted for 17.5% TRR (0.22 mg/kg). Water-soluble conjugated moieties formed the majority of this extract, particularly the conjugated malonic acid derivative of glucoside of hydroxy-N-oxide accounting for 4.4% TRR (0.06 mg/kg), the glucoside of hydroxy-N-oxide (2.6% TRR, 0.03 mg/kg) and the conjugate of hydroxy ketone (2.4% TRR, 0.03 mg/kg).

Post-extraction solids after initial extractions accounted for 49.9% TRR (0.63 mg/kg).

The organic and aqueous phases of wheat straw were further characterised by hydrolysis with hydrochloric acid. The majority of the TRR in the organic and aqueous extract was converted to spiroxamine-ketone (M15) accounting for 25.5% TRR (0.32 mg/kg) and the spiroxamine-hydroxy ketone (M16) (11.6% TRR, 0.015 mg/kg).

Wheat grain

The TRR in wheat grain was 0.05 mg/kg and was not further extracted.

Swiss chard

Organo-extractable radioactivity accounted for 49.3% TRR (0.04 mg/kg). Parent spiroxamine accounted for 8.8% TRR (<0.01 mg/kg). The main metabolites were spiroxamine-despropyl (M02) and the spiroxamine-N-oxide (M03), each accounting for 14.2% TRR (0.01 mg/kg) and the band comprising spiroxamine-hydroxyl and spiroxamine-desethyl (M05 and M01) accounting for 12.1% TRR (<0.01 mg/kg).

The aqueous phase of Swiss chard accounted for 31.5% TRR (0.02 mg/kg). No further analysis was conducted on this sample due to low residues.

The post-extraction solid (PES) accounted for 19.2% TRR (0.01 mg/kg).

The organic and aqueous phases of Swiss chard were further characterised by hydrolysis with hydrochloric acid. The majority of the TRR in the organic and aqueous extract was converted to tert.-

butylketone accounting for 33.6% TRR (0.03 mg/kg) with the spiroxamine-hydroxy ketone accounting for 15.6% TRR (0.01 mg/kg) and the diol comprising 13.2% TRR (<0.01 mg/kg).

Turnips

In leaves, 49.2% TRR (0.07 mg/kg) was extractable with organic solvent. Parent spiroxamine accounted for 11.6% TRR (0.01 mg/kg). The main metabolite was spiroxamine-despropyl (M02) comprising 16.7% TRR (0.02 mg/kg). The band comprising spiroxamine-hydroxyl and spiroxamine-desethyl (M05 and M01) accounted for 11.0% TRR (0.02 mg/kg).

The aqueous phase of turnip leaves accounted for 35.5% TRR (0.04mg/kg). As with other aqueous extracts, the same water-soluble conjugated moieties formed the majority of this extract, with only residues of the glucoside of hydroxy-N-oxide >0.01 mg/kg (8.4% TRR 0.01 mg/kg).

The post-extraction solid (PES) in turnip leaves accounted for 17.3% TRR (0.02 mg/kg).

Further characterisation of the organic and aqueous phases of turnip leaves by hydrolysis with hydrochloric acid indicated the majority of the TRR in the organic and aqueous extract was converted to spiroxamine-ketone (M15) accounting for 38.1% TRR (0.05 mg/kg) with the spiroxamine-hydroxy ketone (M16) accounting for 11.7% TRR (0.02 mg/kg) and spiroxamine-diol (M14) comprising 13.0% TRR (0.02 mg/kg).

In the roots, 37.9% TRR (<0.01 mg/kg) was extractable with organic solvent. Trace amounts of spiroxamine, the band comprising spiroxamine-hydroxyl and spiroxamine-desethyl (M05 and M01), spiroxamine-despropyl (M02) and spiroxamine-N-oxide (M03) were tentatively detected.

The aqueous phase of turnip roots accounted for 28.2% TRR (0.01 mg/kg). No further analysis was conducted on this sample due to low residues.

The post-extraction solid (PES) in turnip roots accounted for 33.9% TRR (<0.01 mg/kg).

Soil

The identity of the metabolites in soil was determined by TLC with comparison to reference materials. The distribution of parent spiroxamine and metabolites from both day 30 and day 161 plant-back intervals is shown in Table CA 6.6.1/01-9.

Results from the two organic phases were combined and at both plant-back intervals, parent spiroxamine was the major metabolite accounting for 37.7% TRR (0.21 mg/kg) at the 30 DAA sampling and 38.8% TRR (0.26 mg/kg) at the 161 DAA sampling. Formation of the tert.-butyl ketone moiety was observed, however, this was considered to be an artefact of the parent compound that was formed during extraction with hot acetonitrile. Spiroxamine-desethyl and spiroxamine-despropyl were detected at both time point accounting for 2% and 1% TRR at 30 DAA (0.01 mg/kg) and 5.5% and 3.7% TRR (0.03 and 0.02 mg/kg) by HPLC DAD.

Table CA 6.6.1/01-7 Distribution of parent spiroxamine and metabolites in rotational crops sown 30 days after treatment with [cyclohexyl-1-^{14}C]spiroxamine

First rotation	% TRR (mg/kg)				
Sample	Wheat		Swiss chard	Turnip	
	Straw	Grain	Leaves	Leaves	Roots
Organic phase	36.7 (0.40)	n/a	66 (0.1)	66.3 (0.30)	55.2 (0.02)
Spiroxamine (KWG 4168)	6.8 (0.08)	-	40.8 (0.06)	29.2 (0.13)	45.8 (0.01)
Spiroxamine-N-formyl-desethyl (M04)	9.2 (0.10)	-	n/d	n/d	n/d

First rotation	% TRR (mg/kg)				
Sample	**Wheat**		**Swiss chard**	**Turnip**	
	Straw	**Grain**	**Leaves**	**Leaves**	**Roots**
Spiroxamine-hydroxyl and spiroxamine-desethyl (M05, M01)	4.5 (0.05)	-	11.6 (0.02)	14.8 (0.07)	4.4 (<0.01)
Spiroxamine-despropyl (M02)	3.5 (0.04)	-	7.5 (<0.01)	17.7 (0.08)	3.6 (<0.01)
Spiroxamine-N-oxide (M03)	12.7 (0.13)	-	6.1 (<0.01)	4.8 (0.02)	2.8 (<0.01)
Unknown	-	-	-	-	-
Aqueous phase	9.5 (0.10)	n/a	20.2 (0.03)	24.0 (0.11)	29.2 (<0.01)
Metabolites of organic phase unknown	0.9 (<0.01)	-	3.1 (<0.01)	0.9 (<0.01)	7.6 (<0.01)
Glucoside of hydroxy-N-oxide (M20)	2.1 (0.01)	-	2.5 (<0.01)	10.4 (0.04)	n/d
Conjugate of spiroxamine-hydroxy ketone (M23)	1.5 (0.01)	-	2.2 (<0.01)	3.8 (<0.01)	8.1 (<0.01)
Conjugate of diol (M24)	2.2 (0.02)	-	3.0 (<0.01)	2.0 (<0.01)	7.8 (<0.01)
Malonic acid derivative of glucoside of hydroxy-N-oxide (M21)	n/d	-	1.6 (<0.01)	1.7 (<0.01)	n/d
Unknown(s)	2.0 (0.02)	-	7.3 (<0.01)	3.6 (<0.01)	5.7 (<0.01)
PES	53.8 (0.58)	100.0 (0.06)	13.8 (0.02)	9.7 (0.05)	15.6 (<0.01)
Total	100 (1.07)	100.0 (0.06)	100 (0.15)	100.0 (0.46)	100.0 (0.04)

n/a not analysed, n/d not detected

Table CA 6.6.1/01-8 Distribution of parent spiroxamine and metabolites in rotational crops sown 161 days after treatment with [cyclohexyl-1-^{14}C]spiroxamine

Second rotation	% TRR (mg/kg)				
Sample	**Wheat**		**Swiss chard**	**Turnip**	
	Straw	**Grain**	**Leaves**	**Leaves**	**Roots**
Organic phase	32.6 (0.42)	3.9[1] (<0.1[1])	49.3 (0.04)	49.2 (0.07)	37.9 (<0.01)
Spiroxamine (KWG 4168)	4.0 (0.05)	-	8.8 (<0.01)	11.6 (0.01)	27.4 (<0.01)
Spiroxamine-N-formyl-desethyl (M04)	7.5 (0.10)	-	n/d	n/d	n/d
Spiroxamine-hydroxyl and spiroxamine-desethyl (M05, M01)	2.4 (0.03)	-	12.1 (<0.01)	11.0 (0.02)	3.7 (trace)
Spiroxamine-despropyl (M02)	2.9 (0.04)	-	14.2 (0.01)	16.7 (0.02)	3.3 (trace)
Spiroxamine-N-oxide (M03)	12.1 (0.15)	-	14.2 (0.01)	9.9 (0.01)	3.5 (trace)
Unknown	3.7 (0.05)	-	n/d	n/d	n/d
Aqueous phase	17.5 (0.22)	3.3[1] (<0.01[1])	31.5[1] (0.02[1])	33.5 (0.04)	28.2[1] (0.01[1])
Metabolites of organic phase unknown	0.7 (<0.01)	-	-	0.8 (trace)	-
Glucoside of hydroxy-N-oxide (M20)	2.6 (0.03)	-	-	8.4 (0.01)	-
Conjugate of spiroxamine-hydroxy ketone (M23)	2.4 (0.03)	-	-	7.9 (<0.01)	-
Conjugate of diol (M24)	1.9 (0.02)	-	-	4.3 (<0.01)	-
Malonic acid derivative of glucoside of hydroxy-N-oxide (M21)	4.4 (0.06)	-	-	3.7 (<0.01)	-

Second rotation	% TRR (mg/kg)				
Sample	Wheat		Swiss chard	Turnip	
	Straw	Grain	Leaves	Leaves	Roots
Unknown(s)	5.7 (0.07)	-	-	8.4 (0.01)	-
PES	49.9 (0.63)	92.8 (0.05)	19.2 (0.01)	17.3 (0.02)	33.9 (<0.01)
Total	100.0 (1.27)	100.0 (0.05)	100 (0.07)	100 (0.13)	

1 – quantitation (by TLC) was not possible due to low radioactive residues
n/d not detected

Table CA 6.6.1/01-9 Distribution of TRR in extracts of soil after treatment with [cyclohexyl-1-^{14}C]spiroxamine

Sample	% TRR (mg/kg)	
	Day 30 (first rotation)	Day 161 (second rotation)
Organic phase 1	8.9 (0.05)	37.3 (0.25)
Spiroxamine (KWG 4168A)	6.4 (0.04)	19.1 (0.13)
Spiroxamine (KWG 4168B)	2.5 (0.01)	10.7 (0.07)
Spiroxamine-desethyl (M01)	n.d.	4.5 (0.03)
Spiroxamine-despropyl (M02)	n.d.	2.7 (0.02)
Unknown material (origin TLC)	n.d.	0.3 (<0.01)
Organic phase 2	54.3 (0.29)	18.3 (0.12)
Spiroxamine (KWG 4168A)	18.3 (0.10)	4.5 (0.03)
Spiroxamine (KWG 4168B)	11.5 (0.06)	4.5 (0.03)
Spiroxamine-ketone (M15)	19.4 (0.10)	7.1[1] (0.05)
Unknown(s)	2.3 (0.01)	
Spiroxamine-desethyl (M01)	2.0 (0.01)	1.0 (<0.01)
Spiroxamine-despropyl (M02)	1.0 (<0.01)	1.0 (<0.01)
Unknown material (origin TLC)	n.d.	0.4 (<0.01)
PES	36.8 (0.19)	44.4 (0.30)
Total	100 (0.53)	100 (0.67)

1 – the exposure of the TLC plate was repeated by some of the components had volatilised from the TLC plate

Table CA 6.6.1/01-10 Distribution of radioactivity in rotational crops sown 30 days after treatment with [cyclohexyl-1-^{14}C]spiroxamine following hydrolysis with 1N HCl

First rotation	Wheat Straw		Swiss chard leaves		Turnip leaves	
Sample	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Organic phase	36.7	0.39	66.0	0.10	66.3	0.30
Spiroxamine-ketone (M15)	31.5	0.33	44.1	0.07	37.4	0.17
Spiroxamine-hydroxy ketone (M16)	5.2	0.06	21.9	0.03	24.0	0.11
Unknown components	n.d.	n.d.	n.d.	n.d.	4.9	0.02
Aqueous phase	9.5	0.10	20.2	0.03	24.0	0.11
Spiroxamine-ketone (M15)	1.4	0.02	2.3	<0.01	0.7	<0.01
Spiroxamine-hydroxy ketone (M16)	3.7	0.04	7.4	0.01	13.3	0.06
Spiroxamine-diol (M14)	3.5	0.04	8.8	0.01	4.4	0.02
Unknown components	0.9	<0.01	1.7	<0.01	5.6	0.03

First rotation	Wheat Straw		Swiss chard leaves		Turnip leaves	
Sample	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Sum of organic and aqueous phases	46.2	0.49	86.2	0.13	90.3	0.41
Spiroxamine-ketone (M15)	32.9	0.35	46.4	0.07	38.1	0.17
Spiroxamine-hydroxy ketone (M16)	8.9	0.10	29.3	0.04	37.3	0.17
Spiroxamine – diol (M14)	3.5	0.04	8.8	0.01	4.4	0.02
Unknown components	0.9	<0.01	1.7	<0.01	10.5	0.05
Solids	53.8	0.58	13.8	0.02	9.7	0.05
Total	100.0	1.07	100.0	0.15	100.0	0.46

Table CA 6.6.1/01-11 Distribution of radioactivity in rotational crops sown 161 days after treatment with [cyclohexyl-1-^{14}C]spiroxamine following hydrolysis with 1N HCl

Second rotation	Wheat Straw		Swiss chard leaves		Turnip leaves	
Sample	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Organic phase	32.6	0.42	49.3	0.04	49.2	0.07
Spiroxamine-ketone (M15)	24.4	0.31	31.9	0.03	36.5	0.05
Spiroxamine-hydroxy ketone (M16)	6.2	0.08	9.7	<0.01	6.7	<0.01
Unknown components	2.0	0.03	7.7	<0.01	6.0	<0.01
Aqueous phase	17.5	0.22	34.5	0.02	33.5	0.04
Spiroxamine-ketone (M15)	1.1	0.01	1.7	<0.01	1.6	<0.01
Spiroxamine-hydroxy ketone (M16)	5.4	0.07	5.9	<0.01	5.0	<0.01
Spiroxamine – diol (M14)	4.0	0.05	13.2	<0.01	13.0	0.02
Unknown components	7.0	0.09	10.7	<0.01	13.9	0.02
Sum of organic and aqueous phases	50.1	0.64	80.8	0.06	82.7	0.11
Spiroxamine-ketone (M15)	25.5	0.32	33.6	0.03	38.1	0.05
Spiroxamine-hydroxy ketone (M16)	11.6	0.15	15.6	0.01	11.7	0.02
Spiroxamine – diol (M14)	4.0	0.05	13.2	<0.01	13.0	0.02
Unknown components	9.0	0.12	18.4	0.01	19.9	0.02
Solids	49.9	0.63	19.2	0.01	17.3	0.02
Total	100.0	1.27	100.0	0.07	100.0	0.13

Table CA 6.6.1/01-12 Distribution radioactivity for the isolation and derivatisation of glucose from wheat grain from the 30-day plant back interval

Sample	kBq	% TRR
Wheat grain (72.9 Bq/g)	5.47 (75.0 g sample)	100
Filtrate from HCl hydrolysis	3.82	70
Eluate from ion exchange column	3.65	67
Crystalline glucosazone	1.59	29

Storage stability

An assessment of storage stability was not required as all plant samples were extracted within 8 days of sampling and stored at approximately -20°C.

III. Conclusions

A single application of ^{14}C-spiroxamine (labelled in the cyclohexyl-1-^{14}C position) was made to bare sandy loam soil in 1 m^2 containers at a target rate of 1.58 kg a.s./ha as an emulsifiable concentrate (EC 500) formulation. This rate is 2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Crops (wheat, Swiss chard and turnips) were planted at 30 days after application (DAA), and 161 DAA. Each crop was harvested at maturity.

The TRR in wheat grain was 0.06 mg/kg (measured as mg spiroxamine equivalents per kg) at the 30 day interval (first rotation) and 0.05 mg/kg at the 161 day interval (second rotation). Corresponding levels in wheat straw were 1.07 mg/kg and 1.27 mg/kg (30 day and 161 day intervals respectively). In Swiss chard, TRRs were 0.15 mg/kg at the 30 day interval declining to 0.07 mg/kg at the 161 day interval. Residues in turnip roots were low at both plant-back

Parent spiroxamine was the main component of the residue in Swiss chard, and turnips harvested from the 30 day plant-back interval, accounting for 29% TRR (0.13 mg/kg) in turnip leaves, 45.8% TRR (0.01 mg/kg in turnip roots) and 40.8% TRR (0.06 mg/kg) in Swiss chard. In wheat straw, the main metabolite was spiroxamine-N-oxide (M03), accounting for 12.7% TRR (0.13 mg/kg). Desalkylated metabolites (M01, M02) were also seen in wheat straw and parent spiroxamine accounted for 6.8% TRR (0.08 mg/kg).

Parent spiroxamine was also seen in rotated crops at the 161 DAA plant-back interval (second rotation), although at lower levels, accounting for between 4% TRR (0.05 mg/kg) in wheat straw and 27% TRR (<0.01 mg/kg) in turnip roots. In wheat straw, the main metabolite was the spiroxamine-N-oxide, accounting for 12.1% TRR (0.15 mg/kg). In Swiss chard, the major metabolites were spiroxamine-despropyl and the spiroxamine-N-oxide, each accounting for 14.2% TRR (0.01 mg/kg). In turnip leaves, the main metabolite was spiroxamine-despropyl comprising 16.7% TRR (0.02 mg/kg). Residues in turnip roots at this interval were very low however, trace amounts of spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-N-oxide (M03) were tentatively detected.

The organic and aqueous phases of wheat straw, Swiss chard and turnip leaves were further characterised by hydrolysis with hydrochloric acid and the majority of the TRR in the organic and aqueous extract was converted to spiroxamine-ketone (M15) (25.5 – 46.5% TRR), the spiroxamine-hydroxy ketone (M16) (8.8 – 37.2% TRR) and spiroxamine-diol (M14) (3.5 - 13.2% TRR).

The nature of the residue in rotated crops when spiroxamine is applied to bare soil is essentially the same as found in the primary wheat metabolism study. Desalkylation of the parent compound occurs forming the desethyl and despropyl moieties. Additionally, oxidation of spiroxamine in the tertiary amine group forming the N-oxide and to a minor extent in the tert.-butyl group. Hydrolysis occurred (ketone formation) which was followed by reduction of the keto group to the hydroxyl group. Conjugation at both hydroxyl positions was also observed and to a minor extent at the secondary amine group (N-formyl formation).

The proposed metabolic pathway for spiroxamine in rotational crops is shown in Figure CA 6.6.1-1.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2010) IIA 6.6.2/01.

It is noted that the longest interval of 270 – 365 days to represent crops sown the following year was not conducted in this study.

The nature of the residue in rotated crops when spiroxamine is applied to bare soil is essentially the same as found in the primary wheat metabolism study. Desalkylation of the parent compound occurs forming the desethyl and despropyl moieties. Additionally, oxidation of spiroxamine in the tertiary amine group forming the N-oxide and to a minor extent in the tert.-butyl group. Hydrolysis occurred (ketone formation) which was followed by reduction of the keto group to the hydroxyl group. Conjugation at both hydroxyl positions was also observed and to a minor extent at the secondary amine group (N-formyl formation).

Data Point:	KCA 6.6.1/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [1,3-dioxolane-4-14C]KWG 4168 in confined rotational crops - First part: 1st rotation
Report No:	MEF-08/120
Document No:	M-303904-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1850; Canadian PMRA Ref.: DACO 6.3; OECD 502
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A single application of ^{14}C-spiroxamine (1,3-dioxolane-4-^{14}C) was made to bare sandy loam soil in a 1 m^2 container at a rate of 1.62 kg a.s./ha as an emulsifiable concentrate (EC 500) formulation. This rate is just below 2.2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Crops were planted 30 days after application (DAA), first rotation, 193 DAA (second rotation) and 294 DAA (third rotation). The planting container was maintained in a greenhouse. This summary describes the magnitude and nature of the residues in crop commodities from first rotation. Details of the crops sampled from second and third rotation of the same study are described in the following summary (CA 6.6.1/03).

Seeds of wheat (*Triticum aestivum,* Thasos), Swiss chard (*Beta vulgaris,* Lukullus) and turnips (*Brassica rapa,* Rondo) were sown into the soil at 30, 193 and 294 DAA and crops grown to maturity. Crop samples collected were immature Swiss chard (sampled at BBCH 35), wheat forage (BBCH 29), wheat hay (BBCH 65 – 71). Additionally at maturity, wheat straw, wheat grain, Swiss chard, turnip root and turnip leaves were also harvested.

The total radioactive residues (TRRs) in plant matrices were determined by summation of the radioactivity in organic solvent extracts and that in the residual post-extraction solids.

In crops harvested from the first plant-back interval (30 DAA), the TRR in wheat forage was 0.593 mg/kg (measured as mg spiroxamine equivalents per kg), 1.983 mg/kg in wheat hay and 3.178 mg/kg in wheat straw. In wheat grain, the TRR was 0.131 mg/kg

Corresponding TRR in immature Swiss chard was 0.846mg/kg and 0.676 mg/kg in mature Swiss chard. The final crop group with turnip as a representative crop saw residue levels of 0.892 mg/kg in leaves and 0.101 mg/kg in roots.

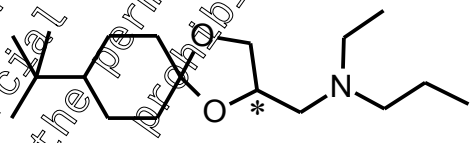
The majority of the radioactive residue in crop matrices was generally readily extractable. In wheat matrices (with the exception of grain), between 65 and 80% TRR was extracted with conventional extracts (acetonitrile:water 8:2 v/v). In grain, only 21% TRR was extractable using this solvent. In both immature and mature Swiss chard, the residue was almost entirely extractable, with between 93 – 100% TRR extractable. The extractability was similar in turnip roots and leaves with 91% TRR readily extractable. The remaining radioactivity in plant matrices could be exhaustively extracted using a range of solvents. Additionally in grain, enzyme digestion with diastase released a significant proportion of the non-extracted residue (70% TRR). Final non-extractable or bound residues (PES) accounted for between 5-9% TRR in all wheat matrices, 0.3 – 7% TRR in Swiss chard and 2 – 10% TRR in turnips.

Parent spiroxamine and the two desalkylated metabolites, spiroxamine-despropyl (M02) and spiroxamine-desethyl (M01)comprised the majority of the radioactive residue in all crop matrices harvested from the 30 day plant-back interval. In wheat, matrices, spiroxamine accounted for between 2.7% TRR in grain to 32.9% TRR (0.195 mg/kg) in wheat forage. Spiroxamine-despropyl (M02)ranged from 4.8% TRR in grain to 23.4% TRR (0.139 mg/kg) in forage. Corresponding levels of spiroxamine-desethyl (M01) were lower, ranging from 2.9% TRR in grain to 20.0% TRR in forage (0.119 mg/kg). The same three metabolites also comprised the majority of the residue in Swiss chard and turnips planted at the first rotation. Parent spiroxamine accounted for between 4 and 9% TRR in these crops, the spiroxamine-despropyl (M02) ranged from 3% TRR in turnip roots to 21.1% TRR (0.188 mg/kg) in turnip leaves. Corresponding levels of spiroxamine-desethyl (M01) were approximately half those seen of the despropyl moiety, ranging from 1.5% TRR in roots to 9.3% TRR in turnip leaves. A number of glycoside conjugates (up to 6) were also detected in all rotated crop matrices, with the exception of wheat grain. In this matrix, and also in the highly starchy turnip root, further digestion of the PES was conducted with diastase enzyme releasing significant additional radioactivity (69.5% TRR, 0.091 mg/kg in grain and 18.9%, 0.019 mg/kg in roots). This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

I. Materials and methods

A. Test material

[1,3-dioxolane-4-^{14}C]-spiroxamine



* Denotes radiolabel position

Specific activity (µCi/mg)	110.58 (4.09 MBq/mg)
Lot/Batch No.:	KATH 6052
Radiochemical purity:	>98% determined by TLC, HPLC, GC-FID
CAS No.:	118134-30-8

B. Study design

Soil

Soil characterisation details are presented below in Table CA 6.6.1/01-1

Table CA 6.6.1/01-1 Soil classification and physico-chemical properties

Soil Type (USDA)	pH ($CaCl_2$)	OM %	Sand %	Silt %	Clay %	Moisture holding capacity mL / 100g)	CEC meq/ 100 g
Sandy loam	6.9	2.12	58.1	27.9	14.0	Not stated	8.1

Test system

The test systems were planted in a container (1m²) containing a sandy loam soil (described above) and located in a greenhouse at the Metabolism / Environmental Fate function, Bayer CropScience AG, Monheim, Germany.

Crops grown following application of the test item were wheat (*Triticum aestivum*, variety 'Thasos'), Swiss chard (*Beta vulgaris* spp, variety 'Lukullus') and turnip (*Brassica rapa*, variety 'Rondo'). These crops represent a cereal crop, a leafy crop and a root crop. The daytime temperature of the greenhouse was kept at 20 - 22°C and the night time temperature (20.00 – 06.00 h) at 15 - 16°C. Addition lighting was automatically switched on when the natural daylight intensity fell below 35 kLx. Relative humidity was maintained at 65%. During the ageing period the sol was watered to maintain adequate moisture content. The plants were irrigated as needed. Plant protection and fertilisation measures were carried out as required and documented. A single container was used for this study.

For each plant back interval, wheat occupied half of the container with Swiss chard and turnips sharing the remaining available space.

Plants were irrigated and fertilised as required for good crop growth.

The experimental work started on October 30, 2007 and was completed on June 11 2008 (first rotation).

The study was conducted at Bayer CropScience AG, Development, Metabolism / Environmental Fate, D-40789 Monheim am Rhein, Germany.

Experimental conditions

The uptake and metabolism of [^{14}C]-spiroxamine in tested rotational crops of wheat, Swiss chard and turnip was studied following single bare-soil applications. The rate employed in this study is higher (2.2N) than the proposed seasonal critical GAP for the use of spiroxamine on field crops but as a worst case is considered representative and appropriate for the objectives.

The dose calculations were based on an application rate of 1.50 kg a.s./ha and a plot size of 1 m² (i.e. 150 mg a.s./m²).

Preparation of radiolabelled dose

The radioactive test item KATH 6032 (59.10 mg, 241.7 MBq) was diluted with 121.46 mg non-radiolabelled test item. The specific activity following radio-dilution was 1.36 MBq/mg (36.76µCi/mg). A portion (173.72 mg) of the radio-diluted test item was mixed with 173.36 mg of the EC 500 blank formulation by swirling. This was then slowly added to 100 mL water by stirring. The radioactivity in the final spray emulsion was determined by liquid scintillation counting and amounted to 2.32 MBq/mL.

Application

The bare soil was treated on November 05, 2007. The planting container was surrounded with protective foil to prevent spray-drift. The application was made using a computer-controlled track sprayer fitted with a flat-fan nozzle. The spray application included an approximate 15% excess to compensate for

any losses. After application of the 100 mL spray emulsion, the protective foil was removed and rinsed with methanol. The spray container was also rinsed with methanol and water. All rinsing solutions were quantified by LSC and the rinses subtracted from the total applied to give final applied material of 1.62 kg a.s./ha (220.93 MBq/m^2).

The homogeneity of the spray solution was checked by determination of the spray application on ten filter papers (1.5 cm diameter) which were randomly placed on the soil prior to application. The spray solution was homogeneous.

The stability of the test item in the application solution was checked before and after application by HPLC. No degradation was observed.

Soil

For ageing, the soil remained undisturbed for 30 days. The soil was watered in order to maintain an adequate moisture content. Before sowing of the crops, the upper soil layer was intensively mixed (approximately 10 cm depth) and soil cores (approximately 15 cm depth) were taken. The radioactivity in the air-dried soil cores was determined by combustion and LSC.

Planting and sampling of following crops

30 DAA: (first rotation)

Wheat

Wheat forage was taken at BBCH 29 (79 DAA). One of five rows of wheat plants was cut at the roots, which remained in the soil. The forage was cut into small pieces and homogenised in liquid nitrogen using a Polytron blender.

Wheat hay was taken at BBCH 65 - 71 (157 DAA). One of five rows of wheat plants was cut at the roots, which remained in the soil. The dried hay sample was cut into small pieces and homogenised in liquid nitrogen.

Wheat straw and grain were harvested together at BBCH 89 (192 DAA). The wheat plants were cut just above the soil surface, with the roots remaining in the soil. The seeds were collected manually yielding the grain sample. The remaining ears and chaff were combined with the straw. The samples were cut into small pieces and homogenised in liquid nitrogen.

Turnip leaves and roots

Turnip leaves and roots were harvested together at maturity (BBCH 45 – 49, 99 DAA). The turnips were pulled out of the soil and the leaves separated from the roots. The roots were cut into slices and the leaves cut into small pieces. Both were homogenised in liquid nitrogen.

Swiss chard

Immature Swiss chard was harvested at BBCH 35 (66 DAA) and again at maturity (BBCH 49, 84 DAA). The samples were cut from the roots which remained in the soil. The samples were cut into small pieces and homogenised in liquid nitrogen.

Aliquots of all plant homogenates were used for extraction. All samples were stored frozen at approximately -20°C.

The sampling schedule for all crops is summarised in Table CA 6.6.1/02-2.

Table CA 6.6.1/02-2 Sampling schedule for rotational crops planted 30 days after application to bare soil

Crop matrix	Sampling occasion (days after application) / BBCH at sampling
Wheat - Forage	79 / BBCH 29
Wheat - Hay	157 / BBCH 65 - 71
Wheat - Straw	192 / BBCH 89 (maturity)
Wheat - Grain	192 / BBCH 89 (maturity)
Swiss chard - immature	66 / BBCH 35
Swiss chard - mature	84 / BBCH 49 (maturity)
Turnip - Roots	99 / BBCH 45 - 49 (maturity)
Turnip - Foliage	99 / BBCH 45 - 49 (maturity)

Sample preparation and extraction

Plants

Aliquots of the homogenised plant samples were extracted three times with acetonitrile/water (8:2 v/v) and once with acetonitrile. The extractions were conducted using a Polytron. The post-extraction solids (PES's) were air-dried and weighed. The total radioactive residues (TRRs) of the plant fractions were determined by summation of the radioactivity in the acetonitrile/water extracts and The post-extraction solids (PES). Residues were expressed as mg/kg spiroxamine equivalents.

Concentrated extracts were examined by TLC and HPLC. Concentration recoveries ranged from 95.2 to 100.9%.

Exhaustive extraction of Post extraction solids (PES)

Aliquots of solids were extracted with mixtures of acetonitrile/water (7:3 v/v), acetonitrile/water (1:1 v/v), acetonitrile/water (1:1 v/v) containing 2.5 mL formic acid (and occasionally 0.5 N hydrochloric acid). Each extraction was conducted for 20 minutes using microwave assistance. The radioactivity in the dried PES was determined. Recoveries of exhaustive extraction ranged from 103 to 105%.

Concentrated combined exhaustive extracts were examined by HPLC. Additional investigations of wheat grain were performed by TLC. Concentration recoveries ranged from 95.3 to 99.3%.

Digestion of solids in wheat grain

An aliquot of the solids of wheat grain was digested with *Diastase* enzyme for 13 days. The radioactivity in the digestion solution and the PES were determined. The recovery of material was 101.9%. The digest was partitioned with ethyl acetate and the radioactive residue characterised.

The radioactivity in the organic phases was determined by liquid scintillation counting (LSC) and in the solids by combustion.

Combustion analysis:

Solid samples were combusted using a Harvey Ox 500 Tissue Oxidiser (Zinsser-Analytic). The resultant $^{14}CO_2$ was absorbed in scintillation cocktail (Oxysolve C-400, Zinsser) and radioactivity measured by LSC.

Liquid scintillation counting (LSC):

Liquid samples were counted in a liquid scintillation counter using a commercial scintillation cocktail (Quick-Safe A, Zinsser Analytic) containing 5% water. Samples were generally measured in triplicate.

Thin layer chromatography (TLC):

The radioactive solutions were analysed by one dimensional TLC using silica gel (60 WF, Merck), which were eluted with the following solvent systems:

System 1	Chloroform/methanol/ammonia (25%)	65:28:8 v/v/v
System 2	Acetonitrile/water/ammonia (25%)	320:18:2 v/v/v

Radioactive components were detected using a Fuji Imaging Analyser with a software peak integration package ('Tina', Raytest). The reference materials used in co-chromatography were detected using iodine vapour or by spraying with vanillin/sulphuric acid and heating the plate to 120°C.

For identification of sugar compounds, the TLC plate was sprayed with a solution of aniline phthalate (Fluka). After heating at 120 °C for approximately 30 minutes, a typical colour reaction proved indication of the presence of sugars.

High performance liquid chromatography (HPLC

Parent compound and metabolites in the combined extracts were quantified, isolated and analysed by HPLC using the following system:

Agilent 1100 liquid chromatograph was used with oven temperature set at 40°C, UV wavelength detector set at 254 nm. The chromatograph was coupled to a radioactivity detector (Raytest, Ramona) with a glass scintillator cell.

Data was evaluated using Raytest GINA-START software.

The column used was a Purospher STAR PR-18e, 250 x 4.6 mm i.d..

Three different mobile phase gradient systems were used:

1. For stability and purity check; (gradient moving from 100% A to 100% B over 30 minutes)

eluent A: water/formic acid (99/1 v/v)
eluent B: acetonitrile/water/formic acid (97/2/1 v/v/v)

2. For profiling, quantitation, isolation and co-chromatography of parent compound and metabolites; (gradient moving from 100% A to 100% B over 90 minutes)

eluent A: .0.05% triethylamine in water
eluent B: 0.05% triethylamine in acetonitrile

3. For isolation of metabolites; (gradient moving from 100% A to 100% B over 90 minutes)

eluent A: water/formic acid (99/1 v/v)
eluent B: acetonitrile/water/formic acid (97/2/1 v/v/v)

Mass spectroscopy (MS)

Selected sample extracts were analysed by MS. The electro-spray ionisation MS spectra (ESI) were obtained with either a TSQ 7000 instrument or with a LTQ Orbitrap XL mass spectrometer (Finnegan).

II. Results and discussion

Total radioactive residues (TRR)

TRR data for rotational crop plant matrices are summarised in Table CA 6.6.1/02-3. Summaries of extractable radioactivity in plant matrices are presented in Table CA 6.6.1/02-4 (wheat), Table CA 6.6.1/02-5 (Swiss chard) and Table CA 6.6.1/02-6 (turnip).

The TRRs in Swiss chard, turnip roots and leaves were determined by the summation of radioactivity in the conventional extracts, exhaustive extracts, diastase enzyme digest (where conducted) and the post-extraction solids (PES). Residues are expressed as mg/kg parent spiroxamine equivalents.

First rotation (30 DAA planted crops)

Wheat

The total radioactive residue (TRR) in wheat planted 30 days after application to bare soil was 0.593 mg/kg in forage, 1.983 mg/kg in hay, 3.178 mg/kg in straw and 0.131 mg/kg in grain. In forage, 80.4% TRR (0.477 mg/kg) was readily extractable with conventional solvents (acetonitrile/water, 8:2 v/v). In hay, 71.1% TRR (1.411 mg/kg) was readily extractable, 64.9% TRR (2.062 mg/kg) in straw and 21.3% (0.028 mg/kg) in grain. Exhaustive extraction released a further 13.8% TRR (0.082 mg/kg) in forage, 24% (0.475 mg/kg) in hay, 29.4% (0.933 mg/kg) in straw and 37.4% (0.064 mg/kg) in grain. The grain PES after conventional extraction was also treated with diastase enzyme. This released 69.5% TRR (0.091 mg/kg) indicating radioactivity that was naturally incorporated into $^{14}CO_2$.

Swiss chard

The total radioactive residue (TRR) in immature Swiss chard planted 30 days after application to bare soil was 0.846 mg/kg. Organo-extractable radioactivity accounted for 99.7% TRR (0.844 mg/kg). The post-extraction solid (PES) accounted for 0.3% TRR (0.003 mg/kg). The total radioactive residue (TRR) in mature Swiss chard was 0.676 mg/kg. Organo-extractable radioactivity accounted for 93.0% TRR (0.629 mg/kg). The PES accounted for 7.0% TRR (0.048 mg/kg).

Turnips

The total radioactive residue (TRR) in turnips planted 30 days after application to bare soil was 0.892 mg/kg in leaves and 0.101 mg/kg in roots. In leaves, 90.9% TRR (0.811 mg/kg) was extractable with organic solvent. Exhaustive extraction released a further 6.8% TRR (0.062 mg/kg). The post-extraction solid (PES) accounted for 2.2% TRR (0.019 mg/kg). In the roots, 90.5% TRR (0.091 mg/kg) was extractable with organic solvent. The PES accounted for 9.5% TRR (0.01 mg/kg).

Table CA 6.6.1/02-3 Total radioactive residues (TRR) in rotational crops sown 30 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

	Wheat[1]				Swiss chard[1]		Turnip[1]	
	Forage (mg/kg)	Hay (mg/kg)	Straw (mg/kg)	Grain (mg/kg)	Immature (mg/kg)	Mature (mg/kg)	Leaves (mg/kg)	Roots (mg/kg)
30 DAA	0.593	1.983	3.178	0.131	0.846	0.676	0.892	0.101

1 - TRR values were determined by summation of radioactivity in extracts and PES

Table CA 6.6.1/02-4 Distribution of TRR in extracts and solids in wheat matrices sown 30 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)							
	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
30 DAA (first rotation)								
Conventional extracts	80.4	0.477	71.1	1.411	64.9	2.062	21.3	0.028
Exhaustive extracts								
Acetonitrile/water (7:3 v/v)	8.4	0.050	12.4	0.246	20.5	0.652	11.7	0.015
Acetonitrile/water (1+1 v/v)	2.9	0.017	9.6	0.190	6.3	0.200	14.7	0.019
Acetonitrile/water (1:1 v/v) + 2.5 mL formic acid	1.3	0.008	2.0	0.039	2.6	0.081	22.7	0.030
0.5 N hydrochloric acid	1.2	0.007	-	-	-	-	-	-

Sample	% TRR (mg/kg)							
	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Incorporated $^{14}CO_2$ Diastase digest (aqueous phase)	-	-	-	-	-	-	69.5	0.091
PES	5.9	0.035	4.9	0.097	5.7	0.182	9.2	0.012
Total	100.0	0.593	100.0	1.983	100.0	3.178	100.0	0.131

n/d not detected

Table CA 6.6.1/02-5 Distribution of TRR in extracts and solids in Swiss chard matrices sown 30 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)			
	Swiss chard (immature)		Swiss chard (maturity)	
	% TRR	mg/kg	% TRR	mg/kg
30 DAA (first rotation)				
Conventional extracts	99.7	0.844	93.0	0.629
PES	0.3	0.003	7.0	0.048
Total	100.0	0.846	100.0	0.676

n/d not detected

Table CA 6.6.1/02-6 Distribution of TRR in extracts and solids in turnip matrices sown 30 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)			
	Turnip leaves		Turnip roots	
	% TRR	mg/kg	% TRR	mg/kg
30 DAA (first rotation)				
Conventional extracts	90.9	0.811	90.5	0.091
Exhaustive extracts				
Acetonitrile/water (7:3 v/v)	3.9	0.035	-	-
Acetonitrile/water (1:1 v/v)	1.7	0.016	-	-
Acetonitrile/water (1:1 v/v) + 2.5 mL formic acid	1.2	0.011	-	-
Incorporated $^{14}CO_2$ Diastase digest (aqueous phase)	-	-	18.9	0.019
PES	2.2	0.019	9.5	0.010
Total	100.0	0.892	100.0	0.101

Characterisation

Available standard compounds for characterisation were parent test substance spiroxamine (KWG 4168) and the following potential metabolites:

WAK 6893 spiroxamine-despropyl-aminodiol (M31)

WAK 6885 spiroxamine-aminodiol-N-oxide (M29)

WAK 5427 spiroxamine-aminodiol (M28)

Spiroxamine-despropyl-acid glycoside (M45)

Spiroxamine-hydroxy-despropyl glycoside (M39)

Spiroxamine-desethyl-acid glycoside (M43)

Spiroxamine-hydroxy-desethyl glycoside (M42)

Spiroxamine-acid glycoside (M44)

Spiroxamine-hydroxy glycoside (M40)

WAK 6301 spiroxamine-N-oxide (M03)

WAK 5868 spiroxamine-hydroxyl (M05)

Spiroxamine-N-formyl-despropyl (M38)

WAK 6782 spiroxamine-N-formyl-desethyl (M04)

KWG 4557 spiroxamine-desethyl (M01)

KWG 4669 spiroxamine-despropyl (M02)

WAK 6894 spiroxamine-desethyl-aminodiol (M30)

Each metabolite was identified by co-chromatography with the standard compounds predominantly by HPLC, but selected samples were also analysed by LC-MS, LC-MS/MS and TLC.

30 DAA crop matrices (first rotation)

The distribution of parent spiroxamine and metabolites in wheat matrices 30 DAA is shown in Table CA 6.6.1/02-7 and the distribution of spiroxamine and metabolites in Swiss chard and turnip matrices 30 DAA is shown in Table CA 6.6.1/02-8.

Wheat forage

The total radioactive residue in wheat forage planted 30 days after a single application of dioxolane labelled spiroxamine to bare soil at a rate of 1.62 kg a.s./ha was 0.593 mg/kg. A total of 94.1% TRR (0.559 mg/kg) was extractable. Almost 84% TRR in wheat forage (0.498 mg/kg) was identified. The main component of the residue in this matrix was parent spiroxamine, accounting for 32.9% TRR (0.195 mg/kg). Desalkylation of the parent compound, formed the two main metabolites in wheat forage, spiroxamine-despropyl (M02) (23.4% TRR; 0.139 mg/kg) and spiroxamine-desethyl (M01) (20% TRR, 0.119 mg/kg). A further six minor metabolites were detected in wheat forage and these were predominantly glycoside conjugates. The largest of these, spiroxamine-hydroxy-despropyl glucoside (M39) accounted for 2.1% TRR (0.012 mg/kg).

Post-extraction solids in wheat forage after conventional and exhaustive extractions accounted for 5.9% TRR (0.035 mg/kg).

Wheat hay

The total radioactive residue in wheat hay planted 30 days after a single application of dioxolane labelled spiroxamine was 1.983 mg/kg. A total of 95.1% TRR (1.886 mg/kg) was extractable. 60.9% TRR in wheat hay (1.207 mg/kg) was identified. Desalkylation of the parent compound, formed the two main metabolites in wheat hay, spiroxamine-despropyl (M02) (15.2% TRR; 0.302 mg/kg) and spiroxamine-desethyl (M01) (10.4% TRR, 0.207 mg/kg). Parent spiroxamine accounted for 8.5% TRR (0.169 mg/kg). Six glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy-desethyl glucoside (M42) accounted for 6.5% TRR (0.129 mg/kg).

Post-extraction solids in wheat hay after conventional and exhaustive extractions accounted for 4.9% TRR (0.097 mg/kg).

Wheat straw

The total radioactive residue in wheat straw planted 30 days after a single application of dioxolane labelled spiroxamine was 3.178 mg/kg. A total of 94.3% TRR (2.996 mg/kg) was extractable. 75.6% TRR in wheat straw (2.402 mg/kg) was identified. As seen in other wheat matrices, desalkylation of the parent compound, formed the two main metabolites in wheat straw, spiroxamine-despropyl (M02) (17.4% TRR; 0.553 mg/kg) and spiroxamine-desethyl (M01) (15.1% TRR, 0.479 mg/kg). Parent spiroxamine accounted for 15.2% TRR (0.485 mg/kg). The N-formyl derivatives of the desalkylated species accounted for 7.6% TRR (0.243 mg/kg), spiroxamine-N-formyl-despropyl (M38) and 6.4% TRR (0.204 mg/kg) spiroxamine -N-formyl-desethyl (M04). Oxidation of spiroxamine in the tertiary amine group formed the N-oxide (M03), accounting for 7.4% TRR (0.235 mg/kg) in wheat straw. Glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine hydroxy glucoside (M40) accounted for 2.8% TRR (0.088 mg/kg).

Post-extraction solids in wheat straw after conventional and exhaustive extractions accounted for 5.7% TRR (0.182 mg/kg).

Wheat grain

The total radioactive residue in wheat grain planted 30 days after a single application of dioxolane labelled spiroxamine was 0.131 mg/kg. Conventional extractions released 21.3% TRR (0.028 mg/kg). Exhaustive extraction of the PES following conventional extraction with diastase enzyme treatment released a further 69.5% TRR (0.091) that was polar in nature and not readily partitioned into organic solvent. This indicated that the majority of the radioactive residue in wheat grain was small carbon chain units that had been naturally incorporated into natural products such as sugars or starch. A total of 18.9% TRR in wheat grain (0.025 mg/kg) was identified. Oxidation of spiroxamine formed the N-oxide (M03), accounting for 4.3% TRR (0.006 mg/kg) in wheat straw. Spiroxamine-despropyl (M02) (4.8% TRR; 0.006 mg/kg) and spiroxamine-desethyl (M01) (2.9% TRR, 0.004 mg/kg) were also detected in grain. Parent spiroxamine accounted for 2.7% TRR (0.004 mg/kg)

Post-extraction solids in wheat grain after conventional and exhaustive extractions accounted for 9.2% TRR (0.012 mg/kg).

Swiss chard (immature)

The total radioactive residue in immature Swiss chard planted 30 days after a single application of dioxolane labelled spiroxamine was 0.846 mg/kg. A total of 99.7% TRR (0.844 mg/kg) was extractable. 65.3% TRR in Swiss chard (0.552 mg/kg) was identified. Spiroxamine-hydroxyl was the most abundant metabolite, comprising 17.2% TRR (0.146 mg/kg). The desalkylated metabolites, spiroxamine-despropyl (M02) (15.0% TRR; 0.127 mg/kg) and spiroxamine-desethyl (M01) (6.6% TRR, 0.056 mg/kg) were also detected. Parent spiroxamine accounted for 9.4% TRR (0.080 mg/kg). The aminodiol moiety, formed by hydrolysis of spiroxamine accounted for 1.8% TRR (0.016 mg/kg). Five glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy glucoside (M40) accounted for 4.7% TRR (0.040 mg/kg).

Post-extraction solids in immature Swiss chard after conventional and exhaustive extractions accounted for 0.3% TRR (0.003 mg/kg).

Swiss chard (mature)

A similar profile of extractability and identification of components was seen in the mature Swiss chard as in the immature plant. The total radioactive residue in mature Swiss chard was 0.676 mg/kg. A total of 93.0% TRR (0.629 mg/kg) was extractable. 64.2% TRR (0.434 mg/kg) was identified. Spiroxamine-hydroxyl (M05) comprised 12.8% TRR (0.086 mg/kg). The desalkylated metabolites, spiroxamine-despropyl (M02) (19.7% TRR; 0.133 mg/kg) and spiroxamine-desethyl (M01)(9.0% TRR, 0.061 mg/kg) were also detected. Parent spiroxamine accounted for 9.4% TRR (0.064 mg/kg). Six glycoside

conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy glucoside (M40) accounted for 3.8% TRR (0.025 mg/kg).

Post-extraction solids in mature Swiss chard after conventional and exhaustive extractions accounted for 7.0% TRR (0.048 mg/kg).

Turnips

The total radioactive residue in turnip leaves was 0.892 mg/kg and 0.101 mg/kg in roots.

In leaves, 97.8% TRR (0.873 mg/kg) was extractable with organic solvents. 72.2% TRR (0.644 mg/kg) was identified. The desalkylated metabolites, spiroxamine-despropyl (M02) (21.1% TRR, 0.188 mg/kg) and spiroxamine-desethyl (M01) (9.0% TRR, 0.061 mg/kg) comprised the main metabolites in turnip leaves. Parent spiroxamine accounted for 6.7% TRR (0.060 mg/kg). Six glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy glucoside (M40) accounted for 7.6% TRR (0.062 mg/kg).

Post-extraction solids in turnip leaves after conventional and exhaustive extractions accounted for 2.2% TRR (0.019 mg/kg).

In roots, 90.5% TRR (0.091 mg/kg) was extractable with organic solvents. 54.5% TRR (0.055 mg/kg) was identified. Exhaustive extraction of the PES following conventional extraction with diastase enzyme treatment released a further 18.9% TRR (0.019) that was polar in nature and not readily partitioned into organic solvent. This indicated that the majority of the radioactive residue in turnip roots was small carbon chain units that had been naturally incorporated into natural products such as sugars or starch. Spiroxamine-despropyl-aminodiol (M31) accounted for 6.1% TRR (0.006 mg/kg), spiroxamine-aminodiol-N-oxide (M29) for 4.8% TRR (0.005 mg/kg) and spiroxamine-aminodiol (M28) 4.9% TRR (0.005 mg/kg). Parent spiroxamine accounted for 3.5% TRR (0.003 mg/kg). Four glycoside conjugates were also detected in turnip roots. The largest of these, spiroxamine-despropyl acid glucoside (M39) accounted for 3.7% TRR (0.004 mg/kg).

Post-extraction solids in turnip roots after conventional and exhaustive extractions accounted for 9.5% TRR (0.010 mg/kg).

Table CA 6.6.1/02-7 Distribution of TRR in extracts and solids in wheat matrices 30 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]-spiroxamine

Sample	% TRR (mg/kg)							
	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine (KWG 4168)	32.9	0.195	8.5	0.169	15.2	0.485	2.7	0.004
Spiroxamine-despropyl-aminodiol (M31)	n/d	n/d	1.7	0.034	n/d	n/d	n/d	n/d
Spiroxamine-aminodiol-N-oxide (M29)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-aminodiol (M28)	n/d	n/d	0.3	0.006	0.2	0.008	n/d	n/d
Spiroxamine-despropyl-acid glycoside (M45)	1.3	0.008	1.5	0.029	0.6	0.018	n/d	n/d
Spiroxamine-hydroxy-despropyl-acid glycoside (M39)	2.1	0.012	1.6	0.033	0.5	0.015	n/d	n/d
Spiroxamine-desethyl-acid glycoside (M43)	0.6	0.004	3.3	0.065	0.5	0.017	n/d	n/d
Spiroxamine-hydroxy-desethyl-acid glycoside (M42)	n/d	n/d	6.5	0.129	0.8	0.025	n/d	n/d
Spiroxamine-acid glycoside (M44)	0.9	0.005	6.4	0.126	1.0	0.032	n/d	n/d
Spiroxamine-hydroxy glycoside (M40)	1.3	0.008	2.9	0.057	2.8	0.088	n/d	n/d
Spiroxamine-N-oxide (M03)	n/d	n/d	n/d	n/d	7.4	0.235	4.3	0.006
Spiroxamine-hydroxy (M05)	1.3	0.008	2.5	0.049	n/d	n/d	n/d	n/d
Spiroxamine-N-formyl-despropyl (M38)	n/d	n/d	n/d	n/d	7.6	0.243	2.7	0.003
Spiroxamine-N-formyl-desethyl (M04)	n/d	n/d	n/d	n/d	6.4	0.204	1.5	0.002
Spiroxamine-despropyl (M02)	23.4	0.139	15.2	0.302	17.4	0.553	4.8	0.006
Spiroxamine-desethyl (M01)	20.0	0.119	10.4	0.207	15.1	0.479	2.9	0.004
Diastase enzymatic digestion (incorporated $^{14}CO_2$)	-	-	-	-	-	-	69.5	0.091
Total identified	83.9	0.498	60.9	1.208	75.6	2.402	18.9	0.025
Total characterised	10.3	0.061	34.2	0.678	18.7	0.594	72.0	0.094
Total extractable	94.1	0.559	95.1	1.886	94.3	2.996	90.8	0.119
Post-extraction solids (PES)	5.9	0.035	4.9	0.097	5.7	0.182	9.2	0.012
Total radioactive residue (TRR)	100.0	0.593	100.0	1.983	100.0	3.178	100.0	0.131

n/d not detected

Table CA 6.6.1/02-8 Distribution of TRR in extracts and solids in Swiss chard and turnip matrices 30 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]-spiroxamine

Sample	% TRR (mg/kg)							
	Swiss chard (immature)		Swiss chard (mature)		Turnip leaves		Turnip roots	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine (KWG 4168)	9.4	0.080	9.4	0.064	6.7	0.060	3.5	0.003
Spiroxamine-despropyl-aminodiol (M31)	n/d	n/d	n/d	n/d	0.4	0.003	6.1	0.006
Spiroxamine-aminodiol-N-oxide (M29)	1.0	0.008	0.8	0.006	n/d	n/d	4.8	0.005
Spiroxamine-aminodiol (M28)	1.8	0.016	n/d	n/d	0.9	0.008	4.9	0.005
Spiroxamine-despropyl-acid glycoside (M45)	3.5	0.029	1.8	0.012	3.6	0.032	3.7	0.004
Spiroxamine-hydroxy-despropyl-acid glycoside (M39)	2.0	0.017	2.8	0.019	3.1	0.028	n/d	n/d
Spiroxamine-desethyl-acid glycoside (M43)	1.8	0.015	0.6	0.004	5.7	0.051	2.6	0.003
Spiroxamine-hydroxy-desethyl-acid glycoside (M42)	n/d	n/d	0.4	0.003	3.2	0.029	2.8	0.003
Spiroxamine-acid glycoside (M44)	2.2	0.019	3.1	0.021	7.0	0.062	1.9	0.002
Spiroxamine-hydroxy glycoside (M40)	4.7	0.040	3.8	0.025	7.6	0.068	n/d	n/d
Spiroxamine-N-oxide (group if of isomers (M03)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-hydroxy (M05)	17.2	0.146	12.8	0.086	3.6	0.032	0.8	0.001
Spiroxamine-N-formyl-despropyl (M38)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-N-formyl-desethyl (M04)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-despropyl (M02)	15.0	0.127	19.7	0.133	21.1	0.188	2.9	0.003
Spiroxamine-desethyl (M01)	6.6	0.056	9.0	0.061	9.3	0.083	1.5	0.001
Diastase enzymatic digestion (incorporated $^{14}CO_2$)	-	-	-	-	n/d	n/d	18.9	0.019
Total identified	65.3	0.552	64.2	0.434	72.2	0.644	54.5	0.055
Total characterised	34.4	0.291	28.9	0.194	25.6	0.228	35.9	0.036
Total extractable	99.7	0.844	93.0	0.629	97.8	0.873	90.5	0.091
Post-extraction solids (PES)	0.3	0.003	7.0	0.048	2.2	0.019	9.5	0.010
Total radioactive residue (TRR)	100.0	0.846	100.0	0.676	100.0	0.892	100.0	0.101

n/d not detected

Storage stability

An assessment of storage stability was not required as all plant samples were extracted within 15 days of sampling and stored at approximately -20°C.

III. Conclusions

A single application of ^{14}C-spiroxamine (1,3-dioxolane-4-^{14}C) was made to bare sandy loam soil in a 1 m^2 container at a rate of 1.62 kg a.s./ha as an emulsifiable concentrate (EC 500) formulation. This rate is just below 2.2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Crops were planted at 30 days after application (DAT), first rotation, 193 DAA (second rotation) and 294 DAA (third rotation). The planting container was maintained in a greenhouse.

Seeds of wheat (*Triticum aestivum,* Thasos), Swiss chard (*Beta vulgaris,* Lukullus) and turnips (*Brassica rapa,* Rondo) were sown into the soil at 30, 193 and 294 DAA and crops grown to maturity. Crop samples collected were immature Swiss chard (sampled at BBCH 35), wheat forage (BBCH 29), wheat hay (BBCH 65 – 71). Additionally at maturity, wheat straw, wheat grain, Swiss chard, turnip root and turnip leaves were also harvested.

In crops harvested from the first rotation, the total radioactive residue (TRR) in wheat forage was 0.593 mg/kg (measured as mg spiroxamine equivalents per kg), 1.983 mg/kg in wheat hay and 3.178 mg/kg in wheat straw. In wheat grain, the TRR was 0.131 mg/kg. Corresponding TRR in immature Swiss chard was 0.846mg/kg and 0.676 mg/kg in mature Swiss chard. In the root crop (turnip) residue levels of 0.892 mg/kg in leaves and 0.101 mg/kg in roots were determined.

The majority of the radioactive residue in most crop matrices was readily extractable with conventional extracts (acetonitrile:water 8:2 v/v), with the exception of wheat grain where only 21% of the TRR was extractable with the initial solvent used. The remaining radioactivity in plant matrices could be exhaustively extracted using a range of solvents. Additionally in grain, enzyme digestion with diastase released a significant proportion of the non-extracted residue (70% TRR). Final non-extractable or bound residues (PES) accounted for <10% TRR in all matrices.

Parent spiroxamine and the two desalkylated metabolites, spiroxamine-despropyl and spiroxamine-desethyl (M02 and M01) comprised the majority of the radioactive residue in all crop matrices harvested from the 30 day plant-back interval. In wheat matrices, spiroxamine accounted for between 2.7% TRR in grain to 32.9% TRR (0.195 mg/kg) in wheat forage. Spiroxamine-despropyl (M02) ranged from 4.8% TRR in grain to 23.4% TRR (0.139 mg/kg) in forage. Corresponding levels of spiroxamine-desethyl (M01) were lower, ranging from 2.9% TRR in grain to 20.0% TRR in forage (0.119 mg/kg). The same three metabolites also comprised the majority of the residue in Swiss chard and turnips planted at the first rotation. Parent spiroxamine accounted for between 4 and 9% TRR in these crops, the spiroxamine-despropyl (M02) ranged from 3% TRR in turnip roots to 21.1% TRR (0.188 mg/kg) in turnip leaves. Corresponding levels of spiroxamine-desethyl (M01) were approximately half those seen of the despropyl moiety, ranging from 1.5% TRR in roots to 9.3% TRR in turnip leaves. A number of glycoside conjugates (up to 6) were also detected in all rotated crop matrices, with the exception of wheat grain. In this matrix, further digestion of the PES was conducted with diastase enzyme releasing significant additional radioactivity (69.5% TRR, 0.091 mg/kg). This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine B6, 2009, Volume 3 RAR, IIA 6.2.2/02.

The nature of the residue in rotated crops when spiroxamine is applied to bare soil is essentially the same as found in the primary wheat metabolism studies. Parent spiroxamine and the two desalkylated metabolites, spiroxamine-despropyl and spiroxamine-desethyl (M02 and M01) comprised the majority of the radioactive residue in all crop matrices harvested from the 30 day plant-back interval. A number of glycoside conjugates were also detected in rotated crop matrices, with the exception of wheat grain. Grain and turnip root residues were further digested with diastase enzyme releasing significant additional radioactivity (up to 70% TRR). This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

Data Point:	KCA 6.6.1/03
Report Author:	[redacted]
Report Year:	2009
Report Title:	Metabolism of [1,3-dioxolane-4-14C]KWG 4168 in confined rotational crops - Second part: 2nd and 3rd rotation
Report No:	MEF-09/131
Document No:	M-344278-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1850; Canadian PMRA Ref.: DACO 6.3; OECD 502
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A single application of ^{14}C-spiroxamine (1,3-dioxolane-4-^{14}C) was made to bare sandy loam soil in a 1 m^2 container at a rate of 1.62 kg a.s./ha as an emulsifiable concentrate (EC 500) formulation. This rate is just below 2.2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Crops were planted at 30 days after application (DAA), first rotation, 193 DAA (second rotation) and 294 DAA (third rotation). The planting container was maintained in a greenhouse. Full details of the field phase and analysis of crops from the first rotation (30 DAA) are given in the previous summary; CA 6.6.1/02. This summary describes the magnitude and nature of the residues in crop commodities from the second and third rotation of the same study.

Seeds of wheat (*Triticum aestivum,* Thasos), Swiss chard (*Beta vulgaris,* Lukullus) and turnips (*Brassica rapa,* Rondo) were sown into the soil at 30, 193 and 294 DAA and crops grown to maturity. Crop samples collected were immature Swiss chard (sampled at BBCH 35), wheat forage (BBCH 29), wheat hay (BBCH 65 – 71). Additionally at maturity, wheat straw, wheat grain, Swiss chard, turnip root and turnip leaves were also harvested.

The total radioactive residues (TRRs) in plant matrices were determined by summation of the radioactivity in organic solvent extracts and that in the residual post-extraction solids. TRRs in all samples (except wheat forage, hay and grain) decreased between the first plant-back interval (30 days)

and the second (193 days). Residues in all matrices had declined further by the third rotation (294 DAA).

The TRR in wheat forage was 0.766 mg/kg (measured as mg spiroxamine equivalents per kg) in crops harvested from the second rotation (193 DAA) and 0.407 mg/kg at the third rotation (294 DAA). In wheat hay, the TRRs were 3.924 and 0.832 mg/kg at each of the 2 rotations (193 and 294 DAA). In wheat straw, the TRRs were 2.631 and 0.986 mg/kg (193 and 294 DAA respectively). In wheat grain, the TRRs were 0.223 and 0.092 mg/kg (193 and 294 DAA respectively).

Corresponding TRRs in immature Swiss chard were 0.410 and 0.204 mg/kg at each rotation (193 and 294 DAA). In mature Swiss chard, TRRs were 0.348 and 0.104 mg/kg (193 and 294 DAT respectively). The final crop group with turnip as a representative crop saw residue levels of 0.298 and 0.237 mg/kg in leaves (193 and 294 DAA) and 0.026 and 0.012 mg/kg in roots (193 and 294 DAA respectively).

The majority of the radioactive residue in most crop matrices was generally readily extractable. In wheat matrices (with the exception of grain), between 62 and 75% TRR was extracted with conventional extracts (acetonitrile:water 8:2 v/v), with extractability generally declining slightly over time. In grain, only 9 - 15% TRR was extractable using these solvents. In both immature and mature Swiss chard, the residue was almost entirely extractable, with approximately 90% TRR extractable at all timepoints. The extractability was similar in turnip roots and leaves with approximately 92% TRR readily extractable in leaves and only slightly lower in roots (79 - 92% TRR). The remaining radioactivity could be exhaustively extracted using a range of solvents. Additionally in grain, enzyme digestion with diastase released a significant proportion of the non-extracted residue (68 - 77% TRR). Final non-extractable or bound residues (PES) accounted for between 6 - 17% TRR in all wheat matrices, 9 - 10% TRR in Swiss chard and approximately 7.5% TRR in turnip leaves and 13 - 21% TRR in roots, although residues in roots were low, with 21% TRR accounting for 0.002 mg/kg.

Amounts of parent spiroxamine decreased in crops harvested following the second and third rotations (crops planted at 193 and 294 DAA). At the second interval, the two desalkylated metabolites, spiroxamine-despropyl and spiroxamine-desethyl (M02 and M01) comprised the majority of the radioactive residue in all crop matrices. In wheat, matrices, spiroxamine-despropyl (M02)ranged from 14.3% TRR in straw to almost 27% TRR in forage. Residues of this metabolite were not detected in grain. Corresponding levels of spiroxamine-desethyl (M01) were lower, ranging from 6.2% TRR in straw to 9.3% TRR in forage. Again, residues of this metabolite were not detected in grain. These two species also comprised the majority of the residue in Swiss chard and turnips planted at the second rotation, with levels of spiroxamine-despropyl (M02) ranging from 11% TRR in immature Swiss chard to 19% TRR in the mature crop. Corresponding levels of spiroxamine-desethyl (M01) were much lower than the despropyl moiety, accounting for <4% TRR. Neither metabolite was determined in turnip roots. A number of glycoside conjugates (up to 6) were again detected in all rotated crop matrices, with the exception of wheat grain. In this matrix and also in the highly starchy turnip root, further digestion of the PES was conducted with diastase enzyme releasing significant additional radioactivity (67.7% TRR, 0.151 mg/kg in grain and 41.8%, 0.011 mg/kg in roots). This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

At the third interval, the two desalkylated metabolites, spiroxamine-despropyl and spiroxamine-desethyl (M02 and M01) still comprised the majority of the radioactive residue in all crop matrices. In wheat, matrices, spiroxamine-despropyl (M02) ranged from 17.4% TRR in straw to almost 47% TRR in forage. Residues of this metabolite were not detected in grain. Corresponding levels of spiroxamine-desethyl (M01) were much lower, ranging from 5.6% TRR in straw to almost 14% TRR in forage. Again, residues of this metabolite were not detected in grain. These two metabolites also comprised the majority of the residue in Swiss chard and turnips planted at the second rotation, with levels of spiroxamine-despropyl (M02) accounting for approximately 50% in Swiss chard and 19% TRR in turnip leaves. Corresponding levels of spiroxamine-desethyl (M01) were much lower than the despropyl moiety, accounting for <13% TRR. Neither metabolite was determined in turnip roots. A number of glycoside conjugates (up to 6) were again detected in all rotated crop matrices, with the exception of

wheat grain and turnip roots. In these matrices, further digestion of the PES was conducted with diastase enzyme releasing significant additional radioactivity in grain, 76.5% TRR, 0.071 mg/kg. Levels were too low in turnip roots to analyse further. This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

I. Materials and methods

Full details of the application to bare soil, soil characteristics are given in the previous summary (CA 6.6.1/02)

A. Study design

The experimental work started on October 30, 2007 with the final samples analysed on February 17 2009 (PES analysis of grain from the third rotation).

The study was conducted at Bayer CropScience AG, Development, Metabolism / Environmental Fate, D-40789 Monheim am Rhein, Germany.

Experimental conditions

Application

The bare soil was treated on November 05 2007.

Planting and sampling of following crops

193 and 294 DAA: (second and third rotation)

Before each sowing of the crops, the upper soil layer was intensively mixed.

Wheat

Wheat forage was taken at BBCH 29 (239 and 325 DAA).

Wheat hay was taken at BBCH 65 - 71 (276 and 395 DAA).

Wheat straw and grain were harvested together at BBCH 89 (294 and 430 DAA).

Turnip leaves and roots

Turnip leaves and roots were harvested together at maturity (BBCH 45 – 49, 267 and 357 DAA).

Swiss chard

Immature Swiss chard was harvested at BBCH 35 (231 and 325 DAA) and again at maturity (BBCH 49, 254 and 340 DAA).

All crop samples were homogenised as described for the 30 DAA rotated samples (see previous summary CA 6.6.1/02).

Aliquots of all plant homogenates were used for extraction. All samples were stored frozen at approximately -20°C.

The sampling schedule for all crops is summarised in Table CA 6.6.1/03-1.

Table CA 6.6.1/03-1 Sampling schedule for rotational crops planted 193 and 294 days after application to bare soil

Sample	Sampling occasion (days after application) / BBCH at sampling	
	Second rotation (193 DAA to bare soil)	Third rotation (294 DAA to bare soil)
Wheat - Forage	239 / BBCH 29	325 / BBCH 29
Wheat - Hay	276 / BBCH 65 - 71	395 / BBCH 65 - 71

Sample	Sampling occasion (days after application) / BBCH at sampling	
	Second rotation (193 DAA to bare soil)	Third rotation (294 DAA to bare soil)
Wheat - Straw	294 / BBCH 89 (maturity)	430 / BBCH 89 (maturity)
Wheat - Grain	294 / BBCH 89 (maturity)	430 / BBCH 89 (maturity)
Swiss chard - immature	231 / BBCH 35	325 / BBCH 35
Swiss chard - mature	254 / BBCH 49 (maturity)	340 / BBCH 49 (maturity)
Turnip - Roots	267 / BBCH 45 - 49 (maturity)	357 / BBCH 45 - 49 (maturity)
Turnip - Foliage	267 / BBCH 45 - 49 (maturity)	357 / BBCH 45 - 49 (maturity)

PBI = plant back interval

Sample preparation and extraction

Plants

Aliquots of the homogenised plant samples were extracted three times with acetonitrile/water (8:2 v/v) and once with acetonitrile. The extractions were conducted using a Polytron. The post-extraction solids (PES's) were air-dried and weighed. The total radioactive residues (TRRs) of the plant fractions were determined by summation of the radioactivity in the acetonitrile/water extracts and The post-extraction solids (PES). Residues were expressed as mg/kg spiroxamine equivalents.

Concentrated extracts were examined by TLC and HPLC. Concentration recoveries ranged from 95.2 to 100.9%.

Exhaustive extraction of PES

Aliquots of solids were extracted with mixtures of acetonitrile/water (7:3 v/v), acetonitrile/water (1:1 v/v), acetonitrile/water (1:1 v/v) containing 2.5 mL formic acid (and occasionally 0.5 N hydrochloric acid). Each extraction was conducted for 20 minutes using microwave assistance. The radioactivity in the dried PES was determined. Recoveries of exhaustive extraction ranged from 103 to 105%.

Concentrated combined exhaustive extracts were examined by HPLC. Additional investigations of wheat grain were performed by TLC. Concentration recoveries ranged from 95.3 to 99.3%.

Digestion of solids in wheat grain

An aliquot of the solids of wheat grain was digested with *Diastase* enzyme for 13 days. The radioactivity in the digestion solution and the PES were determined. The recovery of material was 101.9%. The digest was partitioned with ethyl acetate and the radioactive residue characterised.

The radioactivity in the organic phases was determined by liquid scintillation counting (LSC) and in the solids by combustion.

Combustion analysis:

Solid samples were combusted using a Harvey Ox 500 Tissue Oxidiser (Zinsser-Analytic). The resultant $^{14}CO_2$ was absorbed in scintillation cocktail (Oxysolve C-400, Zinsser) and radioactivity measured by LSC.

Liquid scintillation counting (LSC):

Liquid samples were counted in a liquid scintillation counter using a commercial scintillation cocktail (Quick-Safe A, Zinsser Analytic) containing 5% water. Samples were generally measured in triplicate.

Thin layer chromatography (TLC)

The radioactive solutions were analysed by one dimensional TLC using silica gel (60 WF, Merck), which were eluted with the following solvent systems:

System 1	Chloroform/methanol/ammonia (25%)	65:28:8 v/v/v
System 2	Acetonitrile/water/ammonia (25%)	320:18:2 v/v/v

Radioactive components were detected using a Fuji Imaging Analyser with a software peak integration package ('Tina', Raytest). The reference materials used in co-chromatography were detected using iodine vapour or by spraying with vanillin/sulphuric acid and heating the plate to 120°C.

For identification of sugar compounds, the TLC plate was sprayed with a solution of aniline phthalate (Fluka). After heating at 120°C for approximately 30 minutes, a typical colour reaction proved the presence of sugars.

High performance liquid chromatography (HPLC

Parent compound and metabolites in the combined extracts were quantified, isolated and analysed by HPLC using the following system:

Agilent 1100 liquid chromatograph was used with oven temperature set at 40°C, UV wavelength detector set at 254 nm. The chromatograph was coupled to a radioactivity detector (Raytest, Ramona) with a glass scintillator cell.

Data was evaluated using Raytest GINA-START software.

The column used was a Purospher STAR PR-18e, 250 x 4.6 mm i.d.

Three different mobile phase gradient systems were used:

1. For stability and purity check; (gradient moving from 100% A to 100% B over 30 minutes)

eluent A: water/formic acid (99/1 v/v)
eluent B: acetonitrile/water/formic acid (97/2/1 v/v/v)

2. For profiling, quantitation, isolation and co-chromatography of parent compound and metabolites; (gradient moving from 100% A to 100% B over 90 minutes)

eluent A: .0.05% triethylamine in water
eluent B: 0.05% triethylamine in acetonitrile

3. For isolation of metabolites; (gradient moving from 100% A to 100% B over 90 minutes)

eluent A: water/formic acid (99/1 v/v)
eluent B: acetonitrile/water/formic acid (97/2/1 v/v/v)

Mass spectroscopy (MS)

Selected sample extracts were analysed by MS. The electro-spray ionisation MS spectra (ESI) were obtained with either a TSQ 7000 instrument or with a LTQ Orbitrap XL mass spectrometer (Finnegan).

II. Results and discussion

Total radioactive residues (TRR)

TRR data for rotational crop plant matrices are summarised in Table CA 6.6.1/03-2. Summaries of extractable radioactivity in plant matrices are presented in Table CA 6.6.1/03-3 (wheat), Table CA 6.6.1/03-4 (Swiss chard) and Table CA 6.6.1/03-5 (turnip).

The TRRs in Swiss chard, turnip roots and leaves were determined by the summation of radioactivity in the conventional extracts, exhaustive extracts, diastase enzyme digest (where conducted) and The post-extraction solids (PES). Residues are expressed as mg/kg parent spiroxamine equivalents.

193 DAA samples (second rotation)

Wheat

The total radioactive residue (TRR) in wheat planted 193 days after application to bare soil was 0.766 mg/kg in forage, 3.924 mg/kg in hay, 2.631 mg/kg in straw and 0.223 mg/kg in grain. In forage, 71.8% TRR (0.550 mg/kg) was readily extractable with conventional solvents (acetonitrile:water, 8:2 v/v). In hay, 66.9% TRR (2.624 mg/kg) was readily extractable, 74.1% TRR (1.951 mg/kg) in straw, and 15.4% (0.034 mg/kg) in grain. Exhaustive extraction released a further 21.9% TRR (0.168 mg/kg) in forage, 27.3% (1.07 mg/kg) in hay, 19.5% (0.512 mg/kg) in straw and 41.8% (0.093 mg/kg) in grain. The grain PES after conventional extraction was also treated with diastase enzyme, releasing 67.7% TRR (0.151 mg/kg) indicating radioactivity that was naturally incorporated into $^{14}CO_2$.

Swiss chard

The total radioactive residue (TRR) in immature Swiss chard planted 193 days after application to bare soil was 0.410 mg/kg. Organo-extractable radioactivity accounted for 90.8% TRR (0.372 mg/kg). The post-extraction solid (PES) accounted for 9.2% TRR (0.038 mg/kg). The total radioactive residue (TRR) in mature Swiss chard was 0.348 mg/kg. Organo-extractable radioactivity accounted for 89.9% TRR (0.313 mg/kg). The PES accounted for 10.1% TRR (0.035 mg/kg).

Turnips

The total radioactive residue (TRR) in turnips planted 193 days after application to bare soil was 0.298 mg/kg in leaves and 0.026 mg/kg in roots. In leaves, 92.9% TRR (0.277 mg/kg) was extractable with organic solvent. The post-extraction solid (PES) accounted for 7.1% TRR (0.021 mg/kg). In the roots, 86.9% TRR (0.023 mg/kg) was extractable with organic solvent. The PES accounted for 13.1% TRR (0.003 mg/kg).

294 DAA samples (third rotation)

Wheat

The total radioactive residue (TRR) in wheat planted 294 days after application to bare soil was 0.407 mg/kg in forage, 0.832 mg/kg in hay, 0.986 mg/kg in straw and 0.092 mg/kg in grain. In forage, 66.9% TRR (0.272 mg/kg) was readily extractable with conventional solvents (acetonitrile:water, 8:2 v/v). In hay, 62.3% TRR (0.518 mg/kg) was readily extractable, 75.1% TRR (0.740 mg/kg) in straw and 9.1% (0.008 mg/kg) in grain. Exhaustive extraction released a further 25.4% TRR (0.104 mg/kg) in forage, 27.5% (0.229 mg/kg) in hay, 18.0% (0.178 mg/kg) in straw and 36.3% (0.033 mg/kg) in grain. The grain PES after conventional extraction was also treated with diastase enzyme, releasing 76.5% TRR (0.071mg/kg) indicating radioactivity that was naturally incorporated into $^{14}CO_2$.

Swiss chard

The total radioactive residue (TRR) in immature Swiss chard planted 294 days after application to bare soil was 0.204 mg/kg. Organo-extractable radioactivity accounted for 90.9% TRR (0.185 mg/kg). The post-extraction solid (PES) accounted for 9.1% TRR (0.019 mg/kg). The total radioactive residue (TRR) in mature Swiss chard was 0.104 mg/kg. Organo-extractable radioactivity accounted for 90.7% TRR (0.094 mg/kg). The PES accounted for 9.3% TRR (0.010 mg/kg).

Turnips

The total radioactive residue (TRR) in turnips planted 294 days after application to bare soil was 0.237 mg/kg in leaves and 0.012 mg/kg in roots. In leaves, 91.9% TRR (0.218 mg/kg) was extractable with organic solvent. The post-extraction solid (PES) accounted for 8.1% TRR (0.019 mg/kg). In the

roots, 78.7% TRR (0.009 mg/kg) was extractable with organic solvent. The PES accounted for 21.3% TRR (0.002 mg/kg).

Table CA 6.6.1/03-2 Total radioactive residues (TRR) in rotational crops sown 193 and 294 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

	Wheat[1]				Swiss chard[1]		Turnip[1]	
	Forage (mg/kg)	Hay (mg/kg)	Straw (mg/kg)	Grain (mg/kg)	Immature (mg/kg)	Mature (mg/kg)	Leaves (mg/kg)	Roots (mg/kg)
193 DAA	0.766	3.924	2.631	0.223	0.410	0.348	0.298	0.026
294 DAA	0.407	0.832	0.986	0.092	0.204	0.104	0.263	0.012

1 - TRR values were determined by summation of radioactivity in extracts and PES

Table CA 6.6.1/03-3 Distribution of TRR in extracts and solids in wheat matrices sown 193 and 294 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)							
	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
193 DAA (second rotation)								
Conventional extracts	71.8	0.550	66.9	2.624	74.1	1.951	15.4	0.034
Exhaustive extracts								
Acetonitrile/water (7:3 v/v)	14.2	0.109	19.7	0.773	16.0	0.421	15.2	0.034
Acetonitrile/water (1:1 v/v)	5.3	0.041	5.6	0.218	2.4	0.063	19.4	0.043
Acetonitrile/water (1:1 v/v) + 2.5 mL formic acid	2.4	0.018	2.0	0.079	1.1	0.028	7.2	0.016
Incorporated $^{14}CO_2$ Diastase digest (aqueous phase)	-	-	-	-	-	-	67.7	0.151
PES	6.3	0.048	5.8	0.229	6.4	0.169	16.9	0.038
Total	100.0	0.766	100.0	3.924	100.0	2.631	100.0	0.223
294 DAA (third rotation)								
Conventional extracts	66.9	0.272	62.3	0.518	75.1	0.740	9.1	0.008
Exhaustive extracts								
Acetonitrile/water (7:3 v/v)	15.8	0.065	12.3	0.103	7.9	0.078	36.3	0.033
Acetonitrile/water (1:1 v/v)	5.2	0.021	15.2	0.126	6.8	0.067	n/d	n/d
Acetonitrile/water (1:1 v/v) + 2.5 mL formic acid	4.4	0.018	n/d	n/d	3.3	0.033	36.3	0.033
Incorporated $^{14}CO_2$ Diastase digest (aqueous phase)	-	-	-	-	-	-	76.5	0.071
PES	7.7	0.031	10.2	0.085	6.9	0.068	14.4	0.013
Total	100.0	0.407	100.0	0.832	100.0	0.986	100.0	0.092

n/d not detected

Table CA 6.6.1/03-4 Distribution of TRR in extracts and solids in Swiss chard matrices sown 193 and 294 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)			
	Swiss chard (immature)		Swiss chard (maturity)	
	% TRR	mg/kg	% TRR	mg/kg
193 DAA (second rotation)				
Conventional extracts	90.8	0.372	89.9	0.313
PES	9.2	0.038	10.1	0.035
Total	100.0	0.410	100.0	0.348
294 DAA (third rotation)				
Conventional extracts	90.9	0.185	90.7	0.094
PES	9.1	0.019	9.3	0.010
Total	100.0	0.204	100.0	0.104

n/d not detected

Table CA 6.6.1/03-5 Distribution of TRR in extracts and solids in turnip matrices sown 193 and 294 days after bare soil treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)			
	Turnip leaves		Turnip roots	
	% TRR	mg/kg	% TRR	mg/kg
193 DAA (second rotation)				
Conventional extracts	92.9	0.277	86.9	0.023
PES	7.1	0.021	13.1	0.003
Total	100.0	0.298	100.0	0.026
294 DAA (third rotation)				
Conventional extracts	91.9	0.218	78.7	0.009
PES	8.1	0.019	21.3	0.002
Total	100.0	0.237	100.0	0.012

Characterisation

Available standard compounds for characterisation were parent test substance (spiroxamine; KWG 4168) and the following potential metabolites:

WAK 6893 spiroxamine-despropyl-aminodiol (M31)

WAK 6885 spiroxamine-aminodiol-N-oxide (M29)

WAK 5427 spiroxamine-aminodiol (M28)

Spiroxamine-despropyl-acid glycoside (M45)

Spiroxamine-hydroxy-despropyl glycoside (M39)

Spiroxamine-desethyl-acid glycoside (M43)

Spiroxamine-hydroxy-desethyl glycoside (M42)

Spiroxamine-acid glycoside (M44)

Spiroxamine-hydroxy glycoside (M40)

WAK 6301 spiroxamine-N-oxide (M03)

WAK 5868 spiroxamine-hydroxyl (M05)

Spiroxamine-N-formyl-despropyl (M38)

WAK 6782 spiroxamine-N-formyl-desethyl (M04)

KWG 4557 spiroxamine-desethyl (M01)

KWG 4669 spiroxamine-despropyl (M02)

WAK 6894 spiroxamine-desethyl-aminodiol (M30)

Each metabolite was identified by co-chromatography with the standard compounds using mainly HPLC, but selected samples also analysed by LC-MS, LC-MS/MS and TLC.

193 DAA crop matrices (second rotation)

The distribution of parent spiroxamine and metabolites in wheat matrices 193 DAA is shown in Table CA 6.6.1/03-6 and the distribution of spiroxamine and metabolites in Swiss chard and turnip matrices, 193 DAA is shown in Table CA 6.6.1/03-7.

Wheat forage

The total radioactive residue in wheat forage planted 193 DAA following a single application of dioxolane labelled spiroxamine to bare soil at a rate of 1.62 kg a.s./ha was 0.766 mg/kg. A total of 93.7% TRR (0.718 mg/kg) was extractable. 54.3% TRR in wheat forage (0.416 mg/kg) was identified. The main components of the residue in this matrix were the desalkylated metabolites, spiroxamine-despropyl (M02) (26.8% TRR; 0.205 mg/kg) and spiroxamine-desethyl (M01) (9.3% TRR, 0.072 mg/kg). A further six minor metabolites were detected in wheat forage and these were glycoside conjugates. The largest of these, spiroxamine-hydroxy-desethyl glucoside (M42) accounted for 3.4% TRR (0.026 mg/kg).

Post-extraction solids in wheat forage after conventional and exhaustive extractions accounted for 6.3% TRR (0.048 mg/kg).

Wheat hay

The total radioactive residue in wheat hay planted 193 DAA following a single application of dioxolane labelled spiroxamine was 3.924 mg/kg. A total of 94.2% TRR (3.694 mg/kg) was extractable. Almost 60% TRR in wheat hay (2.297 mg/kg) was identified. Desalkylation of the parent compound, formed the two main metabolites in wheat hay, spiroxamine-despropyl (M02) (19.8% TRR; 0.776 mg/kg) and spiroxamine-desethyl (M01) (7.4% TRR, 0.292 mg/kg). Parent spiroxamine accounted for 4.0% TRR (0.156 mg/kg). The spiroxamine-N-formyl despropyl (M38) moiety comprised 4.8% TRR (0.189 mg/kg). Six glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy-despropyl glucoside (M39) accounted for 5.9% TRR (0.232 mg/kg).

Post-extraction solids in wheat hay after conventional and exhaustive extractions accounted for 5.8% TRR (0.229 mg/kg).

Wheat straw

The total radioactive residue in wheat straw planted 193 DAA following a single application of dioxolane labelled spiroxamine was 2.631 mg/kg. A total of 93.6% TRR (2.462 mg/kg) was extractable. 57% TRR in wheat straw (1.500 mg/kg) was identified. As seen in other wheat matrices, desalkylation of the parent compound, formed the two main metabolites in wheat straw, spiroxamine-despropyl (M02) (14.3% TRR; 0.376 mg/kg) and spiroxamine-desethyl (M01) (6.2% TRR, 0.163 mg/kg). Parent spiroxamine accounted for 3.0% TRR (0.078 mg/kg). The N-formyl derivatives of the desalkylated species accounted for 6.9% TRR (0.181 mg/kg) spiroxamine-N-formyl-despropyl (M38) and 2.8% TRR (0.075 mg/kg) spiroxamine-N-formyl-desethyl (M04). Oxidation of spiroxamine in the tertiary amine group formed the spiroxamine-N-oxide (M03), accounting for 5.0% TRR (0.132 mg/kg) in wheat straw. Glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine hydroxy-desethyl glucoside (M42) accounted for 4.7% TRR (0.124 mg/kg).

Post-extraction solids in wheat straw after conventional and exhaustive extractions accounted for 6.4% TRR (0.169 mg/kg).

Wheat grain

The total radioactive residue in wheat grain planted 193 DAA following a single application of dioxolane labelled spiroxamine was 0.223 mg/kg. Conventional extractions released 15.4% TRR (0.034 mg/kg). Exhaustive extraction of the PES following conventional extraction with diastase enzyme treatment released a further 67.7% TRR (0.151 mg/kg) that was polar in nature and not readily partitioned into organic solvent. This indicated that the majority of the radioactive residue in wheat grain was small carbon chain units that had been naturally incorporated into natural products such as sugars or starch. None of the extractable radioactivity co-chromatographed with reference materials.

Post-extraction solids in wheat grain after conventional and exhaustive extractions accounted for 16.8% TRR (0.038 mg/kg).

Swiss chard (immature)

The total radioactive residue in immature Swiss chard planted 193 DAA following a single application of dioxolane labelled spiroxamine was 0.410 mg/kg. A total of 90.8% TRR (0.372 mg/kg) was extractable. 37.4% TRR in Swiss chard (0.153 mg/kg) was identified. The desalkylated metabolites, spiroxamine-despropyl (M02) (11.0% TRR; 0.045 mg/kg) and spiroxamine-desethyl (M01) (3.1% TRR, 0.012 mg/kg) were the most abundant in this matrix. Parent spiroxamine accounted for 2.4% TRR (0.010 mg/kg). The spiroxamine-aminodiol-N-oxide moiety (M29) accounted for 5.2% TRR (0.021 mg/kg). Three glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-despropyl-acid glucoside (M45) accounted for 6.0% TRR (0.025 mg/kg).

Post-extraction solids in immature Swiss chard after conventional and exhaustive extractions accounted for 9.2% TRR (0.038 mg/kg).

Swiss chard (mature)

A similar profile of extractability and identification of components was seen in the mature Swiss chard as in the immature plant. The total radioactive residue in mature Swiss chard was 0.348 mg/kg. A total of 89.9% TRR (0.313 mg/kg) was extractable. 42.0% TRR (0.146 mg/kg) was identified. The desalkylated metabolites, spiroxamine-despropyl (M02) (19.0% TRR; 0.066 mg/kg) and spiroxamine-desethyl (M01) (4.0% TRR, 0.014 mg/kg) were the major metabolites. Parent spiroxamine accounted for 3.3% TRR (0.011 mg/kg). Four glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-despropyl acid glucoside (M45) accounted for 5.5% TRR (0.019 mg/kg). The spiroxamine-aminodiol (M28) moiety accounted for a further 3.9% TRR (0.014 mg/kg).

Post-extraction solids in mature Swiss chard after conventional and exhaustive extractions accounted for 10.1% TRR (0.035 mg/kg).

Turnips

The total radioactive residue in turnip leaves was 0.298 mg/kg and 0.026 mg/kg in roots.

In leaves, 92.9% TRR (0.277 mg/kg) was extractable with organic solvents. 67.5% TRR (0.201 mg/kg) was identified. Five glycoside conjugates comprised the majority of the identified TRR. The largest of these were spiroxamine-hydroxy-despropyl glucoside (M39) accounting for 21.3% TRR (0.063 mg/kg) and spiroxamine-hydroxy-desethyl glucoside (M42) accounting for 14.6% TRR (0.044 mg/kg). The desalkylated metabolites, spiroxamine-despropyl (M02) (14.5% TRR; 0.043 mg/kg) and spiroxamine-desethyl (M01) (3.3% TRR, 0.010 mg/kg) comprised the majority of the remainder of the identified residue. Parent spiroxamine accounted for 2.2% TRR (0.007 mg/kg).

Post-extraction solids in turnip leaves after conventional and exhaustive extractions accounted for 7.1% TRR (0.021 mg/kg).

In roots, 86.9% TRR (0.023 mg/kg) was extractable with organic solvents. 59.7% TRR (0.016 mg/kg) was identified. Exhaustive extraction of the PES following conventional extraction with diastase enzyme treatment released a further 41.8% TRR (0.011) that was polar in nature and not readily partitioned into organic solvent. This indicated that the majority of the radioactive residue in turnip roots was small carbon chain units that had been naturally incorporated into natural products such as sugars or starch. Parent spiroxamine was not detected. The glycosidase conjugate spiroxamine-despropyl-acid glucoside (M45)accounted for 9.1% TRR (0.002 mg/kg) and the spiroxamine-aminodiol-N-oxide (M29) 4.7% TRR (0.001 mg/kg).

Post-extraction solids in turnip roots after conventional and exhaustive extractions accounted for 13.1% TRR (0.003 mg/kg).

294 DAA crop matrices (third rotation)

The distribution of parent spiroxamine and metabolites in wheat matrices 294 DAA is shown in Table CA 6.6.1/03-8 and the distribution of spiroxamine and metabolites in Swiss chard and turnip matrices 294 DAA is shown in Table CA 6.6.1/03-9.

Wheat forage

The total radioactive residue in wheat forage planted 294 DAA following a single application of dioxolane labelled spiroxamine to bare soil at a rate of 1.62 kg a.s./ha was 0.407 mg/kg. A total of 92.3% TRR (0.376 mg/kg) was extractable. 74.6% TRR in wheat forage (0.304 mg/kg) was identified. The main components of the residue in this matrix were the desalkylated metabolites, spiroxamine-despropyl (M02) (46.6% TRR, 0.190 mg/kg) and spiroxamine-desethyl (M01) (13.7% TRR, 0.056 mg/kg). A further three minor metabolites were detected in wheat forage and these were glycoside conjugates. The largest of these, spiroxamine-despropyl-acid glucoside (M45)accounted for 1.7% TRR (0.007 mg/kg).

Post-extraction solids in wheat forage after conventional and exhaustive extractions accounted for 7.7% TRR (0.031 mg/kg).

Wheat hay

The total radioactive residue in wheat hay planted 294 DAA following a single application of dioxolane labelled spiroxamine was 0.832 mg/kg. A total of 89.8% TRR (0.747 mg/kg) was extractable. Almost 70% TRR in wheat hay (0.574 mg/kg) was identified. Desalkylation of the parent compound, formed the two main metabolites in wheat hay, spiroxamine-despropyl (M02) (30.9% TRR; 0.257 mg/kg) and spiroxamine-desethyl (M01) (7.6% TRR, 0.063 mg/kg). Parent spiroxamine accounted for 6.5% TRR (0.054 mg/kg). The spiroxamine-N-formyl despropyl moiety (M38) comprised 6.8% TRR (0.056 mg/kg). Five glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy-despropyl glucoside (M39) accounted for 4.4% TRR (0.037 mg/kg).

Post-extraction solids in wheat hay after conventional and exhaustive extractions accounted for 10.2% TRR (0.085 mg/kg).

Wheat straw

The total radioactive residue in wheat straw planted 294 DAA following a single application of dioxolane labelled spiroxamine was 0.986 mg/kg. A total of 93.1% TRR (0.918 mg/kg) was extractable. Almost 60% TRR in wheat straw (0.588 mg/kg) was identified. As seen in other wheat matrices, desalkylation of the parent compound, formed two of the main metabolites in wheat straw, spiroxamine-despropyl (M02) (17.4% TRR; 0.172 mg/kg) and spiroxamine-desethyl (M01) (5.6% TRR, 0.055 mg/kg). Parent spiroxamine accounted for 4.2% TRR (0.041 mg/kg). Glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-hydroxy glucoside (M40) accounting for 12.6% TRR (0.124 mg/kg) and spiroxamine-acid glucoside (M44) accounting for 10.5% TRR (0.103 mg/kg).

Post-extraction solids in wheat straw after conventional and exhaustive extractions accounted for 6.9% TRR (0.068 mg/kg).

Wheat grain

The total radioactive residue in wheat grain planted 294 DAA following a single application of dioxolane labelled spiroxamine was 0.092 mg/kg. Conventional extractions released 9.1% TRR (0.008 mg/kg). Exhaustive extraction of the PES following conventional extraction with diastase enzyme treatment released a further 76.5% TRR (0.071 mg/kg) that was polar in nature and not readily partitioned into organic solvent. This indicated that the majority of the radioactive residue in wheat grain was small carbon chain units that had been naturally incorporated into natural products such as sugars or starch. None of the extractable radioactivity co-chromatographed with reference materials.

Post-extraction solids in wheat grain after conventional and exhaustive extractions accounted for 14.4% TRR (0.013 mg/kg).

Swiss chard (immature)

The total radioactive residue in immature Swiss chard planted 294 DAA following a single application of dioxolane labelled spiroxamine was 0.204 mg/kg. A total of 90.9% TRR (0.185 mg/kg) was extractable. 79.3% TRR in Swiss chard (0.162 mg/kg) was identified. The desalkylated metabolites, spiroxamine-despropyl (M02) (50.0% TRR; 0.102 mg/kg) and spiroxamine-desethyl (M01) (12.6% TRR, 0.026 mg/kg) were the most abundant in this matrix. Parent spiroxamine accounted for 8.2% TRR (0.017 mg/kg). Two glycoside conjugates comprised the majority of the remainder of the identified TRR. The largest of these, spiroxamine-acid glucoside (M44) accounted for 3.3% TRR (0.007 mg/kg).

Post-extraction solids in immature Swiss chard after conventional and exhaustive extractions accounted for 9.1% TRR (0.019 mg/kg).

Swiss chard (mature)

The total radioactive residue in mature Swiss chard was 0.104 mg/kg. A total of 90.7% TRR (0.094 mg/kg) was extractable. 76.2% TRR (0.079mg/kg) was identified. The desalkylated metabolites, spiroxamine-despropyl (M02) (51.2% TRR; 0.053 mg/kg) and spiroxamine-desethyl (M01) (12.3% TRR, 0.013 mg/kg) were the major metabolites. Parent spiroxamine accounted for 10.0% TRR (0.010 mg/kg). Spiroxamine-despropyl acid glucoside (M45) accounted for 2.8% TRR (0.003 mg/kg).

Post-extraction solids in mature Swiss chard after conventional and exhaustive extractions accounted for 9.3% TRR (0.010 mg/kg).

Turnips

The total radioactive residue in turnip leaves was 0.237 mg/kg and 0.012 mg/kg in roots.

In leaves, 91.9% TRR (0.218 mg/kg) was extractable with organic solvents. 62.8% TRR (0.149 mg/kg) was identified. Five glycoside conjugates comprised the majority of the identified TRR. The largest of these were spiroxamine-hydroxy-despropyl glucoside (M39) accounting for 13.2% TRR (0.031 mg/kg) and spiroxamine-acid glucoside (M44) accounting for 11.6% TRR (0.027 mg/kg). The desalkylated metabolites, spiroxamine-despropyl (M02) (18.8% TRR; 0.045 mg/kg) and spiroxamine-desethyl (M01) (3.1% TRR, 0.007 mg/kg) comprised the majority of the remainder of the identified residue. Parent spiroxamine was not detected.

Post-extraction solids in turnip leaves after conventional and exhaustive extractions accounted for 8.1% TRR (0.019 mg/kg).

In roots, 78.7% TRR (0.009 mg/kg) was extractable with organic solvents. Exhaustive extraction of the PES following conventional extraction with diastase enzyme treatment was conducted but not analysed due to high matrix loading and very low amounts of radioactivity in the extract. Parent spiroxamine was

not detected. It was not possible to identify the residue in turnip roots from this third rotation, due to very low levels of radioactivity and high amounts of co-extracted material.

Post-extraction solids in turnip roots after conventional and exhaustive extractions accounted for 21.3% TRR (0.002 mg/kg).

Table CA 6.6.1/03-6 Distribution of TRR in extracts and solids in wheat matrices 193 days after treatment with [1,3-dioxolane-4-^{14}C]-spiroxamine

Sample	% TRR (mg/kg)							
	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine (KWG 4168)	2.6	0.020	4.0	0.156	3.0	0.078	n/d	n/d
Spiroxamine-despropyl-aminodiol (M31)	1.6	0.012	1.4	0.056	1.4	0.037	n/d	n/d
Spiroxamine-aminodiol-N-oxide (M29)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-aminodiol (M28)	n/d	n/d	0.6	0.024	n/d	n/d	n/d	n/d
Spiroxamine-despropyl-acid glycoside (M45)	2.5	0.019	3.7	0.145	2.8	0.075	n/d	n/d
Spiroxamine-hydroxy-despropyl-acid glycoside (M39)	1.6	0.013	5.9	0.232	1.6	0.043	n/d	n/d
Spiroxamine-desethyl-acid glycoside (M43)	2.6	0.020	1.3	0.051	3.4	0.088	n/d	n/d
Spiroxamine-hydroxy-desethyl-acid glycoside (M42)	3.4	0.026	3.3	0.131	4.7	0.124		
Spiroxamine-acid glycoside (M44)	2.3	0.018	1.6	0.063	3.9	0.104	n/d	n/d
Spiroxamine-hydroxy glycoside (M40)	1.5	0.011	1.0	0.041	0.9	0.024	n/d	n/d
Spiroxamine-N-oxide (group of isomers (M03)	n/d	n/d	2.1	0.082	5.0	0.132	n/d	n/d
Spiroxamine-hydroxy (M05)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-N-formyl-despropyl (M38)	n/d	n/d	4.8	0.189	6.9	0.181	n/d	n/d
Spiroxamine-N-formyl-desethyl (M04)	n/d	n/d	1.5	0.060	2.8	0.075	n/d	n/d
Spiroxamine-despropyl (M02)	26.8	0.205	19.8	0.776	14.3	0.376	n/d	n/d
Spiroxamine-desethyl (M01)	9.3	0.072	7.4	0.292	6.2	0.163	n/d	n/d
Diastase enzymatic digestion (incorporated $^{14}CO_2$)	-	-	-	-	-	-	67.7	0.151
Total identified	54.2	0.416	58.5	2.297	57.0	1.500	n/d	n/d
Total characterised	39.4	0.302	35.6	1.398	36.6	0.962	83.1	0.185
Total extractable	93.7	0.718	94.2	3.694	93.6	2.462	83.1	0.185
Post-extraction solids (PES)	6.3	0.048	5.8	0.229	6.4	0.169	16.9	0.038
Total radioactive residue (TRR)	100.0	0.766	100.0	3.924	100.0	2.631	100.0	0.223

n/d not detected

Table CA 6.6.1/03-7 Distribution of TRR in extracts and solids in Swiss chard and turnip matrices 193 days after treatment with [1,3-dioxolane-4-^{14}C]spiroxamine

Sample	% TRR (mg/kg)							
	Swiss chard (immature)		Swiss chard (mature)		Turnip leaves		Turnip roots	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine (KWG 4168)	2.4	0.010	3.3	0.011	2.2	0.007	n/d	n/d
Spiroxamine-despropyl-aminodiol (M31)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-aminodiol-N-oxide (M29)	5.2	0.021	n/d	n/d	n/d	n/d	4.7	0.001
Spiroxamine-aminodiol (M28)	2.4	0.010	3.9	0.014	2.3	0.007	n/d	n/d
Spiroxamine-despropyl-acid glycoside (M45)	6.0	0.028	5.5	0.019	2.2	0.006	9.1	0.002
Spiroxamine-hydroxy-despropyl-acid glycoside (M39)	n/d	n/d	n/d	n/d	21.3	0.063	n/d	n/d
Spiroxamine-desethyl-acid glycoside (M43)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-hydroxy-desethyl-acid glycoside (M42)	n/d	n/d	1.6	0.005	14.6	0.044	n/d	n/d
Spiroxamine-acid glycoside (M44)	4.6	0.019	2.4	0.008	6.0	0.018	n/d	n/d
Spiroxamine-hydroxy glycoside (M40)	0.8	0.003	0.8	0.003	1.0	0.003	n/d	n/d
Spiroxamine-N-oxide (group of isomers (M03)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-hydroxy (M05)	2.9	0.008	1.5	0.005	n/d	n/d	n/d	n/d
Spiroxamine-N-formyl-despropyl (M38)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-N-formyl-desethyl (M04)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-despropyl (M02)	11.0	0.045	19.0	0.066	14.5	0.043	n/d	n/d
Spiroxamine-desethyl (M01)	3.1	0.012	4.0	0.014	3.3	0.010	n/d	n/d
Diastase enzymatic digestion (incorporated $^{14}CO_2$)	-				n/d	n/d	41.8	0.011
Total identified	37.4	0.153	42.0	0.146	67.5	0.201	59.7	0.016
Total characterised	53.3	0.218	48.0	0.167	25.4	0.076	27.2	0.007
Total extractable	90.8	0.372	89.9	0.313	92.9	0.277	86.9	0.023
Post-extraction solids (PES)	9.2	0.038	10.1	0.035	7.1	0.021	13.1	0.003
Total radioactive residue (TRR)	100.0	0.410	100.0	0.348	100.0	0.298	100.0	0.026

n/d not detected

Table CA 6.6.1/03-8 Distribution of TRR in extracts and solids in wheat matrices 294 days after treatment with [1,3-dioxolane-4-^{14}C]-spiroxamine

Sample	% TRR (mg/kg)							
	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine (KWG 4168)	9.7	0.039	6.5	0.054	4.2	0.041	n/d	n/d
Spiroxamine-despropyl-aminodiol (M31)	n/d	n/d	n/d	n/d	1.8	0.018	n/d	n/d
Spiroxamine-aminodiol-N-oxide (M29)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-aminodiol (M28)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-despropyl-acid glycoside (M45)	1.7	0.007	1.9	0.016	2.8	0.027	n/d	n/d
Spiroxamine-hydroxy-despropyl-acid glycoside (M39)	n/d	n/d	4.4	0.037	n/d	n/d	n/d	n/d
Spiroxamine-desethyl-acid glycoside (M43)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-hydroxy-desethyl-acid glycoside (M42)	1.6	0.007	2.7	0.023	n/d	n/d	n/d	n/d
Spiroxamine-acid glycoside (M44)	1.3	0.005	3.8	0.032	10.5	0.103	n/d	n/d
Spiroxamine-hydroxy glycoside (M40)	n/d	n/d	2.6	0.022	12.6	0.124	n/d	n/d
Spiroxamine-N-oxide (group of isomers (M03)	n/d	n/d	1.7	0.014	1.4	0.014	n/d	n/d
Spiroxamine-hydroxy (M05)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-N-formyl-despropyl (M38)	n/d	n/d	6.8	0.056	3.4	0.034	n/d	n/d
Spiroxamine-N-formyl-desethyl (M04)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Spiroxamine-despropyl (M02)	46.6	0.190	30.9	0.257	17.4	0.172	n/d	n/d
Spiroxamine-desethyl (M01)	13.7	0.056	7.6	0.063	5.6	0.055	n/d	n/d
Diastase enzymatic digestion (incorporated $^{14}CO_2$)	-	-	-	-	-	-	76.5	0.071
Total identified	74.6	0.304	69.0	0.574	59.6	0.588	n/d	n/d
Total characterised	17.7	0.072	20.8	0.173	33.5	0.330	85.6	0.079
Total extractable	92.3	0.376	89.8	0.747	93.1	0.918	85.6	0.079
Post-extraction solids (PES)	7.7	0.031	10.2	0.085	6.9	0.068	14.4	0.013
Total radioactive residue (TRR)	100.0	0.407	100.0	0.832	100.0	0.986	100.0	0.092

n/d not detected

Table CA 6.6.1/03-9 Distribution of TRR in extracts and solids in Swiss chard and turnip matrices 294 days after treatment with [1,3-dioxolane-4-^{14}C]-spiroxamine

Sample	% TRR (mg/kg)							
	Swiss chard (immature)		Swiss chard (mature)		Turnip leaves		Turnip roots	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Spiroxamine (KWG 4168)	8.2	0.017	10.0	0.010	n/d	n/d	-	-
Spiroxamine-despropyl-aminodiol (M31)	n/d	n/d			n/d	n/d	-	-
Spiroxamine-aminodiol-N-oxide (M29)	n/d	n/d			n/d	n/d	-	-
Spiroxamine-aminodiol (M28)	n/d	n/d			3.3	0.008	-	-
Spiroxamine-despropyl-acid glycoside (M45)	2.6	0.005	2.8	0.003	1.7	0.004	-	-
Spiroxamine-hydroxy-despropyl-acid glycoside (M39)	n/d	n/d			13.2	0.031		-
Spiroxamine-desethyl-acid glycoside (M43)	n/d	n/d			n/d	n/d	-	-
Spiroxamine-hydroxy-desethyl-acid glycoside (M42)	n/d	n/d			8.2	0.019		-
Spiroxamine-acid glycoside (M44)	3.3	0.007			11.6	0.027	-	-
Spiroxamine-hydroxy glycoside (M40)	n/d	n/d			2.8	0.007	-	-
Spiroxamine-N-oxide (group of isomers (M03)	n/d	n/d			n/d	n/d	-	-
Spiroxamine-hydroxy (M05)	2.7	0.005			n/d	n/d	-	-
Spiroxamine-N-formyl-despropyl (M38)	n/d	n/d			n/d	n/d	-	-
Spiroxamine-N-formyl-desethyl (M04)	n/d	n/d			n/d	n/d	-	-
Spiroxamine-despropyl (M02)	50.0	0.102	51.2	0.053	18.8	0.045	-	-
Spiroxamine-desethyl (M01)	12.6	0.026	12.3	0.013	3.1	0.007	-	-
Diastase enzymatic digestion (incorporated $^{14}CO_2$)	-				n/d	n/d	n/a	n/a
Total identified	79.3	0.162	76.2	0.079	62.8	0.149	-	-
Total characterised	11.6	0.024	14.4	0.015	29.1	0.069	78.7	0.009
Total extractable	90.9	0.185	90.7	0.094	91.9	0.218	78.7	0.009
Post-extraction solids (PES)	9.1	0.019	9.3	0.010	8.1	0.019	21.3	0.002
Total radioactive residue (TRR)	100.0	0.204	100.0	0.104	100.0	0.237	100.0	0.012

n/d not detected
n/a not analysed due to high matrix loading and low amount of radioactivity in the extract

Storage stability

An assessment of storage stability was not required as all plant samples were extracted within 15 days of sampling and stored at approximately -20°C.

III. Conclusions

A single application of ^{14}C-spiroxamine (1,3-dioxolane-4-^{14}C) was made to bare sandy loam soil in a 1 m^2 container at a rate of 1.62 kg a.s./ha as an emulsifiable concentrate (EC 500) formulation. This rate is just below 2.2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Crops were planted at 30 days after application (DAA), first rotation; 193 DAA (second rotation) and 294 DAA (third rotation). The planting container was maintained in a greenhouse.

Seeds of wheat (*Triticum aestivum,* Thasos), Swiss chard (*Beta vulgaris,* Lukullus) and turnips (*Brassica rapa,* Rondo) were sown into the soil at 30, 193 and 294 DAA and crops grown to maturity. Crop samples collected were immature Swiss chard (sampled at BBCH 39), wheat forage (BBCH 29), wheat hay (BBCH 65 – 71). Additionally at maturity, wheat straw, wheat grain, Swiss chard, turnip root and turnip leaves were also harvested.

The TRR in wheat forage was 0.766 mg/kg (measured as mg spiroxamine equivalents per kg) in crops harvested from the second rotation (193 DAA) and 0.407 mg/kg at the third rotation (294 DAA). In wheat hay, the TRRs were 3.924 and 0.832 mg/kg at each of the 2 rotations (193 and 294 DAA). In wheat straw, the TRRs were 2.631 and 0.986 mg/kg (193 and 294 DAA respectively). In wheat grain, the TRRs were 0.223 and 0.092 mg/kg (193 and 294 DAA respectively). Corresponding TRRs in immature Swiss chard were 0.410 and 0.204 mg/kg at each rotation (193 and 294 DAA). In mature Swiss chard, TRRs were 0.348 and 0.104 mg/kg (193 and 294 DAA respectively). The final crop group with turnip as a representative crop saw residue levels of 0.298 and 0.237 mg/kg in leaves (193 and 294 DAA) and 0.026 and 0.012 mg/kg in roots (193 and 294 DAT respectively).

The majority of the radioactive residue in most crop matrices was readily extractable with conventional extracts (acetonitrile:water 8:2 v/v), with the exception of wheat grain where only 9 - 15% TRR was extractable with the initial solvent used. The remaining radioactivity in plant matrices could be exhaustively extracted using a range of solvents. Additionally in grain, enzyme digestion with diastase released a significant proportion of the non-extracted residue (70% TRR). Final non-extractable or bound residues (Post Extractable Solids, PES) accounted for up to 21% TRR in turnip roots, although this only corresponded to 0.002 mg/kg.

Amounts of parent spiroxamine had decreased in crops harvested following the second and third rotations (193 and 294 DAA). At the second interval, the two desalkylated metabolites, Spiroxamine-despropyl (M02) and spiroxamine-desethyl (M01) comprised the majority of the radioactive residue in all crop matrices. In wheat matrices, spiroxamine-despropyl (M02) ranged from 14.3% TRR in straw to almost 27% TRR in forage. Residues of this metabolite were not detected in grain. Corresponding levels of spiroxamine-desethyl (M01) were lower, ranging from 6.2% TRR in straw to 9.3% TRR in forage. Again, residues of this metabolite were not detected in grain. These two metabolites also comprised the majority of the residue in Swiss chard and turnips planted at the second rotation, with levels of spiroxamine-despropyl (M02) ranging from 11% TRR in immature Swiss chard to 19% TRR in the mature crop. Corresponding levels of spiroxamine-desethyl (M01) were much lower than the spiroxamine-despropyl metabolite, accounting for <4% TRR. Neither metabolite was determined in turnip roots. A number of glycoside conjugates (up to 6) were again detected in all rotated crop matrices, with the exception of wheat grain. In this matrix, and also in the highly starchy turnip root, further digestion of the PES was conducted with diastase enzyme releasing significant additional radioactivity (67.7% TRR, 0.151 mg/kg in grain and 41.8%, 0.011 mg/kg in roots). This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

At the third interval, the two desalkylated metabolites, spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) still comprised the majority of the radioactive residue in all crop matrices. In wheat, matrices, spiroxamine-despropyl (M02) ranged from 17.4% TRR in straw to almost 47% TRR in forage. Residues of this metabolite were not detected in grain. Corresponding levels of spiroxamine-desethyl (M01) were much lower, ranging from 5.6% TRR in straw to almost 14% TRR in forage. Again, residues of this metabolite were not detected in grain. These two species also comprised the majority of the residue in Swiss chard and turnips planted at the second rotation, with levels of spiroxamine-despropyl (M02) accounting for approximately 50% in Swiss chard and 19% TRR in turnip leaves. Corresponding levels of spiroxamine-desethyl (M01) were much lower than the spiroxamine-despropyl metabolite, accounting for <13% TRR. Neither metabolite was determined in turnip roots. A number of glycoside conjugates (up to 6) were again detected in all rotated crop matrices, with the exception of wheat grain and turnip roots. In these matrices, further digestion of the PES was conducted with diastase enzyme releasing significant additional radioactivity in grain, 76.5% TRR, 0.071 mg/kg. Levels were too low in turnip roots to analyse further. This radioactivity was polar in natural incorporation.

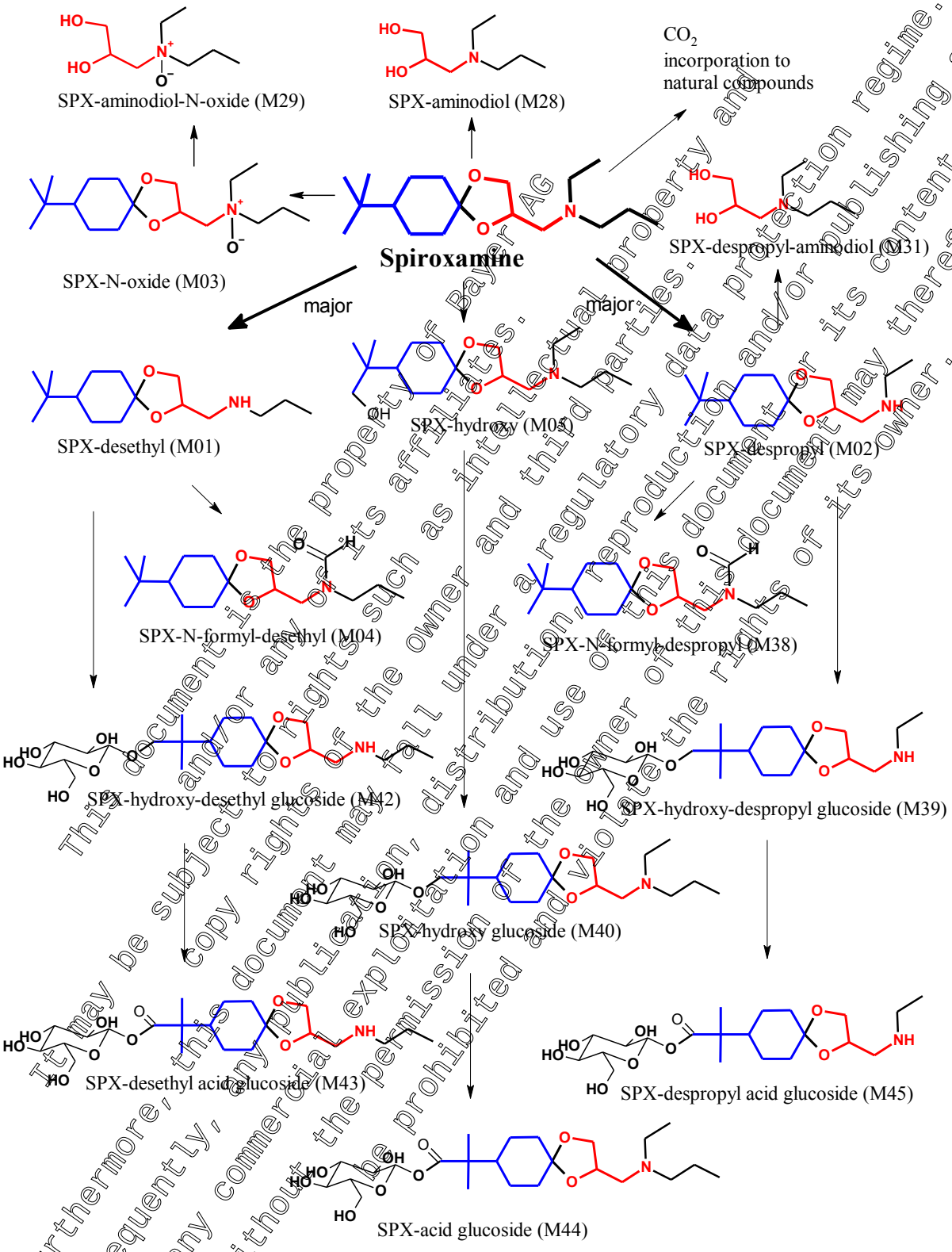
The proposed metabolic pathway for spiroxamine in rotational crops is shown in Figure CA 6.6.1-1.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: Spiroxamine B7, 2010, Volume 3 RAR, IIA 6.2.2/02.

The nature of the residue in rotated crops when spiroxamine is applied to bare soil is essentially the same as found in the primary wheat metabolism studies. The two desalkylated metabolites, spiroxamine-despropyl (M02) and spiroxamine-desethyl (M01) comprised the majority of the radioactive residue in all crop matrices harvested from the second and third rotations (193 and 294 DAA to bare soil). A number of glycoside conjugates were also detected in rotated crop matrices, with the exception of wheat grain. Grain and the highly starchy turnip root were further digested with diastase enzyme releasing significant additional radioactivity (69.5% TRR, 0.091 mg/kg in grain and 18.9%, 0.019 mg/kg in roots). This radioactivity was polar in nature and indicated mineralised CO_2 that had been incorporated into natural products.

Figure CA 6.6.1-1 Proposed metabolic pathway of spiroxamine in rotational crops



Applicant conclusion on metabolic pathway of spiroxamine in rotated crops

The nature of the residue in rotated crops when spiroxamine is applied to bare soil is essentially the same as found in the primary wheat metabolism studies. Desalkylation of the parent compound occurs forming the desethyl and despropyl moieties. Spiroxamine also undergoes oxidation in the tert.-butyl group forming hydroxy products which are then further oxidised forming the carboxylic acid. The hydroxy and acid metabolites then form conjugates with sugars (hexose). Additionally, the desalkyl metabolites also formed conjugates with formic acid. Oxidation of spiroxamine also occurred in the tertiary amine group, resulting in formation of the spiroxamine-N-oxide. Cleavage of the dioxolane ring forming the spiroxamine-aminodiol was observed. Finally, mineralisation of the parent compound in the soil leads to carbon dioxide which is further incorporated into natural products i.e. starch and sugars.

Parent spiroxamine is the major component of the residue in primary crops cereals and fruits as well as in rotational crops.

Therefore, the residue definition in rotational crops for monitoring is: **spiroxamine (parent only)**.

The residue definition for risk assessment in rotational crops is proposed as: **sum of spiroxamine and metabolites containing the tert.-butylcyclohexanone moiety, expressed as spiroxamine**. This is the same as for primary crop cereals.

CA 6.6.2 Magnitude of residues in rotational crops

Data Point:	KCA 6.6.2/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Field rotational crop studies with KWG 4168 500 EC in Germany and Great Britain
Report No:	RA-2120/94
Document No:	M-006097-01-1
Guideline(s) followed in study:	BBA Guideline IV, 3-10
Deviations from current test guideline:	Yes OECD 504 guideline (January 2007). Application of test item not made to bare soil and only a single plant-back interval (30 Days) was evaluated.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

A rotational study on the magnitude of residue of spiroxamine (total residues) was conducted in northern Europe (United Kingdom and Germany). Four field trials were conducted during the 1994 growing season after two applications of Spiroxamine 500 EC formulation (actual concentration 491.4 g/L spiroxamine) were applied to spring barley at a rate of 0.75 kg as/ha (1.5 L/ha) at BBCH 31-61. This rate is 2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. The grains were harvested and removed from the field 30 days after the last application (DALA) to prevent incorporation of germinable grains into the soil. The straw of the spring

barley primary crop was crushed on the plot and incorporated in the soil up to a maximum depth of 15 cm by rotary cultivator.

This rate is more critical than the maximum seasonal application of the representative crops under consideration and is intended to support the use of spiroxamine plant protection products to field crops. The target rate applied per hectare (total seasonal rate 1500 g a.s./ha) is equivalent to a soil PEC of 0.50 mg a.s./kg in the 0-20 cm soil layer (calculation based on a nominal soil density of 1.5 g/cm^3). This value does not include an estimation of the rate when crop interception is considered. Considering EFSA crop intercept values [EFSA Journal 2014;12(5):3662] at the BBCH growth stages for the first and second applications gives a PEC soil calculation for 1x 750 g a.s./ha with 80% interception plus 1x 750 g a.s./ha with 90% interception (with no degradation in-between) of 0.075 mg/kg in the 0-20 cm soil layer.

Immediately after incorporation of the crushed spring barley, rotational crops were sown. Rotational crops were spinach (representative of leafy vegetables), turnip (representative of root and tuber vegetables) and barley (representative of small grain cereals). The total plot was divided into a treated and a control area. The treated and control plots were divided into three subplots, one for each rotational crop.

Rotational crops were planted at a plant back interval (PBI) of 29-37 days. RAC specimens were sampled at BBCH 47-49 (spinach), BBCH 44-49 (turnip) and BBCH 49-59 (barley). The days after application (DAA) were recorded for each crop sampling. Soil samples were taken at 0 to 154 days after application. A summary of the trial sub-plot design is shown in Table CA 6.6.2/01-1 and details of the soils used are summarised in Table CA 6.6.2/01-2.

The trial parameters and residue results are summarised in Table CA 6.6.2/01-3 and Table CA 6.6.2/01-4.

Table CA 6.6.2/01-1 Summary of sub-plot plant back interval design

Trial Reference	Trial number / sub-plot	Application dates	Planting date	Rotated crop, Plant Back Interval/ Days After application
CA 6.6.2/01 (M-006097-01-1) D-51399 Burscheid, Höfchen, Germany 1994	405566 (plot A)	30/05/1994 29/06/1994	n/a	n/a
	405566 (plot B)	30/05/1994 29/06/1994	29/07/1994 Crop failure	Barley PBI = 31 days DAT = 71, 111, 141 days
	405457 (plot C)	30/05/1994 29/06/1994	29/07/1994 Crop failure	Spinach PBI = 30 days DAT = 71, 111 days
	405515 (plot D)	30/05/1994 29/06/1994	29/07/1994 Crop failure	Turnip PBI = 30 days DAT = 71, 111 days
CA 6.6.2/01 (M-006097-01-1) D-67551 Worms-Heppenheim, Germany 1994	405574 (plot A)	24/05/1994 16/06/1994	n/a	n/a
	405574 (plot B)	24/05/1994 16/06/1994	23/07/1994 Crop failure	Barley PBI = 37 days DAT = 71, 154 days
	405485 (plot C)	24/05/1994 16/06/1994	23/07/1994 Crop failure	Spinach PBI = 37 days DAT = 71, 131 days
	405523 (plot D)	24/05/1994 16/06/1994	23/07/1994 Crop failure	Turnip

Trial Reference	Trial number / sub-plot	Application dates	Planting date	Rotated crop, Plant Back Interval/ Days After application
				PBI = 37 days DAT = 71, 132 days
CA 6.6.2/01 (M-006097-01-1) Elm farm, Thurston, Suffolk, United Kingdom 1994	405582 (plot A)	02/06/1994 28/06/1994	n/a	n/a
	405582 (plot B)	02/06/1994 28/06/1994	29/07/1994 Crop failure	Barley PBI = 31 days DAT = 90, 154 days
	405493 (plot C)	02/06/1994 28/06/1994	29/07/1994 Crop failure	Spinach PBI = 31 days DAA = 90, 141 days
	405531 (plot D)	02/06/1994 28/06/1994	29/07/1994 Crop failure	Turnip PBI = 31 days DAA = 90, 141 days
CA 6.6.2/01 (M-006097-01-1) D-40789 Monheim, Laacherhof, Germany 1994	405590 (plot A)	27/05/1994 23/06/1994	22/07/94	n/a
	405590 (plot B)	27/05/1994 23/06/1994	22/07/94	Barley PBI = 29 days DAA = 75, 120, 147 days
	405507 (plot C)	27/05/1994 23/06/1994	22/07/94	Spinach PBI = 29 days DAA = 75, 120 days
	405558 (plot D)	27/05/1994 23/06/1994	22/07/94	Turnip PBI = 29 days DAA = 75, 120 days

Table CA 6.6.2/01-2 Soil classification and physico-chemical properties

Soil Type	pH (in water)	Organic carbon (%)	Sand %	Silt %	Clay %	Moisture holding capacity at pF 2.5 (%)	CEC meq / 100 g
Silt loam (405477, 405515, 405566)	6.5	2.0	nr	nr	nr	nr	nr
Silt loam (405485, 405523, 405574)	7.5	1.89	nr	nr	nr	nr	nr
Sandy clay loam (405493, 405531, 405582)	7.1	2.24	nr	nr	nr	nr	nr
Sandy loam (405507, 405558, 405590)	6.7	1.74	nr	nr	nr	nr	nr

nr- not recorded

Residue analysis for total residues of spiroxamine (4-tert.-butylcyclohexanone moiety) in RAC samples was performed according to validated method 00312 and its supplement E001, report reference MR-114/95 (**see Doc MCA Section 4**). Residue analysis for total residues of spiroxamine (4-tert.-butylcyclohexanone moiety) in soil samples was performed according to validated method 00374, report reference MR-607/94 (**see Doc MCA Section 4**).

The residues of spiroxamine in RAC samples were extracted with a mixture of methanol and hydrochloric acid. After filtration the extract was diluted in water and applied to a reversed phase column. Residues were extracted from the column with dichloromethane, purified by chromatography

on a silica gel column and determined by gas chromatography with a mass selective detector (GC-MSD) in single ion mode.

Soil samples were extracted in a Soxtec hot extraction equipment with boiling methanol / water/ammonia (25%) (800:200:10, v/v/v). After solvent evaporation to the aqueous remainder the internal standard is added. The active ingredient and the metabolites were quantitatively determined by liquid chromatography with MS-MS detection. For calculation of the total residues in soil, the concentrations of the metabolites spiroxamine-desethyl (KWG 4557, M01) and spiroxamine-despropyl (KWG 4669, M02)) were converted to concentrations of spiroxamine equivalents and added to the corresponding concentrations of spiroxamine.

The limit of quantification (LOQ) of the analytical method was 0.05 mg/kg for the spiroxamine total residues for all RAC commodities and 0.005 mg/kg for soil.

II. Results and discussion

No residues above the LOQ were found in control samples, except for the primary crop controls at 0.077 and 0.110 mg/kg in barley forage and barley straw respectively.

In samples of the primary crop, residues of spiroxamine (total residues) were 6.4 to 15.0 mg/kg in barley forage (day 0). Residues declined to 2.8 to 8.5 mg/kg in straw and 0.120 to 0.290 mg/kg in grain 29 to 30 DALA/

In samples of succeeding crops, no residues of spiroxamine (total residues) were detected at or above the limit of quantification (0.05 mg/kg) in any of the treated spinach, turnip (root and leaves) and barley (forage, straw and grain) samples.

In soil samples, residues of spiroxamine were between 0.039 to 0.066 mg/kg at 0 DALA, 0.019 to 0.047 mg/kg 29 to 36 DALA and <0.005 to 0.039 mg/kg 132 to 154 DALA. Levels of total spiroxamine were between 0.053 to 0.088 mg/kg at 0 DALA, 0.041 to 0.076 mg/kg 29 to 36 DALA and <0.005 to 0.059 mg/kg 132 to 154 DALA. These total spiroxamine soil concentrations for 0 DALA are in good agreement with the estimated PEC soil calculation of 0.075 mg/kg considering the crop interception of the primary barley crop. The soil concentrations reported at the first plant back interval, which were after the soil incorporation of the harvested plant material, show that total spiroxamine was still available for potential crop uptake and therefore as this study is conducted at 2N the current critical GAP for cereals and crop destruction for the usual use of the plant protection products is unlikely, the findings in crops of residues below the LOQ are acceptable to show that residues of concern in rotational crops are not expected.

Procedural recoveries for spiroxamine (total) were performed at the LOQ (0.05 mg/kg) and 10xLOQ (0.5 mg/kg) for all commodities, and additionally at 100xLOQ (5 mg/kg) for barley grain and straw and 200xLOQ (10 mg/kg) for barley forage. The overall mean procedural recoveries of spiroxamine (total) were 77 to 84%. Procedural recoveries for spiroxamine were performed at the LOQ (0.005 mg/kg) for soil. The overall mean procedural recovery of spiroxamine (total) was 77%. These data are summarised in Table CA 6.6.2/01-5 and Table CA 6.6.2/01-6.

As shown in Table CA 6.6.2/01-7, the samples were stored for a maximum of 320 days (10.6 months) from sampling to analysis. These storage periods are covered by the available storage stability data for high starch content, high water content, dried commodities which demonstrate stability of spiroxamine for up to 2 years for high starch crops and 517 - 566 days for high water and dry crops (approximately 17 to 19 months, refer to Point CA 6.1).

Table CA 6.6.2/01-3 Field rotational crop trial with spiroxamine in support of the critical GAP – residue results for crops in Northern Europe

Doc. No. Trial Ref Location Crop Year	Application				Crop / part Succeeding crop unless stated	PBI/ DAA (days)	Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.6.2/01 (M-006097-01-1) 405566 (plot A) D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Barley forage Barley grain Barley straw **Primary crop**	- / 0 - / 29 - / 29	6.4 0.12 2.8
CA 6.6.2/01 (M-006097-01-1) 405566 (plot B) D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Barley forage Barley forage Barley grain Barley straw	30 / 71 30 / 111 30 / 141 30 / 141	<0.05 <0.05 <0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405477 (plot C) D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Spinach leaf Spinach leaf	30 / 71 30 / 111	<0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405515 (plot D) D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Turnip leaf Turnip leaf Turnip root	30 / 71 30 / 111 30 / 111	<0.05 <0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405574 (plot A) D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Barley forage Barley grain **Primary crop**	- / 0 - / 30	11.0 <0.05

Doc. No. Trial Ref Location Crop Year	Application				Crop / part Succeeding crop unless stated	PBI DAA (days)	Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.6.2/01 (M-006097-01-1) 405574 (plot B) D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Barley forage Barley ear Barley remainder	37 / 71 37 / 154 37 / 154	<0.05 <0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405485 (plot C) D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Spinach leaf Spinach leaf	37 / 71 37 / 131	<0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405523 (plot D) D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Turnip leaf Turnip leaf Turnip root	37 / 71 37 / 132 37 / 132	<0.05 <0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405582 (plot A) Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Barley forage Barley grain Barley straw **Primary crop**	- / 0 - / 29 - / 29	15.0 0.29 8.50
CA 6.6.2/01 (M-006097-01-1) 405582 (plot B) Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Barley forage Barley forage	31 / 90 31 / 154	<0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405493 (plot C) Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Spinach leaf Spinach leaf	31 / 90 31 / 141	<0.05 <0.05

Doc. No. Trial Ref Location Crop Year	Application				Crop / part Succeeding crop unless stated	PBI DAA (days)	Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	kg a.s./ha	L/ha	Growth stage			
CA 6.6.2/01 (M-006097-01-1) 405531 (plot D) Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Turnip leaf Turnip leaf Turnip root	31 / 99 31 / 141 31 / 141	<0.05 <0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405590 (plot A) D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Barley forage Barley grain Barley straw **Primary crop**	- / 0 - / 29 - / 29	11.0 0.22 6.8
CA 6.6.2/01 (M-006097-01-1) 405590 (plot B) D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Barley forage Barley forage Barley ear Barley remainder	29 / 75 29 / 120 29 / 147 29 / 147	<0.05 <0.05 <0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405507 (plot C) D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Spinach leaf Spinach leaf	29 / 75 29 / 120	<0.05 <0.05
CA 6.6.2/01 (M-006097-01-1) 405558 (plot D) D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Turnip leaf Turnip leaf Turnip root	29 / 75 29 / 120 29 / 120	<0.05 <0.05 <0.05

Table CA 6.6.2/01-4 Field rotational crop trial with spiroxamine in support of the critical GAP – residue results for soil in Northern Europe

Doc. No. Trial Ref Location Crop Year	Application				Soil horizon	DAA (days)	Residue (mg/kg)			
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine	Spiroxamine-desethyl (M01)	Spiroxamine-despropyl (M02)	Spiroxamine (total residues)[1]
CA 6.6.2/01 (M-006097-01-1) 405566 D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	0 29 71 111 141	0.066 0.047 0.047 0.044 0.039	0.010 0.013 0.008 0.007 0.008	0.010 0.013 0.011 0.009 0.010	0.089[2] 0.075 0.068 0.063 0.059
CA 6.6.2/01 (M-006097-01-1) 405477 D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	29 71 111	0.044 0.021 0.031	0.013 <0.005 0.006	0.015 0.006 0.008	0.076 0.030 0.047
CA 6.6.2/01 (M-006097-01-1) 405515 D-51399 Burscheid, Höfchen, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	29 71 111	0.044 0.024 0.035	0.013 <0.005 0.007	0.015 0.007 0.009	0.076 0.035 0.053
CA 6.6.2/01 (M-006097-01-1) 405574 D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	0 36 71 154	0.049 0.019 0.006 <0.005	0.015 0.010 <0.005 <0.005	0.015 0.011 0.005 0.005	0.083[1] 0.043 0.014 0.011
CA 6.6.2/01 (M-006097-01-1) 405485 D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	36 71 131	0.029 0.011 0.008	0.015 0.008 0.007	0.015 0.009 0.009	0.063 0.031 0.027

Doc. No. Trial Ref Location Crop Year	Application				Soil horizon	DAA (days)	Residue (mg/kg)			
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine	Spiroxamine-desethyl (M01)	Spiroxamine-despropyl (M02)	Spiroxamine (total residues)[1]
CA 6.6.2/01 (M-006097-01-1) 405523 D-67551, Worms-Heppenheim, Germany 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	0 71 132	0.023 0.005 <0.005	0.012 <0.005 <0.005	0.014 <0.005 <0.005	0.053 0.011 <0.005
CA 6.6.2/01 (M-006097-01-1) 405582 Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	0 30 90 141	0.051 0.037 0.021 0.018	0.011 0.013 0.006 <0.005	0.011 0.015 0.008 0.006	0.076[2] 0.069 0.037 0.028
CA 6.6.2/01 (M-006097-01-1) 405493 Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	30 90 141	0.039 0.028 0.016	0.012 0.007 <0.005	0.013 0.009 0.007	0.067 0.046 0.030
CA 6.6.2/01 (M-006097-01-1) 405531 Elm farm, Thurston, Suffolk, United Kingdom 1994	2	0.75 0.75	300 300	BBCH 31 BBCH 58-61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	30 90 141	0.038 0.026 0.023	0.015 0.008 0.006	0.015 0.010 0.007	0.072 0.047 0.037
CA 6.6.2/01 (M-006097-01-1) 405590 D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	0 29 75 120 147	0.039 0.022 0.008 0.006 0.008	0.005 0.008 <0.005 <0.005 <0.005	0.007 0.009 0.006 0.006 0.005	0.053[2] 0.041 0.018 0.016 0.017
CA 6.6.2/01 (M-006097-01-1) 405507 D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	29 75 120	0.022 0.006 0.005	0.008 <0.005 <0.005	0.009 0.005 <0.005	0.041 0.015 0.011

Doc. No. Trial Ref Location Crop Year	Application				Soil horizon	DAA (days)	Residue (mg/kg)			
	No.	kg a.s./ha	L/ha	Growth stage			Spiroxamine	Spiroxamine-desethyl (M01)	Spiroxamine-despropyl (M02)	Spiroxamine (total residues)[1]
CA 6.6.2/01 (M-006097-01-1) 405558 D-40789 Monheim, Laacherhof, Germany 1994	2	0.75 0.75	300 300	BBCH 37 BBCH 61	Soil (0-20 cm) Soil (0-20 cm) Soil (0-20 cm)	29 75 120	0.020 0.013 0.007	0.008 0.006 <0.005	0.011 0.009 0.006	0.043 0.029 0.018

1 - For calculation of the total residues the concentrations of the metabolites spiroxamine-desethyl and spiroxamine-despropyl were converted to concentrations of spiroxamine equivalents and added to the corresponding concentrations of spiroxamine.
2 – Day 0 sample contains soil only, samples from day 29 - 36 contain soil and crushed / incorporated straw

Table CA 6.6.2/01-5 Procedural recovery data for the determination of spiroxamine (total) residues in rotated crop matrices

Document	Analytical method	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.6.2/01	00312 and its supplement E001	Spinach leaf	0.05 0.5 overall	4 4 8	90, 89, 99, 82 84, 88, 81, 57 mean: 84 RSD: 14.5
CA 6.6.2/01	00312 and its supplement E001	Turnip leaf	0.05 0.5 overall	4 4 8	80, 83, 82, 78 71, 85, 80, 83 mean: 80 RSD: 5.4
CA 6.6.2/01	00312 and its supplement E001	Turnip root	0.05 0.5 overall	4 4 8	79, 82, 76, 79 75, 81, 80, 75 mean:78 RSD: 3.5
CA 6.6.2/01	00312 and its supplement E001	Barley forage	0.05 0.5 5.0 10.0 overall	4 4 1 1 10	77, 80, 78, 73 92, 80, 82, 75 84 83 mean: 80 RSD: 6.7
CA 6.6.2/01	00312 and its supplement E001	Barley grain	0.05 0.5 5.0 overall	2 4 1 7	73, 75 69, 81, 76, 82 80 mean: 77 RSD: 6.2
CA 6.6.2/01	00312 and its supplement E001	Barley straw	0.05 0.5 5.0 overall	2 2 1 5	82, 77 82, 80 75 mean: 79 RSD: 4.0

Table CA 6.6.2/01-6 Procedural recovery data for the determination of spiroxamine (total), spiroxamine-desethyl and spiroxamine-despropyl residues in soil

Document	Analytical method	Matrix	Fortification level (mg/kg)	Analyte	Recovery rates / no. of replicates (%)
CA 6.6.2/01	00374	Soil	Not stated[1]	Spiroxamine	70 to 88 mean: 76 RSD: 7.9 n = 29
CA 6.6.2/01	00374	Soil	Not stated[1]	Spiroxamine-desethyl (M01)	70 to 92 mean: 80 RSD: 9.8 n = 29
CA 6.6.2/01	00374	Soil	Not stated[1]	Spiroxamine-despropyl (M02)	70 to 97 mean: 85 RSD: 14 n = 29

1 – the fortification levels for concurrent recoveries were not specifically stated in the report. The concurrent recoveries were performed at different fortification levels with a mixture of the standard Speyer soils and with two other control soils.

Table CA 6.6.2/01-7 Storage of rotated crop samples before determination of spiroxamine (total) residues

Crop	Crop part	Minimum to maximum storage period from sampling to analysis [extraction] (days)
Turnip	Root	24 to 235
	Leaf	24 to 235
Spinach	Leaves	14 to 234
Barley	Forage	13 to 320
	Grain	13 to 320
	Straw	13 to 320

III. Conclusions

After two applications of spiroxamine (500 EC) at a rate of 0.75 kg as/ha to a primary cereal crop (30 DALA the straw of the primary crop was crushed on the plot and incorporated in the soil up to a maximum depth of 15 cm by rotary cultivator), total residues of spiroxamine (residues measured as 4-TBCH) were all <0.05 mg/kg, in samples of spinach, turnip root, turnip leaves, barley forage, straw and grain at 30 day PBI intervals in four trials conducted in Northern Europe. This rate is 2N the total seasonal rate for the use of spiroxamine on cereals at the critical GAP for the representative use. Therefore, the application rates and trial design support the proposed critical GAP for the representative uses of spiroxamine and demonstrate that no significant residues will be present in representative rotational crops when target primary crops are treated according to the proposed GAP.

In soil samples, residues of spiroxamine were between 0.039 to 0.066 mg/kg at 0 DALA, 0.019 to 0.047 mg/kg 29 to 36 DALA and <0.005 to 0.039 mg/kg 132 to 154 DALA. Levels of total spiroxamine were between 0.053 to 0.089 mg/kg at 0 DALA, 0.041 to 0.076 mg/kg 29 to 36 DALA and <0.005 to 0.059 mg/kg 132 to 154 DALA. These total spiroxamine soil concentrations for 0 DALA are in good agreement with the estimated PEC soil calculation of 0.075 mg/kg considering the crop interception of the primary barley crop. The soil concentrations reported at the first plant back interval, which were after the soil incorporation of the harvested plant material, show that total spiroxamine was still available for potential crop uptake and therefore this study is acceptable to show that residues of concern in rotational crops are not expected.

Assessment and conclusion by applicant:

Acceptable study to address the data point. Study previously submitted and accepted in the EU: RAR Annex B7 (2010) IIA 6.6.3/01.

Study is acceptable as the study parameters represent a realistic worst case, however in recognition of deficiencies against current OECD guidelines, a new study was initiated in 2020.

Residues of spiroxamine (total residues) were all <0.05 mg/kg after two applications of spiroxamine (500 EC) in samples of spinach, turnip root, turnip leaves, barley forage, straw and grain at 30 day PBI intervals in four trials conducted in Northern Europe. The application rates and trial design are more critical than the proposed critical GAP for the representative uses of spiroxamine and demonstrate that no significant residues will be present in representative rotational crops when target primary crops are treated according to the proposed GAP.

The following new study is currently on-going and an interim report will be submitted at an agreed submission top-up time-point:

Dossier node	Draft title	Study ID	Planned submission
KCA 6.6.2	Field rotational crop trials following one application of Spiroxamine 500 EC to bare soil – Northern and Southern Europe – 2020 to 2021	RDE-20-42206	Interim report Not before May 2021

CA 6.7 Proposed residue definitions and maximum residue levels

Data Point:	KCA 6.7/01
Report Author:	[redacted]; [redacted]
Report Year:	2009
Report Title:	Spiroxamine - Annex I renewal - Further information requested by the BfR to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I
Report No:	M-344146-01-2
Document No:	M-344146-01-2
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2017)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

The Position Paper outlined the Applicants position and response to various requests from the previous RMS-DE relating to rat metabolism and plant metabolites. For the residues aspects the responses were presented by the RMS-DE in the RAR Vol 3 B7 (2010) and where still relevant these positions have been included in the discussions on residue definition in this section.

CA 6.7.1 Proposed residue definitions

Residue definitions are proposed in accordance with the OECD Guidance Document on the Definition of Residue from the Series on Testing and Assessment, no. 63 and the Series on Pesticides No. 31, July 2009.

Table CA 6.7.1-1 Proposed EU residue definitions for spiroxamine

End-Point	Residue definition Proposed EU end point
Definition of the residue in crops (for MRL-setting purposes) RD-Mo	Spiroxamine (parent only)

End-Point	Residue definition Proposed EU end point
Definition of the residue in crops (for risk assessment purposes) RD-RA	Cereals and rotational crops: Sum of spiroxamine and metabolites containing the tert.-butylcyclohexanone moiety, expressed as spiroxamine Fruits: Sum of spiroxamine and metabolites containing the aminodiol (N-ethyl-N-propyl-1,2-dihydroxy-3-amino-propane) moiety, expressed as spiroxamine
Conversion factor (monitoring to risk assessment)	Cereal grain: x4 Grapes: x1.8 *The need for additional safety factors from isomer ratios is not anticipated. Applicant position is that no further correction will be required, based on argumentation and weight of evidence from residue trials.*
Definition of the residue in animal products (for MRL-setting purposes) RD-Mo	Ruminants: Spiroxamine-acid (M06), expressed as spiroxamine Poultry: Sum of spiroxamine and spiroxamine-acid (M06), expressed as spiroxamine
Definition of the residue in animal products (for risk assessment purposes) RD-RA	Ruminants: Sum of spiroxamine, spiroxamine-acid (M06), its glucuronide conjugate (M19) and spiroxamine-hydroxy acid (M07), expressed as spiroxamine (sum of isomers) Poultry: Sum of spiroxamine, spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-acid (M06) expressed as spiroxamine (sum of isomers)
Conversion factor (monitoring to risk assessment)	Ruminant/pig muscle: 1.4 Ruminant/pig liver: 3.0 Ruminant/pig kidney: 3.8 Ruminant/pig fat: 1.8 Milk: 1.2 Poultry liver: 3.0 Poultry muscle: 1.4 Poultry fat: 1.1 Poultry egg: 1.4

Conversion factors for use in risk assessment (crops)

Conversion factors for use in risk assessment as proposed by previous RMS are shown below from Volume 3, Annex B.7, Residue data. Final RMS version August 2017.

RMS proposed to use factors of x0.5 for grapes, x0.23 for cereal grain and x0.17 for straw when summarising older residue trials only reporting total SPX common moiety residues results. These trials were still used in MRL proposals from the last EU evaluation. Reciprocal values of these factors were proposed for consumer risk assessment purposes.

Cereals data: extract below from RAR (2017)

Table B. 7.6-1 Conversion factors for the calculation of parent spiroxamine to or from total spiroxamine residue values in cereals

Plant product	Spiroxamine [mg/kg]	Total residue [mg/kg]	Conversion factor parent:total residue	Reference
Spring wheat plant (14 d)	5.74	8.37	0.69	RIP9600170
Spring wheat grain (63 d)	0.010	0.034	0.29	RIP9600170
Barley grain	0.107	0.312	0.34	RIP1999-919, 1001-98
Barley grain	0.172	0.923	0.19	RIP1999-919, 1628-98
Barley grain	0.055	0.246	0.22	RIP1999-920, 1002-98
Barley grain	0.050	0.169	0.30	RIP1999-920, 1629-98
Barley grain	0.106	0.5	0.21	RIP1999-919, 1003-98
Barley grain	0.127	0.901	0.14	RIP1999-919, 1630-98
Barley grain	0.05	0.268	0.19	RIP1999-920, 1004-98
Barley grain	<0.05 [a]	0.264	0.19	RIP1999-920, 1631-98
Mean (SD)			**0.23 (0.07)**	
Spring wheat straw (63 d)	8.76	25.42	0.34	RIP9600170
Barley straw	0.650	4.400	0.15	RIP1999-919, 1001-98
Barley straw	0.669	3.900	0.17	RIP1999-919, 1628-98
Barley straw	0.967	5.610	0.17	RIP1999-920, 1002-98
Barley straw	1.200	10.200	0.12	RIP1999-920, 1629-98
Barley straw	0.416	2.58	0.16	RIP1999-919, 1003-98
Barley straw	0.635	3.99	0.16	RIP1999-919, 1630-98
Barley straw	0.522	3.68	0.14	RIP1999-920, 1004-98
Barley straw	0.719	5.75	0.13	RIP1999-920, 1631-98
Mean (SD)			**0.17 (0.07)**	

[a] calculated as 0.05 mg/kg

Update for cereals based on currently available data

In the previous evaluation (above) data were used from the wheat plant metabolism and from studies conducted at the high rate of 750 g a.s./ha. Since a total of 14 barley or wheat residue trials are now available at the supported GAP where grain data >LOQ can be used to derive conversion factors with 39 trials for straw, these values are considered more appropriate and are presented in Table CA 6.7.1-2 and Table CA 6.7.1-3.

The factors are derived from these trials by comparison of the total spiroxamine (common moiety) data with the spiroxamine values in mature grain or straw. The common moiety method measures all metabolites that can be converted to the 4-tert-butylcyclohexanone metabolite and represents the Group A metabolites of spiroxamine (and theoretically any Group B metabolites, however metabolism data shows that any Group B metabolites are very minor in cereals). In the previous EU evaluation, the x0.23 factor for total spiroxamine residue (common moiety) to spiroxamine for MRL purposes was also the basis (by applying the reciprocal 1/0.23) for using a conversion factor of x 4.3 for the MRL residue definition to the risk assessment residue definition to account for the total residue of metabolites being higher in concentration than spiroxamine.

Using the latest grain data from cereal residue trials, the factor for spiroxamine residue to total spiroxamine (common moiety) for risk assessment purposes is an average of x4.16 with a median value of x3.42. **It is proposed to use x4.0 for this submission.**

Using the latest straw data from residue trials, the factor for spiroxamine residue to total spiroxamine (common moiety) for risk assessment purposes is an average of x10.13 with a median value of x7.90. **If this factor is required it is proposed to use x9.0 for this submission.**

Table CA 6.7.1-2: Grain conversion factors

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
CA 6.3.2/13 16-2045-01	Barley grain	42	0.025	0.086	3.44	0.291
CA 6.3.2/13 16-2045-04	Barley grain	41	0.011	0.023	2.091	0.478
CA 6.3.2/14 17-2145-02	Barley grain	48	0.028	0.095	3.393	0.295
CA 6.3.2/15 16-2162-03	Barley grain	61	0.011	0.018	1.636	0.611
CA 6.3.2/16 17-2159-01	Barley grain	33	0.010	0.07	7.00	0.143
CA 6.3.2/16 17-2159-02	Barley grain	28	0.140	0.630	4.50	0.222
CA 6.3.2/16 17-2159-03	Barley grain	41	0.029	0.35	12.069	0.083
CA 6.3.2/16 17-2159-04	Barley grain	48	0.038	0.13	3.421	0.292
CA 6.3.2/17 17-2016-01	Barley grain	30	0.024	0.077	3.208	0.312
CA 6.3.2/17 17-2016-02	Barley grain	47	0.043	0.21	4.884	0.205
CA 6.3.2/17 17-2016-03	Barley grain	17	0.027	0.13	4.815	0.208
CA 6.3.2/17 17-2016-04	Barley grain	42	0.029	0.15	5.172	0.193
CA 6.3.2/19 E19RP003-05	Barley grain	39	0.048	0.15	3.15	0.317
CA 6.3.3/16 17-2015-02	Wheat grain	26	0.022	0.036	1.636	0.611
CA 6.3.3/16 17-2015-04	Wheat grain	39	0.011	0.022	2.00	0.500
				Average	**4.159**	0.318
				Median	**3.421**	0.292
				Std Dev	2.897	0.157
				RSD	69.6	49.5

Table CA 6.7.1-3: Straw conversion factors

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
CA 6.3.2/13 16-2045-01	Barley straw	42	0.62	3.0	4.839	0.207
CA 6.3.2/13 16-2045-02	Barley straw	48	0.45	2.0	4.444	0.225
CA 6.3.2/13 16-2045-03	Barley straw	57	0.23	1.9	8.261	0.121
CA 6.3.2/13	Barley straw	41	0.28	1.9	6.786	0.147

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
16-2045-04						
CA 6.3.2/14 17-2145-01	Barley straw	33	0.233	4.0	17.167	0.058
CA 6.3.2/14 17-2145-02	Barley straw	48	0.325	4.0	12.308	0.081
CA 6.3.2/15 16-2162-01	Barley straw	66	0.34	2.8	8.235	0.121
CA 6.3.2/15 16-2162-02	Barley straw	64	0.51	4.6	9.020	0.111
CA 6.3.2/15 16-2162-03	Barley straw	61	0.104	1.6	15.835	0.065
CA 6.3.2/15 16-2162-04	Barley straw	74	0.19	1.4	7.368	0.136
CA 6.3.2/16 17-2159-01	Barley straw	33	0.32	6.5	20.313	0.048
CA 6.3.2/16 17-2159-02	Barley straw	28	0.42	4.7	11.190	0.089
CA 6.3.2/16 17-2159-03	Barley straw	41	0.38	5.1	13.420	0.075
CA 6.3.2/16 17-2159-04	Barley straw	48	0.37	5.3	14.324	0.07
CA 6.3.2/17 17-2016-01	Barley straw	30	0.44	4.1	9.318	0.107
CA 6.3.2/17 17-2016-02	Barley straw	47	0.27	3.3	12.222	0.082
CA 6.3.2/17 17-2016-03	Barley straw	17	0.36	4.7	13.056	0.077
CA 6.3.2/17 17-2016-04	Barley straw	42	0.22	5.6	25.455	0.039
CA 6.3.2/19 E19RP003-01	Barley straw	33	0.283	1.84	6.50	0.154
CA 6.3.2/19 E19RP003-02	Barley straw	64	0.094	1.56	16.50	0.061
CA 6.3.2/19 E19RP003-03	Barley straw	55	0.120	1.33	11.111	0.090
CA 6.3.2/19 E19RP003-04	Barley straw	49	0.406	3.11	7.647	0.131
CA 6.3.2/19 E19RP003-05	Barley straw	39	0.693	4.30	6.207	0.161
CA 6.3.2/19 E19RP003-06	Barley straw	60	0.419	2.33	5.556	0.180
CA 6.3.2/19 E19RP003-07	Barley straw	50	0.060	1.635	27.20	0.037
CA 6.3.3/12 16-2046-01	Wheat straw	50	0.44	2.00	4.545	0.220
CA 6.3.3/12 16-2046-02	Wheat straw	62	0.14	0.44	3.143	0.318
CA 6.3.3/12 16-2046-03	Wheat straw	54	0.15	0.67	4.467	0.224
CA 6.3.3/12	Wheat straw	53	0.30	0.20	6.667	0.150

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
16-2046-04						
CA 6.3.3/13 16-2163-01	Wheat straw	50	0.55	2.50	4.545	0.220
CA 6.3.3/13 16-2163-02	Wheat straw	46	0.74	3.8	5.135	0.195
CA 6.3.3/13 16-2163-03	Wheat straw	43	1.5	9.0	6.00	0.167
CA 6.3.3/13 16-2163-04	Wheat straw	42	1.00	6.70	6.70	0.149
CA 6.3.3/14 17-2144-01	Wheat straw	56	0.16	2.5	15.625	0.064
CA 6.3.3/14 17-2144-02	Wheat straw	42	0.75	4.25	5.667	0.176
CA 6.3.3/15 17-2030-01	Wheat straw	50	0.53	4.50	8.491	0.118
CA 6.3.3/15 17-2030-02	Wheat straw	41	0.50	0.30	6.00	0.167
CA 6.3.3/16 17-2015-01	Wheat straw	61	0.77	3.80	4.935	0.203
CA 6.3.3/16 17-2015-02	Wheat straw	26	0.93	2.2	2.366	0.423
CA 6.3.3/16 17-2015-03	Wheat straw	43	0.72	4.5	6.250	0.160
CA 6.3.3/16 17-2015-04	Wheat straw	39	0.43	1.00	2.326	0.430
CA 6.3.3/17 17-2158-01	Wheat straw	56	0.27	2.20	8.148	0.123
CA 6.3.3/17 17-2158-02	Wheat straw	57	0.29	3.70	12.759	0.078
CA 6.3.3/17 17-2158-03	Wheat straw	33	0.39	7.20	18.462	0.054
CA 6.3.3/17 17-2158-04	Wheat straw	42	1.4	7.6	5.429	0.184
CA 6.3.3/17 17-2158-05	Wheat straw	66	0.13	4.40	33.846	0.030
				Average	**10.126**	0.142
				Median	**7.900**	0.127
				Std Dev	6.678	0.087
				RSD	66.0	61.23

Grapes data: extract below from RAR (2017)

The calculated conversion factors for the short PHI (table grapes, 14 days) and the long PHI (wine grapes, 35 days) are 0.55 (RSD 34 %) and 0.45 (RSD 46 %), respectively. Since any conversion factor is linked with a considerable uncertainty and the conversion factors for 14 and 35 days PHI do not show significant differences, it is considered appropriate to set an **overall mean conversion factor of 0.5** for the evaluation of the critical GAP of wine and table grapes.

Table B. 7.6-12 Conversion factor for the calculation of parent spiroxamine to or from total spiroxamine residue values in grapes

		Day 14 after application			Day 35 after application		
Trial Sub-ID	**Sample material**	**spiroxamine parent [mg/kg]**	**total residue of spirox.[a] [mg/kg]**	**F[b]**	**spiroxamine parent [mg/kg]**	**total residue of spirox.[a] [mg/kg]**	**F[b]**
0193-00	bunch	0.13	0.20	*0.65*	0.07	0.16	*0.44*
0194-00	bunch	0.19	0.16	*1.0*[e]	0.10	0.12	*0.83*
0195-00	bunch	0.13	0.35	*0.37*	<0.05	0.21	*n.a.*
0195-00	berry *(desiemmed grapes)*	0.11	0.27	*n.a.*	0.06	0.26	*0.23*
0225-00	bunch	0.09	0.18	*0.50*	<0.05	0.16	*n.a.*
0653-97[c]	bunch	0.14[d]	0.50[d]	*0.28*	0.09	0.25	*0.36*
0655-97[c]	bunch	0.06	0.16	*0.38*	0.09	0.25	*0.36*
0656-97[c]	bunch	0.22	0.70	*0.31*	0.10	0.48	*0.21*
0657-97[c]	bunch	0.14	0.17	*0.82*	0.05	0.07	*1.00*
0658-97	bunch	0.41	0.50	*0.82*	0.33	0.49	*0.67*
0659-97	bunch	0.37	0.58	*0.64*	0.20	0.36	*0.56*
0660-97	bunch	<0.05	0.09	*n.a.*	<0.05	0.07	*n.a.*
0660-97	berry	0.06	0.12	*0.5*	<0.05	0.05	*n.a.*
0661-97	bunch	0.08	0.21	*0.38*	0.07	0.23	*0.30*
0672-07	bunch	0.21	0.36	*0.58*	0.13	0.40	*0.38*
0673-07	bunch	0.22	0.36	*0.61*	0.12	0.34	*0.35*
0675-07	bunch	0.21	0.30	*0.70*	0.17	0.25	*0.68*
0676-07	bunch	0.20	0.33	*0.61*	0.20	0.31	*0.65*
0701-07	bunch	0.10	0.26	*0.38*	0.06	0.09	*0.67*
0702-07	bunch	1.10	1.40	*0.79*	1.00	1.40	*0.71*
0703-07	bunch	0.24	0.37	*0.65*	0.09	0.32	*0.28*
0704-07	bunch	0.31	0.47	*0.66*	0.14	0.29	*0.48*
0757-97	bunch	0.39	0.85	*0.46*	0.26	0.77	*0.34*
0758-97	bunch	0.37	0.92	*0.40*	0.20	0.57	*0.35*
1143-98	bunch				0.10	0.12	*0.83*
1632-98	bunch				0.12	0.37	*0.32*
1633-98	bunch				0.17	0.29	*0.59*
1634-98	bunch				0.22	0.48	*0.46*
1653-98	bunch				0.10	0.21	*0.48*
1655-98	bunch				0.20	0.47	*0.43*
1656-98	bunch				0.13	0.23	*0.57*
1657-98	bunch				0.17	0.32	*0.53*
1658-98	bunch				0.05	0.12	*0.42*
1659-98	bunch				0.06	0.10	*0.60*
Median conversion factor				*0.56*			*0.45*
RSD (%)				*34*			*46*
Overall mean conversion factor					***0.5***		

[a] determined as aminodiol (after hydrolysis, expressed in spiroxamine equivalents)
[b] F = conversion factor parent:total residue
[c] 4x (EU) as/ha, Southern Europe; trial not used for CXL evaluation
[d] 42 instead 14 days after application
[e] conversion factor restricted to a maximum value of 1

Update for grapes based on currently available data

In the previous evaluation (above) data were used from the then available residue trials. Since a larger number of residue trials are now available where grapes data >LOQ can be used to derive conversion factors, these values are more appropriate and are presented in Table CA 6.7.1-4.

This table shows all data from grapes trials where measurable residues allowed the data to be used for deriving conversion factors. Where residues of total spiroxamine by the common moiety method were below the values reported for spiroxamine, the factors were set to x1.0 (minimum or maximum).

The factor from total spiroxamine residue (common moiety) to spiroxamine for MRL purposes is derived from the large number of trials by comparison of the total spiroxamine (common moiety) data with the spiroxamine values in grapes, sampled between the relevant PHIs of 14 to 35 days after last application of spiroxamine. The common moiety method measures all metabolites that can be converted to the aminodiol metabolite and represents the Group C metabolites of spiroxamine.

In the previous EU evaluation, the x0.5 factor for total spiroxamine residue (common moiety) to spiroxamine for MRL purposes was also the basis (by applying the reciprocal 1/0.5) for using a

conversion factor of x 2 for the MRL residue definition to the risk assessment residue definition to account for the Group C metabolites being approximately equal in concentration to spiroxamine.

Using the latest data from residue trials, the factor for spiroxamine residue to total spiroxamine (common moiety) for risk assessment purposes is an average of x1.80 with a median value of x1.50. **It is proposed to use x1.8 for this submission which represents a realistic factor for estimating exposures.**

Table CA 6.7.1-4: Grapes conversion factors

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
CA 6.3.1/14 16-2111-14	Grapes	13	0.23	0.32	1.391	0.719
	Grapes	26	0.11	0.27	2.455	0.407
CA 6.3.1/14 16-2111-02	Grapes	13	0.271	0.36	1.328	0.753
	Grapes	28	0.17	0.24	1.412	0.708
CA 6.3.1/14 16-2111-05	Grapes	14	0.15	0.24	1.6	0.625
	Grapes	28	0.13	0.24	1.846	0.542
CA 6.3.1/14 16-2111-06	Grapes	13	0.05	0.06	1.2	0.833
	Grapes	26	0.06	0.09	1.5	0.667
CA 6.3.1/14 16-2111-07	Grapes	13	0.171	0.54	3.158	0.317
	Grapes	26	0.09	0.42	4.667	0.214
CA 6.3.1/14 16-2111-08	Grapes	15	0.04	0.08	2.00	0.50
	Grapes	29	0.02	0.06	3.00	0.333
CA 6.3.1/15 16-2078-01	Grapes	14	0.08	0.08	1	1
	Grapes	28	0.09	0.05	1	1
CA 6.3.1/15 16-2078-02	Grapes	13	0.15	0.18	1.2	0.833
	Grapes	29	0.1	0.11	1.1	0.909
CA 6.3.1/15 16-2078-04	Grapes	13	0.27	0.27	1	1
	Grapes	28	0.18	0.26	1.444	0.692
CA 6.3.1/15 16-2078-05	Grapes	14	0.06	0.07	1.167	0.857
CA 6.3.1/16 16-2047-01	Grapes	14	0.17	0.17	1	1
	Grapes	14	0.17	0.16	1.0	1.0
	Grapes	21	0.1	0.12	1.2	0.833
	Grapes	28	0.087	0.098	1.126	0.888
	Grapes	35	0.096	0.12	1.25	0.8
	Grapes	35	0.072	0.088	1.222	0.818
CA 6.3.1/16 16-2047-02	Grapes	14	0.043	0.042	1.0	1.0
	Grapes	14	0.03	0.029	1.0	1.0
	Grapes	21	0.026	0.025	1.0	1.0
	Grapes	28	0.025	0.019	1.0	1.0
	Grapes	35	0.027	0.026	1.0	1.0
	Grapes	35	0.017	0.018	1.059	0.944
CA 6.3.1/16 16-2047-03	Grapes	14	0.17	0.46	2.706	0.370
	Grapes	14	0.17	0.39	2.294	0.436
	Grapes	21	0.14	0.38	2.714	0.368
	Grapes	28	0.11	0.38	3.455	0.289
	Grapes	35	0.12	0.54	4.500	0.222
	Grapes	35	0.075	0.29	3.867	0.259
CA 6.3.1/16 16-2047-04	Grapes	14	0.036	0.036	1.0	1.0
	Grapes	14	0.046	0.049	1.065	0.939

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
	Grapes	21	0.06	0.073	1.207	0.822
	Grapes	28	0.042	0.049	1.167	0.857
	Grapes	35	0.042	0.042	1.0	1.0
	Grapes	35	0.035	0.043	1.229	0.814
CA 6.3.1/17 16-2193-01	Grapes	14	0.11	0.15	1.364	0.733
	Grapes	14	0.087	0.097	1.115	0.897
	Grapes	21	0.089	0.12	1.348	0.742
	Grapes	28	0.079	0.11	1.392	0.718
	Grapes	34	0.079	0.1	1.266	0.79
	Grapes	34	0.071	0.082	1.155	0.866
CA 6.3.1/17 16-2193-02	Grapes	14	0.13	0.2	1.538	0.65
	Grapes	14	0.22	0.36	1.8	0.556
	Grapes	21	0.094	0.15	1.596	0.627
	Grapes	28	0.13	0.28	2.154	0.464
	Grapes	35	0.091	0.18	1.978	0.506
	Grapes	35	0.1	0.18	1.8	0.556
CA 6.3.1/17 16-2193-03	Grapes	14	0.24	0.26	1.083	0.923
	Grapes	14	0.3	0.36	1.2	0.833
	Grapes	21	0.24	0.33	1.375	0.727
	Grapes	28	0.21	0.23	1.095	0.913
	Grapes	35	0.18	0.21	1.167	0.857
	Grapes	35	0.16	0.2	1.25	0.8
CA 6.3.1/17 16-2193-04	Grapes	14	0.14	0.17	1.214	0.824
	Grapes	14	0.16	0.18	1.125	0.889
	Grapes	21	0.1	0.14	1.4	0.714
	Grapes	28	0.092	0.14	1.522	0.657
	Grapes	35	0.11	0.15	1.364	0.733
	Grapes	35	0.094	0.12	1.277	0.783
CA 6.3.1/18 S20-02176-01	Grapes	14	0.03	0.03	1.0	1.0
	Grapes	34	0.02	0.04	2.0	0.5
CA 6.3.1/18 S20-02176-02	Grapes	14	<0.01	<0.01	-	-
	Grapes	34	0.05	0.09	1.8	0.556
CA 6.3.1/18 S20-02176-03	Grapes	14	0.09	0.10	1.111	0.90
	Grapes	34	0.03	0.03	1.0	1.0
CA 6.3.1/18 S20-02176-04	Grapes	14	0.04	0.10	2.5	0.4
	Grapes	34	0.09	0.15	1.667	0.6
CA 6.3.1/18 S20-02176-05	Grapes	14	0.12	0.20	1.667	0.6
	Grapes	34	0.07	0.11	1.571	0.636
CA 6.3.1/18 S20-02176-06	Grapes	14	0.16	0.22	1.357	0.727
	Grapes	34	0.07	0.15	2.143	0.467
CA 6.3.1/18 S20-02176-07	Grapes	14	<0.01	0.02	-	-
	Grapes	34	<0.01	0.01	-	-
CA 6.3.1/18 S20-02176-08	Grapes	14	0.08	0.18	2.25	0.444
	Grapes	34	<0.01	0.03	-	-
CA 6.3.1/19 13-2164-01	Grapes	13	0.84	0.81	1.0	1.0
	Grapes	13	0.55	0.58	1.055	0.948
	Grapes	20	0.66	0.61	1.0	1.0
	Grapes	27	0.59	0.64	1.085	0.922
	Grapes	34	0.6	0.62	1.033	0.968

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
	Grapes	34	0.53	0.57	1.075	0.93
CA 6.3.1/19 13-2164-02	Grapes	13	0.4	0.41	1.025	0.976
	Grapes	13	0.23	0.32	1.391	0.719
	Grapes	20	0.3	0.31	1.033	0.968
	Grapes	27	0.31	0.37	1.194	0.838
	Grapes	34	0.25	0.32	1.28	0.781
	Grapes	34	0.24	0.29	1.208	0.828
CA 6.3.1/20 13-2141-01	Grapes	14	0.08	0.09	1.125	0.889
	Grapes	28	0.08	0.10	1.250	0.800
CA 6.3.1/20 13-2141-03	Grapes	14	0.09	0.21	2.333	0.429
CA 6.3.1/20 13-2141-04	Grapes	14	0.11	0.13	1.182	0.846
	Grapes	21	0.08	0.10	1.25	0.8
	Grapes	28	0.08	0.10	1.25	0.8
	Grapes	35	0.082	0.10	1.205	0.82
CA 6.3.1/21 13-2163-01	Grapes	14	0.12	0.15	1.25	0.8
	Grapes	14	0.082	0.085	1.037	0.965
	Grapes	21	0.075	0.089	1.187	0.843
	Grapes	28	0.071	0.078	1.099	0.91
	Grapes	35	0.058	0.074	1.276	0.784
	Grapes	35	0.075	0.11	1.467	0.682
CA 6.3.1/21 13-2163-02	Grapes	14	0.11	0.18	1.636	0.611
	Grapes	14	0.059	0.091	1.542	0.648
	Grapes	21	0.089	0.15	1.685	0.593
	Grapes	28	0.061	0.11	1.803	0.555
	Grapes	35	0.05	0.1	2.00	0.500
CA 6.3.1/22 13-2161-01	Grapes	14	0.076	0.093	1.224	0.817
	Grapes	21	0.061	0.060	1.0	1.0
	Grapes	28	0.066	0.083	1.258	0.795
CA 6.3.1/22 13-2161-02	Grapes	14	0.076	0.17	2.237	0.447
	Grapes	14	0.11	0.19	1.727	0.579
	Grapes	21	0.059	0.063	1.068	0.937
	Grapes	35	0.056	0.13	2.321	0.431
CA 6.3.1/22 13-2161-04	Grapes	14	0.12	0.16	1.333	0.75
	Grapes	14	0.12	0.23	1.917	0.522
	Grapes	21	0.093	0.16	1.72	0.581
	Grapes	28	0.084	0.15	1.852	0.54
	Grapes	35	0.09	0.15	1.667	0.6
	Grapes	35	0.062	0.14	2.258	0.443
CA 6.3.1/23 13-2134-01	Grapes	14	0.10	0.23	2.300	0.435
	Grapes	21	0.09	0.21	2.333	0.429
CA 6.3.1/23 13-2134-02	Grapes	14	0.1	0.18	1.80	0.556
	Grapes	21	0.09	0.21	2.333	0.429
	Grapes	35	0.09	0.24	2.667	0.375
CA 6.3.1/23 13-2134-03	Grapes	14	0.41	0.35	1.0	1.0
	Grapes	21	0.41	0.42	1.024	0.976
	Grapes	28	0.27	0.33	1.222	0.818
	Grapes	35	0.3	0.27	1.0	1.0
CA 6.3.1/23	Grapes	14	0.3	0.24	1.0	1.0

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
13-2134-04	Grapes	21	0.26	0.26	1.0	1.0
	Grapes	28	0.27	0.26	1.0	1.0
	Grapes	35	0.27	0.26	1.0	1.0
CA 6.3.1/24 09-2036-01	Grapes	14	0.1	0.1	1.0	1.0
	Grapes	14	0.12	0.13	1.083	0.923
	Grapes	21	0.15	0.15	1	1
	Grapes	35	0.11	0.13	1.182	0.846
	Grapes	35	0.1	0.13	1.300	0.769
CA 6.3.1/24 09-2036-02	Grapes	14	0.1	0.16	1.6	0.625
	Grapes	14	0.12	0.17	1.417	0.706
	Grapes	35	0.08	0.16	2.00	0.500
	Grapes	35	0.1	0.21	2.1	0.476
CA 6.3.1/24 09-2036-03	Grapes	14	0.06	0.06	1.0	1.0
	Grapes	14	0.1	0.11	1.1	0.909
	Grapes	21	0.05	0.05	1.0	1.0
CA 6.3.1/24 09-2036-04	Grapes	14	0.05	0.1	2.00	0.50
	Grapes	14	0.05	0.09	1.8	0.556
CA 6.3.1/01 R 2007 0675/8	Grapes	14	0.16	0.23	1.438	0.696
	Grapes	21	0.14	0.23	1.643	0.609
	Grapes	35	0.13	0.2	1.538	0.65
CA 6.3.1/01 R 2007 0676/6	Grapes	14	0.15	0.25	1.667	0.6
	Grapes	35	0.15	0.23	1.533	0.652
CA 6.3.1/01 R 2007 0703/7	Grapes	14	0.18	0.35	1.944	0.514
	Grapes	21	0.12	0.28	2.333	0.429
	Grapes	35	0.07	0.24	3.429	0.292
CA 6.3.1/01 R 2007 0704/5	Grapes	14	0.23	0.35	1.522	0.657
	Grapes	35	0.11	0.22	2.00	0.500
CA 6.3.1/02 R 2007 0672/3	Grapes	14	0.16	0.27	1.688	0.593
	Grapes	20	0.17	0.32	1.882	0.531
	Grapes	35	0.11	0.3	2.727	0.367
CA 6.3.1/02 R 2007 0673/1	Grapes	14	0.17	0.27	1.588	0.63
	Grapes	35	0.09	0.26	2.889	0.346
CA 6.3.1/02 R 2007 0704/6	Grapes	14	0.08	0.21	2.625	0.381
	Grapes	21	0.06	0.21	3.5	0.286
	Grapes	35	0.05	0.23	4.6	0.217
CA 6.3.1/02 R 2007 0702/9	Grapes	14	0.52	0.66	1.269	0.788
	Grapes	35	0.47	0.66	1.404	0.712
CA 6.3.1/03 R 2000 0194/0	Grapes	14	0.14	0.12	0.857	1.167
	Grapes	35	0.07	0.09	1.286	0.778
	Grapes	14	0.12	0.11	1.0	1.0
	Grapes	35	0.05	0.1	2	0.5
CA 6.3.1/03 R 2000 0195/9	Grapes	14	0.1	0.28	2.8	0.357
	Grapes	14	0.09	0.22	2.444	0.409
CA 6.3.1/04 816531	Grapes	35	0.09	0.19	2.111	0.474
	Grapes	35	0.06	0.15	2.50	0.40
CA 6.3.1/04 816558	Grapes	35	0.19	0.44	2.316	0.432
	Grapes	35	0.16	0.38	2.375	0.421
CA 6.3.1/04 816566	Grapes	35	0.12	0.25	2.083	0.48
	Grapes	35	0.08	0.21	2.625	0.381

Trial ID	Sample material	PHI (days)	Spiroxamine residue (mg/kg)	Total spiroxamine residue (mg/kg)	Conversion factor spiroxamine to total residue	Conversion factor total residue to spiroxamine
CA 6.3.1/04 816574	Grapes	35	0.16	0.35	2.188	0.457
	Grapes	35	0.13	0.38	2.923	0.342
CA 6.3.1/04 816582	Grapes	35	0.07	0.17	2.429	0.412
CA 6.3.1/04 816590	Grapes	35	0.09	0.15	1.667	0.6
	Grapes	35	0.08	0.12	1.5	0.667
CA 6.3.1/05 811432	Grapes	35	0.1	0.52	5.2	0.192
	Grapes	35	0.1	0.56	5.6	0.179
CA 6.3.1/05 816329	Grapes	35	0.12	0.79	6.583	0.152
	Grapes	35	0.07	0.52	7.429	0.135
CA 6.3.1/05 816337	Grapes	35	0.14	0.24	1.714	0.583
	Grapes	35	0.14	0.3	2.143	0.467
CA 6.3.1/05 816345	Grapes	35	0.18	0.38	2.111	0.474
	Grapes	35	0.21	0.39	1.857	0.538
CA 6.3.1/06 706582	Grapes	14	0.38	0.47	1.237	0.809
	Grapes	35	0.31	0.46	1.484	0.674
	Grapes	14	0.3	0.43	1.433	0.698
	Grapes	35	0.18	0.39	2.167	0.462
CA 6.3.1/06 706590	Grapes	14	0.22	0.36	1.636	0.611
	Grapes	35	0.12	0.22	1.833	0.545
	Grapes	14	0.16	0.32	2.00	0.500
	Grapes	35	0.09	0.25	2.778	0.360
CA 6.3.1/06 706604	Grapes	14	0.11	0.23	2.091	0.478
CA 6.3.1/06 706612	Grapes	14	0.14	0.4	2.857	0.35
	Grapes	35	0.14	0.42	3	0.333
	Grapes	14	0.162	0.56	3.457	0.289
	Grapes	35	0.152	0.35	2.303	0.434
CA 6.3.1/06 707570	Grapes	14	0.24	0.52	2.167	0.462
	Grapes	35	0.16	0.47	2.938	0.34
	Grapes	14	0.22	0.57	2.591	0.386
	Grapes	35	0.12	0.45	3.75	0.267
CA 6.3.1/06 707589	Grapes	14	0.73	1.72	2.356	0.424
	Grapes	35	0.382	1.06	2.775	0.36
	Grapes	14	0.46	1.5	3.261	0.307
	Grapes	35	0.35	1.18	3.371	0.297
				Average	1.798	0.662
				Median	1.50	0.657
				Std Dev	0.965	0.238
				RSD	53.69	35.99

The RMS-DE for the previous EU evaluation changed the LoEP in 2017 from the EFSA 2010 conclusions and stated that the Group B metabolites of spiroxamine should also be included in the risk assessment for grapes and that as a conservative position, the total contribution from Group B metabolites would be equal to spiroxamine and Group C metabolites, giving a conversion factor before any toxicity exposure factor was applied of spiroxamine x (1 + 2 x 1) = spiroxamine x 3

Although this was a very conservative assumption, the RMS-DE did not see it as double accounting of the contribution to consumer exposure from grapes metabolites since their position at that time was

seen to be justified by the toxicological profile and the higher toxicity at that time (August 2017) attributed to the lead Group B (spiroxamine-cyclohexanol, M13) and lead Group C (spiroxamine-aminodiol, M28) metabolites compared with spiroxamine as shown below. Table CA 6.7.1-5 presents the end-points initially proposed by the RMS-DE and Table CA 6.7.1-6 presents the same end-points after the evaluation of the confirmatory data (EFSA 2018 and EFSA mandate 2020). The toxicity exposure factor (TEF) comparing the end-points with spiroxamine (after expression of the metabolite end-point as spiroxamine equivalents by adjustment for molecular weight) is also included in these tables.

Table CA 6.7.1-5: Endpoints: RMS August 2017

Compound	Endpoint	Value	Safety Factor	TEF cf spiroxamine	Reference
Spiroxamine	Acceptable Daily Intake (ADI)	0.025 mg/kg bw/d	100	-	EFSA Journal 2010;8(10)1719
M13 (Group B)		0.006 mg/kg bw/d [0.01 as spiroxamine]	5400	2.5	Revision RMS April 2017
M28 (Group C)		0.006 mg/kg bw/d [0.01 as spiroxamine]	5400	2.5	Revision RMS April 2017
Spiroxamine	Acute Reference Dose (ARfD)	0.10 mg/kg bw	100	-	EFSA Journal 2010;8(10)1719
M13 (Group B)		0.033 mg/kg bw [0.06 as spiroxamine]	1000	1.667	Revision RMS April 2017
M28 (Group C)		0.033 mg/kg bw [0.06 as spiroxamine]	1000	1.667	Revision RMS April 2017

Table CA 6.7.1-6: Endpoints: EFSA Confirmatory data (EFSA 2018 and EFSA 2021)

Compound	Endpoint	Value	Safety Factor	TEF cf spiroxamine	Reference
Spiroxamine	Acceptable Daily Intake (ADI)	0.025 mg/kg bw/d	100	-	EFSA Journal 2010;8(10)1719
M13 (Group B)		0.03 mg/kg bw/d [0.057 as spiroxamine]	1000	0.439	EFSA Supporting publication 2018:EN-1360; EFSA publication 2021.6385
M28 (Group C)		0.03 mg/kg bw/d [0.055 as spiroxamine]	1000	0.454	EFSA Supporting publication 2018:EN-1360; EFSA publication 2021.6385
Spiroxamine	Acute Reference Dose (ARfD)	0.10 mg/kg bw	100	-	EFSA Journal 2010;8(10)1719

Compound	Endpoint	Value	Safety Factor	TEF cf spiroxamine	Reference
M13 (Group B)		0.10 mg/kg bw [0.19 as spiroxamine]	300	0.526	EFSA Supporting publication 2018:EN-1360; EFSA publication 2021.6385
M28 (Group C)		0.50 mg/kg bw [0.922 as spiroxamine]	300	0.108	EFSA Supporting publication 2018:EN-1360; EFSA publication 2021.6385

From the confirmatory data (EFSA 2018 and EFSA 2021) it is clear that the metabolites M13 and M28 representing Group B and Group C metabolites, respectively, have higher endpoints for ADI and ARfD (both as substances and when expressed as spiroxamine equivalents) and are less toxic than parent compound spiroxamine.

Therefore, the agreed spiroxamine end-points can be used for consumer risk assessment as a worst case. In other words, when the necessary conversion factors for MRL residue definition to risk assessment definition are applied, the exposure is compared with the spiroxamine end-points and no additional toxicity exposure factor (TEF) is required. It should also be noted that, as shown in Table CA 6.7.1-6, the TEF for the Group B and Group C metabolites is actually below 1. It could be considered that a reduction in toxicity effect on consumers would theoretically be applicable and this could be considered when reviewing the potential impact of isomer ratios on risk assessment.

Furthermore, it is no longer considered appropriate or necessary to include Group B metabolites [4-tert.-butylcyclohexanone moiety] as well as the Group C metabolites [aminodiol moiety] in the risk assessment residue definition for grapes. The reason for this is that, despite the discussed different toxicological profile of Group B and Group C lead metabolites in comparison to spiroxamine, the metabolites are of lower overall toxicity and the toxicological burden can be conservatively assessed by use of a conversion factor from the parent spiroxamine MRL based on the total residue of spiroxamine from the aminodiol (M28, Group C) common moiety residue method. By this approach based on grapes metabolism data (M-006104-01-1, CA 6.2.1/04) and a radiovalidation study (M-020937-01-1, see Section MCA 4) almost 80% of the total toxicological burden is included for comparison against the spiroxamine end-points, which is sufficiently protective given the margin of safety for consumer risk assessment shown in Section CA 6.9.

Parent plus Group C for risk assessment is sufficiently protective for the consumer.

From a toxicological perspective, the following information supports this position. Unlike the parent compound, spiroxamine and spiroxamine-N-oxide, which are considered tertiary amines, spiroxamine-cyclohexanol (M13 which has lost the tertiary amine group) and spiroxamine-aminodiol (M28, only containing the aminodiol group) did not display histopathological lesions associated with tertiary amines (hyperkeratosis of the epithelium of the oesophagus and forestomach). It is prudent to acknowledge that for M28 the presence of the two hydroxy groups in the aminodiol vastly reduces the overt pH and pKa values observed with both the parent and M03 (spiroxamine-N-oxide), and therefore the mucosal membrane containing tissues, which would be site of first contact are not targeted. The two hydroxy groups present in M28 vastly increases the water solubility, and with a smaller chemical structure (*i.e.* omission of the cyclohexane dimethyl ethyl group) results in rapid absorption, with a likely scenario of absorption between mucosal cells within the gastric environment, rather than crossing between membranes. Therefore the existence within such an environment is markedly reduced compared to parent and M03.

Livestock

Conversion factors (CFs) for use in products of animal origin are presented in Table CA 6.7.1-7, taking into account the proposed residue definitions for monitoring (MRL) and risk assessment as proposed in this dossier for renewal of Approval. In some cases, particularly for poultry tissues and eggs, these differ from those factors described in the RAR for spiroxamine: Spiroxamine, Volume 3, Annex B.7, Residue data. Final RMS version revised March 2020. In that document, the values presented in section B.7.12.3 show the original CFs proposed that were amended during the January 2010 response to comments received during the peer review. The changes were highlighted in yellow in the document and do not appear to have been re-evaluated since. It is now considered that there was an error in these conversion factors. Table CA 6.7.1-7 below details how these CFs were derived using data from ruminant and poultry metabolism studies.

It should be noted that the values now proposed are not new and were previously considered and evaluated during the 2015 spiroxamine MRL review (EFSA journal 2015;13(1):3992).

The calculations were made as follows:

Ruminant: Sum of residue of spiroxamine (mg/kg) + M06 (mg/kg) + M19 (mg/kg) + M07 (mg/kg) / M06 (mg/kg)

Poultry: Sum of residue of spiroxamine (mg/kg) + M06 (mg/kg) + M01 (mg/kg) + M02 (mg/kg) / spiroxamine (mg/kg) + M06 (mg/kg)

Table CA 6.7.1-7 Derivation of conversion factors for animal products

Ruminant Refer to Table CA 6.2.3/01-11 for details of residues in edible tissues and milk		
Animal/tissue	**Conversion factor**	**Derivation (residues as mg spiroxamine eq./kg)**
Ruminant/pig muscle:	1.4	0.106 + 0.5 + 0.082 / 0.5
Ruminant/pig liver:	3.0	0.38 + 4.33 + 7.22 + 1.1 / 4.33
Ruminant/pig kidney:	3.8	2.27 + 1.48 + 1.89 + 0.03 / 1.48
Ruminant/pig fat:	1.8	0.063 + 0.199 + 0.101 / 0.199
Milk:	1.2	0.101 + 0.496 / 0.496
Poultry Refer to Table CA 6.2.2/01-9 for details of residues in edible tissues and eggs		
Animal/tissue	**Conversion factor**	**Derivation (residues as mg spiroxamine eq./kg)**
Poultry liver:	3.0	3.723 + 3.793 + 1.486 + 0.324 / 1.486 + 2.324
Poultry muscle:	1.4	0.225 + 0.273 + 0.901 + 0.43 / 0.901 + 0.43
Poultry fat:	4.1	1.038 + 0.42 + 0.210 + 9.562 / 0.210 + 9.562
Poultry egg:	1.4	0.097 + 0.086 + 0.317 + 0.1 / 0.317 + 0.1

End-Point	**Residue definition** **Proposed EU end point**
Definition of the residue in animal products (for MRL-setting purposes) RD-Mo	Ruminants: Spiroxamine-acid (M06), expressed as spiroxamine Poultry: Sum of spiroxamine and spiroxamine-acid (M06), expressed as spiroxamine (sum of isomers)

End-Point	Residue definition Proposed EU end point
Definition of the residue in animal products (for risk assessment purposes) RD-RA	Ruminants: Sum of spiroxamine, spiroxamine-acid (M06), its glucuronide conjugate (M19) and spiroxamine-hydroxy acid (M07), expressed as spiroxamine (sum of isomers) Poultry: Sum of spiroxamine, spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-acid (M06) expressed as spiroxamine (sum of isomers)

RESIDUE DEFINITION FOR MRL AND MONITORING (CROPS)

Parent spiroxamine is the major residue component in primary crop cereals (wheat and barley) and fruits (grapes) and also, if required, for rotational crops. The proposed residue definition for monitoring and setting of MRLs is spiroxamine (parent only).

The previously acceptable metabolism studies on cereals, grapes and bananas, summarised again in this dossier (refer to Section CA 6.2.1) support this residue definition and no changes are required.

RESIDUE DEFINITION FOR DIETARY RISK ASSESSMENT (CROPS)

Information presented here is adapted and updated, where necessary, from Applicant Position Paper CA 6.7/01 (M-344146-01-2, 2009) which was also considered in the previous evaluation RAR, Volume 3 B.7, August 2017.

The available plant metabolism studies (refer to Section CA 6.2 and Section CA 6.6) cover the crop groups cereals (wheat) and fruits (grapes, banana) as well as in rotational crops cereals (wheat), leafy crops (Swiss chard, turnip leaves) and root crops (turnip root). The two crop groups covered by primary crop metabolism studies are not intended to propose a global crop residue definition as the supported uses of spiroxamine are only applicable to primary uses on cereals and fruits plus rotational crops following cereal field uses.

The metabolism pathway in the primary crop groups cereals and fruits shows some overlapping of metabolic reactions such as desalkylation and oxidation at the amine and to a lesser extent, at the tert.-butyl group. The main differences in metabolic pathway between cereals and fruits are with regard to the higher stability of the spiro position during the metabolism degradation process in cereals and the nature and the amount of specific metabolites formed in the different commodities. Therefore, a separate residue definition for dietary risk assessment is required for primary uses on cereals (and for rotational field crops) and fruits/grapes.

Primary crops

For the supported representative uses on cereals and grapes, the following data is available from the wheat and grape metabolism studies, covering both tested radiolabels of spiroxamine to give data on both the tert.-butyl cyclohexyl moiety and the aminodiol moiety.

Residues reported at significant levels in consumer edible commodities (≥10% of TRR and ≥0.01 mg/kg) or in potential livestock feed items (≥10% of TRR and ≥0.05 mg/kg) were parent spiroxamine (cereals and fruits) plus the metabolites M03 spiroxamine-N-oxide (cereals), M13 spiroxamine-cyclohexanol (fruits; aglycon or common moiety to the associated glycoside and fatty acid conjugates assigned as M32, M34, M35 and M36 after release by hydrolysis), M14 spiroxamine-diol (fruits, aglycon hydrolysis product of glycoside conjugate M24) and M28 spiroxamine-aminodiol (fruits).

Due to the potential for significant uptake of residues by plants or significant residues from direct foliar application, further minor metabolites (<10 % of TRR) may show the potential to exceed the level of

significance in food (0.01 mg/kg) or feed (0.05 mg/kg) at the supported critical GAP rates. These are metabolites M01 spiroxamine-desethyl (cereals, fruits), M02 spiroxamine-despropyl (cereals, fruits), M04 spiroxamine-N-formyl-desethyl (cereals), M05 spiroxamine-hydroxy (cereals, fruits), M09 spiroxamine-hydroxy-despropyl (cereals), M20 spiroxamine-hydroxy-N-oxide (cereals; common moiety to the associated glucoside assigned as M21), M23 spiroxamine-hydroxy-ketone-conjugate (cereals) and M31 spiroxamine-despropyl-aminodiol (fruits).

For cereals and for fruits, no metabolite specific residue methods are used in the crop residue trials. The two principal analytical methods are used for total residues of spiroxamine, both of which include parent and Group A metabolites (cereals, rotational crops and fruits) and separately determine Group B metabolites (cereals and rotational crops) and Group C metabolites (fruits), are documented in the respective residue trial summaries and fully described in MCA Section 4.

Cereal crops

In the cereal metabolism studies, hydrolysis experiments were performed in order to develop an analytical method based on the tert.-butylketone derivative (4-tert.-butylcyclohexanone, M15) capable to determine the majority of total residue in cereal straw and grain. The tert.-butylketone derivative was the dominant hydrolysis product and common moiety of parent spiroxamine and some of its metabolites and accounted in the [cyclohexyl-1-^{14}C] metabolism study for 94.9% and 78.5% of the TRR in forage (day 0 and 14), 72.8% of the TRR in straw and 48.6% of the TRR in grain. The residue compounds determined by hydrolysis were parent compound, spiroxamine-desethyl (M01), spiroxamine-despropyl (M02), spiroxamine-aminoxide (M03), spiroxamine-N-formyl-desethyl (M04) and minor hydrolysis products determined as tert-butylcyclohexanone.

In a radiovalidation study (M-090998-01-1, see Section MCA 4) using samples of the [cyclohexyl-1-^{14}C] metabolism study in cereals, 88.3% of TRR in straw and 71.1% of TRR in grain were extracted and 68.7 % (straw) and 33.6% (grain) detected as tert.-butylcyclohexanone by the total residue method M00312, which is also used for the residue trials.

In a method validation study (M-018352-02-1, method M00312, see Section MCA 4), recovery experiments in cereal straw and grain were performed with parent compound, the spiroxamine-desethyl (M01), spiroxamine-despropyl (M02), spiroxamine-N-oxide (M03) and spiroxamine-acetyl-despropyl (BNF 5567A is not a reported metabolite in cereals or rotational crops) after hydrolysis to the common moiety. For these compounds acceptable recoveries were determined. In the cereal metabolism study, these compounds represent approximately 52% of the TRR in straw and 36% of the TRR in the grain and are therefore in a good agreement with the results of the radiovalidation study. No method validation was performed for spiroxamine-N-formyl-desethyl (M04) but this metabolite was <10% TRR in cereal matrices and <0.01 mg/kg in cereal grain and is expected to be determined by the total spiroxamine residue method.

Taking into account:

i) a very large portion of total radioactivity will be determined with the developed total residue method for cereals
ii) the toxicological properties of all metabolites occurring in human edible commodities are sufficiently characterised
iii) all major metabolites are covered by the analytical methods for total residues of spiroxamine used in residue trials (and the basis for risk assessment conversion factors)

It is proposed to set the residue definition for dietary risk assessment (cereals) as

sum of spiroxamine and metabolites containing the tert.-butylcyclohexanone moiety, expressed as spiroxamine

The potential relevance of minor metabolites (<10% of TRR) exceeding the trigger of 0.01 mg/kg (food) or 0.05 mg/kg (feed) is considered to be covered by the common moiety total residue proposal.

Fruits (grapes)

In the fruit metabolism studies, hydrolysis experiments were performed in order to develop an analytical method based on the major metabolite and hydrolysis product aminodiol, capable to determine the total residue. In the hydrolysis experiments of the [dioxolane-4-^{14}C] metabolism studies 79.1% and 89.1% of TRR were determined in grapes and bananas, respectively, as aminodiol covering parent compound, minor metabolite spiroxamine-hydroxy (M05) and the cleavage product aminodiol (M28).

In a radiovalidation study (M-020937-01-1, see Section MCA 4) using samples of the [dioxolane-4-^{14}C]-metabolism study in grapes, 86.7 % of TRR were extracted and 78.1 % were detected as spiroxamine-aminodiol (M28) by the total residue method M00407 which is also used for residue trials. In view of the toxicological evaluation of fruit metabolites of Group C (representative M28), it is proposed to set the residue definition for dietary risk assessment (fruits) as

Sum of spiroxamine and metabolites containing the aminodiol (N-ethyl-N-propyl-1,2-dihydroxy-3-amino-propane), expressed as spiroxamine

Rotational crops

In rotational crops, residues detected in two metabolism studies (2N rate) at significant levels (≥10 % of TRR and 0.01 mg/kg for food or 0.05 mg/kg for feed) were primarily parent spiroxamine along with metabolites M01, M02, M03, M05 (including its glycoside M40) and M38.

In a field rotational crop study (M-006097-01-1, CA 6.6.2/01) conducted at 2N the critical field application rate (2 x 0.750 kg a.s./ha) to a primary cereal crop (plant material incorporated to the soil before plant back of rotational crops) and covering northern European growing conditions, analysis of total spiroxamine derived residues (parent and Group A metabolites, detected as the Group B common moiety 4-tert.-butylcyclohexanone) revealed no residues ≥ LOQ except for cereal forage in one trial at the LOQ level. The analytical method was validated in all rotational crop matrices for parent, spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-N-oxide (M03). For all these compounds it is expected that residues are unlikely to be of significance. A new field rotational crop study is ongoing to further investigate the potential for uptake of residues according to the latest OECD 2018 guidance with application to bare soil (Interim report expected for first top-up submission).

Metabolite spiroxamine-hydroxy (M05, including its spiroxamine-hydroxy glycoside (M40)) was detected in both confined rotational crop studies at potentially significant levels at the 30 day plant-back interval (PBI) in human edible (Swiss chard) and livestock feed commodities (wheat straw and hay). As these metabolites would not be included in the total spiroxamine residue method, detectable residues under realistic field conditions cannot be excluded for short PBIs but are in reality not likely to be of concern as the studies were conducted to bare soil at a 2N rate compared to the representative use on cereals.

Furthermore, on the basis of confined rotational crop studies with both cyclohexyl and dioxolane labels, no residues in human edible commodities are expected for 2nd rotation plant back intervals (study PBI of 161 or 193 days). Under the representative critical GAP conditions for the field crop cereals, applications of spiroxamine will typically be made to established and well developed crops, where subsequent failure and short term sowing of a rotated human edible leafy crop is not a realistic case. The typical sowing of a leafy crop after application of spiroxamine to cereals is expected at the earliest to be in the next vegetation period/season.

Residues in livestock feed items could theoretically slightly exceed a level of 0.05 mg/kg at the critical GAP rate from any short PBI (30 days), however as discussed above, this is not a realistic situation.

Generally, these low levels are not likely to contribute significantly to the overall dietary burden of livestock animals. Therefore, residues of M05 or M40 are not considered relevant for inclusion into the residue definition for rotational crops.

Metabolite spiroxamine-N-formyl-despropyl (M38) was found in the CRC metabolism study with dioxolane label in wheat hay and straw at potentially significant levels (approximately 7% TRR, 0.09 mg/kg in straw at 1N rate, 2nd rotation; 193 days PBI). Given the very low contribution of this residue to the calculated maximum dietary burden for livestock animals (<1%), and the fact that this metabolite will be determined by the total spiroxamine residue method, residues of M38 are not considered relevant for specific inclusion in the residue definition.

The proposed residue definition for rotational crops is the same as for cereals

sum of spiroxamine and metabolites containing the tert.-butylcyclohexanone moiety, expressed as spiroxamine

Toxicological relevance of metabolites in human edible commodities

The discussions below are summarised in Table CA 6.7.1-8 and Table CA 6.7.1-9.

Spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02). Group A:

Metabolites M01 and M02 are N-dealkylated metabolites of spiroxamine in primary and rotational crops as well as in livestock animals (identified in poultry, postulated intermediates for ruminants and rats). In cereals and grapes these are minor metabolites (<5% TRR) and were not reported in ruminants. Additional data on the toxicological properties of these metabolites are not available, however, as dealkylation can be considered as a common detoxification step in animal metabolism, M01 and M02 are regarded as of equal or lower toxicity to parent compound. No concerns for consumer exposure.

Spiroxamine-N-oxide (M03). Group A:

Metabolite M03 is minor in grapes (<5% TRR) and significant in cereals (up to 22% TRR in straw). It is not reported in livestock. Appropriate toxicological data indicates an equal toxicity of metabolite M03 to parent compound (Section CA 5.8). No concerns for consumer exposure.

Spiroxamine-hydroxy (M05). Group A:

Metabolite M05 is a hydroxylated metabolite on the t-BCH group and is an intermediate rat and livestock metabolite with less or similar toxic potential when compared to spiroxamine. M05 is not identified in grapes but is a transient metabolite prior to cleavage and glucoside conjugation as the group of metabolites covered by the identified di-conjugate M24. M05 is minor in cereals (<10% TRR) and not reported in grain. No concerns for consumer exposure.

Spiroxamine – N-formyl-despropyl (M38). Group A:

Metabolite M38 can be considered as the formylated derivative of spiroxamine-despropyl (M02).

It can be expected that M38 is hydrolysed into M02 upon ingestion by mammals. M38 is regarded as of equal or lower toxicity when compared to parent compound. No concerns for consumer exposure.

Spiroxamine-cyclohexanol (M13). Group B (lead Group B metabolite):

Conjugates of M13 (4-tert. butyl cyclohexan-1-ol), with sugars or fatty acids identified as M24, M32, M33, M34, M35 and M36, are reported in grapes (approximately 25% of TRR detected as total amount of M13) and banana pulp (approximately 14% of TRR). The respective glucuronide (M22) occurred in a rat metabolism study in amounts of up to 1.7%. The aglycon (M13) is formed in gastric juice of rats from either of spiroxamine or spiroxamine-N-oxide (in amounts of 20 to 27% within 4 h at 37°C). M13 has been specifically assessed in toxicological testing (refer to Section MCA 5.8) and under the

confirmatory data evaluation (EFSA 2018 and EFSA 2021) an agreed ADI and ARfD was set at a higher value than spiroxamine, therefore confirming that it is less toxic than parent spiroxamine.

In the 28-d gavage toxicity studies in rats with spiroxamine and M13, different effects were reported. In the developmental toxicity studies in rats, different effects were reported for spiroxamine and M13 dosed as the acetate.

The toxicological profile of spiroxamine (and therefore also Group A metabolites) is driven by the tertiary amines found in the active substance and Group A metabolites. This is different to the toxicological profile of metabolite M13 (representing Group B) where M13 (which has lost the tertiary amine group) did not display histopathological lesions associated with tertiary amines. The difference in toxicological profile is further evaluated in the MCA Section 5 of this dossier, however as noted above the toxicological effects of the M13 (Group B) metabolites are less of a concern for consumers compared with those of the active substance spiroxamine and the toxicological reference end-points of the group B metabolites are now accepted following peer review of the confirmatory data [EFSA 2018 and EFSA 2021] to be of lower toxicity than spiroxamine. EFSA Journal 2021;19(1):6385 states

*Based on an acute oral study in rats and an acute dermal study in rabbits, M13 was concluded of low acute toxicity. M13 was negative in a bacterial reverse mutation assay and in an in vitro chromosomal aberration assay. A quantitative structure-activity relationship (QSAR) analysis was performed and no alerts were reported for genotoxicity. It was concluded that M13 is unlikely to be genotoxic in vitro. In a 28-day oral toxicity study in rats performed with M13, a no-observed adverse effect level (NOAEL) of 50 mg/kg bw per day was set based on clinical signs and transient signs of neurotoxicity. An oral developmental toxicity study in rat conducted with M13 acetate was also submitted to assess the general toxicity of M13. In this study, a maternal toxicity NOAEL of 40 mg/kg bw per day was identified based on clinical signs, corresponding to 31.5 mg/kg bw per day for M13 after correction of the difference in molecular weight between M13 and M13 acetate. For metabolite M13, **the agreed ADI is 0.03 mg/kg bw per day**, based on the maternal toxicity NOAEL of 31.5 mg/kg bw per day for clinical signs from the developmental toxicity study in rats performed with M13 acetate, applying an UF of 1000 (100 standard UF and an additional factor of 10 due to incomplete data set). **The acute reference dose (ARfD) is 0.1 mg/kg bw**, based on the same maternal toxicity NOAEL of 31.5 mg/kg bw per day from the developmental toxicity study in rat performed with M13 acetate and supported by the NOAEL of 50 mg/kg bw per day for transient signs of neurotoxicity observed in the 28-day rat study performed with M13. It was agreed by all experts including the RMS to apply an UF of 300, including an additional factor of 3 due to the lack of a complete data package.*

Therefore, despite separate end-points being available for M13 and by extrapolation to all Group B metabolites, there are no concerns for consumer exposure of total spiroxamine residues from grapes (where group B metabolites are significant) and a protective approach to consumer risk assessment for grapes will use the proposed residue definition considering spiroxamine, Group A metabolites and Group C metabolites only. Including Group B metabolites is double accounting of total exposure when concentrations are expressed as total spiroxamine equivalents. The ADI and ARfD end-points for spiroxamine are lower (higher toxicity) than M13 and therefore a conservative and conventional approach to consumer risk assessment can still be used despite separate end-points being available for the Group B and Group C metabolites.

<u>Spiroxamine-diol-diglycoside (M24). Group B:</u>

Not identified in cereals. In grapes M24 was the major metabolite in a multi-component group of polar conjugates and post hydrolysis will form M14. This glycoside conjugate is regarded as of equal toxicity as M13 (less toxic than spiroxamine). No concerns for consumer exposure.

<u>Spiroxamine-aminodiol (M28). Group C (lead Group C metabolite):</u>

Metabolite M28 (free form) occurred in primary crop fruit (grapes and banana) metabolism studies at levels of 31 to 38% TRR; it was not significant in rotational crops (<5% TRR and ≤0.01 mg/kg in any

edible commodities) and not reported in cereals. In a rat metabolism study, it occurred in urine at 2.2 to 5.7% of the applied dose.

M28 has been specifically assessed in toxicological testing (refer to Section MCA 5.8) and under the confirmatory data evaluation (EFSA 2018 and EFSA 2021) an agreed ADI and ARfD was set at a higher value than spiroxamine, therefore confirming that it is less toxic than parent spiroxamine.

Based on the specific study design of the 28-d dietary toxicity study in rats with M28, a direct comparison of the effects induced by spiroxamine and M28 is not possible. In the developmental toxicity studies in rats, different effects were reported for spiroxamine and M28.

The toxicological profile of spiroxamine (and therefore also Group A metabolites) is driven by the tertiary amines found in the active substance and Group A metabolites. This is different to the toxicological profile of metabolite M28 (representing Group C) where M28 (only containing the aminodiol group) did not display histopathological lesions associated with tertiary amines. The difference in toxicological profile is further evaluated in the MCA Section 5 of this dossier, however the toxicological effects of the M28 (Group C) metabolites are less of a concern for consumers compared with those of the active substance spiroxamine and the toxicological reference end-points of the metabolites are now accepted following peer review of the confirmatory data [EFSA 2018 and EFSA 2021] to be of lower toxicity than spiroxamine. EFSA Journal 2021;19(1):6385 states:

Metabolite M28 is of moderate acute toxicity on the basis of an acute oral toxicity study in rat. A bacterial reverse mutation assay, a Hypoxanthine-Guanine Phospho Ribosyl Transferase (HGPRT) gene mutation assay and an in vitro micronucleus test in human lymphocytes were negative. Therefore, it was concluded that M28 is unlikely to be genotoxic in vitro. In a 28-day study in rats, no adverse effects were observed up to the highest dose tested. The top dose of 28.4/31.4 mg/kg bw per day was considered to be the NOAEL. In a developmental toxicity study in rats, the developmental toxicity NOAEL of 30 mg/kg bw per day was based on changes in ossification at 150 mg/kg bw per day and the maternal toxicity NOAEL was 150 mg/kg bw per day based on mortality, clinical observations (gasping and rales), gaseous contents and gas filled parts of the gastrointestinal tract, and individual body weight decreases at 500 mg/kg bw per day. For the metabolite M28, the ***ADI is 0.03 mg/kg bw per day****, based on the developmental toxicity NOAEL of 30 mg/kg bw per day for changes in ossification in the developmental toxicity study in rats, supported by the NOAEL of 30 mg/kg bw per day (top dose) in the 28-day study in rats and applying an UF of 1000 (including an additional factor of 10 due to incomplete data set).* ***The ARfD is 0.5 mg/kg bw****, based on the maternal toxicity NOAEL of 150 mg/kg bw per day for mortality and clinical signs observed in the developmental toxicity study in rats, applying an UF of 300 (including an additional factor of 3 due to the lack of a complete data package). All experts, including the RMS agreed with this conclusion, confirming the outcome indicated in the technical report on the same confirmatory data (EFSA 2018).*

Therefore, despite separate end-points being available for M28 and by extrapolation to all Group C metabolites, there are no concerns for consumer exposure of total spiroxamine residues from grapes (where group C metabolites are significant) and a protective approach to consumer risk assessment for grapes will use the proposed residue definition considering spiroxamine, Group A metabolites and Group C metabolites only. The ADI and ARfD end-points for spiroxamine are lower (higher toxicity) than M28 and therefore a conservative and conventional approach to consumer risk assessment can still be used despite separate end-points being available for the Group B and Group C metabolites.

<u>Spiroxamine-aminodiol-N-oxide (M29). Group C:</u>

Metabolite M29 is minor in grapes (<1% TRR). This metabolite is regarded as of equal toxicity as M28 (less toxic than spiroxamine). No concerns for consumer exposure.

Spiroxamine-desethyl-aminodiol (M30) and spiroxamine-despropyl-aminodiol (M31). Group C:

Metabolite M30 and M31 are minor in grapes (both <5% TRR). These metabolites are regarded as being of equal toxicity as M28 (less toxic than spiroxamine). No concerns for consumer exposure.

RESIDUE DEFINITION FOR MRL AND MONITORING (FOOD OF ANIMAL ORIGIN)

Ruminants

Metabolite spiroxamine-acid (M06) is the major residue component for all edible tissues in ruminant metabolism. Parent spiroxamine would not contribute to any potential MRLs monitoring for routine analysis, therefore the proposed residue definition for monitoring and setting of MRLs in ruminants is:

Spiroxamine-acid (M06), expressed as spiroxamine

The previously acceptable metabolism studies on the goat, summarised again in this dossier (refer to Section CA 6.4.2), support this residue definition and no changes are required from the previous RMS proposal. The acceptable ruminant (cattle) feeding study determined residues in all samples on this basis.

According to EFSA 2015 following the peer-review of MRLs (EFSA Journal 2015;13(1):3992), the residue definition for monitoring (animal matrices) should be extended to cover also parent spiroxamine ("Sum of spiroxamine and spiroxamine carboxylic acid (M06), expressed as spiroxamine (sum of isomers)"). For the previous evaluation of spiroxamine, the RMS-DE did not support this change of the residue definition proposed by EFSA for ruminants.

Previous RMS-DE justification for ruminants:

Spiroxamine itself as parent compound is not reported from goat metabolism in ruminant fat, muscle or milk. Spiroxamine was reported in trace, not significant amounts in kidney (<1% TRR) and only in low abundance in liver (5% TRR, 1.10 mg/kg), noting that an exaggerated dose of 10 mg/kg bw or 250 mg/kg feed was used in the goat study. The available and acceptable ruminant (cattle) feeding study with data generation method for analysis of residues of the major metabolite M06 [spiroxamine-acid] allows the assessment of consumer dietary risk but it would not provide complete data according to the previous residue definition proposal from EFSA. The EFSA 2015 proposal would mean that a new ruminant feeding study could be required and the previous RMS-DE considered that such a study would not give any added value in terms of human health assessment or monitoring.

The Applicant agrees with this position and furthermore notes that this would be an unnecessary and unacceptable use of vertebrate animals, given the availability of a satisfactory cattle feeding study (M-006159-02-1, CA 6.4.2/01).

Poultry

For poultry, the possibility for transfer of significant residues from feed items to edible commodities cannot be excluded according to the dietary burden calculations of EFSA 2015 (EFSA Journal 2015;13(1):3992) and also as presented in this dossier under Annex point CA 6.4. The available poultry feeding study is considered to be acceptable since the data generated according to the residue method determines spiroxamine, spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) as a total spiroxamine equivalents basis and even at the highest dose level (approximately 2N the highest dietary burden) residues in all edible commodities are below the analytical LOQ (0.05 mg/kg). Therefore, even though the data are not generated fully in compliance with the current and proposed residue definition for monitoring (see below) the hen metabolism data show that in all poultry edible commodities residues would be detectable if significant transfer from feed occurred. Therefore no positive MRLs above the LOQ are proposed and no new poultry feeding study is required.

Therefore, and in accordance with EFSA (2015) and the RMS-DE LoEP 2017, the following residue definition for monitoring in poultry is proposed:

sum of spiroxamine and spiroxamine-acid (M06), expressed as spiroxamine (sum of isomers)

RESIDUE DEFINITION FOR DIETARY RISK ASSESSMENT (FOOD OF ANIMAL ORIGIN)

Information presented here is adapted and updated, where necessary, from Applicant Position Paper CA 6.7/01 (M-344146-01-2, 2009) which was also considered in the previous evaluation RAR, Volume 3 B.7, August 2017.

Note that the use on grapes does not contribute to livestock feed. Besides parent compound, several potentially toxicologically relevant Group A metabolites occur in the ruminant and/or poultry livestock feed items from the use on cereals at significant relative and/or absolute levels.

From metabolism data on cereals, apart from parent spiroxamine which comprises the majority of all cereal residues, there are the major metabolite spiroxamine-N-oxide (M03) and Group A minor metabolites spiroxamine-desethyl (M01), spiroxamine-despropyl (M02), spiroxamine-N-formyl-desethyl (M04), spiroxamine-hydroxy (M05), spiroxamine-hydroxy-despropyl (M09), spiroxamine-hydroxy-N-oxide glucoside (M20) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21).

Group B spiroxamine-hydroxy-ketone-conjugate (M23) and spiroxamine-diol-diglycoside-conjugate (M24) are postulated minor metabolites in cereal commodities.

The Group C metabolites spiroxamine-aminodiol (M28), spiroxamine-aminodiol-N-oxide (M29), spiroxamine-desethly-aminodiol (M30) and spiroxamine-despropyl-aminodiol (M31) were only seen after acid hydrolysis and are not cereal metabolites in feed.

None of these metabolites were detected after administration of parent spiroxamine in ruminants, poultry or rat except the major metabolite spiroxamine-N-oxide (M03), which was not detected in the excreta of rats, but as an intermediate in the liver at maximum plasma concentrations after 100 mg/kg application at reported low levels (0.11% of liver radioactivity) and then as desalkylated metabolites spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) in poultry.

The previous RMS-DE considered that some unique fruit metabolites could become relevant for livestock feed items if further uses of spiroxamine were approved for crops that can be used as feed, namely spiroxamine-aminodiol (M13, plus associated conjugates or long chain fatty acid esters; M32, M33, M34, M35 and M36), spiroxamine-diol-diglycoside (M24 as the major identified component in a group of polar metabolites) and M28 (3.0 to 5.7% of Applied Dose detected in rat excreta). The Applicant dismisses this possibility as currently no other uses in fruit crops for the EU market other than grapes are considered and no products from grapes processing are relevant for EU livestock diets. The authorised uses of spiroxamine on bananas is also not relevant for livestock.

Metabolism of parent compound

The metabolism of parent spiroxamine in lactating ruminants and poultry can be considered as different with regard to the routes and rate of metabolism.

In lactating ruminants, the metabolism of parent spiroxamine follows two major routes:

1. Oxidation of the tert-butyl moiety to the alcohol and carboxylic acid metabolites. The alcohol group is only found in conjugated, i.e. sulphated form spiroxamine-sulphate (M25), while the acid metabolites exist as free spiroxamine-acid (M06) as well as spiroxamine-acid glucuronide (M19).

2. Desalkylation of the amino moiety under formation of the despropyl- and desethyl metabolites spiroxamine-despropyl acid (M12), spiroxamine-desethyl acid (M11), spiroxamine-despropyl-sulfate (M27) and spiroxamine-desethyl-sulfate (M26).

Residues occurring in ruminant tissues and milk at maximum plasma concentrations at significant levels (>10% of the TRR) are spiroxamine-acid (M06) and its spiroxamine-acid-glucuronide (M19) as well as spiroxamine-hydroxy acid (M07). The very polar metabolite M07 does not occur in the rat, however, due to its polarity there is no evidence of a possible cumulative behaviour in edible tissues or milk. Taking into account the exaggerated dose in the metabolism study compared with the potential 1N residues in feeding stuffs no detectable residues of this metabolite are expected. The maximum total spiroxamine diet for ruminants was for sheep at 0.187 mg/kg bw or 4.41 mg/kg in diet which is 57N compared with the dose level in the goat metabolism study. In the ruminant cattle feeding study only liver and kidney showed significant residues in the closest feeding level (refer to M-006159-02-1, CA 6.4.2/01).

In poultry, a combination of these two degradation routes is not observed. Parent spiroxamine is the major residue component in all analysed matrices. Spiroxamine is adsorbed and metabolised to three metabolites in poultry tissues via oxidation of the t-butyl moiety to yield spiroxamine-acid (M06) or via desalkylation of the amino group to give spiroxamine-desethyl (M01) spiroxamine-despropyl (M02).

Livestock metabolism of plant metabolite spiroxamine-N-oxide (M03)

Metabolite M03 occurs in cereal straw up to 22% TRR (7.7 mg/kg at 1.1N rate from wheat metabolism, estimated 1.98 mg/kg from HR total spiroxamine straw value used in dietary burden; 9.0 x 22% maximum formation from metabolism) and in smaller amounts in grapes (up to 4.7% of TRR, 0.61 mg/kg at 1.8N rate). As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed.

In-vivo investigations with radiolabelled spiroxamine and *in-vitro* tests under simulated hydrolysis conditions, using the gastric juice of rats, 0.1N HCl and sheep rumen fluid with both radiolabelled parent spiroxamine and synthesised radiolabelled spiroxamine-N-oxide have been conducted (Refer to Section MCA 5, M-005044-01-1, CA 5.1.2/01). The investigations demonstrated that:

i) metabolite M03 can be formed in small quantities in livestock rumen fluid after administration of parent spiroxamine (approximately 1.3 % of the incubated radioactivity)
ii) metabolite M03 is likely to be stable under conditions simulating the rumen environment (incubation of M03 reference compound with sheep rumen juice at 37°C for 4 h)
iii) metabolite M03 and parent compound are both partially hydrolysed (approximately 20-27%) to the spiroxamine-tert-butylketone metabolite (M15) when incubated with gastric juice of rats under physiological conditions for 4 h at 37°C, simulating the environment of the stomach of rats and the abomasum of ruminants.

The testing supports the conclusion that it is likely that the majority of M03 will be reduced to parent compound during digestion in the rumen of livestock.

In conclusion, residues of M03 can be considered as parent compound equivalents. Since a higher toxicological potential of M03 (including any metabolites) can be excluded and an accumulation in animal matrices compared to parent compound is very unlikely, the calculation of the dietary burden of livestock animals on the basis of parent compound equivalents (noting that M03 will be included by using the total spiroxamine common moiety residue method for cereals and rotational crops) can be regarded as a realistic worst case. No further livestock metabolism studies with M03 are necessary for the representative uses on cereals.

Livestock metabolism of plant metabolite spiroxamine-desethyl (M01) Group A

The desalkylated metabolite M01 occurs in cereal straw at 2% of TRR or up to 5% of TRR together with metabolite M05 from the dioxolane label (maximum 4.32 mg/kg at 1.1N rate from wheat metabolism, estimated 0.45 mg/kg from HR total spiroxamine straw value used in dietary burden; 9.0 x 5% maximum formation from metabolism) and fruits (1 to 3% of TRR). As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed. Exposure to M01 is included by using the total spiroxamine common moiety residue method for cereals and rotational crops. As M01 is a hen metabolite and a proposed intermediate in the ruminant and rat, its metabolic pathway is considered as covered by spiroxamine. Its toxicological properties are covered by parent compound. No further livestock metabolism studies with M01 are considered necessary for the representative uses.

Livestock metabolism of plant metabolite spiroxamine-despropyl (M02) Group A

The desalkylated metabolite M02 occurs in cereal straw at 3-4% of TRR (maximum 3.48 mg/kg at 1.1N rate from wheat metabolism, estimated 0.36 mg/kg from HR total spiroxamine straw value used in dietary burden; 9.0 x 4% maximum formation from metabolism) and fruits (1-3% of TRR). As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed. Exposure to M02 included by using the total spiroxamine common moiety residue method for cereals and rotational crops. As it is a hen metabolite and a proposed intermediate in the ruminant and rat, its metabolic pathway is considered as covered by spiroxamine. Its toxicological properties are covered by parent compound. No further livestock metabolism studies with M02 are considered necessary for the representative uses.

Livestock metabolism of plant metabolite spiroxamine –N-formyl-desethyl (M04) Group A

Metabolite M04 occurs in cereal straw at 7.5-9.7% TRR (maximum 8.0 mg/kg at 1.1N rate from wheat metabolism, estimated 0.87 mg/kg from HR total spiroxamine straw value used in dietary burden; 9.0 x 9.7% maximum formation from metabolism). No information is available about the metabolic fate of this metabolite in rat or livestock animals however M04 can be regarded as similar to livestock metabolite spiroxamine-desethyl (M01, identified in poultry, postulated for ruminant and rat) bearing a very small substituent as the formyl group. After hydrolysis of M04 the corresponding remainder would be M01, or smaller moieties, if the spiroketal bridge were to be also hydrolysed. The RMS-DE considered this to be reasonable and regarded the metabolic fate in livestock as well as the toxicological properties of M04 (and its metabolites) as covered by available studies and similar or less toxic than parent spiroxamine. No further livestock metabolism studies with M04 are considered necessary for the representative uses.

Livestock metabolism of plant metabolite spiroxamine–hydroxyl (M05) Group A

Metabolite M05, a hydroxylated parent compound residue, occurs in wheat straw at 2% of TRR or up to 5% of TRR together with metabolite M01 from the dioxolane label (maximum 4.32 mg/kg at 1.1N rate from wheat metabolism; estimated 0.45 mg/kg from HR total spiroxamine straw value used in dietary burden; 9.0 x 5% maximum formation from metabolism). It can be regarded as an intermediate in the rat and livestock metabolism pathway leading to spiroxamine-sulfate (M25) and / or spiroxamine-acid (M06). The metabolic pathway and toxicological properties are therefore covered by rat and livestock metabolism studies. No further livestock metabolism studies with M05 are considered necessary for the representative uses.

The total residue method developed for cereals does not include the analysis of M05. However, due to the fact that the residue method accounts for the major part of total residues in straw (68.7% of TRR in straw were detected as tert.-butylcyclohexanone by the total residue method in the radiovalidation study), no further data are required.

Livestock metabolism of plant metabolite spiroxamine–hydroxy-despropyl (M09) Group A

Metabolite M09, (hydroxylated spiroxamine–despropyl, M02), occurs in wheat straw at very low levels, 0.3% of TRR (0.11 mg/kg at 1.1N rate from wheat metabolism, estimated 0.03 mg/kg from HR total spiroxamine straw value used in dietary burden; 9.0 x 0.3% maximum formation from metabolism). Metabolism of M09 can be regarded as equal to M02. No further livestock metabolism or residue data are considered necessary.

Livestock metabolism of plant metabolite spiroxamine-hydroxy-N-oxide glucoside (M20) and spiroxamine-hydroxy-N-oxide malonyl glucoside (M21) Group A

These M20/M21 metabolites, glucosides of metabolite M03, occur in wheat straw at low levels 2-3% of TRR (maximum 2.6 mg/kg at 1.1N rate for both from wheat metabolism, estimated 0.27 mg/kg for both from HR total spiroxamine straw value used in dietary burden; 9.0 x 3% maximum formation from metabolism). The conclusions drawn for M03 (see above) also apply to M20 and M21.

Livestock metabolism of plant metabolite spiroxamine-hydroxy-ketone-conjugate (M23) Group B

The hydroxy-ketone conjugate M23 was a postulated metabolite in cereal straw at low amounts (4.8 % of TRR; 0.63 mg/kg). Post hydrolysis, it is structurally similar to spiroxamine-cyclohexanol (M13), the aglycon of the minor goat and rat metabolite spiroxamine-cyclohexanol-glucuronide (M22, 0.4 % of TRR in goat kidney; 1.7 % of applied dose in rat ADME studies). As the toxicological profile of M13 is known and concluded to be less toxic than spiroxamine, the toxicological properties of M23 and its aglycon can be clarified. No further livestock metabolism studies are required. The total residue method developed for cereals does not include the analysis of M23 (or the aglycon M13) but due to the fact that this method accounts for the predominant part of total residues in straw (68.7% of TRR in straw were detected as tert.-butylcyclohexanone by the total residue method in the radiovalidation study), no further data are required.

Livestock metabolism of plant metabolite spiroxamine-diol-diglycoside (M24) Group B

M24 was found in significant levels in grapes (14.8 % TRR, 0.50 mg/kg at 1.8N rate). As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed. M24 might be found at low levels in cereals and would not be significant for the overall dietary burden. Therefore, metabolite M24 is not of further relevance in animal feed items for the representative uses.

Livestock metabolism of plant metabolite spiroxamine – aminodiol (M28) Group C

Cleavage product aminodiol (M28) is a major metabolite of parent spiroxamine in fruits (37.5% of TRR in grapes). As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed. Therefore, metabolite M28 is not of relevance in animal feed items for the representative uses.

Livestock metabolism of plant metabolites spiroxamine-desethyl - aminodiol (M30) and spiroxamine-despropyl - aminodiol (M31) Group C

Metabolites M30 and M31 are minor desalkylated aminodiol (M28) metabolites found in fruits at levels of just 1% of TRR. As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed. Therefore, metabolite M28 is not of relevance in animal feed items for the representative uses.

Livestock metabolism of plant metabolites M32, M33, M34, M35, M36 Group B

A number of sugar and fatty acid conjugates of the tert.-butylcyclohexanol derivative were found at potentially significant levels in fruits. As already noted, the use on grapes or any authorised fruit crops does not contribute to livestock feed. Therefore, these metabolites are not of relevance in animal feed items for the representative uses.

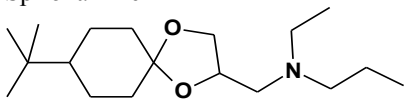
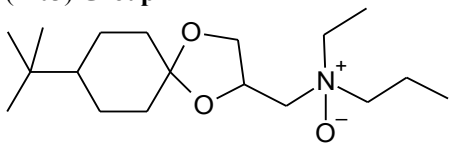
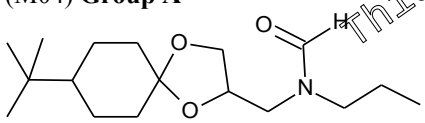
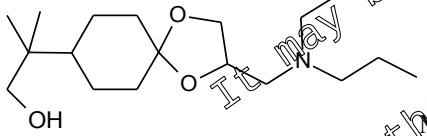
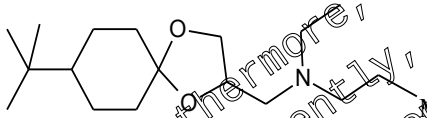
The following residue definition for risk assessment is, according to EFSA 2015 (ASB2015-1094), proposed for ruminants and pigs on basis of the metabolism studies with parent spiroxamine

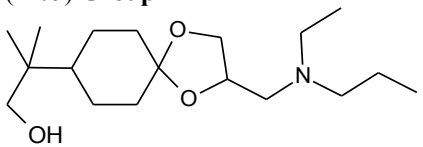
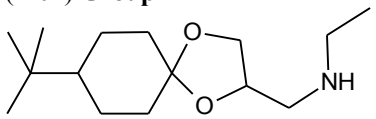
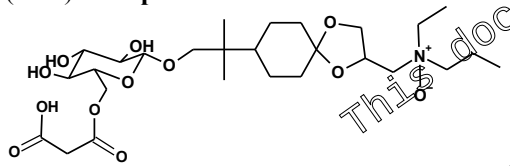
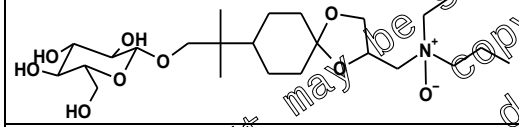
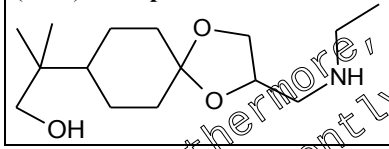
Sum of spiroxamine, spiroxamine-acid (M06), its glucuronide conjugate (M19) and spiroxamine-hydroxy acid (M07), expressed as spiroxamine (sum of isomers)

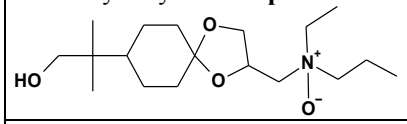
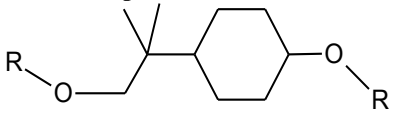
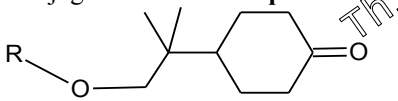
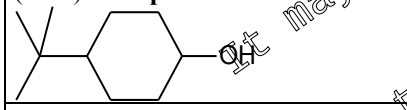
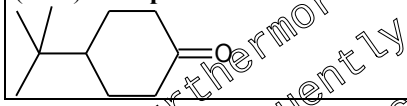
and for poultry

Sum of spiroxamine, spiroxamine-desethyl (M01), spiroxamine-despropyl (M02) and spiroxamine-acid (M06) expressed as spiroxamine (sum of isomers)

Table CA 6.7.1-8 Relevance of metabolites detected in cereal (wheat) plant metabolism studies

Cereal crops	% of TRR (mg/kg)	Comments	Toxicology understood
Primary (free) plant metabolites	Note that all primary metabolites determined in cereal crops are Group A metabolites		
Spiroxamine 	64 - 76% 0 DALA forage 41 - 45% 14 DALA forage 21 - 25% 61 DALA straw 3 - 14% 61 DALA grain	Major component of cereal commodities even at harvest, 61 days after last application.	Yes, parent compound
Spiroxamine-N-oxide (M03) **Group A** 	8 - 11% 0 DALA forage 9 - 13% 14 DALA forage 21 - 22% 61 DALA straw 1 - 18% 61 DALA grain	Main metabolite in cereal crops detected in all matrices. Rapidly formed and found in similar proportions to parent SDX in mature straw and grain	Yes, covered by parent compound
Spiroxamine-N-formyl-desethyl (M04) **Group A** 	2 - 5% 0 DALA forage 5 - 6% 14 DALA forage 8 - 10% 61 DALA straw n/d - 7% 61 DALA grain	Small amounts of this metabolite formed quickly after application to cereals. Seen in all crop commodities.	Yes, covered by parent compound
Spiroxamine – hydroxy + Spiroxamine-desethyl (M05 + M01) (unresolved) **Group A**  M05  M01	3 - 5% 0 DALA forage 5 - 7% 14 DALA forage n/d - 5% 61 DALA straw n/d 61 DALA grain	These two metabolites could not be resolved in the chromatographic systems employed in the study. Combined totals still relatively low. Not detected in grain.	Yes, covered by parent compound

Cereal crops	% of TRR (mg/kg)	Comments	Toxicology understood
Spiroxamine – hydroxy (M05) **Group A** 	n/d 0 DALA forage n/d 14 DALA forage 2% 61 DALA straw 2% 61 DALA grain	Minor metabolite in mature straw and grain	Yes, covered by parent compound
Spiroxamine-despropyl (M02) **Group A** 	3 - 4% 0 DALA forage 5% 14 DALA forage 3 - 4% 61 DALA straw n/d - 3% 61 DALA grain	Minor metabolite in all cereal matrices	Yes, covered by parent compound
Spiroxamine – hydroxy-N-oxide malonyl glucoside (M21) **Group A** 	0.2% 0 DALA forage 1 - 2% 14 DALA forage 2 - 3% 61 DALA straw n/d 61 DALA grain	Very minor metabolite in cereal matrices. Not detected in grain	Yes, covered by parent compound
Spiroxamine – hydroxy-N-oxide glucoside (M20) **Group A** 	n/d 0 DALA forage 1% 14 DALA forage 2% 61 DALA straw n/d 61 DALA grain	Very minor metabolite in cereal matrices. Not detected in grain	Yes, covered by parent compound
Spiroxamine – hydroxy-despropyl (M09) **Group A** 	n/d 0 DALA forage n/d 14 DALA forage 0.3% 61 DALA straw n/d 61 DALA grain	Extremely minor metabolite in cereal matrices. Not detected in grain	Yes, covered by parent compound

Cereal crops	% of TRR (mg/kg)	Comments	Toxicology understood
Metabolites based on identified/characterised hydrolysis products			
Spiroxamine-hydroxy-despropyl (M09) and unknown metabolite 9 (based on tert.-butylketone) - before hydrolysis **Group A** 	0.2 - 2% 0 DALA forage 0.6 - 1.6% 14 DALA forage 0.4% 61 DALA straw n/d 61 DALA grain	Very minor metabolite(s) in cereal matrices. Not detected in grain	Yes, covered by parent compound
Spiroxamine-diol-diglycoside (M24) Conjugate of M14 **Group A** 	0.2% 0 DALA forage 1% 14 DALA forage 1.3% 61 DALA straw n/d 61 DALA grain	Very minor metabolite in cereal matrices. Not detected in grain	Yes, covered by parent compound
Spiroxamine-hydroxy-ketone-conjugate (M23) Conjugate of M16 **Group A** 	n/d 0 DALA forage 1% 14 DALA forage 1.3% 61 DALA straw n/d 61 DALA grain	Very minor metabolite in cereal matrices. Not detected in grain	Yes, covered by parent compound
Identified components released after exhaustive acid hydrolysis	**Note: these are not primary plant metabolites**		
Spiroxamine-cyclohexanol (M13) **Group B** 	n/d 0 DALA forage n/d 14 DALA forage n/d 61 DALA straw 2% 61 DALA grain		Yes, lead Group B metabolite [EFSA 2018 and EFSA 2021]
Spiroxamine-ketone (M15) **Group B** 	n/d 0 DALA forage n/d 14 DALA forage 6% 61 DALA straw 5% 61 DALA grain		Yes, covered by M13

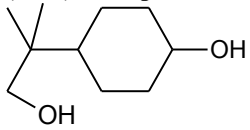
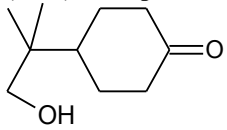
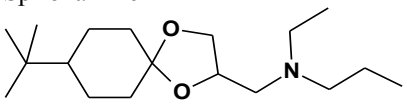
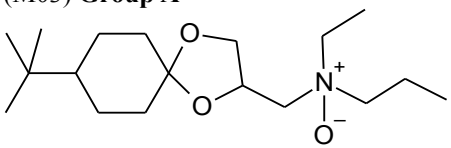
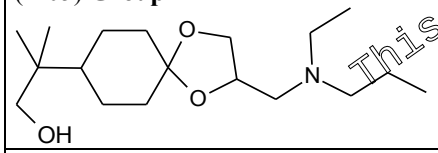
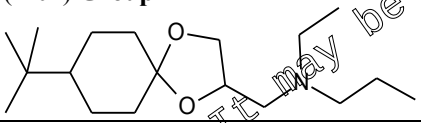
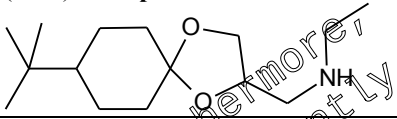
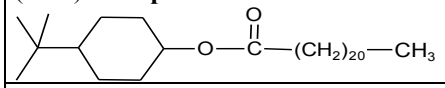
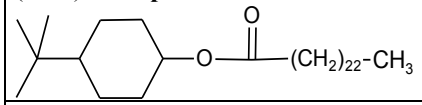
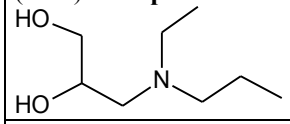
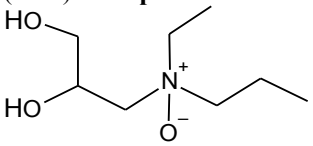
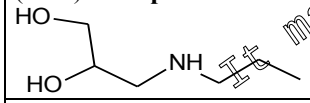
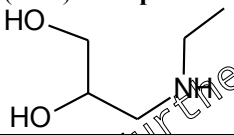
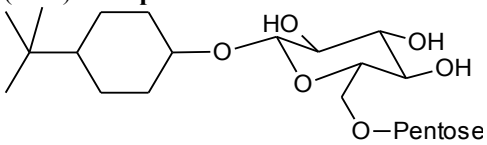
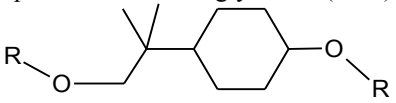
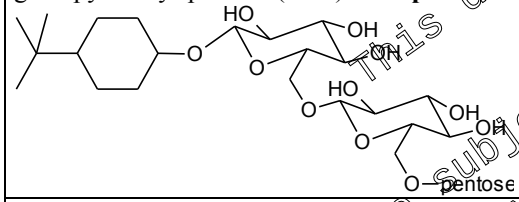
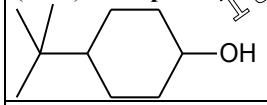
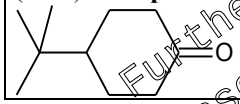
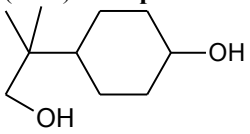
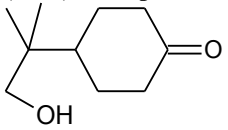
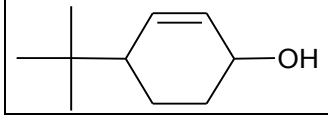
Cereal crops	% of TRR (mg/kg)	Comments	Toxicology understood
Spiroxamine-diol (M14) **Group B** 	n/d 0 DALA forage n/d 14 DALA forage 0.2% 61 DALA straw 3% 61 DALA grain		Yes, covered by M13
Spiroxamine-hydroxy-ketone (M16) **Group B** 	n/d 0 DALA forage n/d 14 DALA forage 1% 61 DALA straw 8% 61 DALA grain		Yes, covered by M13

Table CA 6.7.1-9 Relevance of metabolites detected in fruit (grape) plant metabolism studies

Grapes	% of TRR (mg/kg)	Comments	Toxicology understood
Primary (free) plant metabolites			
Spiroxamine 	25 – 46% 35 DALA	Major component of grapes at cGAP PHI of 14 or 35 days	Yes, parent compound
Spiroxamine-N-oxide (M03) **Group A** 	3 – 5% 35 DALA	Minor metabolite in grapes	Yes, covered by parent compound
Spiroxamine-hydroxy + (M05) **Group A** 	0.3% 35 DALA	Very minor metabolite in grapes	Yes, covered by parent compound
Spiroxamine-desethyl (M01) **Group A** 	1 - 2% 35 DALA	Minor metabolite in grapes	Yes, covered by parent compound
Spiroxamine-despropyl (M02) **Group A** 	1 - 2% 35 DALA	Minor metabolite in grapes	Yes, covered by parent compound

Grapes	% of TRR (mg/kg)	Comments	Toxicology understood
Spiroxamine-docosanoic acid ester (M35) **Group B** 	13% 35 DALA	Major component of grapes at cGAP PHI of 14 or 35 days	Yes, covered by M13
Spiroxamine-tetracosanoic acid ester (M36) **Group B** 	4%	Minor metabolite in grapes	Yes, covered by M13
Spiroxamine-aminodiol (M28) **Group C** 	38% 35 DALA	Major component of grapes at cGAP PHI of 14 or 35 days	Yes, lead Group C metabolite [EFSA 2018 and EFSA 2021]
Spiroxamine-aminodiol-N-oxide (M29) **Group C** 	0.1% 35 DALA	Very minor metabolite in grapes	Yes, covered by M28
Spiroxamine-desethyl-aminodiol (M30) **Group C** 	1% 35 DALA	Minor metabolite in grapes	Yes, covered by M28
Spiroxamine-despropyl-aminodiol (M31) **Group C** 	1% 35 DALA	Minor metabolite in grapes	Yes, covered by M28

Grapes	% of TRR (mg/kg)	Comments	Toxicology understood
Metabolite group 11[2], main component spiroxamine-cyclohexanol glucopyrosyl-pentose (M33) **Group B** 	Maximum 19.1% TRR	Groups of metabolites in grapes were poorly resolved, probably due to the chemical similarity of the sugar conjugates. Therefore, the components of aqueous phase 1 were listed as metabolite groups. Separate hydrolysis experiments showed, that metabolite groups 11 and 13 were mainly based on *cis*-tert.-butylcyclohexanol, with the main component of group 11 designated as spiroxamine-cyclohexanol glucopyrosyl-pentose (M33), the main component of group 13 as spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) and the main component of metabolite group 12 based on the diol as spiroxamine-diol-diglycoside (M24).	Conjugates of M13 (4-tert-butyl cyclohexan-1-ol), with sugars or fatty acids identified as M24, M33 and M34, M35 and M36, are reported in grapes. The aglycon (M13) is formed in gastric juice of rats from either of spiroxamine or spiroxamine-N-oxide (M03) (in amounts of 20 to 27% within 4 h at 37°C). M13 has been specifically assessed in toxicological testing (refer to Section MCA 5.8) and under the confirmatory data evaluation (EFSA 2018 and EFSA 2021) an agreed ADI and ARfD was set at a higher value than spiroxamine, therefore confirming that it is less toxic than parent spiroxamine. In conclusion, these metabolites are covered by M13
Metabolite group 12[2], main component spiroxamine-diol-diglycoside (M24) **Group B** 	Maximum 14.8% TRR		
Metabolite group 13[2], main component spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) **Group B** 	Maximum 3.8% TRR		
Identified components released after exhaustive acid hydrolysis	**Note: these are not primary plant metabolites**		
Spiroxamine-cyclohexanol (M13) **Group B** 	25%	-	Yes, lead Group B metabolite [EFSA 2018 and EFSA 2021]
Spiroxamine-ketone (M15) **Group B** 	1.3%	-	Yes, covered by M13

Grapes	% of TRR (mg/kg)	Comments	Toxicology understood
Spiroxamine-diol (M14) **Group B** 	13%		Yes, covered by M13
Spiroxamine-hydroxy-ketone (M16) **Group B** 	0.5%	-	Yes, covered by M13
Spiroxamine-cyclohexenol (M37) **Group B** 	3.2%	Refer to MCA Section 5, CA 5.8.1.27, M-761884-01-1	Yes, covered by M13

IMPACT OF ISOMERS ON RISK ASSESSMENT [EFSA JOURNAL 2019;17(8):5804]

Data Point:	KCA 6.7.1/01
Report Author:	[redacted]; [redacted]
Report Year:	2013
Report Title:	Spiroxamine: Confirmatory data according to the Commission Implementing Regulation (EU) N° 797/2011 of 9 August 2011. Spiroxamine: Consideration of the stereochemistry of metabolites M13 and M28
Report No:	M-472579-01-1
Document No:	M-472579-01-1
Guideline(s) followed in study:	Commission Implementing Regulation (EU) N° 797/2011
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

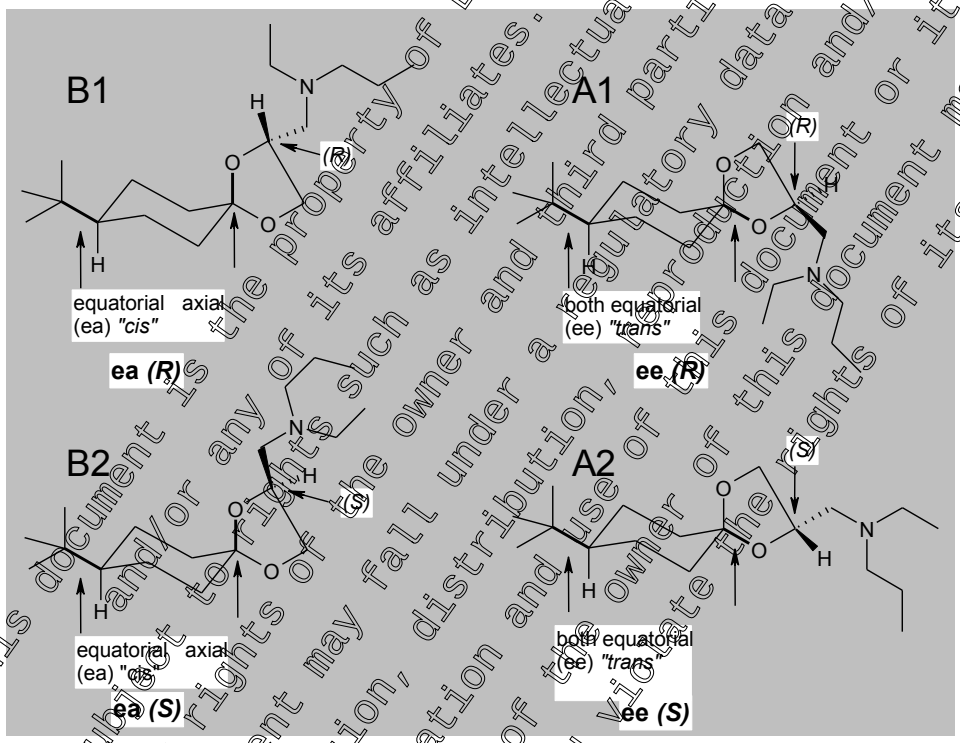
The Position Paper considers the stereochemistry of M13 and M28 in order to address the toxicity of these metabolites. For the residues aspects, the responses were not presented by the RMS-DE in the RAR Vol 3 B7 since the impact of isomers guidance was not available. Where still relevant this paper has been considered in the discussions on residue definition and impact of isomers ratio in this section.

The following information summarises the currently available information on the isomers of spiroxamine and its metabolites for crops and the impact on risk assessment. This data is mainly taken from crop residue trials employing full chiral analysis on spiroxamine active substance. Additional information from metabolism studies is also considered.

Spiroxamine consists of two diastereomers, each with 1:1 mixture of enantiomers.

From a chemistry perspective, there is no interconversion of diastereomers unless harsh chemical process conditions are applied which do not occur under environmental conditions post application to crops.

Interconversion of the enantiomers is considered to be even more unlikely and this is supported by the analytical data from residue trials.



Diastereoisomers	
Enantiomers "trans" A	Enantiomers "cis" B
ee (R) and ee (S)	ea (R) and ea (S)
49 - 56%	44 - 51%
1:1 mixture of 2 enantiomers	1:1 mixture of 2 enantiomers

Impact of isomers on risk assessment - crops

Grapes

Spiroxamine enantiomer ratio in grapes [A1:A2:B1:B2 or A:B] does show a trend to vary up to 14 or 35 days PHI. Although the HPLC profile of the 4 enantiomers can be seen visually to be similar at harvest compared with 0 days after last application, evaluation of the mg/kg results of A1:A2:A3:A4 or

A:B as isomer ratios and as % difference from day 0 shows a trend; with trans isomers (A1:A2) increasing by up to around +10 to +15% with a corresponding decrease in the cis isomers (B1:B2).

When A (trans) and B (cis) isomers are considered as diastereomers and the EFSA guidance stereoisomer excess (se) values are determined, the difference in se values (compared to 0 DALA) is often >10% for grapes at intervals of 14-35 days PHI but with multiple data points for grapes there are also many trials where this se difference is <10%.

Also, the % difference in the A (trans) diastereomer in grapes compared to 0 DALA with time is mostly <10%. The A isomers are the most fungicidally active.

As interconversion of isomers is not expected under environmental conditions, this observed trend is attributed to preferential degradation of the B1 and B2 enantiomers compared to A1 and A2. In some trials on grapes this difference in se is not significant.

In conclusion for grapes, although the ratio of A (trans) and B (cis) isomers is shown to vary between application and harvest, with an observed increase in the contribution of the A (trans) isomers, the large number of trials where measurement of the isomers by chiral analysis is available shows that the stereoisomeric excess is most often <10% and that the change in the A (trans) isomer itself is in a majority of trials also <10% from the last day of application.

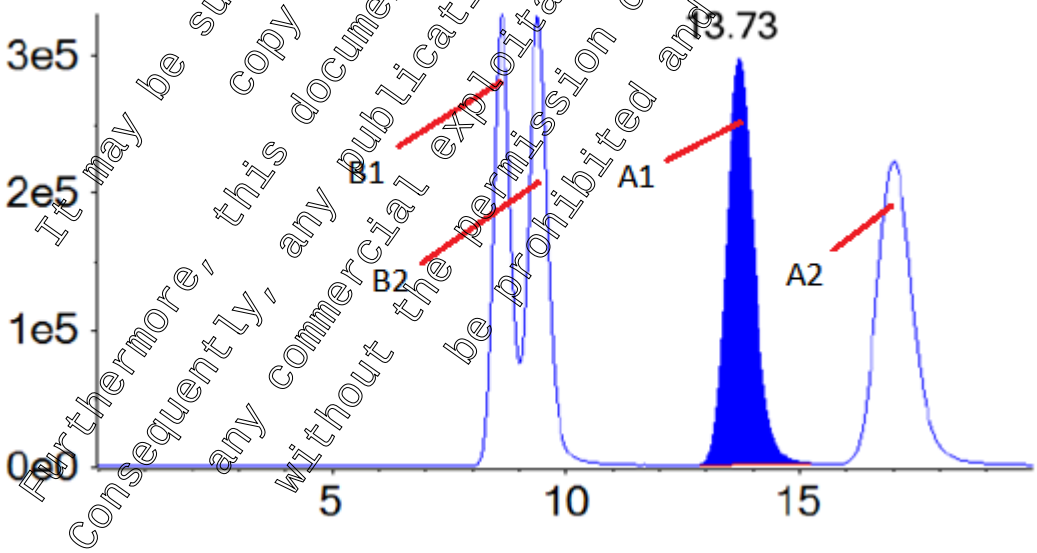
For chronic consumer risk assessment, application of an uncertainty factor to the contribution of exposure from parent spiroxamine will not impact the overall acceptable risk assessment for spiroxamine as it is already apparent that any additional isomer uncertainty factor, if required, would be less than x 1.5 and the grapes worst case consumer risk is just 14% of the ADI for total spiroxamine exposure which includes the Group C metabolites.

For the grapes acute consumer risk assessment, an uncertainty factor of up to x1.5 for the spiroxamine contribution to consumer exposure would not result in an unacceptable consumer risk.

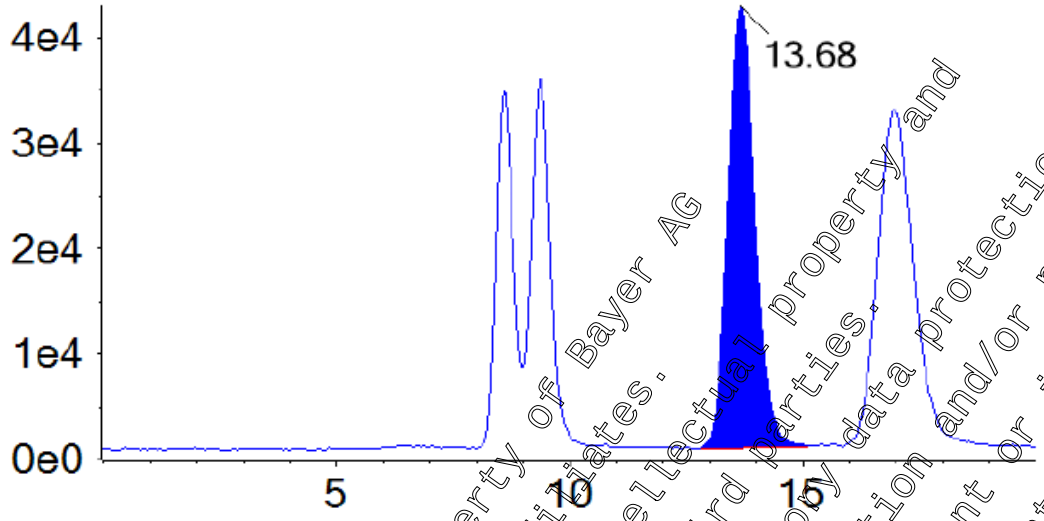
However, the Applicant proposes that, based on the weight of evidence, the spiroxamine isomers ratio is not significantly altered after application to grapes and therefore no additional uncertainty factors are required for the risk assessment.

A typical chiral HPLC profile of grapes spiroxamine data is shown below.

Grapes 0 DALA [M-026160-01-1 SEU]



Grapes 14 DALA [M-626160-01-1 SEU]



As A1:A2 and B1:B2 ratios do not change the A:B stereoisomeric excess (se) can be determined and compared. For this study [M-626160-01-1 SEU, CA 6.3.1/16] on grapes the average data from all 4 trials can be assessed to show the trend for change in se or in the A isomers (highest fungicidal activity). This example of average isomer ratios in grapes is shown in Table CA 6.7.1-10. Fully tabulated data from the available grape residue trials with chiral analysis are shown in Table CA 6.7.1-12 to Table CA 6.7.1-44.

Table CA 6.7.1-10 Typical average isomer ratio data for grapes (M-626160-01-1)

Grapes Sample	%A	%B	% diff A	% diff B	% se (A-B)	se change from 0 DALA	% increase in A from 0 DALA
Pre last appln	62.52	37.48	9.47	-12.61	25.04	10.82	5.4
0 DALA	57.11	42.89	0.00	0.00	14.22	0.00	0.0
7 DALA	61.29	38.71	7.31	-9.74	22.58	8.35	4.2
14 DALA	62.03	37.97	8.60	-11.46	24.05	9.83	4.9
14 DALA berry	62.75	37.25	9.87	-13.14	25.50	11.27	5.6
21 DALA	63.42	36.58	11.05	-14.71	26.84	12.62	6.3
28 DALA	64.23	35.77	12.47	-16.60	28.47	14.24	7.1
35 DALA	64.46	35.54	12.86	-17.12	28.91	14.69	7.3
35 DALA berry	64.59	35.41	13.09	-17.43	29.18	14.95	7.5

Cereals

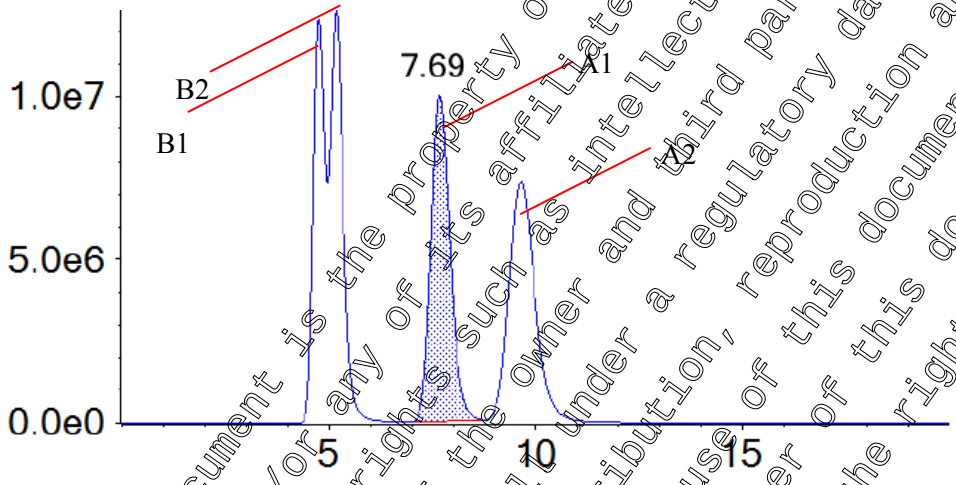
Spiroxamine enantiomer ratio [A1:A2:B1:B2 or A:B] does not vary significantly in barley and wheat straw at harvest compared with day 0 plant material. In grain, much of the data are inconclusive due to very low residues or residues <LOQ. Where the grain data are >LOQ, and have been used to compare ratios, these support the straw data.

When A (trans) and B (cis) isomers are considered as diastereomers and the EFSA guidance stereoisomer excess (se) values are determined, the difference in se values is consistently <10% for straw at harvest compared with the plant at day 0. Also, the % difference in A (trans) diastereomers in straw compared to whole plant with time is <10%. The A isomers are known to be the most fungicidally active.

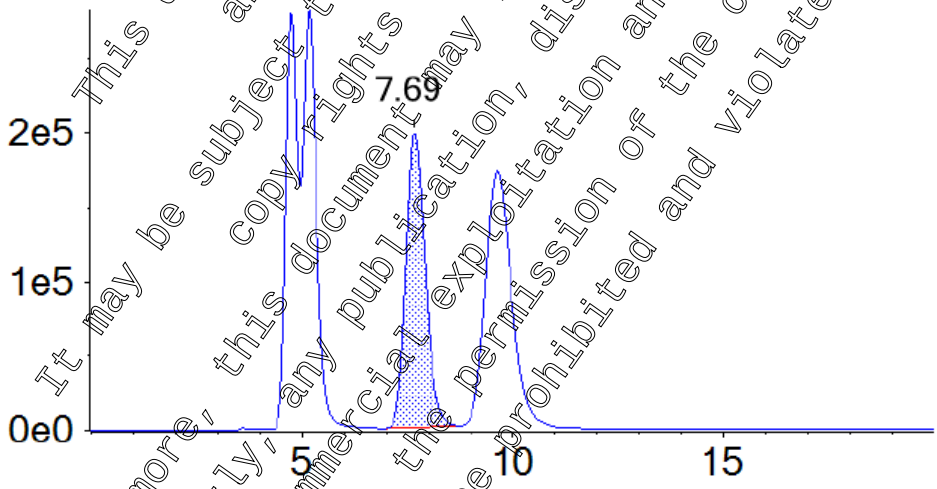
Conclusion: parent spiroxamine isomers do not vary significantly in cereal plants up to harvest based on field residue trials data. There is no impact on risk assessment and no isomer ratio uncertainty factor is required.

A typical chiral HPLC profile of barley plant / straw spiroxamine isomer data is shown below. Visual comparisons of these chromatograms support that there is no significant change in the ratios. For this study [M-631606-01-1 SEU, CA 6.3.2/15] on barley, the average data from all 4 trials can be assessed to show the trend for change in se or in the A isomers (highest fungicidal activity) and this example of average isomer ratios in cereal commodities is shown in Table CA 6.7.1-11. Fully tabulated data from the available cereal residue trials with chiral analysis are shown in Table CA 6.7.1-45 to Table CA 6.7.1-105.

Barley 0 DALA whole plant [M-631606-01-1 SEU]



Barley mature straw plant [M-631606-01-1 SEU]



As A1:A2 and B1:B2 ratios do not change the A:B stereoisomeric excess (se) can be determined and compared. For this study [M-631606-01-1, CA 6.3.2/15] on barley the average data from all 4 trials can be assessed to show no significant change (<10%) in se or in the A isomers (highest fungicidal activity).

Table CA 6.7.1-11 Typical average isomer ratio data for barley (M-631606-01-1)

Sample	%A	%B	% diff A	% diff B	% se (A-B)	se change from 0 DALA	% increase in A from 0 DALA
Plant 0 DALA	55.08	44.92	0.00	0.00	10.16	0.00	0.0
Mature grain	58.31	41.69	5.86	-7.19	16.62	6.46	3.2
Mature straw	57.98	42.02	5.26	-6.45	15.96	5.80	2.9

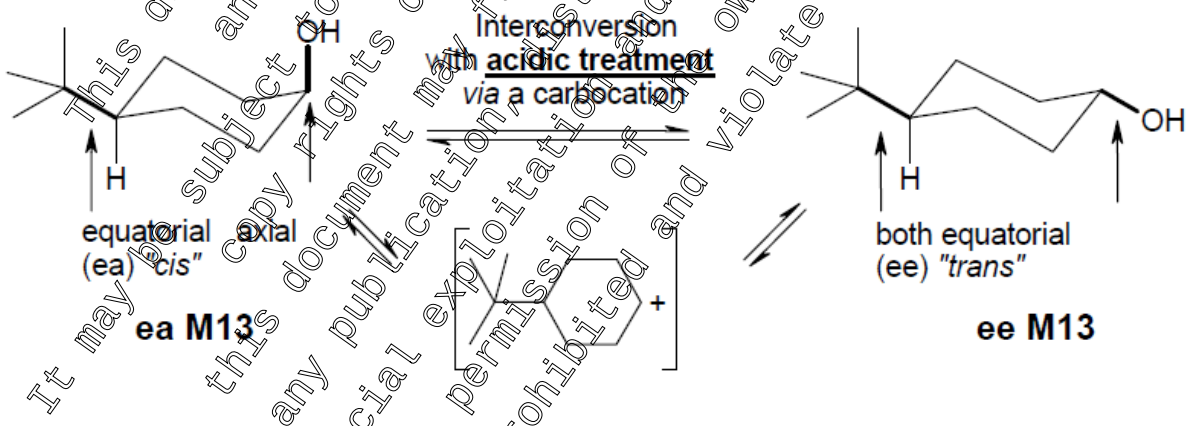
Metabolites

Grapes

Group B metabolite spiroxamine-cyclohexanol (M13) was discussed in Position Paper CA 6.7.1/01 (M-472579-01-1) which proposes that a worst case safety factor of x3 can be applied to M13 to cover potential worst case change in isomer ratio.

For M13, the tert-butyl group is in the equatorial position. This means that for this metabolite, only 2 isomers are possible, the ea (cis) and the ee (trans).

The isomers can convert under acidic conditions, via a carbocation intermediate.



However, the reliability of a proposed 3 fold safety factor is questionable. The isomer ratio of M13 in grapes was from TLC plate information in the grape metabolism study following HCl hydrolysis. M13 was not identified as a free metabolite but as long chain fatty acid esters. Therefore, M13 will have been released or formed post hydrolysis from these conjugates or potentially from spiroxamine or Group A metabolites and this is not necessarily any indication of the isomer ratio to which consumers are exposed for M13, either as an individual metabolite (as conjugates) or representative of Group B. The M13 isomer ratio as a standard reference item was not available from the grape metabolism study but was reported for the certificates of analysis from two toxicology studies at the time of preparing the Position Paper [CA 5.8.1/08; M-471123-01-1 and CA 5.8.1/09; M-471125-01-1].

The proposal for this renewal submission is that the Group B (M13) metabolites do not need to be considered in the grapes risk assessment residue definition and no uncertainty factor from isomer ratio is necessary.

To support this position for Group B there are two areas of justification.

1) Ongoing 2020 grapes trials will attempt to develop a method and analyse samples for the metabolite M13 using chiral methods pre-hydrolysis, so the isomer ratio results from this study are key before any conclusions are made. The method development for this is ongoing and is proving to be technically difficult and no results are available at the time of submission of this dossier. Free metabolite M13 is not expected to be significant so it is possible that hydrolysis will be required as was performed in the grape metabolism study. As noted already, acidic conditions are known to potentially alter the isomer ratio by interconversion, therefore it is likely that conjugates of M13 would either be eliminated rapidly after consumption or when under gastric conditions the free M13 could be released and potentially absorbed
2) The confirmatory data (EFSA 2018 and EFSA 2021) for Group B representative metabolite M13 shows that the ADI and ARfD end-point TRVs are higher than for parent spiroxamine, i.e. Group B metabolites are of lower toxicity than spiroxamine. Table CA 6.7.1-6 shows that the TEF compared to spiroxamine for M13 is 1/0.439 = x2.3 for the ADI and 1/0.526 = x1.9 for the ARfD. Therefore, chronic and acute risk assessments for grapes with respect to the contribution to consumer exposure for Group B metabolites would already include a worst case x 2 safety factor for comparison to the toxic end-points for spiroxamine. The setting of a further isomers uncertainty factor in risk assessment would be overly conservative and not justified.

Group C metabolite spiroxamine-aminodiol (M28) was discussed in Position Paper CA 6.7.1/01 (M-472579-01-1) and proposed that no safety factor is required for M28 based on an expected stable 1:1 isomer ratio in test item and in fruits.

In the absence of trials data the Applicant appreciates that a worst case would be an additional x2 uncertainty factor for M28 and Group C metabolites. However, for the same second reason as explained above for Group B metabolites, applying an uncertainty factor for Group C metabolites is not justified:

2) The confirmatory data for Group C metabolite M28 shows that the ADI and ARfD are higher than for parent spiroxamine, i.e. Group C metabolites are of lower toxicity than spiroxamine. Table CA 6.7.1-6 shows that the TEF compared to spiroxamine for M28 is 1/0.454 = x2.2 for the ADI and 1/0.108 = x9.3 for the ARfD. Therefore, chronic and acute risk assessments for grapes with respect to the contribution to consumer exposure for Group C metabolites are already including a worst case x 2 factor for chronic and x9 factor for acute comparison to the end-points for spiroxamine. The setting of a further isomer ratio uncertainty factor in risk assessment would be overly conservative and is not justified.

Cereals

For the predominant Group A metabolites in cereals, it is justified to extrapolate the isomer ratios from parent spiroxamine data. No significant change in isomer ratios is seen from the large database of parent trials data with chiral analysis, therefore there is no impact on risk assessment and no additional uncertainty factors are required. There are no significant Group B metabolites in cereal matrices and no reported Group C metabolites for cereal metabolism of spiroxamine. Therefore, it can be concluded that no isomer uncertainty factors are necessary for consumer risk assessment of cereals including transfer from livestock feed.

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626160-01-1, 2018 'Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 500 in Italy, Greece and France (South)'

Table CA 6.7.1-12 Trial 01 Summarised under CA 6.3.1/16

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.038	0.038	0.026	0.027	0.295	0.295	0.202	0.209	6.05	1.00	-9.30	0.47
0	0.200	0.210	0.160	0.150	0.278	0.292	0.222	0.208	0.00	0.00	0.00	0.00
7	0.060	0.059	0.039	0.042	0.300	0.295	0.195	0.210	8.00	1.14	-12.25	0.80
14	0.052	0.05	0.032	0.035	0.308	0.296	0.189	0.207	10.77	1.44	-14.79	-0.59
14 berry	0.053	0.053	0.033	0.036	0.303	0.303	0.189	0.206	9.03	3.84	-15.14	-1.26
21	0.031	0.031	0.018	0.019	0.313	0.313	0.182	0.192	12.73	7.36	-18.18	-7.88
28	0.027	0.027	0.016	0.017	0.310	0.310	0.184	0.195	11.72	6.40	-17.24	-6.21
35	0.022	0.022	0.013	0.014	0.310	0.310	0.183	0.197	11.55	6.24	-17.61	-5.35
35 berry	0.031	0.03	0.017	0.018	0.323	0.313	0.177	0.188	16.25	7.14	-20.31	-10.00

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	58.91	41.09	3.46	-4.58	17.83	3.94	2.0
0	56.94	43.06	0.00	0.00	13.89	0.00	0.0
7	59.50	40.50	4.49	-5.94	19.00	5.11	2.6
14	60.36	39.64	5.99	-7.92	20.71	6.82	3.4
14 berry	60.57	39.43	6.37	-8.42	21.14	7.25	3.6
21	62.63	37.37	9.98	-13.20	25.25	11.36	5.7
28	62.07	37.93	9.00	-11.90	24.14	10.25	5.1
35	61.97	38.03	8.83	-11.68	23.94	10.05	5.0
35 berry	63.54	36.46	11.59	-15.32	27.08	13.19	6.6

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626160-01-1, 2018 ‘Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 500 in Italy, Greece and France (South)’

Table CA 6.7.1-13 Trial 02 Summarised under CA 6.3.1/16

Grapes	Isomer residue (mg/kg)					Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2		A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.008	0.008	0.005	0.005		0.308	0.308	0.192	0.192	10.20	7.69	-10.26	-12.90
0	0.043	0.044	0.033	0.034		0.279	0.286	0.214	0.221	0.00	0.00	0.00	0.00
7	0.015	0.014	0.009	0.01		0.313	0.292	0.188	0.208	11.92	2.08	-12.50	-5.64
14	0.009	0.009	0.006	0.006		0.300	0.300	0.200	0.200	7.44	5.00	-6.67	-9.41
14 berry	0.013	0.013	0.008	0.009		0.302	0.302	0.186	0.209	8.27	5.81	-13.18	-5.20
21	0.008	0.008	0.005	0.005		0.308	0.308	0.192	0.192	10.20	7.69	-10.26	-12.90
28	0.008	0.008	0.005	0.005		0.308	0.308	0.192	0.192	10.20	7.69	-10.26	-12.90
35	0.005	0.005	0.003	0.003		0.313	0.313	0.188	0.188	11.92	9.38	-12.50	-15.07
35 berry	0.009	0.008	0.005	0.005		0.333	0.296	0.185	0.185	19.38	3.70	-13.58	-16.12

Grapes	enantiomers sum		% diff from day 0			stereoisomer excess (se) overview		
DALA	%A	%B	A	B		se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	61.54	38.46	8.93	-11.60		23.08	10.09	5.0
0	56.49	43.51	0.00	0.00		12.99	0.00	0.0
7	60.42	39.58	6.94	-9.02		20.83	7.85	3.9
14	60.00	40.00	6.21	-8.06		20.00	7.01	3.5
14 berry	60.47	39.53	7.03	-9.13		20.93	7.94	4.0
21	61.54	38.46	8.93	-11.60		23.08	10.09	5.0
28	61.54	38.46	8.93	-11.60		23.08	10.09	5.0
35	62.50	37.50	10.63	-13.81		25.00	12.01	6.0
35 berry	62.96	37.04	11.45	-14.87		25.93	12.94	6.5

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626160-01-1, 2018 'Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 500 in Italy, Greece and France (South)'

Table CA 6.7.1-14 Trial 03 Summarised under CA 6.3.1/16

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.046	0.046	0.023	0.023	0.333	0.333	0.167	0.167	14.29	14.29	-20.00	-20.00
0	0.210	0.210	0.150	0.150	0.292	0.292	0.208	0.208	0.00	0.00	0.00	0.00
7	0.045	0.044	0.025	0.026	0.321	0.314	0.179	0.186	10.20	7.76	-14.29	-10.86
14	0.056	0.054	0.029	0.031	0.329	0.318	0.171	0.182	12.94	8.91	-18.12	-12.47
14 berry	0.056	0.055	0.028	0.029	0.333	0.327	0.167	0.173	14.29	12.24	-20.00	-17.14
21	0.046	0.046	0.023	0.024	0.331	0.331	0.165	0.173	13.46	13.46	-20.58	-17.12
28	0.04	0.039	0.017	0.017	0.354	0.345	0.150	0.150	21.37	18.33	-27.79	-27.79
35	0.043	0.043	0.018	0.018	0.352	0.352	0.148	0.148	20.84	20.84	-29.18	-29.18
35 berry	0.025	0.025	0.012	0.013	0.338	0.338	0.162	0.162	15.83	15.83	-22.16	-22.16

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	66.67	33.33	14.29	-20.00	33.33	16.67	8.3
0	58.33	41.67	0.00	0.00	16.67	0.00	0.0
7	63.57	36.43	8.98	-12.57	27.14	10.48	5.2
14	64.71	35.29	10.92	-15.29	29.41	12.75	6.4
14 berry	66.07	33.93	13.27	-18.57	32.14	15.48	7.7
21	66.19	33.81	13.46	-18.85	32.37	15.71	7.9
28	69.91	30.09	19.85	-27.79	39.82	23.16	11.6
35	70.49	29.51	20.84	-29.18	40.98	24.32	12.2
35 berry	67.57	32.43	15.83	-22.16	35.14	18.47	9.2

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626160-01-1, 2018 'Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 500 in Italy, Greece and France (South)'

Table CA 6.7.1-15 Trial 04 Summarised under CA 6.3.1/16

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.009	0.008	0.005	0.005	0.333	0.296	0.185	0.185	16.88	5.22	-11.56	-17.26
0	0.079	0.078	0.058	0.062	0.285	0.282	0.209	0.224	0.00	0.00	0.00	0.00
7	0.019	0.018	0.011	0.012	0.317	0.300	0.183	0.200	11.03	6.54	-12.44	-10.65
14	0.015	0.014	0.008	0.009	0.326	0.304	0.174	0.196	14.34	8.08	-16.94	-12.59
14 berry	0.011	0.012	0.006	0.007	0.306	0.333	0.167	0.194	-7.14	18.38	-20.40	-13.13
21	0.019	0.019	0.011	0.011	0.317	0.317	0.183	0.183	11.03	12.46	-12.44	-18.09
28	0.013	0.013	0.007	0.008	0.317	0.317	0.171	0.195	11.18	12.60	-18.46	-12.82
35	0.011	0.011	0.006	0.007	0.314	0.314	0.171	0.200	10.20	11.61	-18.13	-10.65
35 berry	0.014	0.013	0.007	0.008	0.333	0.310	0.167	0.196	16.88	9.92	-20.40	-14.90

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	62.96	37.04	11.09	-14.51	25.93	12.57	6.3
0	56.68	43.32	0.00	0.00	13.36	0.00	0.0
7	61.67	38.33	8.80	-11.51	23.33	9.98	5.0
14	63.04	36.96	11.23	-14.69	26.09	12.73	6.4
14 berry	63.89	36.11	12.72	-16.64	27.78	14.42	7.2
21	63.33	36.67	11.74	-15.36	26.67	13.31	6.7
28	63.41	36.59	11.88	-15.55	26.83	13.47	6.7
35	62.86	37.14	10.90	-14.26	25.71	12.36	6.2
35 berry	64.29	35.71	13.42	-17.56	28.57	15.21	7.6

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626160-01-1, 2018 'Determination of the residues of spiroxamine in/on grape after spray and low-volume spray application of KWG 4168 EC 500 in Italy, Greece and France (South)'

Table CA 6.7.1-16 Average of all 4 trials

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.02525	0.025	0.01475	0.015	0.317	0.308	0.186	0.188	11.912	7.062	-12.704	-12.518
0	0.133	0.136	0.100	0.099	0.283	0.288	0.214	0.215	0.0	0.0	0.0	0.0
7	0.035	0.03375	0.021	0.0225	0.313	0.300	0.186	0.201	10.3	4.4	-12.9	-6.6
14	0.033	0.03175	0.01875	0.02025	0.316	0.304	0.183	0.196	11.4	5.8	-14.1	-8.8
14 berry	0.03325	0.03325	0.01875	0.02025	0.311	0.316	0.177	0.196	9.7	10.0	-17.1	-9.2
21	0.026	0.026	0.01425	0.01475	0.317	0.317	0.181	0.185	11.9	10.2	-15.4	-14.1
28	0.022	0.02175	0.01125	0.01175	0.322	0.320	0.174	0.183	13.7	11.3	-18.4	-14.9
35	0.02025	0.02025	0.01	0.0105	0.322	0.322	0.172	0.183	13.7	12.0	-19.3	-15.0
35 berry	0.01975	0.019	0.01025	0.01075	0.332	0.314	0.173	0.181	17.1	9.2	-19.1	-15.8

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	62.52	37.48	9.47	-12.61	25.04	10.82	5.4
0	57.11	42.89	0.00	0.00	14.23	0.00	0.0
7	61.29	38.71	7.31	-9.74	22.58	8.35	4.2
14	62.03	37.97	8.60	-11.46	24.05	9.83	4.9
14 berry	62.75	37.25	9.87	-13.14	25.50	11.27	5.6
21	63.42	36.58	11.05	-14.71	26.84	12.62	6.3
28	64.23	35.77	12.47	-16.60	28.47	14.24	7.1
35	64.46	35.54	12.86	-17.12	28.91	14.69	7.3
35 berry	64.59	35.41	13.09	-17.43	29.18	14.95	7.5

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-623069-01-1, 2018 'Determination of the residues of spiroxamine in/on Grape after spray and low-volume spray application of KWG 4168 EC 500 in France (North), Belgium and Germany'

Table CA 6.7.1-17 Trial 01 Summarised under CA 6.3.1/17

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.023	0.024	0.016	0.016	0.291	0.304	0.203	0.203	7.54	10.13	-10.10	-9.17
0	0.120	0.120	0.098	0.097	0.276	0.276	0.225	0.223	0.00	0.00	0.00	0.00
7	0.080	0.081	0.053	0.053	0.300	0.303	0.199	0.199	8.61	9.97	-11.89	-10.98
14	0.034	0.034	0.022	0.021	0.306	0.306	0.198	0.189	11.04	11.04	-12.02	-15.16
14 berry	0.027	0.027	0.017	0.017	0.307	0.307	0.193	0.193	11.22	11.22	-14.25	-13.37
21	0.028	0.027	0.017	0.017	0.315	0.303	0.191	0.191	14.04	9.97	-15.21	-14.34
28	0.025	0.024	0.015	0.015	0.316	0.304	0.190	0.190	14.72	10.13	-15.72	-14.85
34	0.024	0.024	0.015	0.015	0.308	0.308	0.192	0.192	11.54	11.54	-14.64	-13.76
35 berry	0.022	0.021	0.014	0.014	0.310	0.296	0.197	0.197	12.32	7.22	-12.47	-11.57

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	59.49	40.51	7.83	-9.64	18.99	18.99	8.64
0	55.17	44.83	0.00	0.00	10.34	10.34	0.00
7	60.30	39.70	9.29	-11.44	20.60	20.60	10.25
14	61.26	38.74	11.04	-13.58	22.52	22.52	12.18
14 berry	61.36	38.64	11.22	-13.81	22.73	22.73	12.38
21	61.80	38.20	12.01	-14.78	23.60	23.60	13.25
28	62.03	37.97	12.42	-15.29	24.05	24.05	13.71
34	61.54	38.46	11.54	-14.20	23.08	23.08	12.73
35 berry	60.56	39.44	9.77	-12.03	21.13	21.13	10.78

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-623069-01-1, 2018 'Determination of the residues of spiroxamine in/on Grape after spray and low-volume spray application of KWG 4168 EC 500 in France (North), Belgium and Germany'

Table CA 6.7.1-18 Trial 02 Summarised under CA 6.3.1/17

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.048	0.047	0.022	0.022	0.345	0.338	0.158	0.158	14.82	12.43	-19.04	-22.04
0	0.120	0.120	0.078	0.081	0.301	0.301	0.195	0.203	0.00	0.00	0.00	0.00
7	0.061	0.061	0.03	0.03	0.335	0.335	0.165	0.165	11.44	11.44	-15.68	-18.80
14	0.043	0.043	0.021	0.02	0.339	0.339	0.165	0.157	12.58	12.58	-15.41	-22.43
14 berry	0.07	0.07	0.032	0.032	0.343	0.343	0.157	0.157	14.09	14.09	-19.76	-22.73
21	0.032	0.033	0.015	0.014	0.340	0.351	0.160	0.149	13.19	16.73	-18.37	-26.64
28	0.046	0.045	0.019	0.018	0.359	0.352	0.148	0.141	19.49	16.89	-24.07	-30.73
35	0.032	0.033	0.013	0.013	0.352	0.363	0.143	0.143	16.92	20.58	-26.92	-29.63
35 berry	0.037	0.038	0.015	0.015	0.352	0.362	0.143	0.143	17.17	20.33	-26.92	-29.63

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	68.35	31.65	13.62	-20.56	36.69	16.39	8.2
0	60.18	39.85	0.00	0.00	20.30	0.00	0.0
7	67.03	32.97	11.44	-17.27	34.07	13.77	6.9
14	67.72	32.28	12.58	-18.99	35.43	15.13	7.6
14 berry	68.63	31.37	14.09	-21.27	37.25	16.95	8.5
21	69.15	30.85	14.96	-22.58	38.30	18.00	9.0
28	71.09	28.91	18.19	-27.46	42.19	21.89	10.9
35	71.43	28.57	18.75	-28.30	42.86	22.56	11.3
35 berry	71.43	28.57	18.75	-28.30	42.86	22.56	11.3

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-623069-01-1, 2018 'Determination of the residues of spiroxamine in/on Grape after spray and low-volume spray application of KWG 4168 EC 500 in France (North), Belgium and Germany'

Table CA 6.7.1-19 Trial 03 Summarised under CA 6.3.1/17

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.043	0.044	0.027	0.027	0.305	0.312	0.191	0.191	-7.40	-9.90	-8.76	-13.83
0	0.230	0.230	0.170	0.180	0.284	0.284	0.210	0.222	0.00	0.00	0.00	0.00
7	0.120	0.12	0.072	0.071	0.313	0.313	0.188	0.185	10.34	10.34	-10.43	-16.58
14	0.076	0.079	0.047	0.042	0.311	0.324	0.193	0.172	9.69	14.02	-8.22	-22.54
14 berry	0.092	0.1	0.058	0.054	0.303	0.329	0.191	0.178	6.58	15.85	-9.09	-20.07
21	0.077	0.081	0.042	0.04	0.321	0.338	0.175	0.167	12.99	18.86	-16.62	-25.00
28	0.067	0.07	0.037	0.035	0.321	0.335	0.177	0.167	12.90	17.95	-15.65	-24.64
35	0.057	0.058	0.032	0.033	0.317	0.322	0.178	0.183	11.52	13.48	-15.29	-17.50
35 berry	0.05	0.052	0.029	0.025	0.321	0.333	0.186	0.166	12.88	17.39	-11.43	-27.88

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	61.70	38.30	8.65	-11.37	23.40	9.82	4.9
0	56.79	43.21	0.00	0.00	13.58	0.00	0.0
7	62.66	37.34	10.34	-13.59	25.33	11.75	5.9
14	63.52	36.48	11.86	-15.59	27.05	13.47	6.7
14 berry	63.16	36.84	11.21	-14.74	26.32	12.74	6.4
21	65.83	34.17	15.92	-20.93	31.67	18.09	9.0
28	65.55	34.45	15.43	-20.27	31.10	17.52	8.8
35	63.89	36.11	12.50	-16.43	27.78	14.20	7.1
35 berry	65.38	34.62	15.13	-19.89	30.77	17.19	8.6

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-623069-01-1, 2018 'Determination of the residues of spiroxamine in/on Grape after spray and low-volume spray application of KWG 4168 EC 500 in France (North), Belgium and Germany'

Table CA 6.7.1-20 Trial 04 Summarised under CA 6.3.1/17

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.037	0.038	0.026	0.024	0.296	0.304	0.208	0.192	13.85	1.33	-5.45	-12.73
0	0.130	0.150	0.110	0.110	0.260	0.300	0.220	0.220	0.00	0.00	0.00	0.00
7	0.074	0.076	0.048	0.049	0.300	0.308	0.194	0.198	15.23	2.56	-11.67	-9.83
14	0.041	0.043	0.026	0.026	0.301	0.316	0.191	0.191	15.95	5.39	-13.10	-13.10
14 berry	0.048	0.052	0.03	0.03	0.300	0.325	0.188	0.188	15.38	8.33	-14.77	-14.77
21	0.032	0.033	0.018	0.017	0.320	0.330	0.180	0.170	23.08	10.00	-18.18	-22.73
28	0.028	0.032	0.016	0.015	0.308	0.352	0.176	0.165	18.34	17.22	-20.08	-25.07
35	0.034	0.035	0.02	0.019	0.315	0.324	0.185	0.176	21.08	8.02	-15.82	-20.03
35 berry	0.029	0.031	0.017	0.017	0.309	0.330	0.181	0.181	18.66	9.93	-17.79	-17.79

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	60.00	40.00	7.14	-9.09	20.00	8.00	4.0
0	56.00	44.00	0.00	0.00	12.00	0.00	0.0
7	60.73	39.27	8.44	-10.75	21.46	9.46	4.7
14	61.76	38.24	10.29	-13.10	23.53	11.53	5.8
14 berry	62.50	37.50	11.61	-14.77	25.00	13.00	6.5
21	65.00	35.00	16.07	-20.45	30.00	18.00	9.0
28	65.93	34.07	17.74	-22.58	31.87	19.87	9.9
35	63.89	36.11	14.09	-17.93	27.78	15.78	7.9
35 berry	63.83	36.17	13.98	-17.79	27.66	15.66	7.8

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-623069-01-1, 2018 'Determination of the residues of spiroxamine in/on Grape after spray and low-volume spray application of KWG 4168 EC 500 in France (North), Belgium and Germany'

Table CA 6.7.1-21 Average of all 4 trials

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.038	0.038	0.023	0.022	0.309	0.314	0.190	0.186	-0.429	8.394	-10.622	-14.273
0	0.150	0.155	0.114	0.117	0.280	0.290	0.213	0.217	0	0	0	0
7	0.084	0.085	0.051	0.051	0.312	0.315	0.186	0.187	11.3	8.5	-12.3	-14.0
14	0.049	0.050	0.029	0.027	0.314	0.321	0.187	0.177	12.3	10.7	-12.1	-18.2
14 berry	0.059	0.062	0.034	0.033	0.313	0.326	0.182	0.179	11.8	12.4	-14.4	-17.6
21	0.042	0.044	0.023	0.022	0.324	0.330	0.176	0.169	15.6	13.9	-17.1	-22.1
28	0.042	0.043	0.022	0.021	0.326	0.335	0.173	0.166	16.4	15.6	-18.7	-23.7
34-35	0.037	0.038	0.020	0.020	0.323	0.329	0.175	0.174	15.2	13.4	-17.9	-20.0
35 berry	0.035	0.036	0.019	0.018	0.323	0.330	0.177	0.176	15.2	13.8	-16.9	-21.5

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	62.39	37.61	9.39	-12.47	24.77	10.71	5.4
0	57.03	42.97	0.00	0.00	14.06	0.00	0.0
7	62.68	37.32	9.91	-13.15	25.36	11.31	5.7
14	63.57	36.43	11.47	-15.22	27.13	13.08	6.5
14 berry	63.91	36.09	12.07	-16.02	27.82	13.77	6.9
21	65.45	34.55	14.76	-19.59	30.89	16.83	8.4
28	66.15	33.85	16.00	-21.23	32.30	18.25	9.1
35	65.19	34.81	14.31	-18.98	30.37	16.32	8.2
35 berry	65.30	34.70	14.51	-19.25	30.60	16.55	8.3

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627030-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grape (red and white varieties) after spray application of Fluopyram & Spiroxamine SE 275 in Northern France and Austria'

Table CA 6.7.1-22 Trial 01 Summarised under CA 6.3.1/15

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.011	0.011	0.007	0.007	0.306	0.306	0.194	0.194	8.08	6.11	-9.42	-9.42
0	0.054	0.055	0.041	0.041	0.283	0.288	0.215	0.215	0.00	0.00	0.00	0.00
3	0.024	0.025	0.017	0.017	0.289	0.301	0.205	0.205	2.28	4.60	-4.58	-4.58
7	0.019	0.018	0.013	0.013	0.302	0.286	0.206	0.206	6.67	-0.78	-3.87	-3.87
14	0.016	0.016	0.01	0.01	0.308	0.308	0.192	0.192	8.83	6.85	-10.41	-10.41
28	0.02	0.019	0.012	0.012	0.317	0.302	0.190	0.190	12.29	4.73	-11.27	-11.27

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se(%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	61.11	38.89	7.08	-9.42	22.35	8.09	4.0
0	57.07	42.93	0.00	0.00	14.14	0.00	0.0
3	59.04	40.96	3.45	-4.58	18.07	3.94	2.0
7	58.73	41.27	2.91	-3.87	17.46	3.32	1.7
14	61.54	38.46	7.83	-10.41	23.08	8.94	4.5
28	61.90	38.10	8.48	-11.27	23.81	9.67	4.8

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627030-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grape (red and white varieties) after spray application of Fluopyram & Spiroxamine SE 275 in Northern France and Austria'

Table CA 6.7.1-23 Trial 02 Summarised under CA 6.3.1/15

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.016	0.015	0.011	0.011	0.302	0.283	0.208	0.208	-8.98	-2.17	-7.50	-6.34
0	0.100	0.100	0.081	0.080	0.577	0.277	0.224	0.222	0.00	0.00	0.00	0.00
3	0.031	0.033	0.02	0.02	0.298	0.317	0.192	0.192	-7.61	-14.55	-14.29	-13.22
7	0.022	0.022	0.014	0.014	0.306	0.306	0.194	0.194	10.31	10.31	-13.34	-12.26
13	0.03	0.031	0.019	0.018	0.306	0.316	0.194	0.184	10.51	14.19	-13.59	-17.12
29	0.02	0.019	0.012	0.012	0.317	0.302	0.190	0.190	14.60	8.87	-15.11	-14.05

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	58.49	41.51	5.58	-6.93	16.98	6.18	3.1
0	55.40	44.60	0.00	0.00	10.80	0.00	0.0
3	61.54	38.46	11.08	-13.76	23.08	12.27	6.1
7	61.11	38.89	10.31	-12.80	22.22	11.42	5.7
13	62.24	37.76	12.35	-15.34	24.49	13.69	6.8
29	61.90	38.10	10.74	-14.58	23.81	13.01	6.5

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627030-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grape (red and white varieties) after spray application of Fluopyram & Spiroxamine SE 275 in Northern France and Austria'

Table CA 6.7.1-24 Trial 04 Summarised under CA 6.3.1/15

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.045	0.045	0.028	0.027	0.310	0.310	0.193	0.186	2.80	9.66	-6.96	-10.28
0	0.160	0.150	0.110	0.110	0.302	0.283	0.208	0.208	8.98	2.17	-7.50	-6.34
3	0.075	0.074	0.05	0.051	0.300	0.296	0.200	0.204	8.30	6.86	-10.86	-7.94
7	0.072	0.074	0.046	0.045	0.304	0.312	0.194	0.190	9.67	12.72	-13.50	-14.32
13	0.055	0.055	0.033	0.033	0.313	0.313	0.188	0.188	12.81	12.81	-16.44	-15.39
28	0.039	0.039	0.022	0.022	0.320	0.320	0.180	0.180	15.40	15.40	-19.63	-18.63

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	62.07	37.93	6.12	-8.62	24.14	7.16	3.6
0	58.49	41.51	0.00	0.00	16.98	0.00	0.0
3	59.60	40.40	1.90	-2.67	19.20	2.22	1.1
7	61.60	38.40	5.32	-7.50	23.21	6.23	3.1
14	62.50	37.50	6.85	-9.66	25.00	8.02	4.0
28	63.93	36.07	9.31	-13.11	27.87	10.89	5.4

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627030-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grape (red and white varieties) after spray application of Fluopyram & Spiroxamine SE 275 in Northern France and Austria'

Table CA 6.7.1-25 Trial 05 Summarised under CA 6.3.1/15

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.01	0.01	0.007	0.006	0.303	0.303	0.212	0.182	4.90	4.90	0.48	-13.88
0	0.026	0.026	0.019	0.019	0.289	0.289	0.211	0.211	0.00	0.00	0.00	0.00
3	0.015	0.015	0.01	0.01	0.300	0.300	0.200	0.200	3.85	3.85	-5.26	-5.26
7	0.017	0.018	0.012	0.011	0.293	0.310	0.207	0.190	1.46	7.43	-2.00	-10.16
14	0.012	0.012	0.008	0.008	0.300	0.300	0.200	0.200	3.85	3.85	-5.26	-5.26

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	60.61	39.39	4.90	-6.70	21.21	5.66	2.8
0	57.78	42.22	0.00	0.00	15.56	0.00	0.0
3	60.00	40.00	3.85	-5.26	20.00	4.44	2.2
7	60.34	39.66	4.44	-6.08	20.69	5.13	2.6
14	60.00	40.00	3.85	-5.26	20.00	4.44	2.2

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627030-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grape (red and white varieties) after spray application of Fluopyram & Spiroxamine SE 275 in Northern France and Austria'

Table CA 6.7.1-26 Average of all 4 trials

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.021	0.020	0.013	0.013	0.305	0.300	0.202	0.193	6.111	5.724	-5.885	-9.932
0	0.085	0.083	0.063	0.063	0.288	0.284	0.214	0.214	0	0	0	0
3	0.036	0.037	0.024	0.025	0.297	0.304	0.199	0.200	3.2	6.8	-7.1	-6.3
7	0.033	0.033	0.021	0.021	0.301	0.303	0.200	0.195	4.7	6.8	-6.5	-8.7
13-14	0.028	0.029	0.018	0.017	0.307	0.309	0.193	0.191	6.6	8.8	-9.8	-10.7
28-29	0.026	0.026	0.015	0.015	0.318	0.308	0.187	0.187	10.6	8.2	-12.7	-12.5

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	60.57	39.43	5.92	-7.91	21.14	6.77	3.4
0	57.18	42.82	0.00	0.00	14.37	0.00	0.0
3	60.04	39.96	5.00	-6.68	20.09	5.72	2.9
7	60.45	39.55	5.71	-7.62	20.89	6.53	3.3
14	61.57	38.43	7.67	-10.24	23.14	8.77	4.4
28	62.58	37.42	9.44	-12.60	25.16	10.79	5.4

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-27 Trial 01 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.051	0.051	0.036	0.034	0.297	0.297	0.209	0.198	4.86	4.86	-6.42	-6.23
0	0.110	0.110	0.087	0.082	0.283	0.283	0.224	0.211	0.00	0.00	0.00	0.00
3	0.084	0.083	0.062	0.058	0.293	0.289	0.216	0.202	3.50	2.27	-3.41	-4.13
7	0.05	0.05	0.036	0.034	0.294	0.294	0.212	0.200	4.01	4.01	-5.31	-5.12
13	0.046	0.046	0.032	0.03	0.299	0.299	0.208	0.195	5.63	5.63	-7.09	-7.59
26	0.024	0.023	0.014	0.013	0.324	0.311	0.189	0.176	14.69	9.91	-15.41	-16.66

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	59.30	40.70	4.86	-6.32	18.60	5.49	2.7
0	56.56	43.44	0.00	0.00	13.11	0.00	0.0
3	58.19	41.81	2.89	-3.76	16.38	3.27	1.6
7	58.82	41.18	4.01	-5.22	17.65	4.54	2.3
13	59.74	40.26	5.63	-7.33	19.48	6.37	3.2
26	63.51	36.49	12.30	-16.02	27.03	13.92	7.0

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-28 Trial 02 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.069	0.068	0.055	0.053	0.282	0.278	0.224	0.216	0.79	-0.67	1.77	-1.93
0	0.190	0.190	0.150	0.150	0.279	0.279	0.221	0.221	0.00	0.00	0.00	0.00
3	0.120	0.12	0.099	0.093	0.278	0.278	0.229	0.215	-0.58	-0.58	3.89	-2.41
7	0.12	0.12	0.091	0.085	0.288	0.288	0.219	0.204	3.24	3.24	-0.83	-7.37
13	0.053	0.053	0.04	0.038	0.288	0.288	0.217	0.207	3.09	3.09	-1.45	-6.38
28	0.032	0.032	0.024	0.022	0.291	0.291	0.218	0.200	4.11	4.11	-1.09	-9.33

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	55.92	44.08	0.06	-0.08	11.84	0.07	0.04
0	55.88	44.12	0.00	0.00	11.76	0.00	0.0
3	55.56	44.44	-0.58	0.74	11.11	-0.65	-0.3
7	57.69	42.31	3.24	-4.10	15.38	3.62	1.8
13	57.61	42.39	3.09	-3.91	15.22	3.45	1.7
28	58.18	41.82	4.11	-5.21	16.36	4.60	2.3

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-29 Trial 03 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	LOQ	LOQ	LOQ	LOQ	-	-	-	-	-	-	-	-
0	0.016	0.015	0.014	0.013	0.376	0.259	0.241	0.224	0.00	0.00	0.00	0.00
3	0.007	0.007	0.005	0.004	0.304	0.304	0.217	0.174	-10.33	-17.68	-9.94	-22.41
7	0.003	0.003	LOQ	LOQ	-	-	-	-	-	-	-	-
14	LOQ	LOQ	LOQ	LOQ	-	-	-	-	-	-	-	-
28	0.011	0.011	0.007	0.007	0.306	0.306	0.194	0.194	10.76	18.15	-19.44	-13.25

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	-	-	-	-	-	-	-
0	53.45	46.55	0.00	0.00	6.90	0.00	0.0
3	60.87	39.13	13.88	-15.94	21.74	14.84	7.4
7	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
28	61.11	38.89	14.34	-16.46	22.22	15.33	7.7

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-30 Trial 04 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.009	0.009	0.006	0.006	0.300	0.300	0.200	0.200	6.96	6.96	-8.89	-8.89
0	0.023	0.023	0.018	0.018	0.280	0.280	0.220	0.220	0.00	0.00	0.00	0.00
3	0.014	0.014	0.011	0.011	0.280	0.280	0.220	0.220	-0.17	-0.17	0.22	0.22
7	0.016	0.015	0.011	0.011	0.302	0.283	0.208	0.208	7.63	0.90	-5.45	-5.45
14	0.004	0.004	0.003	0.003	0.286	0.286	0.214	0.214	1.86	1.86	-2.38	-2.38
29	0.011	0.011	0.008	0.007	0.297	0.297	0.216	0.189	5.99	5.99	-1.50	-13.81

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	60.00	40.00	6.96	-8.89	20.00	7.80	3.9
0	56.10	43.90	0.00	0.00	12.20	0.00	0.0
3	56.00	44.00	-0.17	0.22	12.00	-0.20	-0.1
7	58.49	41.51	4.27	-5.45	16.98	4.79	2.4
14	57.14	42.86	1.86	-2.38	14.29	2.09	1.0
29	59.46	40.54	5.99	-7.66	18.92	6.72	3.4

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-31 Trial 05 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.056	0.056	0.038	0.038	0.298	0.298	0.202	0.202	3.44	3.44	-4.68	-4.68
0	0.110	0.110	0.081	0.081	0.288	0.288	0.212	0.212	0.00	0.00	0.00	0.00
3	0.047	0.046	0.032	0.033	0.297	0.291	0.203	0.209	3.30	1.10	-4.49	-1.50
7	0.043	0.042	0.027	0.027	0.309	0.302	0.194	0.194	7.43	4.93	-8.39	-8.39
14	0.033	0.034	0.019	0.019	0.314	0.324	0.181	0.181	9.14	12.45	-14.66	-14.66
25	0.028	0.028	0.014	0.014	0.333	0.333	0.167	0.167	15.76	15.76	-21.40	-21.40

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	59.57	40.43	3.44	-4.68	19.15	3.97	2.0
0	57.59	42.41	0.00	0.00	15.18	0.00	0.0
3	58.86	41.14	2.20	-2.99	17.72	2.54	1.3
7	61.15	38.85	6.18	-8.39	22.30	7.12	3.6
14	63.81	36.19	10.80	-14.66	27.62	12.44	6.2
25	66.67	33.33	15.76	-21.40	33.33	18.15	9.1

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-32 Trial 06 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.008	0.008	0.006	0.006	0.286	0.286	0.214	0.214	2.71	2.71	-4.91	-1.84
0	0.079	0.079	0.064	0.062	0.278	0.278	0.225	0.218	0.00	0.00	0.00	0.00
3	0.021	0.021	0.015	0.015	0.292	0.292	0.208	0.208	4.85	4.85	-7.55	-4.57
7	0.017	0.017	0.011	0.012	0.298	0.298	0.193	0.211	7.22	7.22	-14.36	-3.57
13	0.009	0.009	0.006	0.006	0.300	0.300	0.200	0.200	7.85	7.85	-11.25	-8.39
25	0.012	0.012	0.007	0.007	0.316	0.316	0.184	0.184	13.52	13.52	-18.26	-15.62

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	57.14	42.86	2.71	-3.40	14.29	3.02	1.5
0	55.63	44.37	0.00	0.00	11.27	0.00	0.0
3	58.33	41.67	4.85	-6.08	16.67	5.40	2.7
7	59.65	40.35	7.22	-9.05	19.30	8.03	4.0
13	60.00	40.00	7.85	-9.84	20.00	8.73	4.4
25	63.16	36.84	13.52	-16.96	26.32	15.05	7.5

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-33 Trial 07 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.034	0.034	0.017	0.016	0.337	0.337	0.168	0.158	10.34	10.34	-13.65	-18.73
0	0.072	0.072	0.046	0.046	0.305	0.305	0.195	0.195	0.00	0.00	0.00	0.00
3	0.058	0.058	0.031	0.03	0.328	0.328	0.175	0.169	7.41	7.41	-10.14	-13.04
7	0.036	0.035	0.019	0.017	0.336	0.327	0.178	0.159	10.28	7.22	-8.90	-18.49
14	0.035	0.035	0.019	0.021	0.318	0.318	0.173	0.191	4.29	4.29	-11.38	-2.06
28	0.019	0.019	0.01	0.01	0.328	0.328	0.172	0.172	7.38	7.38	-11.54	-11.54

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	67.33	32.67	10.34	-16.19	34.65	12.62	6.3
0	61.02	38.98	0.00	0.00	22.03	0.00	0.0
3	65.54	34.46	7.41	-11.59	31.07	9.04	4.5
7	66.36	33.64	8.75	-13.69	32.71	10.68	5.3
14	63.64	36.36	4.29	-6.72	27.29	5.24	2.6
28	65.52	34.48	7.38	-11.54	31.03	9.00	4.5

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-34 Trial 08 Summarised under CA 6.3.1/14

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.012	0.012	0.007	0.007	0.3158	0.3158	0.1842	0.1842	12.97	14.14	-18.05	-15.89
0	0.097	0.096	0.078	0.076	0.280	0.277	0.225	0.219	0.00	0.00	0.00	0.00
3	0.054	0.054	0.036	0.035	0.302	0.302	0.201	0.196	7.92	9.04	-10.53	-10.72
7	0.025	0.025	0.016	0.016	0.305	0.305	0.195	0.195	9.06	10.20	-13.20	-10.91
15	0.007	0.007	0.005	0.004	0.304	0.304	0.217	0.174	8.87	10.01	-3.29	-20.59
29	0.005	0.005	0.003	0.003	0.313	0.313	0.188	0.188	11.79	12.96	-16.59	-14.39

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	63.16	36.84	13.55	-16.99	26.32	15.08	7.5
0	55.62	44.38	0.00	0.00	11.24	0.00	0.0
3	60.34	39.66	8.48	-10.63	20.67	9.43	4.7
7	60.98	39.02	9.63	-12.07	21.95	10.71	5.4
15	60.87	39.13	9.44	-11.83	21.74	10.50	5.2
29	62.50	37.50	12.37	-15.50	25.00	13.76	6.9

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-629509-01-1, 2018 'Determination of the residues of AE C656948 and spiroxamine in/on grapes and table grapes after spray application of Fluopyram & Spiroxamine SE 275 in Italy, Greece and Spain'

Table CA 6.7.1-35 Average of all 8 trials

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
pre 0	0.034	0.034	0.024	0.023	0.302	0.301	0.200	0.196	6.473	7.218	-9.028	-8.732
0	0.087	0.087	0.067	0.066	0.284	0.281	0.220	0.215	0	0	0	0
3	0.051	0.050	0.036	0.035	0.297	0.295	0.209	0.199	4.6	5.1	-5.2	-7.3
7	0.039	0.038	0.030	0.029	0.305	0.300	0.200	0.196	7.4	6.6	-9.3	-8.9
13-15	0.027	0.027	0.018	0.017	0.301	0.303	0.202	0.194	6.2	7.7	-8.5	-9.5
25-29	0.018	0.018	0.011	0.010	0.313	0.312	0.191	0.184	10.5	10.9	-13.2	-14.5

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
pre 0	60.35	39.65	6.84	-8.88	20.69	7.73	3.9
0	56.48	43.52	0.00	0.00	12.96	0.00	0.0
3	59.21	40.79	4.83	-6.27	18.42	5.46	2.7
7	60.45	39.55	7.02	-9.12	20.90	7.94	4.0
13-15	60.40	39.60	6.94	-9.01	20.80	7.84	3.9
25-29	62.51	37.49	10.68	-13.86	25.03	12.07	6.0

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-36 Trial 01 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.0450	0.0450	0.0380	0.0370	0.273	0.273	0.230	0.224	0.00	0.00	0.00	0.00
14	0.0086	0.0086	0.0052	0.0050	0.314	0.314	0.190	0.182	15.09	15.09	-17.60	-18.62
34	0.0079	0.0075	0.0049	0.0046	0.317	0.301	0.197	0.185	16.33	10.44	-14.55	-17.62

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	54.55	45.45	0.00	0.00	9.09	0.00	0.0
14	62.77	37.23	15.09	-18.10	25.55	16.46	8.2
34	61.85	38.15	13.39	-16.06	23.69	14.60	7.3

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-37 Trial 02 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.0730	0.0730	0.0510	0.0630	0.281	0.281	0.196	0.242	0.00	0.00	0.00	0.00
14	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
34	0.0140	0.0140	0.0086	0.0082	0.313	0.313	0.192	0.183	+11.30	+11.30	-2.14	-24.46

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	56.15	43.85	0.00	0.00	12.31	0.00	0.00
14	-	-	-	-	-	-	-
34	62.50	37.50	+11.30	-14.47	25.00	12.69	6.3

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-38 Trial 03 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.0950	0.0920	0.0810	0.0740	0.278	0.269	0.237	0.216	0.00	0.00	0.00	0.00
14	0.0280	0.0280	0.0200	0.0190	0.295	0.295	0.211	0.200	6.11	9.57	-11.11	-7.57
34	0.0082	0.0083	0.0054	0.0053	0.301	0.305	0.199	0.195	8.53	13.44	-16.18	-9.95

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	54.68	45.32	0.00	0.00	9.36	0.00	0.0
14	58.95	41.05	7.81	-9.42	17.89	8.54	4.3
34	60.66	39.34	10.94	-13.20	21.32	11.97	6.0

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-39 Trial 04 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.0930	0.0920	0.0740	0.0660	0.286	0.283	0.228	0.203	0.00	0.00	0.00	0.00
14	0.0150	0.0140	0.0063	0.0061	0.362	0.338	0.152	0.147	26.62	19.46	-33.17	-27.44
34	0.0310	0.0300	0.0140	0.0140	0.348	0.337	0.187	0.157	21.72	19.08	-30.91	-22.54

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	56.92	43.08	0.00	0.00	13.85	0.00	0.0
14	70.05	29.95	23.06	-30.47	40.10	26.25	13.1
34	68.54	31.46	20.41	-26.97	37.08	23.23	11.6

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-40 Trial 05 Summarised under CA 6.3.1/018

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.2100	0.2100	0.1600	0.1500	0.288	0.288	0.219	0.205	0.00	0.00	0.00	0.00
14	0.0390	0.0370	0.0230	0.0250	0.315	0.298	0.185	0.202	9.33	3.73	-15.37	-1.88
34	0.0210	0.0210	0.0140	0.0150	0.296	0.296	0.197	0.211	2.82	2.82	-10.04	2.82

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	57.53	42.47	0.00	0.00	15.07	0.00	0.0
14	61.29	38.71	6.53	-8.84	22.58	7.51	3.8
34	59.15	40.85	2.82	-3.82	18.31	3.24	1.6

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-41 Trial 06 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.200	0.200	0.150	0.170	0.278	0.278	0.208	0.236	0.00	0.00	0.00	0.00
14	0.0530	0.0520	0.0300	0.0290	0.323	0.317	0.183	0.177	16.34	14.15	-12.20	-25.11
34	0.0220	0.0210	0.0110	0.0110	0.338	0.323	0.169	0.169	21.85	16.31	-18.77	-28.33

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	55.56	44.44	0.00	0.00	11.11	0.00	0.0
14	64.02	35.98	15.24	-19.05	28.05	16.94	8.5
34	66.15	33.85	19.08	-23.85	32.31	21.20	10.6

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-42 Trial 07 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.0180	0.0170	0.0150	0.0150	0.277	0.262	0.231	0.231	0.00	0.00	0.00	0.00
14	0.0035	0.0035	<0.00229	<0.00229	0.500	0.500	LOQ	LOQ	80.56	91.18	LOQ	LOQ
34	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	53.85	46.15	0.00	0.00	7.69	0.00	0.0
14	100.00	LOQ	85.71	LOQ	LOQ	LOQ	LOQ
34	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-43 Trial 08 Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.1200	0.1200	0.0920	0.0990	0.2784	0.2784	0.2135	0.2297	0.00	0.00	0.00	0.00
14	0.0290	0.0270	0.0140	0.0130	0.349	0.325	0.169	0.157	25.49	16.84	-20.98	-31.81
34	0.0041	0.0041	<0.00229	<0.00229	0.500	0.500	LOQ	LOQ	79.58	79.58	LOQ	LOQ

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	55.68	44.32	0.00	0.00	11.37	0.00	0.0
14	67.47	32.53	21.16	-26.59	34.94	23.57	11.8
34	100.00	LOQ	81.30	LOQ	LOQ	LOQ	44.3

Grapes isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763137-01-1, 2021 'Determination of residues of spiroxamine and its metabolites after one or two applications of Spiroxamine EC 500 in grapes (outdoor) at 4 sites in Northern Europe and 4 sites in Southern Europe 2020'

Table CA 6.7.1-44 Average of all 8 trials Summarised under CA 6.3.1/18

Grapes	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0	0.107	0.106	0.083	0.084	0.281	0.279	0.218	0.222	0.00	0.00	0.00	0.00
14	0.025	0.024	0.016	0.016	0.307	0.296	0.200	0.197	9.1	6.0	-8.0	-11.1
34	0.015	0.015	0.010	0.010	0.310	0.303	0.193	0.194	10.2	8.4	-11.2	-12.6

Grapes	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0	56.06	43.94	0.00	0.00	12.11	0.00	0.0
14	60.27	39.73	7.52	-9.59	20.54	8.42	4.2
34	61.27	38.73	9.30	-11.87	22.54	10.43	5.2

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-618660-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, the Netherlands, the United Kingdom and Belgium'

Table CA 6.7.1-45 Trial 01 Summarised under CA 6.3.2/13

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.4	1.4	1.1	1.1	0.280	0.280	0.220	0.220	0	0	0	0
42 grain	0.006	0.008	0.006	0.004	0.350	0.333	0.250	0.167	-10.7	19.0	13.6	-24.2
42 straw	0.170	0.17	0.14	0.13	0.279	0.279	0.230	0.213	-0.5	-0.5	4.3	-3.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.00	44.00	0.00	0.00	12.00	0.00	0.0
42 grain	58.33	41.67	4.17	-5.30	16.67	4.67	2.3
42 straw	55.74	44.26	-0.47	0.60	11.48	-0.52	-0.3

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-618660-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, the Netherlands, the United Kingdom and Belgium'

Table CA 6.7.1-46 Trial 02 Summarised under CA 6.3.2/13

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.4	1.3	1.1	1.1	0.286	0.265	0.224	0.224	0	0	0	0
48 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
48 straw	0.130	0.13	0.098	0.097	0.286	0.286	0.215	0.213	0.0	7.7	-4.1	-5.0

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.10	44.90	0.00	0.00	10.20	0.00	0.0
48 grain	-	-	-	-	-	-	-
48 straw	57.14	42.86	3.70	-4.55	14.29	4.08	2.0

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-618660-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, the Netherlands, the United Kingdom and Belgium'

Table CA 6.7.1-47 Trial 03 Summarised under CA 6.3.2/13

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.6	2.6	2.2	2.1	0.274	0.274	0.232	0.221	0	0	0	0
57 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
57 straw	0.065	0.071	0.051	0.047	0.278	0.303	0.218	0.201	1.5	10.9	-5.9	-9.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.74	45.26	0.00	0.00	9.47	0.00	0.0
57 grain	-	-	-	-	-	-	-
57 straw	58.12	41.88	6.18	-7.47	16.24	6.77	3.4

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-618660-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, the Netherlands, the United Kingdom and Belgium'

Table CA 6.7.1-48 Trial 04 Summarised under CA 6.3.2/13

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.4	2.4	1.9	1.9	0.279	0.279	0.221	0.221	0	0	0	0
41 grain	0.003	0.003	<0.00247	<0.00229	0.500	0.500	LOQ	LOQ	79.2	79.2	-	-
41 straw	0.079	0.082	0.06	0.058	0.285	0.294	0.215	0.208	1.5	5.3	-2.7	-5.9

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.81	44.19	0.00	0.00	11.63	0.00	0.0
41 grain	100.00	-	79.17	-	-	-	44.2
41 straw	57.71	42.29	3.39	-4.28	15.41	3.78	1.9

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-618660-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, the Netherlands, the United Kingdom and Belgium'

Table CA 6.7.1-49 Average of all 4 trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.950	1.925	1.575	1.550	0.280	0.275	0.224	0.222	0	0	0	0
41-57 grain	0.005	0.006	0.006	0.004	0.375	0.417	0.250	0.167	34.1	51.8	11.5	-24.8
41-57 straw	0.111	0.113	0.087	0.083	0.281	0.290	0.209	0.209	0.6	5.8	-2.1	-5.8

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.41	44.59	0.00	0.00	10.83	0.00	0.0
41-57 grain	79.17	41.67	42.87	-6.55	37.50	26.67	23.8
41-57 straw	57.18	42.82	3.18	-3.95	14.35	3.53	1.8

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-630826-02-1, 2018 'Amendment No. 1 to Final Report: Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on barley after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in southern France and Portugal'

Table CA 6.7.1-50 Trial 01 Summarised under CA 6.3.2/14

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.76	0.75	0.64	0.63	0.273	0.270	0.230	0.227	0	0	0	0
33 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
33 straw	0.027	0.026	0.02	0.025	0.276	0.265	0.204	0.255	0.8	-1.7	-11.4	12.6

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.32	45.68	0.00	0.00	8.63	0.00	0.0
33 grain	-	-	-	-	-	-	-
33 straw	54.08	45.92	-0.43	0.51	8.16	-0.47	-0.2

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-630826-02-1, 2018 'Amendment No. 1 to Final Report: Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on barley after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in southern France and Portugal'

Table CA 6.7.1-51 Trial 02 Summarised under CA 6.3.2/14

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.71	0.69	0.61	0.58	0.274	0.266	0.236	0.224	0	0	0	0
48 grain	0.003	0.003	<0.00229	<0.00229	0.500	0.500	LOQ	LOQ	82.4	87.7	-	-
48 straw	0.039	0.037	0.029	0.025	0.390	0.285	0.223	0.192	9.4	6.8	-5.3	-14.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.05	45.95	0.00	0.00	8.11	0.00	0.0
48 grain	100.00	-	85.00	-	-	-	45.9
48 straw	58.46	41.54	8.15	-9.59	16.92	8.81	4.4

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-630826-02-1, 2018 'Amendment No. 1 to Final Report: Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on barley after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in southern France and Portugal'

Table CA 6.7.1-52 Average of both trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.735	0.720	0.625	0.605	0.274	0.268	0.233	0.225	0	0	0	0
33-48 grain	0.003	0.003	<0.00229	<0.00229	0.500	0.500	LOQ	LOQ	~~82.6~~	~~86.5~~	-	-
33-48 straw	0.033	0.032	0.025	0.025	0.288	0.275	0.214	0.224	5.1	2.6	-8.3	-0.7

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.19	45.81	0.00	0.00	8.37	0.00	0.0
33-48 grain	100.00	0.00	84.59	-100.00	100.00	91.63	45.8
33-48 straw	56.27	43.73	3.85	-4.55	12.54	4.17	2.1

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-631606-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spraying of JAU 6476 & KWG 4168 EC 460 in the field in France (South), Spain, Italy and Portugal'

Table CA 6.7.1-53 Trial 01 Summarised under CA 6.3.2/15

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.0	2.0	1.6	1.6	0.278	0.278	0.222	0.222	0	0	0	0
66 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
66 straw	0.085	0.1	0.076	0.075	0.253	0.298	0.226	0.223	-8.9	7.1	1.8	0.4

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.56	44.44	0.00	0.00	11.11	0.00	0.0
66 grain	-	-	-	-	-	-	-
66 straw	55.06	44.94	-0.89	1.12	10.12	-0.99	-0.5

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-631606-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spraying of JAU 6476 & KWG 4168 EC 460 in the field in France (South), Spain, Italy and Portugal'

Table CA 6.7.1-54 Trial 02 Summarised under CA 6.3.2/15

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	3.9	3.9	3.2	3.2	0.275	0.275	0.225	0.225	0	0	0	0
64 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
64 straw	0.160	0.15	0.11	0.099	0.308	0.289	0.212	0.191	12.2	5.2	-5.9	-15.4

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.93	45.07	0.00	0.00	9.86	0.00	0.0
64 grain	-	-	-	-	-	-	-
64 straw	59.73	40.27	8.74	-10.65	19.46	9.60	4.8

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-631606-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spraying of JAU 6476 & KWG 4168 EC 460 in the field in France (South), Spain, Italy and Portugal'

Table CA 6.7.1-55 Trial 03 Summarised under CA 6.3.2/15

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.4	2.4	1.9	1.9	0.279	0.279	0.221	0.221	0	0	0	0
61 grain	0.0030	0.0030	0.0023	0.0020	0.292	0.292	0.223	0.194	4.5	4.5	0.7	-12.0
61 straw	0.032	0.03	0.022	0.02	0.308	0.288	0.212	0.192	10.3	3.4	-4.3	-13.0

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.81	44.19	0.00	0.00	11.63	0.00	0.0
61 grain	58.31	41.69	6.15	-7.50	16.62	4.99	2.5
61 straw	59.62	40.38	6.81	-8.60	19.23	7.60	3.8

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-631606-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spraying of JAU 6476 & KWG 4168 EC 460 in the field in France (South), Spain, Italy and Portugal'

Table CA 6.7.1-56 Trial 04 Summarised under CA 6.3.2/15

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.4	2.3	2	2	0.276	0.264	0.230	0.230	0	0	0	0
74 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
74 straw	0.055	0.056	0.041	0.041	0.285	0.290	0.212	0.212	3.3	9.8	-7.6	-7.6

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.02	45.98	0.00	0.00	8.05	0.00	0.0
74 grain	-	-	-	-	-	-	-
74 straw	57.51	42.49	6.46	-7.59	15.03	6.98	3.5

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-631606-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spraying of JAU 6476 & KWG 4168 EC 460 in the field in France (South), Spain, Italy and Portugal'

Table CA 6.7.1-57 Average of all 4 trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.675	2.650	2.175	2.175	0.277	0.274	0.225	0.225	0	0	0	0
61-74 grain	0.00300	0.00300	0.00229	0.00200	0.2915	0.2915	0.2225	0.1944	5.3	6.4	-0.9	-13.5
61-74 straw	0.083	0.084	0.062	0.059	0.288	0.291	0.216	0.205	4.2	6.3	-4.0	-8.9

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.08	44.92	0.00	0.00	10.16	0.00	0.0
61-74 grain	58.31	41.69	5.86	-7.19	16.62	6.46	3.2
61-74 straw	57.98	42.02	5.26	-6.45	15.96	5.80	2.9

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-656996-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Italy, Spain and Portugal'

Table CA 6.7.1-58 Trial 01 Summarised under CA 6.3.2/16

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.4	1.4	1.2	1.1	0.275	0.275	0.235	0.216	0	0	0	0
33 grain	0.0027	0.0027	0.0023	0.0023	0.270	0.270	0.230	0.230	-1.6	-1.6	-2.3	6.6
33 straw	0.090	0.094	0.068	0.066	0.283	0.296	0.214	0.208	3.1	7.7	-9.1	-3.8

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.90	45.10	0.00	0.00	9.80	0.00	0.0
33 grain	54.00	46.00	-1.64	2.00	8.00	-1.80	-0.9
33 straw	57.86	42.14	5.39	-6.56	15.72	5.92	3.0

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-656996-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Italy, Spain and Portugal'

Table CA 6.7.1-59 Trial 02 Summarised under CA 6.3.2/16 [data not used for MRL purposes but are appropriate for isomer ratio assessment]

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.4	2.4	2	2	0.273	0.273	0.227	0.227	0	0	0	0
28 grain	0.0360	0.0360	0.0360	0.0360	0.250	0.250	0.250	0.250	-8.3	-8.3	10.0	10.0
28 straw	0.120	0.12	0.091	0.086	0.288	0.288	0.218	0.206	5.5	5.5	-4.0	-9.3

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.55	45.45	0.00	0.00	9.09	0.00	0.0
28 grain	50.00	50.00	-8.33	10.00	0.00	-9.09	-4.5
28 straw	57.55	42.45	5.52	-6.62	15.11	6.02	3.0

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-656996-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Italy, Spain and Portugal'

Table CA 6.7.1-60 Trial 03 Summarised under CA 6.3.2/16 [data not used for MRL purposes but are appropriate for isomer ratio assessment]

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.6	2.5	2.1	2	0.283	0.272	0.228	0.217	0	0	0	0
41 grain	0.0078	0.0081	0.0070	0.0064	0.266	0.276	0.239	0.218	-5.8	1.7	4.7	0.5
41 straw	0.110	0.11	0.078	0.075	0.295	0.295	0.209	0.201	4.4	8.5	-8.4	-7.5

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.43	44.57	0.00	0.00	10.87	0.00	0.0
41 grain	54.27	45.73	-0.51	0.61	8.53	-2.34	-1.2
41 straw	58.98	41.02	6.40	-7.96	17.96	7.09	3.5

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-656996-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Italy, Spain and Portugal'

Table CA 6.7.1-61 Trial 04 Summarised under CA 6.3.2/16

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.0	2.0	1.6	1.5	0.282	0.282	0.225	0.211	0	0	0	0
33 grain	0.0091	0.0110	0.0100	0.0080	0.239	0.289	0.262	0.210	-15.2	2.5	16.5	-0.6
33 straw	0.110	0.11	0.077	0.076	0.295	0.295	0.206	0.204	4.7	4.7	-8.4	-3.6

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.34	43.66	0.00	0.00	12.68	0.00	0.0
33 grain	52.76	47.24	-6.36	8.20	5.51	-7.16	-3.6
33 straw	58.98	41.02	4.69	-6.05	17.96	5.29	2.6

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-656996-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on barley after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Italy, Spain and Portugal'

Table CA 6.7.1-62 Average of all 4 trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.100	2.075	1.725	1.650	0.278	0.275	0.229	0.218	0	0	0	0
33 grain	0.0139	0.0145	0.0138	0.0132	0.256	0.271	0.245	0.227	-7.8	-1.4	7.1	4.2
33 straw	0.108	0.109	0.079	0.076	0.290	0.293	0.212	0.205	4.4	6.6	-7.5	-6.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.31	44.69	0.00	0.00	10.61	0.00	0.0
33 grain	52.76	47.24	-4.61	5.70	5.51	-5.10	-2.5
33 straw	58.34	41.66	5.50	-6.80	16.69	6.08	3.0

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-663690-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Hungary, the United Kingdom and northern France'

Table CA 6.7.1-63 Trial 01 Summarised under CA 6.3.2/17

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.5	1.4	1.2	1.2	0.283	0.264	0.226	0.226	0	0	0	0
30 grain	0.0066	0.0067	0.0056	0.0051	0.275	0.279	0.233	0.213	-2.8	5.7	3.1	-6.1
30 straw	0.130	0.13	0.096	0.091	0.291	0.291	0.215	0.204	2.8	10.1	-5.1	-10.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.72	45.28	0.00	0.00	9.43	0.00	0.0
30 grain	55.42	44.58	1.28	-1.55	10.83	1.40	0.7
30 straw	58.17	41.83	6.30	-7.62	16.33	6.90	3.4

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-663690-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Hungary, the United Kingdom and northern France'

Table CA 6.7.1-64 Trial 02 Summarised under CA 6.3.2/17

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	3.2	3.2	2.5	2.5	0.281	0.281	0.219	0.219	0	0	0	0
47 grain	0.0120	0.0120	0.0100	0.0100	0.273	0.273	0.227	0.227	-2.8	-2.8	3.6	3.6
47 straw	0.086	0.081	0.064	0.061	0.295	0.277	0.219	0.209	4.9	-1.2	-0.1	-4.7

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.14	43.86	0.00	0.00	12.28	0.00	0.0
47 grain	54.55	45.45	-2.84	3.64	9.09	-3.19	-1.6
47 straw	57.19	42.81	1.87	-2.40	14.38	2.10	1.1

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-663690-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Hungary, the United Kingdom and northern France'

Table CA 6.7.1-65 Trial 03 Summarised under CA 6.3.2/17

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.6	1.6	1.3	1.2	0.281	0.281	0.228	0.211	0	0	0	0
17 grain	0.0071	0.0076	0.0065	0.0057	0.264	0.283	0.242	0.212	-6.0	0.7	5.9	0.7
17 straw	0.100	0.1	0.078	0.076	0.282	0.282	0.220	0.215	0.0	0.6	-3.4	2.0

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.14	43.86	0.00	0.00	12.28	0.00	0.0
17 grain	54.65	45.35	-2.66	3.41	9.29	-2.99	-1.5
17 straw	56.50	43.50	0.64	-0.81	12.99	0.71	0.4

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-663690-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Hungary, the United Kingdom and northern France'

Table CA 6.7.1-66 Trial 04 Summarised under CA 6.3.2/17

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.1	2	1.7	1.6	0.284	0.270	0.230	0.216	0	0	0	0
42 grain	0.0070	0.0073	0.0080	0.0068	0.241	0.251	0.275	0.234	-15.2	-7.2	19.7	8.1
42 straw	0.065	0.062	0.044	0.044	0.392	0.288	0.205	0.205	6.5	6.7	-10.9	-5.3

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.41	44.59	0.00	0.00	10.81	0.00	0.0
42 grain	49.14	50.86	-11.31	14.05	-1.72	-12.53	-6.3
42 straw	59.07	40.93	6.61	-8.22	18.14	7.33	3.7

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-663690-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on winter barley and spring barley after spray application of JAU 6476 & KWG 4168 EC 460 in Hungary, the United Kingdom and northern France'

Table CA 6.7.1-67 Average of all 4 trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.100	2.050	1.675	1.625	0.282	0.274	0.226	0.218	0	0	0	0
17-47 grain	0.0082	0.0084	0.0075	0.0069	0.263	0.271	0.244	0.221	-6.7	-1.0	8.2	1.5
17-47 straw	0.095	0.093	0.071	0.068	0.293	0.285	0.215	0.208	3.7	3.9	-4.9	-4.7

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.60	44.40	0.00	0.00	11.20	0.00	0.0
17-47 grain	53.44	46.56	-3.89	4.87	6.87	-4.33	-2.2
17-47 straw	57.73	42.27	3.83	-4.80	15.46	4.26	2.1

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-638944-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on spring barley and winter barley after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in Hungary and northern France'

Table CA 6.7.1-68 Trial 01 Summarised under CA 6.3.2/18

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.83	0.81	0.68	0.68	0.277	0.270	0.227	0.227	0	0	0	0
17 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
17 straw	0.033	0.033	0.022	0.022	0.300	0.300	0.200	0.200	8.4	11.1	-11.8	-11.8

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.67	45.33	0.00	0.00	9.33	0.00	0.0
17 grain	-	-	-	-	-	-	-
17 straw	60.00	40.00	9.76	-11.76	20.00	10.67	5.3

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-638944-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on spring barley and winter barley after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in Hungary and northern France'

Table CA 6.7.1-69 Trial 02 Summarised under CA 6.3.2/18

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.46	0.46	0.38	0.38	0.274	0.274	0.226	0.226	0	0	0	0
54 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
54 straw	0.026	0.026	0.018	0.018	0.295	0.295	0.205	0.205	7.9	7.9	-9.6	-9.6

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.76	45.24	0.00	0.00	9.52	0.00	0.0
54 grain	-	-	-	-	-	-	-
54 straw	59.09	40.91	7.91	-9.57	18.18	8.66	4.3

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-638944-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on spring barley and winter barley after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in Hungary and northern France'

Table CA 6.7.1-70 Average of both trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.645	0.635	0.530	0.530	0.275	0.272	0.226	0.226	0	0	0	0
17-54 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
17-54 straw	0.030	0.030	0.020	0.020	0.298	0.298	0.202	0.202	8.2	9.5	-10.7	-10.7

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.71	45.29	0.00	0.00	9.43	0.00	0.0
17-54 grain	-	-	-	-	-	-	-
17-54 straw	59.55	40.45	8.83	-10.67	19.09	9.66	4.8

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-71 Trial 01 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.321	1.321	1.085	1.061	0.276	0.276	0.227	0.222	0	0	0	0
17 plant	0.203	0.208	0.156	0.116	0.279	0.286	0.215	0.220	1.2	3.7	-5.3	-0.7
33 grain	n.a.	n.a.	n.a.	n.a.	LOQ	LOQ	LOQ	LOQ		-	-	-
33 straw	0.073	0.08	0.071	0.060	0.252	0.276	0.245	0.228	-8.8	0.0	8.0	2.7

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.18	44.82	0.00	0.00	10.36	0.00	0.0
17 plant	56.53	43.47	2.45	-3.02	13.07	2.71	1.4
33 grain	-	-	-	-	-	-	-
33 straw	52.76	47.24	-4.39	5.40	5.52	-4.84	-2.4

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-72 Trial 02 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.250	1.250	1.014	0.991	0.277	0.277	0.225	0.220	0	0	0	0
8 plant	0.160	0.160	0.123	0.127	0.281	0.281	0.216	0.223	1.2	1.2	-4.1	1.3
14 plant	0.156	0.156	0.120	0.120	0.283	0.283	0.217	0.217	1.852	1.852	-3.417	-1.176
21 plant	0.097	0.099	0.071	0.073	0.285	0.291	0.209	0.215	2.820	4.940	-7.224	-2.397
64 grain	n.a.	n.a.	n.a.	n.a.	LOQ	LOQ	LOQ	LOQ	-	-	-	-
64 straw	0.028	0.028	0.019	0.019	0.298	0.298	0.202	0.202	7.4	7.4	-10.2	-8.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.49	44.51	0.00	0.00	10.99	0.00	0.0
8 plant	56.14	43.86	1.16	-1.45	12.28	1.29	0.6
14 plant	56.52	43.48	1.85	-2.31	13.04	2.06	1.0
21 plant	57.65	42.35	3.88	-4.84	15.29	4.31	2.2
64 grain	-	-	-	-	-	-	-
64 straw	59.57	40.43	7.35	-9.17	19.15	8.16	4.1

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-73 Trial 03 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.575	1.575	1.331	1.309	0.272	0.272	0.230	0.226	0	0	0	0
16 plant	0.155	0.158	0.124	0.126	0.275	0.281	0.220	0.224	1.210	3.168	-4.189	-1.008
55 grain	n.a.	n.a.	n.a.	n.a.	LOQ	LOQ	LOQ	LOQ	-	-	-	-
55 straw	0.033	0.036	0.027	0.024	0.275	0.300	0.225	0.200	1.1	10.3	-2.1	-11.5

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.40	45.60	0.00	0.00	8.81	0.00	0.0
16 plant	55.60	44.40	2.19	-2.61	11.19	2.38	1.2
55 grain	-	-	-	-	-	-	-
55 straw	57.50	42.50	5.69	-6.79	15.00	6.19	3.1

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-74 Trial 04 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.863	1.863	1.529	1.553	0.274	0.274	0.225	0.228	0	0	0	0
14 plant	0.234	0.236	0.174	0.174	0.286	0.289	0.213	0.213	4.537	5.430	-5.287	-6.751
49 grain	n.a.	n.a.	n.a.	n.a.	LOQ	LOQ	LOQ	LOQ	-	-	-	-
49 straw	0.115	0.117	0.091	0.084	0.283	0.287	0.224	0.206	3.3	5.1	-0.4	-9.5

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.73	45.27	0.00	0.00	9.46	0.00	0.0
14 plant	57.46	42.54	4.98	-6.02	14.91	5.45	2.7
49 grain	-	-	-	-	-	-	-
49 straw	57.00	43.00	4.15	-5.02	14.00	4.55	2.3

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-75 Trial 05 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.389	2.365	1.935	1.935	0.277	0.274	0.224	0.224	0	0	0	0
7 plant	0.454	0.454	0.358	0.358	0.280	0.280	0.220	0.220	0.917	1.941	-1.752	-1.752
14 plant	0.287	0.311	0.215	0.215	0.279	0.303	0.209	0.209	0.782	10.318	-6.788	-6.788
22 plant	0.222	0.225	0.172	0.167	0.282	0.286	0.219	0.212	1.958	4.385	-2.471	-5.306
39 grain	0.014	0.014	0.010	0.010	0.292	0.292	0.208	0.208	5.3	6.4	-7.1	-7.1
39 straw	0.196	0.210	0.143	0.136	0.286	0.307	0.209	0.199	3.3	11.8	-7.0	-11.5

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.13	44.87	0.00	0.00	10.25	0.00	0.0
7 plant	55.91	44.09	1.43	-1.75	11.82	1.57	0.8
14 plant	58.17	41.83	5.53	-6.79	16.34	6.09	3.0
22 plant	56.87	43.13	3.17	-3.89	13.74	3.49	1.7
39 grain	58.33	41.67	5.82	-7.15	16.67	6.42	3.2
39 straw	59.27	40.73	7.52	-9.24	18.54	8.29	4.1

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-76 Trial 06 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	3.028	3.028	2.562	2.562	0.271	0.271	0.229	0.229	0	0	0	0
7 plant	0.280	0.280	0.217	0.224	0.280	0.280	0.217	0.224	3.278	3.278	-5.401	-2.349
13 plant	0.210	0.212	0.161	0.165	0.281	0.283	0.218	0.221	3.658	4.045	-6.074	-3.740
60 grain	n.a.	n.a.	n.a.	n.a.	LOQ	LOQ	LOQ	LOQ	-	-	-	-
60 straw	0.123	0.126	0.089	0.084	0.291	0.299	0.211	0.199	7.6	10.2	-8.0	-13.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.17	45.83	0.00	0.00	8.34	0.00	0.0
7 plant	55.94	44.06	3.28	-3.87	11.89	3.55	1.8
13 plant	56.42	43.58	4.15	-4.91	12.83	4.50	2.2
60 grain	-	-	-	-	-	-	-
60 straw	59.00	41.00	8.93	-10.55	18.01	9.67	4.8

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-77 Trial 07 Summarised under CA 6.3.2/19

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.284	2.284	1.827	1.779	0.279	0.279	0.224	0.218	0	0	0	0
7 plant	0.361	0.361	0.264	0.288	0.283	0.283	0.207	0.226	1.409	1.409	-7.289	3.868
14 plant	0.216	0.219	0.159	0.166	0.284	0.288	0.209	0.218	1.714	3.126	-6.399	0.358
22 plant	0.111	0.115	0.077	0.082	0.288	0.299	0.200	0.213	3.181	6.899	-10.52	-2.139
50 grain	0.007	0.007	n.a.	n.a.	0.500	0.500	LOQ	LOQ	78.9	78.9	-	-
50 straw	0.019	0.019	0.012	0.012	0.306	0.306	0.194	0.194	9.7	9.7	-13.4	-11.1

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.88	44.12	0.00	0.00	11.77	0.00	0.0
7 plant	56.67	43.33	1.41	-1.78	13.34	1.57	0.8
14 plant	57.24	42.76	2.42	-3.07	14.47	2.70	1.4
22 plant	58.70	41.30	5.04	-6.39	17.40	5.63	2.8
50 grain	100.00	-	78.94	-	-	-	-
50 straw	61.29	38.71	9.67	-12.25	22.58	10.81	5.4

Barley isomer ratio data (from chiral analysis of spiroxamine)

Report: M-685313-01-1, 2020 'Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on barley after spray application of PTZ & SPX & TFS EC 280.3 in the field in France (South), Italy, Greece and Spain'

Table CA 6.7.1-78 Average of all seven trials

Barley	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.959	1.955	1.612	1.599	0.275	0.274	0.226	0.224	0	0	0	0
plant 1	0.264	0.265	0.202	0.208	0.281	0.282	0.215	0.222	2.149	2.882	-4.843	-1.274
plant 2	0.217	0.225	0.164	0.167	0.281	0.291	0.212	0.216	2.361	5.963	-6.250	-3.884
plant 3	0.143	0.146	0.107	0.107	0.285	0.291	0.212	0.213	3.514	5.865	-6.397	-5.029
33-64 grain	0.014	0.014	0.010	0.010	0.292	0.292	0.208	0.208	6.1	6.3	-7.9	-7.2
33-64 straw	0.084	0.088	0.065	0.061	0.282	0.296	0.217	0.204	2.7	7.9	-4.0	-8.9

Barley	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.94	45.06	0.00	0.00	9.87	0.00	0.0
plant 1	56.32	43.68	2.51	-3.07	12.63	2.76	1.4
plant 2	57.22	42.78	4.16	-5.07	14.44	4.57	2.3
plant 3	57.51	42.49	4.69	-5.72	15.02	5.15	2.6
33-64 grain	58.33	41.67	6.18	-7.54	16.67	6.79	3.4
33-64 straw	57.84	42.16	5.28	-6.44	15.67	5.80	2.9

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626175-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on spring wheat and winter wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the United Kingdom, Germany and the Netherlands'

Table CA 6.7.1-79 Trial 01 Summarised under CA 6.3.3/12

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.5	1.5	1.3	1.3	0.268	0.268	0.232	0.232	0	0	0	0
50 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
50 straw	0.120	0.13	0.094	0.098	0.271	0.294	0.213	0.222	1.4	9.8	-8.4	-4.5

Wheat	enantiomers sum		% diff from da y0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	53.57	46.43	0.00	0.00	7.14	0.00	0.0
50 grain	-	-	-	-	-	-	-
50 straw	56.56	43.44	5.58	-6.44	13.12	5.98	3.0

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626175-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on spring wheat and winter wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the United Kingdom, Germany and the Netherlands'

Table CA 6.7.1-80 Trial 02 Summarised under CA 6.3.3/12

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.6	1.6	1.4	1.3	0.271	0.271	0.237	0.220	0	0	0	0
62 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
62 straw	0.038	0.039	0.033	0.031	0.270	0.277	0.234	0.220	-0.6	2.0	-1.4	-0.2

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.24	45.76	0.00	0.00	8.47	0.00	0.0
62 grain	-	-	-	-	-	-	-
62 straw	54.61	45.39	0.69	-0.81	9.22	0.75	0.4

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626175-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on spring wheat and winter wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the United Kingdom, Germany and the Netherlands'

Table CA 6.7.1-81 Trial 03 Summarised under CA 6.3.3/12

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.2	1.2	0.93	0.97	0.279	0.279	0.216	0.226	0	0	0	0
54 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
54 straw	0.043	0.044	0.033	0.034	0.279	0.286	0.214	0.221	0.1	2.4	-0.9	-2.1

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.81	44.19	0.00	0.00	11.63	0.00	0.0
54 grain	-	-	-	-	-	-	-
54 straw	56.49	43.51	1.22	-1.54	12.99	1.36	0.7

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626175-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on spring wheat and winter wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the United Kingdom, Germany and the Netherlands'

Table CA 6.7.1-82 Trial 04 Summarised under CA 6.3.3/12

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.7	2.7	2.2	2.3	0.273	0.273	0.222	0.232	0	0	0	0
53 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
53 straw	0.079	0.086	0.067	0.07	0.262	0.285	0.222	0.232	-4.1	4.4	-0.2	-0.2

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.55	45.45	0.00	0.00	9.09	0.00	0.0
53 grain	-	-	-	-	-	-	-
53 straw	54.64	45.36	0.17	-0.20	9.27	0.18	0.1

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-626175-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on spring wheat and winter wheat after spray application of JAU 6476 & KWG 4168 EC 460 in the United Kingdom, Germany and the Netherlands'

Table CA 6.7.1-83 Average of all 4 trials

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.750	1.750	1.458	1.468	0.273	0.273	0.227	0.228	0	0	0	0
50-62 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
50-62 straw	0.070	0.075	0.057	0.058	0.270	0.285	0.221	0.224	-0.8	4.6	-2.8	-1.8

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.54	45.46	0.00	0.00	9.08	0.00	0.0
50-62 grain	-	-	-	-	-	-	-
50-62 straw	55.58	44.42	1.89	-2.27	11.15	2.07	1.0

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627149-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Italy and Greece'

Table CA 6.7.1-84 Trial 01 Summarised under CA 6.3.3/13

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.6	1.6	1.3	1.3	0.276	0.276	0.224	0.224	0	0	0	0
50 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
50 straw	0.210	0.21	0.16	0.16	0.284	0.284	0.216	0.216	2.9	2.9	-3.5	-3.5

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.17	44.83	0.00	0.00	10.34	0.00	0.0
50 grain	-	-	-	-	-	-	-
50 straw	56.76	43.24	2.87	-3.53	13.51	3.17	1.6

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627149-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Italy and Greece'

Table CA 6.7.1-85 Trial 02 Summarised under CA 6.3.3/13

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.6	1.6	1.3	1.3	0.276	0.276	0.224	0.224	0	0	0	0
46 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
46 straw	0.210	0.21	0.16	0.16	0.284	0.284	0.216	0.216	2.9	2.9	-3.5	-3.5

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.17	44.83	0.00	0.00	10.34	0.00	0.0
46 grain	-	-	-	-	-	-	-
46 straw	56.76	43.24	2.87	-3.57	13.51	3.17	1.6

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627149-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Italy and Greece'

Table CA 6.7.1-86 Trial 03 Summarised under CA 6.3.3/13

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.2	1.2	0.97	1.1	0.268	0.268	0.217	0.246	0	0	0	0
43 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
43 straw	0.440	0.44	0.31	0.32	0.291	0.291	0.205	0.212	8.5	8.5	-5.4	-13.9

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	53.69	46.31	0.00	0.00	7.38	0.00	0.0
43 grain	-	-	-	-	-	-	-
43 straw	58.28	41.72	8.54	-9.90	16.56	9.17	4.6

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627149-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Italy and Greece'

Table CA 6.7.1-87 Trial 04 Summarised under CA 6.3.3/13

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	3.1	3.1	2.5	2.5	0.277	0.277	0.223	0.223	0	0	0	0
42 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
42 straw	0.300	0.31	0.21	0.2	0.294	0.304	0.206	0.196	6.3	9.8	-7.8	-12.2

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.36	44.64	0.00	0.00	10.71	0.00	0.0
42 grain	-	-	-	-	-	-	-
42 straw	59.80	40.20	8.03	-9.96	19.61	8.89	4.4

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-627149-01-1, 2018 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Italy and Greece'

Table CA 6.7.1-88 Average of all 4 trials

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.775	1.775	1.435	1.475	0.274	0.274	0.222	0.228	0	0	0	0
42-50 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
42-50 straw	0.275	0.280	0.200	0.200	0.286	0.293	0.211	0.211	4.3	6.8	-4.6	-8.7

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.79	45.21	0.00	0.00	9.57	0.00	0.0
42-50 grain	-	-	-	-	-	-	-
42-50 straw	57.80	42.20	5.50	-6.67	15.60	6.03	3.0

Wheat isomer ratio data (from chiral analysis of spiroxamine)

Report: M-633801-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on wheat after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in Spain and Italy'

Table CA 6.7.1-89 Trial 01 Summarised under CA 6.3.3/14

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.7	1.7	1.4	1.4	0.274	0.274	0.226	0.226	0	0	0	0
56 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
56 straw	0.018	0.018	0.014	0.015	0.277	0.277	0.215	0.231	1.0	1.0	-4.6	2.2

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.84	45.16	0.00	0.00	9.68	0.00	0.0
56 grain	-	-	-	-	-	-	-
56 straw	55.38	44.62	1.00	-1.21	10.77	1.09	0.5

Report: M-633801-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on wheat after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in Spain and Italy'

Table CA 6.7.1-90 Trial 02 Summarised under CA 6.3.3/14

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.55	0.52	0.46	0.44	0.279	0.264	0.234	0.223	0	0	0	0
42 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
42 straw	0.085	0.087	0.065	0.061	0.285	0.292	0.218	0.205	2.2	10.6	-6.6	-8.4

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.31	45.69	0.00	0.00	8.63	0.00	0.0
42 grain	-	-	-	-	-	-	-
42 straw	57.72	42.28	6.27	-7.45	15.44	6.81	3.4

Report: M-633801-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on wheat after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in Spain and Italy'

Table CA 6.7.1-91 Average of both trials

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.125	1.110	0.930	0.920	0.277	0.269	0.230	0.225	0	0	0	0
42-56 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
42-56 straw	0.052	0.053	0.040	0.038	0.281	0.284	0.217	0.218	1.6	5.7	-5.6	-3.0

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.58	45.42	0.00	0.00	9.15	0.00	0.0
42-56 grain	-	-	-	-	-	-	-
42-56 straw	56.55	43.45	3.62	-4.35	13.10	3.95	2.0

Report: M-634111-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on winter wheat after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in The United Kingdom and Germany'

Table CA 6.7.1-92 Trial 01 Summarised under CA 6.3.3/15

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.5	0.48	0.42	0.41	0.276	0.265	0.232	0.227	0	0	0	0
50 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
50 straw	0.058	0.059	0.045	0.046	0.279	0.284	0.216	0.221	0.9	7.0	-6.8	-2.4

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.14	45.86	0.00	0.00	8.29	0.00	0.0
50 grain	-	-	-	-	-	-	-
50 straw	56.25	43.75	3.89	-4.59	12.50	4.21	2.1

Report: M-634111-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on winter wheat after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in The United Kingdom and Germany'

Table CA 6.7.1-93 Trial 02 Summarised under CA 6.3.3/15

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.62	0.6	0.53	0.51	0.274	0.265	0.235	0.226	0	0	0	0
47 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
47 straw	0.053	0.055	0.045	0.048	0.268	0.278	0.227	0.227	-2.4	4.6	-3.1	0.7

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	53.98	46.02	0.00	0.00	7.96	0.00	0.0
47 grain	-	-	-	-	-	-	-
47 straw	54.55	45.45	1.04	1.22	9.09	1.13	0.6

Report: M-634111-01-1, 2018 'Determination of the residues of trifloxystrobin, BYF 00587 and spiroxamine in/on winter wheat after spray application of Bixafen & Spiroxamine & Trifloxystrobin EC 325 in The United Kingdom and Germany'

Table CA 6.7.1-94 Average of both trials

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	0.560	0.540	0.475	0.460	0.275	0.265	0.233	0.226	0	0	0	0
50 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
50 straw	0.056	0.057	0.045	0.046	0.273	0.281	0.222	0.224	-0.7	5.8	-4.9	-0.8

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.06	45.94	0.00	0.00	8.13	0.00	0.0
50 grain	-	-	-	-	-	-	-
50 straw	55.40	44.60	2.47	-2.91	10.80	2.67	1.3

Report: M-659920-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, northern France and the Netherlands'

Table CA 6.7.1-95 Trial 01 Summarised under CA 6.3.3/16

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.6	1.5	1.3	1.3	0.281	0.263	0.228	0.228	0	0	0	0
61 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
61 straw	0.210	0.21	0.17	0.17	0.276	0.276	0.224	0.224	-1.6	5.0	-1.9	-1.9

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.39	45.61	0.00	0.00	8.77	0.00	0.0
61 grain	-	-	-	-	-	-	-
61 straw	55.26	44.74	1.61	-1.92	10.53	1.75	0.9

Report: M-659920-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, northern France and the Netherlands'

Table CA 6.7.1-96 Trial 02 Summarised under CA 6.3.3/16

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.2	1.2	0.97	0.96	0.277	0.277	0.224	0.222	0	0	0	0
26 grain	0.006	0.006	0.005	0.005	0.273	0.273	0.227	0.227	-1.6	-1.6	1.5	2.5
26 straw	0.250	0.26	0.21	0.21	0.269	0.280	0.226	0.226	-3.0	0.9	0.8	1.8

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.43	44.57	0.00	0.00	10.85	0.00	0.0
26 grain	54.55	45.45	-1.59	1.98	9.09	-1.76	-0.9
26 straw	54.84	45.16	-1.06	1.32	9.68	-1.18	-0.6

Report: M-659920-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, northern France and the Netherlands'

Table CA 6.7.1-97 Trial 03 Summarised under CA 6.3.3/16

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.3	1.3	1.1	1.1	0.271	0.271	0.229	0.229	0	0	0	0
43 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
43 straw	0.200	0.2	0.16	0.17	0.274	0.274	0.219	0.233	1.2	1.2	-4.4	1.6

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.17	45.83	0.00	0.00	8.33	0.00	0.0
43 grain	-	-	-	-	-	-	-
43 straw	54.79	45.21	1.16	-1.37	9.59	1.26	0.6

Report: M-659920-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, northern France and the Netherlands'

Table CA 6.7.1-98 Trial 04 Summarised under CA 6.3.3/16

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.2	1.1	0.91	0.89	0.292683	0.268293	0.221951	0.217073	0	0	0	0
39 grain	0.003	0.003	0.002	0.002	0.282	0.282	0.220	0.216	-3.6	5.2	-0.8	-0.7
39 straw	0.120	0.12	0.094	0.093	0.281	0.281	0.220	0.218	-4.0	4.7	-0.8	0.3

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.10	43.90	0.00	0.00	12.20	0.00	0.0
39 grain	56.42	43.58	0.58	-0.74	12.85	0.65	0.3
39 straw	56.21	43.79	0.19	-0.25	12.41	0.22	0.1

Report: M-659920-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in Germany, northern France and the Netherlands'

Table CA 6.7.1-99 Average of all 4 trials

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.325	1.275	1.070	1.063	0.280	0.270	0.226	0.224	0	0	0	0
26-61 grain	0.00450	0.00450	0.00367	0.00365	0.2774	0.2774	0.2238	0.2214	-1.0	2.8	-0.9	-1.2
26-61 straw	0.195	0.198	0.159	0.161	0.275	0.278	0.222	0.225	-1.9	2.9	-1.6	0.5

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.02	44.98	0.00	0.00	10.04	0.00	0.0
26-61 grain	55.48	44.52	0.84	-1.03	10.97	0.93	0.5
26-61 straw	55.28	44.72	0.47	-0.57	10.55	0.51	0.3

Report: M-660174-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal'

Table CA 6.7.1-100 Trial 01 Summarised under CA 6.3.3/17

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.4	1.4	1.2	1.1	0.275	0.275	0.235	0.216	0	0	0	0
56 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
56 straw	0.081	0.078	0.056	0.061	0.293	0.283	0.203	0.221	6.9	3.0	-13.8	2.5

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	54.90	45.10	0.00	0.00	9.80	0.00	0.0
56 grain	-	-	-	-	-	-	-
56 straw	57.61	42.39	4.93	-6.00	15.22	5.41	2.7

Report: M-660174-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal'

Table CA 6.7.1-101 Trial 02 Summarised under CA 6.3.3/17

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	2.2	2.3	1.7	1.7	0.278	0.291	0.215	0.215	0	0	0	0
57 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
57 straw	0.082	0.083	0.061	0.062	0.285	0.288	0.212	0.215	2.2	1.0	-1.6	0.0

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.96	43.04	0.00	0.00	13.92	0.00	0.0
57 grain	-	-	-	-	-	-	-
57 straw	57.29	42.71	0.58	-0.77	14.58	0.66	0.3

Report: M-660174-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal'

Table CA 6.7.1-102 Trial 03 Summarised under CA 6.3.3/17

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.7	1.7	1.3	1.3	0.283	0.283	0.217	0.217	0	0	0	0
33 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
33 straw	0.110	0.11	0.086	0.084	0.282	0.282	0.221	0.215	-0.5	-0.5	1.8	-0.6

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	56.67	43.33	0.00	0.00	13.33	0.00	0.0
33 grain	-	-	-	-	-	-	-
33 straw	56.41	43.59	-0.45	0.59	12.82	-0.51	-0.3

Report: M-660174-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal'

Table CA 6.7.1-103 Trial 04 Summarised under CA 6.3.3/17

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.1	1.1	0.95	0.94	0.269	0.269	0.232	0.230	0	0	0	0
42 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
42 straw	0.380	0.38	0.3	0.3	0.279	0.279	0.221	0.221	3.9	3.9	-5.0	-4.0

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	53.79	46.21	0.00	0.00	7.58	0.00	0.0
42 grain	-	-	-	-	-	-	-
42 straw	55.88	44.12	3.89	-4.53	11.76	4.19	2.1

Report: M-660174-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal'

Table CA 6.7.1-104 Trial 05 Summarised under CA 6.3.3/17

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.9	1.8	1.5	1.5	0.284	0.269	0.224	0.224	0	0	0	0
66 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
66 straw	0.200	0.2	0.14	0.13	0.299	0.299	0.209	0.194	5.3	11.1	-6.7	-13.3

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.22	44.78	0.00	0.00	10.45	0.00	0.0
66 grain	-	-	-	-	-	-	-
66 straw	59.70	40.30	8.11	-10.00	19.40	8.96	4.5

Report: M-660174-01-1, 2019 'Determination of the residues of prothioconazole and spiroxamine in/on wheat after spray application of JAU 6476 & KWG 4168 EC 460 in southern France, Spain, Greece, Italy and Portugal'

Table CA 6.7.1-105 Average of all 5 trials

Wheat	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from day 0			
DALA	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
0 plant	1.660	1.660	1.330	1.308	0.278	0.277	0.225	0.220	0	0	0	0
61 grain	<0.00271	<0.00271	<0.00229	<0.00229	LOQ	LOQ	LOQ	LOQ	-	-	-	-
61 straw	0.171	0.170	0.129	0.127	0.288	0.286	0.213	0.213	3.6	3.2	-5.2	-3.2

Wheat	enantiomers sum		% diff from day 0		stereoisomer excess (se) overview		
DALA	%A	%B	A	B	se (%A-%B)	Change of se from 0 DALA	% increase for A
0 plant	55.51	44.49	0.00	0.00	11.02	0.00	0.0
61 grain	-	-	-	-	-	-	-
61 straw	57.38	42.62	3.37	-4.20	14.76	3.74	1.9

Impact of isomers on risk assessment – food of animal origin

Poultry

Based on the overall conclusions from the poultry metabolism study (CA 6.2.2/01), dietary burden estimations (CA 6.4) and the hen feeding study (CA 6.4.1/01), the potential residues in food of poultry origin are known to be parent spiroxamine in all commodities plus varying contributions from spiroxamine-acid (M06), spiroxamine-desethyl (M01) and spiroxamine-despropyl (M01). From the hen feeding study, which was conducted with the top dose group representing 2N compared with the worst case dietary burden for laying hens or over 40N for broiler chickens, it is clear that residues will be below the analytical LOQ of 0.02 mg/kg for muscle and eggs or 0.05 mg/kg for liver and fat even when it is acknowledged that the analytical method would not have included spiroxamine-acid (M06) which, from metabolism testing, is only significant (>10% TRR) in muscle and eggs.

Therefore, given the insignificant residues expected in food of poultry origin, no additional uncertainty factor for isomer ratios is necessary for consumer risk assessment. It is noted that the contribution to the total TMDI for consumer risk from these commodities is minimal, <0.5% of ADI. The maximum acute exposure from poultry is also just 0.5% of the ARfD.

Ruminant

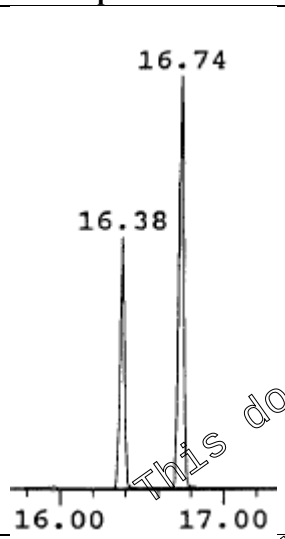
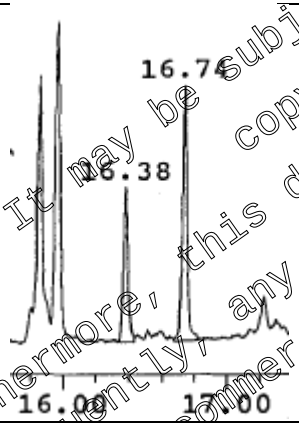
Based on the overall conclusions from the ruminant goat metabolism study (CA 6.2.3/01), dietary burden estimations (CA 6.4) and the cow feeding study (CA 6.4.2/01), the potential major residues in food of ruminant origin are known to be spiroxamine-acid (M06) and its spiroxamine-acid glucuronide conjugate (M19) in all commodities plus varying contributions from spiroxamine-desethyl acid (M11), spiroxamine-despropyl acid (M12), spiroxamine-hydroxy acid (M07) and other minor metabolites. From the cow feeding study, which was conducted with the top dose group representing 7N compared with the worst case dietary burden for dairy cattle or 11N for beef cattle, it is clear that residues will potentially be above the LOQ for edible commodities from ruminants and larger species with the exception of swine for all tissues except liver.

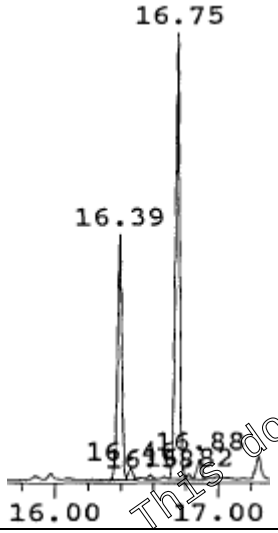
The analytical method used for the cow feeding study (CA 6.4.2/01, M-006159-02-1) determines the total residue of spiroxamine-acid (M06) which represents the majority of expected residues. The data generation method uses LC-MS/MS under non chiral conditions and the spiroxamine-acid (M06) gives a single peak for this data. However, the study also analysed selected samples using the enforcement method 00395; report reference M-019323-02-1 (**see Doc MCA Section 4**) which uses GC/MS in the single ion monitoring mode. Under the chromatography conditions employed for spiroxamine-acid (M06) using a standard OV-1701 capillary column, this gives two peaks for the diastereomers which based on the observed chemistry for spiroxamine, are presumed to be the cis / ee (B) and trans / ea (A) isomers of M06 in order of elution. The corresponding enantiomers are expected to be in the 1:1 ratio and resistant to inter-conversion or change. Although isomer ratio evaluation was not an objective of this study, from the example chromatograms in the cow feeding study report it is possible to make a visual assessment and also to compare the peak areas as shown in Table CA 6.7.1-106.

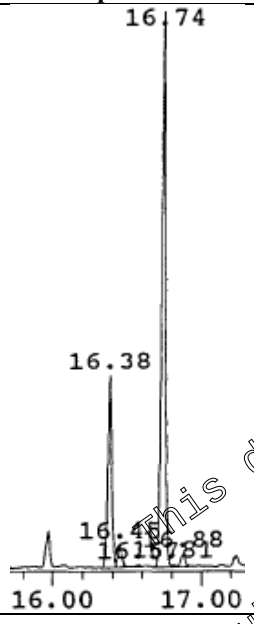
Based on this assessment of the stereoisomeric excess (se) it can be concluded that with the possible exception of liver, there is not a significant change in the derived se values for fat, milk, kidney or muscle.

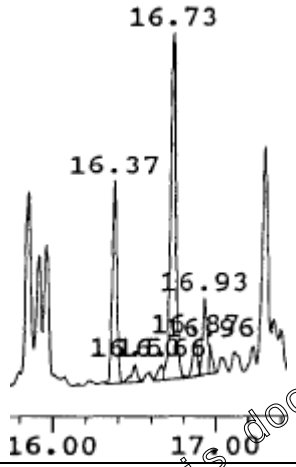
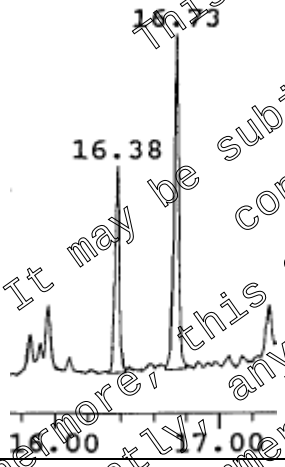
As an overall conclusion for food of ruminant origin, the data available from the cow feeding study supports a proposal that no additional uncertainty factor is required for consumer risk assessment. It is noted that the contribution to the total TMDI for consumer risk from these commodities is low, only 6% of ADI. The maximum acute exposure from ruminants is only 3% of the ARfD.

Table CA 6.7.1-106 Food of ruminant origin spiroxamine-acid (M06) isomer ratio data from M-006159-02-1

Sample ID	Representative chromatogram from report	Area of peak 1	Area of peak 2	Ratio peak 1:peak 2	sc% (peak 2-peak1)	Change in sc from ref std (%)
Reference standard of spiroxamine-acid (M06)		702146	1212159	0.367 : 0.633	26.6	0.0
Fat treated sample		33788	60494	0.358 : 0.642	28.3	1.7

Sample ID	Representative chromatogram from report	Area of peak 1	Area of peak 2	Ratio peak 1:peak 2	se % (peak 2-peak1)	Change in se from ref std (%)
Kidney treated sample		442030	770141	0.365: 0.635	27.1	0.4

Sample ID	Representative chromatogram from report	Area of peak 1	Area of peak 2	Ratio peak 1:peak 2	se % (peak 2-peak1)	Change in se from ref std (%)
Liver treated sample		586336	1629681	0.265: 0.735	47.1	20.4

Sample ID	Representative chromatogram from report	Area of peak 1	Area of peak 2	Ratio peak 1:peak 2	se % (peak 2-peak1)	Change in se from ref std (%)
Milk treated sample		72904	136529	0.348 : 0.652	30.4	3.7
Muscle treated sample		44776	78878	0.362 : 0.638	27.6	0.9

The EFSA Conclusion on Pesticide Peer Review for Spiroxamine (December 2020)

Following consideration of the comments received for the spiroxamine confirmatory data relating to the previous renewal of approval evaluation, the European Commission requested EFSA to organise a further peer review of the previous RMS's evaluation on the confirmatory data, including expert discussion where appropriate and to deliver its conclusions on the update of the consumer risk assessment taking into account the change of the toxicological reference values for metabolites M13 and M28 and the risk assessment for fish, taking into account general risk mitigation measures.

The revised assessment and the reporting table were discussed at the Pesticides Peer Review Meetings on mammalian toxicology and ecotoxicology in June 2020. Details of the issues discussed, together with the outcome of these discussions were recorded in the meeting reports.

A final consultation on the conclusions arising from the peer review took place with Member States via a written procedure in November 2020.

The conclusions of EFSA were presented in document 'Conclusion on the peer review of the pesticide risk assessment for the active substance spiroxamine in light of confirmatory data. EFSA Journal 2021;18(12):6385'. The updated version considered the submitted comments of the Applicant and an overview of the EFSA conclusions is presented in Table CA 6.7.1-107.

Table CA 6.7.1-107 Discussion on EFSA Journal 2021;19(1):6385

EFSA comment / proposal	Implications	Applicant comment	Undertaking made
A new hydrolysis study simulating industrial and household food processing conditions indicated that spiroxamine may degrade pH dependently, although to a moderate extend. The main hydrolysis products (up to 23% applied radioactivity (AR)) were M28 (spiroxamine aminodiol) and M15 (tert-butyl-cyclohexanone) while significant proportions of unknown metabolites are not formed. M28 is the representative of group C and M15 is covered by Group B. Therefore, a different residue definition for processed fruit commodities is not necessary.	None	Applicant agrees with conclusions for this study, no impact on residue definition for processed commodities. See CA 6.5.1/01: M-441801-01-1	No, not required
The RMS-DE proposed to set the residue definition for dietary risk assessment in fruit as • Sum of spiroxamine and metabolites containing the aminodiol (N-ethyl-N-propyl-1,2-dihydroxy-3-amino-propane) and the tert-butylcyclohexanone moiety, expressed as spiroxamine (Germany, 2017, 2020). However, since the toxicological effects of spiroxamine, M13 and	As noted by EFSA, without the use of assumptions or conversion factors, new and currently unavailable residue data for	From the confirmatory data (EFSA 2018 and EFSA 2021) it is clear that the metabolites M13 and M28 representing Group B and Group C metabolites, respectively, have higher endpoints for ADI and ARfD (both as substances and when expressed as spiroxamine equivalents) and are therefore less toxic than parent spiroxamine.	Method development investigations for the individual Group B and Group C metabolites of spiroxamine (as identified in plant metabolism) will be initiated to assess the possibility to accurately measure residue concentrations

EFSA comment / proposal	Implications	Applicant comment	Undertaking made
M28 have proven to be qualitatively different based on the available studies, separate toxicological reference values were derived for these three compounds. EFSA acknowledges the view of the RMS that co-occurrence of residues with a different toxicological profile requires a type of hazard index (HI) approach to account for potential combination effects for the total residues that consumers will be exposed to. Yet, this is a novel (non-standard) approach introduced to the peer review, while following the conventional approach, the residue definition for risk assessment would consist of three different elements and require three separate risk assessment calculations due to the different toxicological profiles: • Spiroxamine • Sum of 4-tert-butylcyclohexanol and its hydroxy-metabolites, their esters and conjugates, expressed as 4-tert-butylcyclohexanol (M13) • Sum of metabolites containing the aminodiol (N-ethyl-N-propyl-1,2-dihydroxy-3-amino-propane) moiety expressed as spiroxamine aminodiol (M28) In the available residue field trials a 'total residue' method was employed, in line with the provisional residue definition for risk assessment in fruit of 2010 (EFSA, 2010). This 'total residue' method for fruit determines all residues that are hydrolysable into M28, including spiroxamine and group A metabolites, while the new residue definition for fruit is composed of spiroxamine and two different breakdown structures that are counterparts of each other and are representing group B and group C metabolites, respectively. In the case where residues are analysed by a 'total residue' method, attribution of different residues to the individual toxicological reference values in order to conduct the consumer risk assessment is only possible with assumptions and approximations. Ideally, a sufficient number of residue trials should be available to determine separately the residues corresponding to the different parts of the residue definition for	grapes would be required.	As such, a conventional approach to consumer risk assessment has been adopted in this dossier for the renewal of approval using both grapes residue data for spiroxamine alone and the common moiety method data for Group C metabolites plus spiroxamine and Group A metabolites, converted to and measured as aminodiol (M28), expressed as total spiroxamine equivalents. This allows a justified conversion factor from RD-Mo to RD-RA to be derived based on a large and robust database and therefore not based on assumptions. As the spiroxamine parent substance TRVs for ADI and ARfD are both lower (i.e. more toxic) than those agreed for M13 (Group B) and M28 (Group C) this is considered to be a conservative and protective approach to consumer risk assessment.	in grapes. Depending on the success of method development and validation, new residue trials in grapes can be initiated if required.

EFSA comment / proposal	Implications	Applicant comment	Undertaking made
risk assessment in order conduct quantitative assessments against the different TRVs for each of the parts of the residue definition. In the absence of such residue trial data, RMS estimated residue levels of group B metabolites in fruits by application of a conversion factor of 0.5 (grapes) and 0.33 (banana) to the 'total residue' determined in the field trials, and levels of group C metabolites based on the assumption that they were formed in equal amounts as group B metabolites.			
In a conservative assessment (Tier 1), consumer exposure to all compounds included in the residue definition for risk assessment was calculated and compared to the ADI and ARfD of parent spiroxamine. In a novel assessment approach (Tier 2), that was described as similar to the hazard index (HI) approach, RMS derived group specific exposure values, using several assumptions, which were compared with the corresponding TRVs. The Tier 1 long-term dietary risk assessment conducted with PRIMo rev. 2 and rev. 3 indicated consumer exposure well below the ADI of spiroxamine (less than 20%), and a Tier 2 assessment was therefore not presented. The Tier 1 acute dietary risk assessment conducted with PRIMo rev.2 and rev.3 indicated an acute risk for consumers arising from the representative use of spiroxamine in table grapes (108 % and 120 % ARfD of spiroxamine respectively). The ARfD of spiroxamine is not exceeded for uses in wine grapes. For the representative use in table grapes, an indicative refined acute risk assessment was conducted, considering the specific reference values for spiroxamine, group B and C metabolites in the HI approach (Tier 2). According to the RMS, the risk assessment with PRIMo rev. 2 and rev. 3 indicated acute consumer exposure to residue in table grapes below the individual ARfD and a HI less than 1. EFSA remarks that the different calculations/estimations and conversion factors derived with regard to the novel assessment approach (Tier 2) are not reproducible down to the last detail, and	None for consumer risk. EFSA notes that the new proposal is unlikely to result in acute (or chronic) consumer risk issues.	The Applicant notes that the proposed Hazard Index approach was not fully implemeted by RMS-DE or EFSA to conduct the full consumer risk assessment. Also, the Applicant agrees that the recently proposed residue definitions will obviously present no unacceptable risks to the consumer. This is because for both chronic and short term assessment, the TRVs for the M13 (Group B) and M28 (Group C) are higher (less toxic) than for spiroxamine itself and the derived exposure values for each EFSA proposed component of the residue definition must be lower than the previous RMS-DE approach. **The approach to residue definitions and consumer risk assessment is discussed and implemented in this dossier; refer to Sections CA 6.7 and CA 6.9.**	As above

EFSA comment / proposal	Implications	Applicant comment	Undertaking made
that the results obtained by this approach are surrounded by several non-standard uncertainties. Based on available information from metabolism studies it had previously been concluded that in fruit, group B and group C metabolites were observed in similar proportions as spiroxamine (EFSA, 2010). It is therefore reasonable to assume that the acute intake assessment for table grapes, when considering group B, group C and spiroxamine residues separate, is unlikely to result in the exceedance of the respective ARfDs, however confirmation of this assumption by residue data is missing. Moreover, a general expert discussion on the acceptability of the HI concept applied by the RMS in the new assessment approach in routine assessments, using the example of spiroxamine, is desirable.			

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The current EU MRLs for spiroxamine are given within Commission Regulation (EU) 2016/432 of 29 March 2016.

A summary of the current and proposed EU MRLs for the crops supported in this renewal of approval dossier and also for food of animal origin are shown in Table CA 6.7.2-1.

Spiroxamine residue trials history

A. Initial development of spiroxamine residues data involved trials in the period 1993 to 1997 on small grain cereals and grapes employing residue methods measuring total spiroxamine by means of a common moiety approach. Residues of spiroxamine itself (current MRL residue definition) were not measured or reported. LOQs were typically 0.05 mg/kg
    a. Total SPX for cereals measured as 4-tert-butylcyclohexanone moiety and represents the majority Group A metabolites of SPX found in cereals
    b. Total SPX for grapes measured as ammodinol moiety and represents the majority Group C metabolites of SPX found in grapes
B. Subsequent development of spiroxamine residues data involved trials in the period 1998 to 2016 on small grain cereals and grapes employing residue methods measuring either spiroxamine itself or both spiroxamine and total spiroxamine by means of a common moiety approach. Residues of spiroxamine were measured and reported. LOQs were originally 0.05 mg/kg but this was then reduced to 0.01 mg/kg
C. From 2016 onwards, spiroxamine residues data involved trials on small grain cereals and grapes employing residue methods measuring both spiroxamine itself with a fully chiral method (reporting each of the four enantiomers and a sum as spiroxamine) and total spiroxamine by means of a common moiety approach. Residues of spiroxamine were measured and reported. LOQs were 0.01 mg/kg

For previous EU evaluations of spiroxamine, it was considered necessary by both the Applicant and RMS to rely on the residue trials conducted during point A above where only total spiroxamine was reported. For MRL purposes these trials could only be used with application of a conversion factor to convert total spiroxamine residues to spiroxamine itself.

The Applicant has conducted many trials since the previous evaluation which can be used to support the EU GAP for cereals and grapes. The total number of reliable and acceptable trials, especially for grapes, is extensive and for all crops is greater than the minimum number of trials required per crop per region. Therefore it is no longer necessary to rely on the oldest residue trials conducted for spiroxamine (only measured as total spiroxamine equivalents) for MRL or risk assessment purposes. The residue data referred to below for MRLs does not use these oldest trials for grapes or cereals.

Additionally, for cereals only the grain MRL proposals do not use those trials with the higher LOQ of 0.05 mg/kg. This is justified since the data from studies with LOQ = 0.01 mg/kg show that when positive grain residues are measured in trials supporting the cGAP the residues are always <0.05 mg/kg and for wheat grain the majority of all residues are ≤0.01 mg/kg.

Table CA 6.7.2-1: Current and proposed EU MRLs for spiroxamine in representative crops and food commodities of animal origin

Food commodity	Code[1]	Current MRL (mg/kg)[2]	Proposed MRL (mg/kg)
Crops [Current / proposed EU MRL residue definition: Spiroxamine]			
Grapes - table	0151010	0.6	**0.4**
Grapes - wine	0151020	0.5	**0.8**
Barley grain	0500010	0.05	**0.07**
Oats (extrapolation)	0500050	0.05	**0.07**
Wheat grain	0500090	0.05	**0.03**
Rye (extrapolation)	0500070	0.05	**0.03**
Commodities of animal origin [Current / proposed EU MRL residue definition: Ruminants - Spiroxamine carboxylic acid (M06), expressed as spiroxamine Poultry - Sum of spiroxamine and spiroxamine carboxylic acid (M06), expressed as spiroxamine (sum of isomers)]			
Swine muscle	1011010	0.02	**0.02**
Swine fat tissue	1011020	0.02	**0.02**
Swine liver	1011030	0.02	**0.05**
Swine kidney	1011040	0.02	**0.02**
Swine edible offals	1011050	0.02	**0.02**
Bovine muscle	1012010	0.03	**0.03**
Bovine fat tissue	1012020	0.05	**0.03**
Bovine liver	1012030	0.3	**0.1**
Bovine kidney	1012040	0.15	**0.08**
Bovine edible offals	1012050	0.3	**0.08**
Sheep muscle	1013010	0.03	**0.03**
Sheep fat tissue	1013020	0.05	**0.05**
Sheep liver	1013030	0.3	**0.2**
Sheep kidney	1013040	0.15	**0.15**
Sheep edible offals	1013050	0.3	**0.15**
Goat muscle	1014010	0.03	**0.03**
Goat fat tissue	1014020	0.05	**0.05**
Goat liver	1014030	0.3	**0.2**
Goat kidney	1014040	0.15	**0.15**
Goat edible offals	1014050	0.3	**0.15**
Equine muscle	1015010	0.03	**0.03**
Equine fat tissue	1015020	0.05	**0.03**
Equine liver	1015030	0.3	**0.1**
Equine kidney	1015040	0.15	**0.08**
Equine edible offals	1015050	0.3	**0.08**
Poultry muscle	1016010	0.05	**0.02**
Poultry fat tissue	1016020	0.05	**0.05**
Poultry liver	1016030	0.2	**0.05**
Poultry kidney[3]	1016040	0.02	**0.05**
Poultry edible offals	1016050	0.2	**0.05**
Milk except sheep	1020000	0.015	**0.02**
Milk sheep	1020020	0.015	**0.03**
Birds eggs	1030000	0.05	**0.02**
Honey and other apiculture product [Proposed EU MRL residue definition: Spiroxamine]			
Honey	1040000	0.05	**0.2**

1 - As given in Commission Regulation (EU) No 2018/62, 17 January 2018, Annex I
2 - Commission Regulation (EU) 2016/452 of 29 March 2016
3 - poultry kidney extrapolated from liver

Proposed MRLs (crops)

Based on the reported residue levels in the supported crops from supervised field trials conducted according to the critical GAPs and presented in Point CA 6.3, it is apparent that in the edible crop commodities, the expected residue levels of spiroxamine lead to changes from the current EU MRLs. The residue definition for spiroxamine for MRL and enforcement purposes is active substance spiroxamine alone, with no metabolites. There is a requirement to modify the current MRLs for spiroxamine following the renewal of approval evaluation.

The available residue trials data were evaluated against current EU MRLs.

According to Article 8 paragraph 1(g) of Regulation (EC) No. 1107/2009, the information presented in this Section of the dossier, considered with the analytical methods summarised in Document MCA Section 4, meets the requirements of Article 7 of Regulation (EC) No 396/2005 for a comparison of current MRLs to support the uses of spiroxamine on grapes (table and wine), barley and wheat with extrapolation to oats and rye, respectively.

Table grapes

An overview of the table grapes residue data relevant for MRL purposes are presented in Table CA 6.7.2-2. These data are summarised in Point CA 6.3.1. Results of MRL calculations using the OECD MRL Calculator are discussed below.

Table CA 6.7.2-2 Summary of table grapes data according to the critical GAP

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH			
CA 6.3.1/03 RA-2142/00 R 2000 0194/0 M-083248-01-1	SEU	3	0.400 0.400 0.400	BBCH 85 BBCH 87 BBCH 87	Grapes	14	0.14
CA 6.3.1/03 RA-2142/00 R 2000 0195/9 M-083248-01-1	SEU	3	0.400 0.400 0.374	BBCH 81 BBCH 83 BBCH 85	Grapes	14	0.10
CA 6.3.1/01 RA-2650/07 R 2007 0675/8 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 81 BBCH 85 BBCH 85	Grapes	14	0.16
CA 6.3.1/01 RA-2650/07 R 2007 0676/6 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 79 BBCH 81 BBCH 83	Grapes	14	0.15
CA 6.3.1/01 RA-2650/07 R 2007 0703/7 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 77 BBCH 79 BBCH 81	Grapes	14	0.18
CA 6.3.1/01 RA-2650/07 R 2007 0704/5 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 75 BBCH 77 BBCH 81	Grapes	13	0.23
CA 6.3.1/20 13-2141 13-2141-01 M-508894-01-1	SEU	2	0.200 0.200	BBCH 85 BBCH 85	Grapes	14	0.08

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine, total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH			
CA 6.3.1/20 13-2141 13-2141-03 M-508894-01-1	SEU	2	0.200 0.200	BBCH 81 BBCH 83	Grapes	14	0.09
CA 6.3.1/20 13-2141 13-2141-04 M-508894-01-1	SEU	2	0.200 0.200	BBCH 79 BBCH 79	Grapes	14	0.11
CA 6.3.1/22 13-2161 13-2161-01 M-507373-02-1	SEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	14	0.08
CA 6.3.1/22 13-2161 13-2161-02 M-507373-02-1	SEU	2	0.300 0.300	BBCH 79 BBCH 81	Grapes	14	0.11
CA 6.3.1/22 13-2161 13-2161-03 M-507373-02-1	SEU	2	0.300 0.300	BBCH 79 BBCH 81	Grapes	14	0.11
CA 6.3.1/22 13-2161 13-2161-04 M-507373-02-1	SEU	2	0.300 0.300	BBCH 77 BBCH 79	Grapes	14	0.12
CA 6.3.1/16 16-2047 16-2047-01 M-626160-01-1	SEU	2	0.300 0.300	BBCH 81 BBCH 83	Grapes	14	0.17
CA 6.3.1/16 16-2047 16-2047-02 M-626160-01-1	SEU	2	0.300 0.300	BBCH 79 BBCH 79	Grapes	14	0.04
CA 6.3.1/16 16-2047 16-2047-03 M-626160-01-1	SEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	14	0.17
CA 6.3.1/16 16-2047 16-2047-04 M-626160-01-1	SEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	21	0.06 Higher value at later PHI
CA 6.3.1/24 09-2036-01 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 83 BBCH 85 BBCH 85	Grapes	21	0.15 Higher value at later PHI
CA 6.3.1/24 09-2036-02 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 79 BBCH 81 BBCH 85	Grapes	14	0.12
CA 6.3.1/24 09-2036-03 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 81 BBCH 83 BBCH 85	Grapes	14	0.10
CA 6.3.1/24 09-2036-04 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 79 BBCH 79 BBCH 81	Grapes	14	0.05

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine, total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH			
CA 6.3.1/14 16-2111-01 M-629509-01-1	SEU	2	0.200 0.200	83 85	Grapes	13	0.23
CA 6.3.1/14 16-2111-02 M-629509-01-1	SEU	2	0.200 0.200	83 85	Grapes	13	0.27
CA 6.3.1/14 16-2111-05 M-629509-01-1	SEU	2	0.200 0.200	83 85	Grapes	14	0.15
CA 6.3.1/14 16-2111-06 M-629509-01-1	SEU	2	0.200 0.200	83 85	Grapes	25	0.06 Higher value at later PHI
CA 6.3.1/14 16-2111-07 M-629509-01-1	SEU	2	0.200 0.200	83 83	Grapes	13	0.17
CA 6.3.1/14 16-2111-08 M-629509-01-1	SEU	2	0.200 0.200	81 83	Grapes	15	0.04
CA 6.3.1/18 S20-02176-05 M-763137-01-1	SEU	2	0.300 0.300	83 85	Grapes	14	0.12
CA 6.3.1/18 S20-02176-06 M-763137-01-1	SEU	2	0.300 0.300	79-81 83	Grapes	13	0.17
CA 6.3.1/18 S20-02176-07 M-763137-01-1	SEU	2	0.300 0.300	81 83	Grapes	13	<0.01
CA 6.3.1/18 S20-02176-08 M-763137-01-1	SEU	2	0.300 0.300	75-77 89	Grapes	14	0.08

1 – Residue data used for MRL are the reported or scaled value for spiroxamine (parent compound), total enantiomers for studies which used chiral chromatography

The MRL has been calculated according to the OECD MRL Calculator Spreadsheet Version 2, with the STMR as the median residue. In these calculations a single data point from each trial supporting the GAP has been considered and the data set from SEU has been considered individually as no use on table grapes is supported for NEU in this dossier. The residue values used in the MRL and STMR calculations are underlined in Table CA 6.7.2-2. The calculated results are presented in Table CA 6.7.2-3 including the highest residue (HR).

Table CA 6.7.2-3 MRL and STMR calculations for spiroxamine in table grapes

Region	Commodity	Residues (mg/kg)	Proposed OECD MRL (mg/kg)[1]	STMR (mg/kg	HR (mg/kg)
SEU	Table grape	<0.01, 2 x 0.04, 0.05, 2 x 0.06, 3 x 0.08, 0.09, 2 x 0.10, 3 x 0.11, 3 x 0.12, 0.14, 3 x 0.15, 0.16, 4 x 0.17, 0.18, 2 x 0.23, 0.27	**0.4**	0.12	0.27

1 - MRL class shown is the rounded MRL

Sufficient residue trial data are available for table grapes to calculate an EU MRL, STMR and HR. The residue trials data have a corresponding maximum STMR and HR of 0.12 and 0.27 mg/kg, respectively for the use of spiroxamine on table grapes at the critical GAP. As the SEU and NEU datasets are not similar, an EU MRL of 0.4 mg/kg for table grapes is proposed based on the SEU EU data set and this is lower than the currently published EU MRL, therefore an MRL application will be associated with this renewal dossier.

The output from the OECD calculator is shown in Figure CA 6.7.2-1.

Figure CA 6.7.2-1 Output data from OECD MRL calculator (table grapes)

Compound	Spiroxamine
Crop	Table Grape
Region / Country	SEU
GAP	14 days PHI
Total number of data (n)	31
Percentage of censored data	3%
Number of non-censored data	30
Lowest residue	0.014
Highest residue	0.270
Median residue	0.120
Mean	0.123
Standard deviation (SD)	0.060
Correction factor for censoring (CF)	0.978
Proposed MRL estimate	
- Highest residue	0.270
- Mean + 4 SD	0.364
- CF x 3 Mean	0.368
Unrounded MRL	0.364
Rounded MRL	0.4

Residues (mg/kg)	*
0.014	
0.040	
0.040	
0.050	
0.060	
0.060	
0.060	
0.080	
0.080	
0.090	
0.100	
0.100	
0.110	
0.110	
0.110	
0.120	
0.120	
0.120	
0.140	
0.150	
0.150	
0.150	
0.160	
0.170	
0.170	
0.170	
0.170	
0.180	
0.230	
0.230	
0.270	

Wine grapes

An overview of the wine grapes residue data relevant for MRL purposes are presented in Table CA 6.7.2-4. These data are summarised in Point CA 6.3.1. Results of MRL calculations using the OECD MRL Calculator are discussed below.

Table CA 6.7.2-4 Summary of wine grapes data according to the critical GAP

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine total isomers (mg/kg)
		No.	kg a.s./ha	BBCH			
CA 6.3.1/06 RA-2168/97 706612 M-010796-01-1	NEU	4	0.160 0.150 0.160 0.160	BBCH 75 BBCH 77 BBCH 81 BBCH 83	Grapes	35	0.15
CA 6.3.1/06 RA-2168/97 707589 M-010796-01-1	NEU	6	0.160 0.160 0.160 0.160	BBCH 73-75 BBCH 75 BBCH 78 BBCH 78-80	Grapes	35	0.38
CA 6.3.1/05 RA-2160/98 816329 M-015466-01-2	NEU	6	0.150 0.150 0.300 0.300 0.300 0.300	BBCH 52 BBCH 56 BBCH 71 BBCH 75 BBCH 77 BBCH 79	Grapes	35	0.12
CA 6.3.1/05 RA-2160/98 816345 M-015466-01-2	NEU	6	0.150 0.159 0.375 0.375 0.375 0.375	BBCH 55 BBCH 61 BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grapes	35	0.21
CA 6.3.1/04 RA-2169/98 816558 M-024140-01-1	NEU	4	0.320 0.320 0.320 0.320	BBCH 73-75 BBCH 77-79 BBCH 79 BBCH 81	Grapes	35	0.19
CA 6.3.1/04 RA-2169/98 816582 M-024140-01-1	NEU	6	0.080 0.160 0.220 0.220 0.220 0.220	BBCH 55 BBCH 65 BBCH 75 BBCH 77 BBCH 79 BBCH 81	Grapes	35	0.07
CA 6.3.1/04 RA-2169/98 816590 M-024140-01-1	NEU	6	0.080 0.160 0.180 0.200 0.180 0.200	BBCH 55 BBCH 61 BBCH 75 BBCH 79 BBCH 79 BBCH 81	Grapes	35	0.09
CA 6.3.1/02 RA-2649/07 R 2007 0672/3 M-301984-01-1	NEU	3	0.400 0.400 0.400	BBCH 81 BBCH 85 BBCH 85	Grapes	35	0.11
CA 6.3.1/02 RA-2649/07 R 2007 0673/1 M-301984-01-1	NEU	3	0.400 0.400 0.400	BBCH 81 BBCH 85 BBCH 85	Grapes	35	0.09

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine, total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH			
CA 6.3.1/02 RA-2649/07 R 2007 0701/0 M-301984-01-1	NEU	3	0.400 0.400 0.376	BBCH 81 BBCH 85 BBCH 85	Grapes	35	0.05
CA 6.3.1/02 RA-2649/07 R 2007 0702/9 M-301984-01-1	NEU	3	0.640 0.640 0.640	BBCH 81 BBCH 85 BBCH 85	Grapes	35	0.47
CA 6.3.1/23 13-2134 13-2134-02 M-506727-01-1	NEU	2	0.200 0.200	BBCH 81 BBCH 85	Grapes	35	0.09
CA 6.3.1/23 13-2134 13-2134-03 M-506727-01-1	NEU	2	0.200 0.200	BBCH 81 BBCH 85	Grapes	35	0.30
CA 6.3.1/23 13-2134 13-2134-04 M-506727-01-1	NEU	2	0.200 0.200	BBCH 81 BBCH 85	Grapes	35	0.27
CA 6.3.1/21 13-2163 13-2163-01 M-508892-01-1	NEU	2	0.300 0.300	BBCH 81 BBCH 85	Grapes	35	0.08
CA 6.3.1/21 13-2163 13-2163-02 M-508892-01-1	NEU	2	0.300 0.300	BBCH 81 BBCH 85	Grapes	35	0.05
CA 6.3.1/19 13-2164 13-2164-02 M-510227-01-1	NEU	2	0.300 0.300	BBCH 81 BBCH 83	Grapes	34	0.60
CA 6.3.1/19 13-2164 13-2164-02 M-510227-01-1	NEU	2	0.300 0.300	BBCH 81 BBCH 83	Grapes	34	0.25
CA 6.3.1/17 16-2193 16-2193-01 M-623069-01-1	NEU	2	0.300 0.300	BBCH 79 BBCH 83	Grapes	34	0.08
CA 6.3.1/17 16-2193 16-2193-02 M-623069-01-1	NEU	2	0.300 0.300	BBCH 73 BBCH 79	Grapes	35	0.10
CA 6.3.1/17 16-2193 16-2193-03 M-623069-01-1	NEU	2	0.300 0.300	BBCH 81 BBCH 83	Grapes	35	0.18
CA 6.3.1/17 16-2193 16-2193-04 M-623069-01-1	NEU	2	0.300 0.300	BBCH 83 BBCH 85	Grapes	35	0.11

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine, total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH			
CA 6.3.1/18 S20-02176-01 M-763137-01-1	NEU	2	0.300 0.300	BBCH 79-81 BBCH 83	Grapes	35	0.02
CA 6.3.1/18 S20-02176-02 M-763137-01-1	NEU	2	0.300 0.300	BBCH79 BBCH 83	Grapes	36	0.05
CA 6.3.1/18 S20-02176-03 M-763137-01-1	NEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	35	0.03
CA 6.3.1/18 S20-02176-04 M-763137-01-1	NEU	2	0.300 0.300	BBCH 83 BBCH 85	Grapes	35	0.09
CA 6.3.1/03 RA-2142/00 R 2000 0194/0 M-083248-01-1	SEU	3	0.400 0.400 0.400	BBCH 85 BBCH 87 BBCH 87	Grapes	35	0.07
CA 6.3.1/03 RA-2142/00 R 2000 0195/9 M-083248-01-1	SEU	3	0.400 0.400 0.374	BBCH 81 BBCH 83 BBCH 85	Grapes	33	<0.05
CA 6.3.1/01 RA-2650/07 R 2007 0675/8 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 81 BBCH 85 BBCH 85	Grapes	35	0.13
CA 6.3.1/01 RA-2650/07 R 2007 0676/6 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 79 BBCH 81 BBCH 83	Grapes	35	0.15
CA 6.3.1/01 RA-2650/07 R 2007 0703/7 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 77 BBCH 79 BBCH 81	Grapes	35	0.07
CA 6.3.1/01 RA-2650/07 R 2007 0704/5 M-301988-01-1	SEU	3	0.400 0.400 0.400	BBCH 75 BBCH 77 BBCH 81	Grapes	35	0.11
CA 6.3.1/20 13-2141 13-2141-04 M-508894-01-1	SEU	2	0.200 0.200	BBCH 79 BBCH 79	Grapes	35	0.08
CA 6.3.1/22 13-2161 13-2161-01 M-507373-02-1	SEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	35	<0.05
CA 6.3.1/22 13-2161 13-2161-02 M-507373-02-1	SEU	2	0.300 0.300	BBCH 79 BBCH 81	Grapes	35	0.06
CA 6.3.1/22 13-2161 13-2161-03 M-507373-02-1	SEU	2	0.300 0.300	BBCH 79 BBCH 81	Grapes	35	<0.05

Reference	Region	Application			Commodity	PHI (days)	Scaled spiroxamine, total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH			
CA 6.3.1/22 13-2161 13-2161-04 M-507373-02-1	SEU	2	0.300 0.300	BBCH 77 BBCH 79	Grapes	35	0.09
CA 6.3.1/16 16-2047 16-2047-01 M-626160-01-1	SEU	2	0.300 0.300	BBCH 81 BBCH 83	Grapes	35	0.10
CA 6.3.1/16 16-2047 16-2047-02 M-626160-01-1	SEU	2	0.300 0.300	BBCH 79 BBCH 79	Grapes	35	0.03
CA 6.3.1/16 16-2047 16-2047-03 M-626160-01-1	SEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	35	0.12
CA 6.3.1/16 16-2047 16-2047-04 M-626160-01-1	SEU	2	0.300 0.300	BBCH 85 BBCH 85	Grapes	35	0.04
CA 6.3.1/24 09-2036-01 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 83 BBCH 85 BBCH 85	Grapes	35	0.11
CA 6.3.1/24 09-2036-02 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 79 BBCH 81 BBCH 85	Grapes	35	0.10
CA 6.3.1/24 09-2036-03 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 81 BBCH 83 BBCH 85	Grapes	35	<0.05
CA 6.3.1/24 09-2036-04 M-390949-01-1	SEU	3	0.300 0.300 0.300	BBCH 79 BBCH 79 BBCH 81	Grapes	35	<0.05
CA 6.3.1/18 S20-02176-05 M-763137-01-1	SEU	2	0.300 0.300	BBCH 83 BBCH 85	Grapes	34	0.07
CA 6.3.1/18 S20-02176-06 M-763137-01-1	SEU	2	0.300 0.300	BBCH 79-81 BBCH 83	Grapes	34	0.07
CA 6.3.1/18 S20-02176-07 M-763137-01-1	SEU	2	0.300 0.300	BBCH 81 BBCH 83	Grapes	34	<0.01
CA 6.3.1/18 S20-02176-08 M-763137-01-1	SEU	2	0.300 0.300	BBCH 75-77 BBCH 89	Grapes	35	<0.01

1 – Residue data used for MRL are the reported or scaled value for spiroxamine (parent compound), total enantiomers for studies which used chiral chromatography

The MRL has been calculated according to the OECD MRL Calculator Spreadsheet Version 2, with the STMR as the median residue. In these calculations a single data point from each trial supporting the GAP has been considered and the data sets from NEU and SEU have been considered individually and

combined. The residue values used in the MRL and STMR calculations are underlined in Table CA 6.7.2-4. The calculated results are presented in Table CA 6.7.2-5 including the highest residue (HR).

Table CA 6.7.2-5 MRL and STMR calculations for spiroxamine in wine grapes

Region	Commodity	Residues (mg/kg)	Proposed OECD MRL (mg/kg)[1]	STMR (mg/kg	HR (mg/kg)
NEU	Wine grape	0.02, 0.03, 3 x0.05, 0.07, 2 x 0.08, 4 x 0.09, 0.10, 2 x 0.11, 0.12, 0.15, 0.18, 0.19, 0.21, 0.25, 0.27, 0.30, 0.38, 0.47, 0.60	**0.8**	0.105	0.60
SEU	Wine grape	2 x <0.01, 0.03, 0.04, 5 x <0.05, 0.06, 4 x 0.07, 0.08, 0.09, 2 x 0.10, 2 x 0.11, 0.12, 0.13, 0.15	**0.3**	0.07	0.15
EU	Wine grape	2 x <0.01, 0.02, 2 x 0.03, 2 x 0.04, 5 x <0.05, 3 x0.05, 0.06, 5 x 0.07, 3 x 0.08, 4 x 0.09, 3 x 0.10, 4 x 0.11, 2 x 0.12, 0.13, 2 x 0.15, 0.18, 0.19, 0.21, 0.25, 0.27, 0.30, 0.38, 0.47, 0.60	**0.6**	0.09	0.60

1 – MRL class shown is the rounded MRL

Sufficient residue trial data are available for wine grapes to calculate an EU MRL, STMR and HR. The residue trials data have a corresponding maximum STMR and HR of 0.105 and 0.60 mg/kg, respectively for the use of spiroxamine on table grapes at the critical GAP. As the SEU and NEU datasets are not similar, an EU MRL of 0.8 mg/kg for wine grapes is proposed based on the NEU EU data set and this is higher than the currently published EU MRL, therefore an MRL application will be associated with this renewal dossier.

The output from the OECD calculator is shown in Figure CA 6.7.2-2

Figure CA 6.7.2-2 Output data from OECD MRL calculator (wine grapes)

Compound	Spiroxamine	Spiroxamine	Spiroxamine
Crop	Wine Grape	Wine Grape	Wine Grape
Region / Country	NEU	SEU	EU
GAP	35 days PHI	35 days PHI	35 days PHI
Total number of data (n)	26	23	49
Percentage of censored data	0%	30%	14%
Number of non-censored data	26	16	42
Lowest residue	0.020	0.010	0.010
Highest residue	0.600	0.150	0.600
Median residue	0.105	0.070	0.090
Mean	0.163	0.073	0.120
Standard deviation (SD)	0.142	0.037	0.115
Correction factor for censoring (CF)	1.000	0.797	0.905
Proposed MRL estimate			
- Highest residue	0.600	0.150	0.600
- Mean + 4 SD	0.731	0.219	0.580
- CF x 3 Mean	0.488	0.174	0.327
Unrounded MRL	0.731	0.219	0.600
Rounded MRL	0.8	0.3	0.6

Residues (mg/kg)	Residues (mg/kg)		Residues (mg/kg)	
0.020	0.010	*	0.010	*
0.030	0.010	*	0.010	*
0.050	0.030		0.020	*
0.050	0.040		0.030	
0.070	0.050	*	0.030	
0.080	0.050	*	0.040	
0.080	0.050	*	0.050	*
0.090	0.050	*	0.050	*
0.090	0.060	*	0.050	*
0.090	0.070		0.050	*
0.100	0.070		0.050	
0.110	0.070		0.050	
0.110	0.080		0.050	
0.150	0.090		0.060	
0.180	0.100		0.070	
0.190	0.100		0.070	
0.210	0.110		0.070	
0.250	0.120		0.070	
0.270	0.130		0.080	
0.300	0.150		0.080	
0.380			0.080	
0.470			0.090	
0.600			0.090	
			0.090	
			0.090	
			0.090	
			0.100	
			0.100	
			0.100	
			0.110	
			0.110	
			0.110	
			0.110	
			0.120	
			0.120	
			0.130	
			0.150	
			0.150	
			0.180	
			0.190	
			0.210	
			0.250	
			0.270	
			0.300	
			0.380	
			0.470	
			0.600	

Barley grain

An overview of the barley grain residue data relevant for MRL purposes are presented in Table CA 6.7.2-6. These data are summarised in Point CA 6.3.2. Results of MRL calculations using the OECD MRL Calculator are discussed below.

Table CA 6.7.2-6 Summary of barley grain data according to the critical GAP

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.2/08 RA-2042/07 R 2007 0448/8 M-298147-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, grain	0.01
CA 6.3.2/08 RA-2042/07 R 2007 0449/6 M-298147-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, grain	0.02
CA 6.3.2/08 RA-2042/07 R 2007 0527/1 M-298147-01-1	NEU	2	0.375 0.3975	BBCH 37 BBCH 61	Barley, grain	<0.01
CA 6.3.2/08 RA-2042/07 R 2007 0529/8 M-298147-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, grain	0.01
CA 6.3.2/13 16-2045-01 M-618660-01	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, grain	0.03
CA 6.3.2/13 16-2045-02 M-618660-01	NEU	2	0.375 0.375	BBCH 32 BBCH 59	Barley, grain	<0.01
CA 6.3.2/13 16-2045-03 M-618660-01	NEU	2	0.375 0.375	BBCH 32 BBCH 61	Barley, grain	<0.01
CA 6.3.2/13 16-2045-04 M-618660-01	NEU	2	0.375 0.375	BBCH 33 BBCH 59	Barley, grain	0.01
CA 6.3.2/17 17-2016-01 M-663690-01-1	NEU	2	0.375 0.375	BBCH 55 BBCH 59	Barley, grain	0.02
CA 6.3.2/17 17-2016-02 M-663690-01-1	NEU	2	0.375 0.401	BBCH 33 BBCH 59	Barley, grain	0.040
CA 6.3.2/17 17-2016-03 M-663690-01-1	NEU	2	0.375 0.375	BBCH 49 BBCH 58	Barley, grain	0.03
CA 6.3.2/17 17-2016-04 M-663690-01-1	NEU	2	0.375 0.375	BBCH 39 BBCH 61	Barley, grain	0.03
CA 6.3.2/09 RA-2043/07 R 2007 0451/8 M-298412-01-1	SEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, grain	0.01

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.2/09 RA-2043/07 R 2007 0452/6 M-298412-01-1	SEU	2	0.375 0.375	BBCH 39 BBCH 61	Barley, grain	0.01
CA 6.3.2/09 RA-2043/07 R 2007 0530/1 M-298412-01-1	SEU	2	0.375 0.375	BBCH 39 BBCH 61	Barley, grain	0.02
CA 6.3.2/09 RA-2043/07 R 2007 0532/8 M-298412-01-1	SEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, grain	0.01
CA 6.3.2/15 16-2162-01 M-631606-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 58	Barley, grain	<0.01
CA 6.3.2/15 16-2162-02 M-631606-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 59	Barley, grain	<0.01
CA 6.3.2/15 16-2162-03 M-631606-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 59	Barley, grain	0.01
CA 6.3.2/15 16-2162-04 M-631606-01-1	SEU	2	0.375 0.375	BBCH 34 BBCH 51	Barley, grain	<0.01
CA 6.3.2/14 17-2145-02 M-630826-02-1	SEU	2	0.150 0.150	BBCH 32 BBCH 61	Barley, grain	0.03
CA 6.3.2/16 17-2159-01 M-656996-01-1	SEU	2	0.375 0.375	BBCH 30 BBCH 61	Barley, grain	0.01
CA 6.3.2/16 17-2159-04 M-656996-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 61	Barley, grain	0.04
CA 6.3.2/10 810045 M-010906-01-1	SEU	2	0.750 0.750	BBCH 33 BBCH 61	Barley, grain	0.03
CA 6.3.2/19 E19RP003-05 M-685312-01-1	SEU	2	0.164 0.157	BBCH 35 BBCH 61	Barley, grain	0.048

1 – Residue data used for MRL are the reported or scaled value for spiroxamine (parent compound), total enantiomers for studies which used chiral chromatography

The MRL has been calculated according to the OECD MRL Calculator Spreadsheet Version 2, with the STMR as the median residue. In these calculations a single data point from each trial supporting the GAP has been considered and the data sets from NEU and SEU have been considered both individually and combined. The residue values used in the MRL and STMR calculations are underlined in Table CA 6.7.2-6. The calculated results are presented in Table CA 6.7.2-7 including the highest residue (HR).

Table CA 6.7.2-7 MRL and STMR calculations for spiroxamine in barley grain

Region	Commodity	Residues (mg/kg)	Proposed OECD MRL (mg/kg)[1]	STMR (mg/kg	HR (mg/kg)
NEU	Barley, grain	3 x <0.01, 3 x 0.01, 2 x 0.02, 3 x 0.03, 0.04	0.07	0.015	0.04
SEU	Barley, grain	3 x <0.01, 5 x 0.01, 0.02, 2 x 0.03, 0.04, 0.048	0.08	0.01	0.048
EU	Barley, grain	6 x <0.01, 8 x 0.01, 3 x 0.02, 5 x 0.03, 2 x 0.04, 0.048	**0.07**	0.01	0.048

1 – MRL class shown is the rounded MRL

Sufficient residue trial data are available for barley, grain to calculate an EU MRL, STMR and HR. The residue trials data have a corresponding maximum STMR and HR of 0.015 and 0.048 mg/kg, respectively for the use of spiroxamine on barley at the critical GAP. An EU MRL of 0.07 mg/kg for barley grain is proposed based on the combined EU data set as both datasets can be shown to be similar based on the Mann-Whitney U-Test. As this is higher than the currently published EU MRL, therefore an MRL application will be associated with this renewal dossier. Under the extrapolation rules covered in SANTE/2019/12752, this data for barley allows extrapolation of the proposed grain MRL also to oats.

Figure CA 6.7.2-3 Output from Mann-Whitney U-Test for barley grain datasets

Mann-Whitney U-Test (α: 0.05)

Data set	Barley NEU	Barley SEU	Rank Set 1	Rank Set 2
1	0.01	0.01	6.5	6.5
2	0.01	0.01	6.5	6.5
3	0.01	0.01	6.5	6.5
4	0.01	0.01	6.5	6.5
5	0.01	0.01	6.5	6.5
6	0.01	0.01	13.5	6.5
7	0.02	0.01	15.5	6.5
8	0.02	0.01	17	13.5
9	0.03	0.02	18	15.5
10	0.03	0.04	19	21
11	0.03	0.05	20	23
12	0.04		22	
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
Mean	0.02	0.02		
STMR	0.02	0.01		
Number of values:			12	11
Sum Rank:			158	119
U_1 and U_2 values:			52.5	79.5
Critical value:			33	(α = 0.05)
n_a = 11			n_b = 12	
Result:			Populations similar	

The output from the OECD calculator is shown in Figure CA 6.7.2-3.

Figure CA 6.7.2-4 Output data from OECD MRL calculator (barley grain)

Reset

Compound	Spiroxamine	Spiroxamine	Spiroxamine
Crop	Barley grain	Barley grain	Barley grain
Region / Country	NEU	NEU	NEU
GAP	2 x 375	2 x 375	2 x 375
Total number of data (n)	12	13	25
Percentage of censored data	25%	23%	24%
Number of non-censored data	9	10	19
Lowest residue	0.010	0.010	0.010
Highest residue	0.040	0.048	0.048
Median residue	0.015	0.010	0.010
Mean	0.019	0.019	0.019
Standard deviation (SD)	0.011	0.011	0.012
Correction factor for censoring (CF)	0.833	0.846	0.840
Proposed MRL estimate			
- Highest residue	0.040	0.048	0.048
- Mean + 4 SD	0.063	0.073	0.067
- CF x 3 Mean	0.048	0.048	0.048
Unrounded MRL	0.063	0.073	0.067
Rounded MRL	0.07	0.08	0.07

Residues (mg/kg)		Residues (mg/kg)		Residues (mg/kg)	
0.010		0.010	*	0.010	*
0.010		0.010	*	0.010	*
0.010		0.010	*	0.010	*
0.010		0.010		0.010	*
0.010		0.010		0.010	*
0.010		0.010		0.010	*
0.020		0.010		0.010	
0.020		0.010		0.010	
0.030		0.020		0.010	
0.030		0.030		0.010	
0.030		0.030		0.010	
0.040		0.040		0.010	
		0.048		0.010	
				0.010	
				0.020	
				0.020	
				0.020	
				0.030	
				0.030	
				0.030	
				0.030	
				0.030	
				0.040	
				0.040	
				0.048	

Barley straw

An overview of the barley straw residue data relevant for MRL purposes are presented in Table CA 6.7.2-8. These data are summarised in Point CA 6.3.2. Results of pseudo MRL calculations using the OECD MRL Calculator are discussed below.

Table CA 6.7.2-8 Summary of barley straw data according to the critical GAP

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.2/01 RA-2002/97 703796 M-010782-01-1	NEU	2	0.400 0.400	BBCH 32 BBCH 61	Barley, straw	0.55
CA 6.3.2/01 RA-2002/97 703826 M-010782-01-1	NEU	2	0.400 0.400	BBCH 32 BBCH 67-63	Barley, straw	0.27
CA 6.3.2/05 RA-2096/00 R 2000 0083/9 M-088981-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.29
CA 6.3.2/05 RA-2096/00 R 2000 0425/7 M-088981-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.25
CA 6.3.2/05 RA-2096/00 R 2000 0426/5 M-088981-01-1	NEU	2	0.375 0.375	BBCH 39 BBCH 61	Barley, straw	0.23
CA 6.3.2/05 RA-2096/00 R 2000 0427/3 M-088981-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 63	Barley, straw	0.41
CA 6.3.2/06 RA-2571/05 R 2005 0030/0 M-272012-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 61	Barley, straw	0.59
CA 6.3.2/06 RA-2571/05 R 2005 0031/9 M-272012-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 61	Barley, straw	0.14
CA 6.3.2/06 RA-2571/05 R 2005 0801/8 M-272012-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 61	Barley, straw	0.11
CA 6.3.2/06 RA-2571/05 R 2005 0802/6 M-272012-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 61	Barley, straw	0.68
CA 6.3.2/07 RA-3042/07 R 2007 0448/8 M-298147-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.36

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.2/08 RA-2042/07 R 2007 0449/6 M-298147-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.47
CA 6.3.2/08 RA-2042/07 R 2007 0527/1 M-298147-01-1	NEU	2	0.375 0.3975	BBCH 37 BBCH 61	Barley, straw	0.06
CA 6.3.2/08 RA-2042/07 R 2007 0529/8 M-298147-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.13
CA 6.3.2/13 16-2045 M-618660-01	NEU	2	0.375 0.375	BBCH 32 BBCH 61	Barley, straw	0.62
CA 6.3.2/13 16-2045 M-618660-01	NEU	2	0.375 0.375	BBCH 32 BBCH 59	Barley, straw	0.45
CA 6.3.2/13 16-2045 M-618660-01	NEU	2	0.375 0.375	BBCH 32 BBCH 61	Barley, straw	0.23
CA 6.3.2/13 16-2045 M-618660-01	NEU	2	0.375 0.375	BBCH 33 BBCH 59	Barley, straw	0.28
CA 6.3.2/17 17-2016-01 M-663690-01-1	NEU	2	0.375 0.375	BBCH 55 BBCH 59	Barley, straw	0.44
CA 6.3.2/17 17-2016-02 M-663690-01-1	NEU	2	0.375 0.401	BBCH 33 BBCH 59	Barley, straw	0.27
CA 6.3.2/17 17-2016-03 M-663690-01-1	NEU	2	0.375 0.375	BBCH 49 BBCH 58	Barley, straw	0.36
CA 6.3.2/17 17-2016-04 M-663690-01-1	NEU	2	0.375 0.375	BBCH 39 BBCH 61	Barley, straw	0.22
CA 6.3.2/18 17-2075-01 M-638944-01-1	NEU	2	0.150 0.150	BBCH 51 BBCH 59	Barley, straw	0.28
CA 6.3.2/18 17-2075-02 M-638944-01-1	NEU	2	0.150 0.150	BBCH 51 BBCH 59	Barley, straw	0.22
CA 6.3.2/05 RA-2096/00 R 2000 0084/5 M-088981-01-1	SEU	2	0.375 0.375	BBCH 37 BBCH 69	Barley, straw	0.35
CA 6.3.2/05 RA-2096/00 R 2000 0428/1 M-088981-01-1	SEU	2	0.375 0.375	BBCH 37 BBCH 69	Barley, straw	0.54

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.2/07 RA-2572/05 R 2005 0468/3 M-272115-01-1	SEU	2	0.3125 0.3125	BBCH 47 BBCH 61	Barley, straw	0.12
CA 6.3.2/07 RA-2572/05 R 2005 0469/1 M-272115-01-1	SEU	2	0.3125 0.3125	BBCH 51 BBCH 59	Barley, straw	0.37
CA 6.3.2/09 RA-2043/07 R 2007 0451/8 M-298412-01-1	SEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.44
CA 6.3.2/09 RA-2043/07 R 2007 0452/6 M-298412-01-1	SEU	2	0.375 0.375	BBCH 30 BBCH 61	Barley, straw	0.42
CA 6.3.2/09 RA-2043/07 R 2007 0530/1 M-298412-01-1	SEU	2	0.375 0.375	BBCH 39 BBCH 61	Barley, straw	0.97
CA 6.3.2/09 RA-2043/07 R 2007 0532/8 M-298412-01-1	SEU	2	0.375 0.375	BBCH 37 BBCH 61	Barley, straw	0.73
CA 6.3.2/15 16-2162-01 M-631606-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 58	Barley, straw	0.34
CA 6.3.2/15 16-2162-02 M-631606-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 53	Barley, straw	0.51
CA 6.3.2/15 16-2162-03 M-631606-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 59	Barley, straw	0.10
CA 6.3.2/15 16-2162-04 M-631606-01-1	SEU	2	0.375 0.375	BBCH 34 BBCH 51	Barley, straw	0.19
CA 6.3.2/14 17-2145 M-630826-02-1	SEU	2	0.150 0.150	BBCH 30 BBCH 61	Barley, straw	0.23
CA 6.3.2/14 17-2145 M-630826-02-1	SEU	2	0.150 0.150	BBCH 52 BBCH 61	Barley, straw	0.33
CA 6.3.2/16 17-2159-01 M-656996-01-1	SEU	2	0.375 0.3750	BBCH 30 BBCH 61	Barley, straw	0.32
CA 6.3.2/16 17-2159-04 M-656996-01-1	SEU	2	0.375 0.3750	BBCH 51 BBCH 61	Barley, straw	0.37
CA 6.3.2/10 RA-2158/98 810045 M-010906-01-1	SEU	2	0.750 0.750	BBCH 33 BBCH 61	Barley, straw	0.26

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.2/10 RA-2158/98 816299 M-010906-01-1	SEU	2	0.750 0.750	BBCH 35 BBCH 61	Barley, straw	0.60
CA 6.3.2/10 RA-2158/98 816310 M-010906-01-1	SEU	2	0.750 0.750	BBCH 33 BBCH 61	Barley, straw	0.36
CA 6.3.2/19 E19RP003-01 M-685313-01-1	SEU	2	0.162 0.159	BBCH 39 BBCH 61	Barley, straw	0.28
CA 6.3.2/19 E19RP003-02 M-685313-01-1	SEU	2	0.158 0.159	BBCH 37 BBCH 61	Barley, straw	0.09
CA 6.3.2/19 E19RP003-03 M-685313-01-1	SEU	2	0.166 0.169	BBCH 32 BBCH 61	Barley, straw	0.12
CA 6.3.2/19 E19RP003-04 M-685313-01-1	SEU	2	0.154 0.157	BBCH 32 BBCH 61	Barley, straw	0.41
CA 6.3.2/19 E19RP003-05 M-685313-01-1	SEU	2	0.164 0.157	BBCH 36 BBCH 61	Barley, straw	0.69
CA 6.3.2/19 E19RP003-06 M-685313-01-1	SEU	2	0.155 0.161	BBCH 37 BBCH 61	Barley, straw	0.42
CA 6.3.2/19 E19RP003-07 M-685313-01-1	SEU	2	0.161 0.156	BBCH 35 BBCH 61	Barley, straw	0.06

1 – Residue data used for MRL are the reported or scaled value for spiroxamine (parent compound), total enantiomers for studies which used chiral chromatography

The pseudo MRL has been calculated according to the OECD MRL Calculator Spreadsheet Version 2, with the STMR as the median residue. In these calculations a single data point from each trial supporting the GAP has been considered and the data sets from NEU and SEU have been considered both individually and combined. The residue values used in the MRL and STMR calculations are underlined in Table CA 6.7.2-8. The calculated results are presented in Table CA 6.7.2-9 including the highest residue (HR).

Table CA 6.7.2-9 MRL and STMR calculations for spiroxamine in barley straw

Region	Commodity	Residues (mg/kg)	Proposed OECD MRL (mg/kg)[1]	STMR (mg/kg	HR (mg/kg)
NEU	Barley, straw	0.06, 0.11, 0.13, 0.14, 2 x 0.22, 2 x 0.23, 0.25, 2 x 0.27, 2 x 0.28, 0.29, 2 x 0.36, 0.41, 0.44, 0.45, 0.47, 0.55, 0.59, 0.62, 0.68	**1.0**	0.28	0.68
SEU	Barley, straw	0.06, 0.07, 0.09, 0.10, 2 x 0.12, 0.19, 0.23, 0.26, 0.28, 0.32, 0.33, 0.34, 0.35, 0.36, 2 x 0.37, 0.41, 2 x 0.42, 0.44, 0.51, 0.54, 0.60, 0.69, 0.73	**1.50**	0.345	0.73
EU	Barley, straw	2 x 0.06, 0.07, 0.09, 0.10, 0.11, 2 x 0.12, 0.13, 0.14, 0.19, 2 x 0.22, 3 x 0.23, 0.25, 0.26, 2 x 0.27, 3 x 0.28, 0.29, 0.32, 0.33, 0.34, 0.35, 3 x 0.36, 2 x 0.37, 2 x 0.41, 2 x 0.42, 2 x 0.44, 0.45, 0.47, 0.51, 0.54, 0.55, 0.59, 0.60, 0.62, 0.68, 0.69, 0.73	**1.0**	0.325	0.73

1 – MRL class shown is the rounded MRL

Sufficient residue trial data are available for barley straw to calculate a pseudo EU MRL, STMR and HR. The residue trials data have a corresponding maximum STMR and HR of 0.345 and 0.73 mg/kg, respectively for the use of spiroxamine on barley at the critical GAP. A pseudo EU MRL of 1.50 mg/kg for barley straw could be proposed based on the SEU data set however EU MRLs in animal feedstuff commodities are not currently published under EU MRL Regulation 396/2005. These values can be used in dietary burden calculations with extrapolation to oats straw.

The output from the OECD calculator is shown in Figure CA 6.7.2-4.

Figure CA 6.7.2-4 Output data from OECD MRL calculator (barley straw)

Compound	Spiroxamine	Spiroxamine	Spiroxamine
Crop	Barley straw	Barley straw	Barley straw
Region / Country	NEU	SEU	EU
GAP	2 x 375	2 x 375	2 x 375
Total number of data (n)	24	26	50
Percentage of censored data	0%	0%	0%
Number of non-censored data	24	26	50
Lowest residue	0.060	0.060	0.060
Highest residue	0.680	0.730	0.730
Median residue	0.280	0.345	0.325
Mean	0.330	0.335	0.332
Standard deviation (SD)	0.167	0.185	0.175
Correction factor for censoring (CF)	1.000	1.000	1.000
Proposed MRL estimate			
- Highest residue	0.680	0.730	0.730
- Mean + 4 SD	0.996	1.075	1.031
- CF x 3 Mean	0.989	1.005	0.998
Unrounded MRL	0.996	1.075	1.031
Rounded MRL	1	1.5	2

Residues (mg/kg)	Residues (mg/kg)	Residues (mg/kg)
0.060	0.060	0.060
0.110	0.070	0.060
0.130	0.080	0.070
0.140	0.090	0.090
0.220	0.120	0.090
0.220	0.120	0.110
0.230	0.190	0.120
0.230	0.200	0.120
0.250	0.220	0.130
0.260	0.280	0.140
0.270	0.320	0.190
0.280	0.330	0.220
0.280	0.340	0.220
0.290	0.360	0.230
0.360	0.360	0.230
0.360	0.370	0.230
0.410	0.370	0.250
0.440	0.410	0.260
0.450	0.420	0.270
0.470	0.420	0.270
0.550	0.440	0.280
0.590	0.510	0.280
0.620	0.540	0.280
0.680	0.600	0.290
	0.680	0.320
	0.690	0.330
		0.340
		0.350
		0.360
		0.360
		0.360
		0.370
		0.370
		0.410
		0.410
		0.420
		0.420
		0.440
		0.440
		0.450
		0.470
		0.510
		0.540
		0.550
		0.590
		0.600
		0.620
		0.680
		0.690
		0.730

Wheat grain

An overview of the wheat grain residue data relevant for MRL purposes are presented in Table CA 6.7.2-10. These data are summarised in Point CA 6.3.3. Results of MRL calculations using the OECD MRL Calculator are discussed below.

Table CA 6.7.2-10 Summary of wheat grain data according to the critical GAP

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.3/08 RA-2040/07 R 2007 0443/7 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/08 RA-2040/07 R 2007 0444/5 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	0.01
CA 6.3.3/08 RA-2040/07 R 2007 0523/9 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/08 RA-2040/07 R 2007 0524/7 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	0.01
CA 6.3.3/12 16-2046-01 M-626175-01-1	NEU	2	0.375 0.375	BBCH 43 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/12 16-2046-02 M-626175-01-1	NEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/12 16-2046-03 M-626175-01-1	NEU	2	0.375 0.375	BBCH 39 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/12 16-2046-04 M-626175-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/16 17-2015-01 M-659920-01-1	NEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/16 17-2015-02 M-659920-01-1	NEU	2	0.375 0.375	BBCH 51 BBCH 69	Wheat, grain	0.02
CA 6.3.3/16 17-2015-03 M-659920-01-1	NEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, grain	<0.01

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)
		No.	kg a.s./ha	BBCH		
CA 6.3.3/16 17-2015-04 M-659920-01-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	0.01
CA 6.3.3/09 RA-2041/07 R 2007 0445/3 M-298650-02-1	SEU	2	0.375 0.375	BBCH 52 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/09 RA-2041/07 R 2007 0446/1 M-298650-02-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/09 RA-2041/07 R 2007 0525/5 M-298650-02-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/09 RA-2041/07 R 2007 0526/3 M-298650-02-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	0.01
CA 6.3.3/13 16-2163-01 M-627149-01-1	SEU	2	0.375 0.375	BBCH 43 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/13 16-2163-02 M-627149-01-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/13 16-2163-03 M-627149-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 65	Wheat, grain	<0.01
CA 6.3.3/13 16-2163-04 M-627149-01-1	SEU	2	0.375 0.375	BBCH 49 BBCH 65	Wheat, grain	<0.01
CA 6.3.3/17 17-2158-01 M-660174-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/17 17-2158-02 M-660174-01-1	SEU	2	0.375 0.375	BBCH 49 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/17 17-2158-03 M-660174-01-1	SEU	2	0.375 0.375	BBCH 39 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/17 17-2158-04 M-660174-01-1	SEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, grain	<0.01
CA 6.3.3/17 17-2158-05 M-660174-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 69	Wheat, grain	<0.01

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		

1 – Residue data used for MRL are the reported or scaled value for spiroxamine (parent compound), total enantiomers for studies which used chiral chromatography

The MRL has been calculated according to the OECD MRL Calculator Spreadsheet Version 2, with the STMR as the median residue. In these calculations a single data point from each trial supporting the GAP has been considered and the data sets from NEU and SEU have been considered both individually and combined. The residue values used in the MRL and STMR calculations are underlined in Table CA 6.7.2-10. The calculated results are presented in Table CA 6.7.2-11 including the highest residue (HR).

Table CA 6.7.2-11 MRL and STMR calculations for spiroxamine in wheat grain

Region	Commodity	Residues (mg/kg)	Proposed OECD MRL (mg/kg)[1]	STMR (mg/kg	HR (mg/kg)
NEU	Wheat, grain	8 x <0.01, 3 x 0.01, 0.02	**0.03**	0.01	0.02
SEU	Wheat, grain	12 x <0.01, 0.01	0.015	0.01	0.01
EU	Wheat, grain	20 x <0.01, 4 x 0.01, 0.02	0.02	0.01	0.02

1 – MRL class shown is the rounded MRL

Sufficient residue trial data are available for wheat, grain to calculate an EU MRL, STMR and HR. The residue trials data have a corresponding maximum STMR and HR of 0.01 and 0.02 mg/kg, respectively for the use of spiroxamine on wheat at the critical GAP. An EU MRL of 0.03 mg/kg for wheat grain is proposed based on the NEU data set and this is lower than the currently published EU MRL, therefore an MRL application will be associated with this renewal dossier. Under the extrapolation rules covered in SANTE/2019/12752, this data for wheat allows extrapolation of the proposed grain MRL also to rye.

The output from the OECD calculator is shown in Figure CA 6.7.2-5.

Figure CA 6.7.2-5 Output data from OECD MRL calculator (wheat grain)

Reset

Compound	Spiroxamine	Spiroxamine	Spiroxamine
Crop	Wheat grain	Wheat grain	Wheat grain
Region / Country	NEU	NEU	NEU
GAP	2 x 375	2 x 375	2 x 375
Total number of data (n)	12	12	12
Percentage of censored data	67%	92%	80%
Number of non-censored data	4	1	5
Lowest residue	0.010	0.010	0.010
Highest residue	0.020	0.010	0.020
Median residue	0.010	0.010	0.010
Mean	0.011	0.010	0.014
Standard deviation (SD)	0.004	0.000	0.002
Correction factor for censoring (CF)	0.556	0.385	0.467
Proposed MRL estimate			
- Highest residue	0.020	0.010	0.020
- Mean + 4 SD	0.022	0.010	0.018
- CF x 3 Mean	0.018	0.015	0.015
Unrounded MRL	0.02	0.02	0.020
Rounded MRL	0.03	0.015	0.02
	High uncertainty of MRL estimate due to high level of censoring.	High uncertainty of MRL estimate due to high level of censoring.	High uncertainty of MRL estimate due to high level of censoring.

Residues (mg/kg)		Residues (mg/kg)		Residues (mg/kg)	
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.010	*	0.010	*	0.010	*
0.020	*	0.010	*	0.010	*
		0.010		0.010	*
				0.010	*
				0.010	*
				0.010	*
				0.010	*
				0.010	*
				0.010	*
				0.010	*
				0.010	*
				0.010	
				0.010	
				0.010	
				0.020	

Wheat straw

An overview of the wheat straw residue data relevant for MRL purposes are presented in Table CA 6.7.2-12. These data are summarised in Point CA 6.3.3. Results of pseudo MRL calculations using the OECD MRL Calculator are discussed below.

Table CA 6.7.2-12 Summary of wheat straw data according to the critical GAP

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)[1]
		No.	kg a.s./ha	BBCH		
CA 6.3.3/01 RA-2002/97 703834 M-010782-01-1	NEU	2	0.400 0.400	BBCH 37 BBCH 69	Wheat, straw	0.76
CA 6.3.3/05 RA-2092/00 R 2000 0081/2 M-087669-01-1	NEU	2	0.375 0.375	BBCH 32 BBCH 69	Wheat, straw	0.32
CA 6.3.3/05 RA-2092/00 R 2000 0430/3 M-087669-01-1	NEU	2	0.375 0.375	BBCH 32 BBCH 69	Wheat, straw	0.35
CA 6.3.3/05 RA-2092/00 R 2000 0431/1 M-087669-01-1	NEU	2	0.375 0.375	BBCH 32 BBCH 69	Wheat, straw	0.70
CA 6.3.3/05 RA-2092/00 R 2000 0433/8 M-087669-01-1	NEU	2	0.375 0.375	BBCH 32 BBCH 69	Wheat, straw	0.27
CA 6.3.3/06 RA-2573/05 R 2005 0470/5 M-271973-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 69	Wheat, straw	0.56
CA 6.3.3/06 RA-2573/05 R 2005 0471/3 M-271973-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 69	Wheat, straw	0.83
CA 6.3.3/06 RA-2573/05 R 2005 0803/4 M-271973-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 69	Wheat, straw	1.32
CA 6.3.3/06 RA-2573/05 R 2005 0804/2 M-271973-01-1	NEU	2	0.3125 0.3125	BBCH 47 BBCH 69	Wheat, straw	1.32
CA 6.3.3/08 RA-2040/07 R 2007 0443/7 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.05
CA 6.3.3/08 RA-2040/07 R 2007 0444/5 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.24

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)
		No.	kg a.s./ha	BBCH		
CA 6.3.3/08 RA-2040/07 R 2007 0523/9 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.39
CA 6.3.3/08 RA-2040/07 R 2007 0524/7 M-298182-02-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.55
CA 6.3.3/12 16-2046-01 M-626175-01-1	NEU	2	0.375 0.375	BBCH 43 BBCH 69	Wheat, straw	0.44
CA 6.3.3/12 16-2046-02 M-626175-01-1	NEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, straw	0.14
CA 6.3.3/12 16-2046-03 M-626175-01-1	NEU	2	0.375 0.375	BBCH 39 BBCH 69	Wheat, straw	0.15
CA 6.3.3/12 16-2046-04 M-626175-01-1	NEU	2	0.375 0.375	BBCH 37 BBCH 69	Wheat, straw	0.30
CA 6.3.3/16 17-2015-01 M-659920-01-1	NEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, straw	0.77
CA 6.3.3/16 17-2015-02 M-659920-01-1	NEU	2	0.375 0.375	BBCH 51 BBCH 69	Wheat, straw	0.93
CA 6.3.3/16 17-2015-03 M-659920-01-1	NEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, straw	0.72
CA 6.3.3/16 17-2015-04 M-659920-01-1	NEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.43
CA 6.3.3/05 RA-2092/00 R 2000 0082/0 M-087669-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 69	Wheat, straw	1.30
CA 6.3.3/05 RA-2092/00 R 2000 0434/6 M-087669-01-1	SEU	2	0.375 0.375	BBCH 32 BBCH 69	Wheat, straw	0.36
CA 6.3.3/07 RA-2574/05 R 2005 0472/1 M-271989-02-1	SEU	2	0.3125 0.3125	BBCH 47 BBCH 69	Wheat, straw	0.85
CA 6.3.3/07 RA-2574/05 R 2005 0474/8 M-271989-02-1	SEU	2	0.3125 0.3125	BBCH 47 BBCH 69	Wheat, straw	0.37
CA 6.3.3/09 RA-2041/07 R 2007 0445/3 M-298650-02-1	SEU	2	0.375 0.375	BBCH 52 BBCH 69	Wheat, straw	0.45

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)
		No.	kg a.s./ha	BBCH		
CA 6.3.3/09 RA-2041/07 R 2007 0446/1 M-298650-02-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.55
CA 6.3.3/09 RA-2041/07 R 2007 0525/5 M-298650-02-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.65
CA 6.3.3/09 RA-2041/07 R 2007 0526/3 M-298650-02-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.51
CA 6.3.3/13 16-2163-01 M-627149-01-1	SEU	2	0.375 0.375	BBCH 43 BBCH 69	Wheat, straw	0.55
CA 6.3.3/13 16-2163-02 M-627149-01-1	SEU	2	0.375 0.375	BBCH 47 BBCH 69	Wheat, straw	0.74
CA 6.3.3/13 16-2163-03 M-627149-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 65	Wheat, straw	1.50
CA 6.3.3/13 16-2163-04 M-627149-01-1	SEU	2	0.375 0.375	BBCH 49 BBCH 65	Wheat, straw	1.00
CA 6.3.3/15 17-2030-01 M-634111-01-1	SEU	2	0.150 0.150	BBCH 51 BBCH 69	Wheat, straw	0.53
CA 6.3.3/15 17-2030-02 M-634111-01-1	SEU	2	0.150 0.150	BBCH 37 BBCH 69	Wheat, straw	0.50
CA 6.3.3/14 17-2144-01 M-633801-01-1	SEU	2	0.150 0.150	BBCH 49 BBCH 69	Wheat, straw	0.16
CA 6.3.3/14 17-2144-02 M-633801-01-1	SEU	2	0.150 0.150	BBCH 45 BBCH 69	Wheat, straw	0.75
CA 6.3.3/17 17-2158-01 M-660174-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 69	Wheat, straw	0.27
CA 6.3.3/17 17-2158-02 M-660174-01-1	SEU	2	0.375 0.375	BBCH 49 BBCH 69	Wheat, straw	0.29
CA 6.3.3/17 17-2158-03 M-660174-01-1	SEU	2	0.375 0.375	BBCH 39 BBCH 69	Wheat, straw	0.39
CA 6.3.3/17 17-2158-04 M-660174-01-1	SEU	2	0.375 0.375	BBCH 45 BBCH 69	Wheat, straw	1.40
CA 6.3.3/17 17-2158-05 M-660174-01-1	SEU	2	0.375 0.375	BBCH 51 BBCH 69	Wheat, straw	0.13

Reference	Region	Application			Commodity	Scaled spiroxamine total isomers (mg/kg)
		No.	kg a.s./ha	BBCH		
CA 6.3.3/10 RA-2018/97 702935 M-010746-01-1	SEU	2	0.750 0.750	BBCH 34 BBCH 69	Wheat, straw	0.21
CA 6.3.3/10 RA-2018/97 702943 M-010746-01-1	SEU	2	0.750 0.750	BBCH 34 BBCH 67	Wheat, straw	0.40
CA 6.3.3/10 RA-2018/97 702978 M-010746-01-1	SEU	2	0.790 0.790	BBCH 33 BBCH 69	Wheat, straw	0.12

1 – Residue data used for MRL are the reported or scaled value for spiroxamine (parent compound), total enantiomers for studies which used chiral chromatography

The MRL has been calculated according to the OECD MRL Calculator Spreadsheet Version 2, with the STMR as the median residue. In these calculations a single data point from each trial supporting the GAP has been considered and the data sets from NEU and SEU have been considered both individually and combined. The residue values used in the MRL and STMR calculations are underlined in Table CA 6.7.2-12. The calculated results are presented in Table CA 6.7.2-13 including the highest residue (HR).

Table CA 6.7.2-13 MRL and STMR calculations for spiroxamine in wheat straw

Region	Commodity	Residues (mg/kg)	Proposed MRL OECD (mg/kg)[1]	STMR (mg/kg	HR (mg/kg)
NEU	Wheat, straw	0.05, 0.14, 0.15, 0.24, 0.27, 0.30, 0.31, 032, 0.39, 0.43, 0.44, 0.55, 0.56, 0.70, 0.72, 0.76, 0.77, 0.83, 0.93, 2 x 1.32	**2.0**	0.440	1.32
SEU	Wheat, straw	0.12, 0.13, 0.16, 0.21, 0.27, 0.29, 0.36, 0.37, 0.39, 0.40, 0.45, 0.50, 0.51, 0.53, 2 x 0.55, 0.65, 0.74, 0.75, 0.85, 1.00, 1.30, 1.40, 1.50	**3.0**	0.505	1.50
EU	Wheat, straw	0.05, 0.12, 0.13, 0.14, 0.15, 0.16, 0.21, 0.24, 2 x 0.27, 0.29, 0.30, 0.31, 0.32, 0.36, 0.37, 2 x 0.39, 0.40, 0.43, 0.44, 0.45, 0.50, 0.51, 0.53, 3 x 0.55, 0.56, 0.65, 0.70, 0.72, 0.74, 0.75, 0.76, 0.77, 0.83, 0.85, 0.93, 1.00, 1.30, 2 x 1.32 1.40, 1.50	**2.0**	0.500	1.50

1 – MRL class shown is the rounded MRL

Sufficient residue trial data are available for wheat straw to calculate a pseudo EU MRL, STMR and HR. The residue trials data have a corresponding maximum STMR and HR of 0.505 and 1.5 mg/kg, respectively for the use of spiroxamine on wheat at the critical GAP. A pseudo EU MRL of 3.0 mg/kg for wheat straw could be proposed based on the SEU data set however EU MRLs in animal feedstuff commodities are not currently published under EU MRL Regulation 396/2005. These values can be used in dietary burden calculations with extrapolation to rye straw.

The output from the OECD calculator is shown in Figure CA 6.7.2-6.

Figure CA 6.7.2-6 Output data from OECD MRL calculator (wheat straw)

Compound	Spiroxamine	Spiroxamine	Spiroxamine
Crop	Wheat straw	Wheat straw	Wheat straw
Region / Country	NEU	SEU	EU
GAP	2 x 375	2 x 375	2 x 375
Total number of data (n)	21	24	45
Percentage of censored data	0%		0%
Number of non-censored data	21		45
Lowest residue	0.050	0.120	0.050
Highest residue	1.320	1.500	1.500
Median residue	0.440	0.505	0.500
Mean	0.548	0.583	0.566
Standard deviation (SD)	0.356	0.387	0.368
Correction factor for censoring (CF)	1.000	1.000	1.000
Proposed MRL estimate			
- Highest residue	1.320	1.500	1.500
- Mean + 4 SD	1.972	2.131	2.042
- CF x 3 Mean	1.643	1.748	1.699
Unrounded MRL	1.970	2.131	2.042
Rounded MRL	2	3	2

Residues (mg/kg)	Residues (mg/kg)	Residues (mg/kg)
0.050	0.120	0.050
0.110	0.130	0.120
0.150	0.180	0.130
0.240	0.210	0.140
0.270	0.270	0.150
0.300	0.290	0.160
0.310	0.360	0.220
0.320	0.370	0.240
0.390	0.380	0.270
0.430	0.400	0.270
0.440	0.450	0.290
0.500	0.500	0.300
0.500	0.510	0.310
0.700	0.530	0.320
0.720	0.550	0.360
0.760	0.550	0.370
0.770	0.650	0.390
0.830	0.740	0.390
0.850	0.750	0.400
0.930	0.850	0.430
1.320	1.000	0.440
1.320	1.300	0.450
	1.400	0.500
	1.500	0.510
		0.530
		0.550
		0.550
		0.550
		0.560
		0.650
		0.700
		0.720
		0.740
		0.750
		0.760
		0.770
		0.830
		0.850
		0.930
		1.000
		1.300
		1.320
		1.320
		1.400
		1.500

MRLs for food of animal origin (excluding honey)

MRLs for food of animal origin have been considered according to the latest EFSA Guidance [Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, September 2015] and by using the MRL output from the EFSA Excel dietary burden calculator (pesticides_mrl_guidelines_animal_model_2017.xls).

The proposed MRL classes for food of animal origin are shown in Table CA 6.7.2-1. A requested change to the MRL for those commodities requiring new MRL will be made in parallel to this renewal of approval evaluation.

Figure CA 6.7.2-7 MRL and STMR calculations for spiroxamine residues in food of animal origin (representative uses)

Animal commodity	Residues at the closet feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	STMR (mg/kg)	HR (mg/kg)
	Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
Cattle (all diets)								
Closest feeding level[a]:	0.07	mg/kg bw	0.77 N Dairy cattle (highest diet)					
Meat	-	-	-	-	-	-	0.03	0.03
Muscle	0.02	0.02	0.02	0.02	**0.03**	1.40	0.03	0.03
Fat	0.02	0.02	0.02	0.02	**0.03**	1.80	0.04	0.04
Liver	0.05	0.06	0.05	0.10	**0.10**	3.00	0.16	0.29
Kidney	0.05	0.05	0.04	0.07	**0.08**	3.80	0.14	0.28
Cattle (dairy only)								
Closest feeding level[a]:	0.07	mg/kg bw	0.77 N Dairy cattle					
Milk[b]	0.02	0.02	0.02	0.02	**0.02**	1.20	0.02	0.03
Sheep (all diets)								
Closest feeding level[a]:	0.19	mg/kg bw	1.04 N Lamb (highest diet)					
Meat	-	-	-	-	-	-	0.03	0.05
Muscle	0.02	0.02	0.02	0.03	**0.03**	1.40	0.03	0.04
Fat	0.02	0.02	0.02	0.05	**0.05**	1.80	0.04	0.08
Liver	0.16	0.18	0.07	0.17	**0.20**	3.00	0.22	0.51
Kidney	0.09	0.11	0.06	0.11	**0.15**	3.80	0.22	0.41
Sheep (dairy only)								
Closest feeding level[a]:	0.19	mg/kg bw	1.32 N Ewe					
Milk[b]	0.02	0.03	0.02	0.02	**0.03**	1.20	0.02	0.03
Swine								
Closest feeding level[a]:	0.00	mg/kg bw	0.00 N Finishing (highest diet)					
Meat	-	-	-	-	-	-	0.03	0.03
Muscle	0.02	0.02	0.02	0.02	**0.02**	1.40	0.03	0.03
Fat	0.02	0.02	0.02	0.02	**0.02**	1.80	0.04	0.04
Liver	0.02	0.02	0.02	0.04	**0.05**	3.00	0.06	0.12
Kidney	0.02	0.02	0.02	0.02	**0.02**	3.80	0.08	0.08
Poultry (all diets)								
Closest feeding level[a]:	0.05	mg/kg bw	0.65 N Layer (highest diet)					
Meat	-	-	-	-	-	-	0.03	0.03
Muscle	0.02	0.02	0.02	0.02	**0.02**	n.c.	0.02	0.02
Fat	0.05	0.05	0.05	0.05	**0.05**	n.c.	0.05	0.05
Liver	0.05	0.05	0.05	0.05	**0.05**	n.c.	0.05	0.05
Poultry (layer only)								
Closest feeding level[a]:	0.05	mg/kg bw	0.65 N Layer					
Eggs[c]	0.02	0.02	0.02	0.02	**0.02**	n.c.	0.02	0.02

(a): Closest feeding level and N dose rate related to the maximum dietary burden.
(b): Highest residue level from day D1 to day D2 (daily mean of X cows).
(c): Highest residue level from day D1 to day D2 (daily mean of Y laying hens).

Confirmatory data gaps from Article 12 evaluation

Data gaps listed in the EC 396/2005 amending regulation Commission Regulation (EU) 2016/452 of 29 March 2016 following the EFSA Journal 2015;13(1):3992 "EFSA (European Food Safety Authority), 2015. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for spiroxamine according to Article 12 of Regulation (EC) No 396/2005" are shown below with comments on status or how they have been addressed.

1. The EU reference labs identified the reference standard for spiroxamine carboxylic acid metabolite M06 as commercially not available. When re-viewing the MRL, the Commission will take into account the commercial availability of the reference standard referred to in the first sentence by 30 March 2017, or, if that reference standard is not commercially available by that date, the unavailability of it.
    a. The Applicant has confirmed that the reference standard for metabolite M06 can be supplied on request from EU reference laboratories
2. The European Food Safety Authority identified some information on storage stability and toxicological data of plant metabolites as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 30 March 2018, or, if that information is not submitted by that date, the lack of it. 0151010 Table grapes 0151020 Wine grapes
    a. The storage stability study, submitted as part of this dossier, was conducted to address this point for spiroxamine in high acid (grapes) and high starch (grain). See CA 6.1/06.
    b. Toxicological data of plant metabolites M13 and M28 (Group B and Group C) was submitted and evaluated as part of the confirmatory data for spiroxamine. Refer to EFSA Supporting publication 2018:EN-1360 and EFSA Journal 2021;19(1):6385.
3. The European Food Safety Authority identified some information on toxicological data of plant metabolites as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 30 March 2018, or, if that information is not submitted by that date, the lack of it. 0163020 Bananas
    a. Toxicological data of plant metabolites M13 and M28 (Group B and Group C) was submitted and evaluated as part of the confirmatory data for spiroxamine. Refer to EFSA Supporting publication 2018:EN-1360 and EFSA Journal 2021;19(1):6385.
4. The European Food Safety Authority identified some information on storage stability as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 30 March 2018, or, if that information is not submitted by that date, the lack of it. 0500010 Barley 0500050 Oat 0500070 Rye 0500090 Wheat
    a. The storage stability study, submitted as part of this dossier, was conducted to address this point for spiroxamine in high acid (grapes) and high starch (grain). See CA 6.1/06.
5. The European Food Safety Authority identified some information on feeding studies in accordance with the proposed residue definition as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 30 March 2018, or, if that information is not submitted by that date, the lack of it. 1016010 Muscle 1016020 Fat tissue 1016030 Liver 1030000 Birds eggs 1030010 Chicken 1030020 Duck 1030030 Geese 1030040 Quail 1030990 Others'
    a. These poultry commodities are considered by the Applicant to be covered by the existing poultry feeding study and the previous RMS-DE agreed that it was not necessary to conduct a new study involving unnecessary use of animals. The existing

study is considered to be acceptable since the data generated according to the residue method determines spiroxamine, spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) as a total spiroxamine equivalents basis and even at the highest dose level (approximately 2N the highest dietary burden for laying hens) residues in all edible commodities are below the analytical LOQ (0.02 or 0.05 mg/kg). Therefore, even though the data are not generated fully in compliance with the current and proposed residue definition for monitoring, data from the hen metabolism study shows that in all poultry edible commodities, residues would be detectable if significant transfer from feed occurred. Therefore no positive MRLs above the LOQ are proposed and no new poultry feeding study is required.

All of the requested confirmatory data related to the Article 12 MRL evaluation for spiroxamine for points 1 to 4 above has been responded to or data generated and evaluated at EU level.

For point 5 no new data have been generated as this is not considered necessary by the Applicant and would represent an unnecessary use of animals.

The RMS is asked to consider the above points in the renewal of approval evaluation and to document this in the Renewal Assessment Report.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

Not applicable.

CA 6.8 Proposed safety intervals

Pre-harvest intervals

Proposed minimum pre-harvest intervals for the use of spiroxamine on the representative crops are detailed in Table CA 6.8-1.

Table CA 6.8-1 Proposed minimum pre-harvest intervals

Crop	Application method	Minimum pre-harvest interval (days)
Grapes (table) cGAP	Foliar spray	14
Grapes (wine) cGAP	Foliar spray	35
Grapes early season (BBCH 13-19)	Foliar spray	Not applicable (BBCH dependent)
Barley / oats	Foliar spray	Not applicable (BBCH dependent)
Wheat / rye / triticale	Foliar spray	Not applicable (BBCH dependent)

Withholding periods or storage periods, in the case of post-harvest uses.

Not applicable.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Acceptable Daily Intake (ADI) and dietary exposure calculation

For spiroxamine, an Acceptable Daily Intake (ADI) of 0.025 mg/kg bw/day is proposed (no change from current LoEP). Refer to Document N1 of this submission for details. This is the reference value which is used here in consumer risk assessment.

Acute Reference Dose (ARfD) and dietary exposure calculation

For spiroxamine, an Acute Reference Dose (ARfD) of 0.1 mg/kg bw/day is proposed (no change from current LoEP). Refer to Document N1 of this submission for details. This is the reference value which is used here in consumer risk assessment.

Input values for consumer risk assessment are shown in Table CA 6.9-1. Proposed MRLs are shown in Table CA 6.7.2-1.

Table CA 6.9-1 Input values for the consumer risk assessment (first tier no refinement)

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Grapes - table	0.72	MRL x CF (1.8)	0.72	MRL x CF (1.8)
Grapes - wine	1.44	MRL x CF (1.8)	1.44	MRL x CF (1.8)
Barley grain	0.28	MRL x CF (4)	0.28	MRL x CF (4)
Oats (extrapolation)	0.28	MRL x CF (4)	0.28	MRL x CF (4)
Wheat grain	0.12	MRL x CF (4)	0.12	MRL x CF (4)
Rye (extrapolation)	0.12	MRL x CF (4)	0.12	MRL x CF (4)
Products of animal origin				
Swine muscle	0.028	MRL x CF (1.4)	0.028	MRL x CF (1.4)
Swine fat tissue	0.036	MRL x CF (1.8)	0.036	MRL x CF (1.8)
Swine liver	0.15	MRL x CF (3.0)	0.15	MRL x CF (3.0)
Swine kidney	0.076	MRL x CF (3.8)	0.076	MRL x CF (3.8)
Swine edible offals	0.076	MRL x CF (3.8)	0.076	MRL x CF (3.8)
Bovine muscle	0.042	MRL x CF (1.4)	0.042	MRL x CF (1.4)
Bovine fat tissue	0.054	MRL x CF (1.8)	0.054	MRL x CF (1.8)
Bovine liver	0.30	MRL x CF (3.0)	0.30	MRL x CF (3.0)
Bovine kidney	0.304	MRL x CF (3.8)	0.304	MRL x CF (3.8)
Bovine edible offals	0.304	MRL x CF (3.8)	0.304	MRL x CF (3.8)
Sheep muscle	0.042	MRL x CF (1.4)	0.042	MRL x CF (1.4)
Sheep fat tissue	0.09	MRL x CF (1.8)	0.09	MRL x CF (1.8)
Sheep liver	0.60	MRL x CF (3.0)	0.60	MRL x CF (3.0)
Sheep kidney	0.57	MRL x CF (3.8)	0.57	MRL x CF (3.8)
Sheep edible offals	0.57	MRL x CF (3.8)	0.57	MRL x CF (3.8)
Goat muscle	0.042	MRL x CF (1.4)	0.042	MRL x CF (1.4)

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Goat fat tissue	0.09	MRL x CF (1.8)	0.09	MRL x CF (1.8)
Goat liver	0.60	MRL x CF (3.0)	0.60	MRL x CF (3.0)
Goat kidney	0.57	MRL x CF (3.8)	0.57	MRL x CF (3.8)
Goat edible offals	0.57	MRL x CF (3.8)	0.57	MRL x CF (3.8)
Equine muscle	0.042	MRL x CF (1.4)	0.042	MRL x CF (1.4)
Equine fat tissue	0.054	MRL x CF (1.8)	0.054	MRL x CF (1.8)
Equine liver	0.30	MRL x CF (3.0)	0.30	MRL x CF (3.0)
Equine kidney	0.304	MRL x CF (3.8)	0.304	MRL x CF (3.8)
Equine edible offals	0.304	MRL x CF (3.8)	0.304	MRL x CF (3.8)
Poultry muscle	0.028	MRL x CF (1.4)	0.028	MRL x CF (1.4)
Poultry fat tissue	0.055	MRL x CF (1.1)	0.055	MRL x CF (1.1)
Poultry liver	0.07	MRL x CF (1.4)	0.07	MRL x CF (1.4)
Poultry kidney	0.07	MRL x CF (1.4)	0.07	MRL x CF (1.4)
Poultry edible offals	0.07	MRL x CF (1.4)	0.07	MRL x CF (1.4)
Milk except sheep	0.024	MRL x CF (1.2)	0.024	MRL x CF (1.2)
Milk sheep	0.036	MRL x CF (1.2)	0.036	MRL x CF (1.2)
Birds eggs	0.028	MRL x CF (1.4)	0.028	MRL x CF (1.4)
Honey	0.2	MRL	0.2	MRL

TMDI CALCULATIONS

Long-term consumer exposure to potential residues resulting from the representative uses of spiroxamine is estimated according to the current EFSA PRIMo model revision 3.1 for chronic risk assessment (approved 22 March 2019) and the EFSA guidance document.

The TMDI values are calculated based on the proposed MRL values with application of the proposed conversion factors (CF) for risk assessment. The residues as entered into the EFSA model are for the supported grape and small grain cereal crop uses at the proposed or current MRL level, including food of animal origin and honey, refer to Table CA 6.9-1 for the inputs. For honey, a guideline study has been conducted (refer to CA 6.10.1/01), and based on these results a conservative MRL of 0.20 mg/kg has been proposed with no CF required.

The TMDI calculations give unrealistic worst-case estimates of intake because they assume that all commodities with proposed uses will contain residues at the MRL. No account is taken of the potential reduction in residues during transport and storage or during commercial and domestic processing. In practice, the actual intake is likely to be much lower than the calculated worst-case values.

The TMDI calculations for spiroxamine using the EFSA PRIMo model are presented in Table CA 6.9-2. With the current EFSA model the chronic risk assessment results show a low exposure against the EU reference value and ranges from 1 to 17% of ADI. The diet with the highest TMDI is "PT general" with 17% of ADI. For this diet, the highest contributor is wine grapes with 14% of ADI. The second diet with the highest TMDI is "FR adult" with 16% of ADI where wine grapes is again the major contributor at 13% of ADI.

The results indicate that there is no unacceptable chronic risk to human health from the consumption of crop commodities treated with spiroxamine including food of animal origin from potential transfer to livestock, according to the representative GAP considered. There is an acceptable margin of safety for the consumer risk assessment.

Table CA 6.9-2 TMDI for spiroxamine using the EFSA PRIMo Model Rev. 3.1 (MRLs, no refinement)

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Spiroxamine

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	0.025	ARfD (mg/kg bw):	0.1
Source of ADI:	EFSA/RMS	Source of ARfD:	EFSA/RMS
Year of evaluation:	2017	Year of evaluation:	2017

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment, children | Details - acute risk assessment, adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI: ---

TMDI/NEDI/IEDI calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contribution to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
	17%	PT general	4.29	14%	Wine grapes	2%	Wheat	0.8%	Table grapes		
	16%	FR adult	3.90	13%	Wine grapes	1%	Wheat	0.4%	Milk: Cattle		
	14%	RO general	3.53	10%	Wine grapes	2%	Wheat	1%	Milk: Cattle		
	14%	GEMS/Food G07	3.44	9%	Wine grapes	2%	Wheat	1%	Table grapes		
	13%	NL toddler	3.35	6%	Milk: Cattle	4%	Table grapes	2%	Wheat		
	11%	GEMS/Food G08	2.85	6%	Wine grapes	2%	Wheat	1.0%	Barley		
	11%	GEMS/Food G15	2.82	6%	Wine grapes	2%	Wheat	1.0%	Table grapes		
	11%	GEMS/Food G11	2.74	6%	Wine grapes	2%	Wheat	1%	Table grapes		
	11%	IE adult	2.69	7%	Wine grapes	1%	Wheat	0.7%	Table grapes		
	9%	DE child	2.24	4%	Table grapes	2%	Wheat	2%	Milk: Cattle		
	9%	DE general	2.23	5%	Wine grapes	1%	Milk: Cattle	0.9%	Wheat		
	9%	DE women 14-50 yr	2.19	5%	Wine grapes	1%	Milk: Cattle	1%	Wheat		
	8%	FR child 3 15 yr	2.09	2%	Wheat	2%	Milk: Cattle	2%	Wine grapes		
	8%	NL child	2.01	3%	Table grapes	2%	Milk: Cattle	2%	Wheat		
	8%	UK adult	1.94	6%	Wine grapes	0.8%	Wheat	0.3%	Milk: Cattle		
	8%	DK child	1.92	3%	Rye	2%	Wheat	1%	Milk: Cattle		
	8%	DK adult	1.91	5%	Wine grapes	1%	Wheat	0.5%	Milk: Cattle		
	8%	GEMS/Food G06	1.89	3%	Wheat	3%	Table grapes	0.4%	Wine grapes		
	7%	GEMS/Food G10	1.76	2%	Wine grapes	2%	Wheat	0.9%	Table grapes		
	7%	NL general	1.66	3%	Wine grapes	0.9%	Wheat	0.8%	Milk: Cattle		
	6%	FR toddler 2 3 yr	1.59	3%	Milk: Cattle	1%	Wheat	1%	Wine grapes		
	6%	UK vegetarian	1.58	5%	Wine grapes	1%	Wheat	0.3%	Milk: Cattle		
	6%	UK infant	1.55	4%	Milk: Cattle	1%	Wheat	0.3%	Oat		
	5%	UK toddler	1.33	2%	Milk: Cattle	2%	Wheat	0.7%	Table grapes		
	5%	ES adult	1.28	2%	Wine grapes	1%	Wheat	0.6%	Barley		
	4%	ES child	1.05	2%	Wheat	1%	Milk: Cattle	0.2%	Bovine: Muscle/meat		
	4%	SE general	0.93	2%	Wheat	1%	Milk: Cattle	0.7%	Bovine: Muscle/meat		
	4%	IT toddler	0.89	3%	Wheat	0.3%	Table grapes	0.0%	Barley		
	3%	FI adult	0.81	2%	Wine grapes	0.3%	Rye	0.2%	Table grapes		
	2%	FR infant	0.80	2%	Milk: Cattle	0.4%	Wheat	0.2%	Wine grapes		
	2%	IT adult	0.59	2%	Wheat	0.4%	Table grapes	0.0%	Barley		
	2%	FI 3 yr	0.57	0.7%	Table grapes	0.6%	Oat	0.6%	Wheat		
	2%	LT adult	0.47	0.5%	Rye	0.5%	Wheat	0.4%	Milk: Cattle		
	2%	FI 6 yr	0.42	0.5%	Table grapes	0.5%	Wheat	0.4%	Oat		
	1%	IE child	0.30	0.6%	Wheat	0.3%	Milk: Cattle	0.2%	Table grapes		
	1%	PL general	0.23	0.9%	Table grapes		Grapefruits				

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Spiroxamine is unlikely to present a public health concern.

NEDI CALCULATIONS

The TMDI values are significantly less than the ADI for spiroxamine so it is not necessary to calculate NEDI values to give a more realistic estimate of intake.

IESTI CALCULATIONS

Acute dietary risk assessments are presented for potential residues arising from the proposed representative use of spiroxamine. Short-term consumer exposure to potential spiroxamine residues is estimated according to the current EFSA PRIMo model revision 3.1 for acute risk assessment (approved 22 March 2019) and the EFSA guidance document[1][2].

IESTI (International Estimate of Short-Term Intake) values are generally calculated assuming that residues are present at the HR, except for commodities bulked or blended during processing, where the STMR-P is used. In this case the MRL for the representative crops including food of animal origin is used; this exhibits a worst-case scenario and does not take into account that some commodities can be considered as blended. The IESTI values are calculated based on current or proposed (where different) MRL values as presented in Table CA 6.7.2-1, with application of the proposed conversion factors (CF) for risk assessment, refer to Table CA 6.9-1 for the inputs.

The IESTI results for spiroxamine obtained using the EFSA PRIMo model 3.1 are presented in Table CA 6.9-3. The highest estimated short-term intake is for the consumption of table grapes by children (FI child 3 years) and represents 53% of the ARfD.

The results indicate that there is no unacceptable acute risk to human health from the consumption of supported crop commodities including food of animal origin treated with spiroxamine according to the representative GAP considered. There is an acceptable margin of safety for the consumer risk assessment.

[1] EFSA (European Food Safety Authority), Brancato A, Brocca D, Ferreira L, Greco L, Jarrah S, Leuschner R, Medina P, Miron I, Nougadere A, Pedersen R, Reich H, Santos M, Stanek A, Tarazona J, Theobald A and Villamar-Bouza L, 2018. Guidance on use of EFSA Pesticide Residue Intake Model (EFSA PRIMo revision 3). EFSA Journal 2018;16(1):5147, 43 pp. https://doi.org/10.2903/j.efsa.2018.5147

[2] EFSA (European Food Safety Authority), Anastassiadou M., Brancato A, Cabrera, L. C., Ferreira L, Greco L, Jarrah S, Kazocina, A., Leuschner R., Magrans J. O., Miron I, Pedersen R, Raczyk M., Reich H, Ruocco S., Sacchi A, Santos M, Stanek A, Tarazona J, Theobald A and Verani A., 2018. Technical Report Pesticide Residue Intake Model - EFSA PRIMo revision 3.1 (update of EFSA PRIMo revision 3). EFSA Supporting Publication 2019:EN-1605 pp 1-15

Table CA 6.9-3 IESTI for spiroxamine using the EFSA PRIMo Rev. 3.1.(MRLs, no refinement)

Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

Show results for all crops

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
53%	Table grapes	0.4 / 0.72	53
13%	Wine grapes	0.8 / 1.44	13
3%	Milk: Cattle	0.02 / 0.02	3.0
2%	Bovine: Liver	0.1 / 0.3	2.4
2%	Bovine: Edible offals (other	0.08 / 0.3	2.2
2%	Wheat	0.03 / 0.12	1.7
2%	Barley	0.07 / 0.28	1.6
1%	Bovine: Kidney	0.08 / 0.3	1.1
0.9%	Milk: Goat	0.03 / 0.04	0.87
0.8%	Rye	0.03 / 0.12	0.76
0.7%	Honey and other apicultural	0.2 / 0.2	0.72
0.5%	Poultry: Muscle/meat	0.02 / 0.03	0.48
0.3%	Eggs: Chicken	0.02 / 0.03	0.35
0.3%	Swine: Muscle/meat	0.02 / 0.03	0.34
0.3%	Oat	0.07 / 0.28	0.31

Expand/collapse list

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
34%	Wine grapes	0.8 / 1.44	34
24%	Table grapes	0.4 / 0.72	24
2%	Sheep: Liver	0.2 / 0.56	1.6
1%	Barley	0.07 / 0.28	1.4
1%	Bovine: Liver	0.1 / 0.3	1.2
1%	Bovine: Edible offals (other	0.08 / 0.3	1.0
1%	Wheat	0.03 / 0.12	1.0
0.9%	Milk: Cattle	0.02 / 0.02	0.93
0.7%	Milk: Goat	0.03 / 0.04	0.66
0.6%	Bovine: Kidney	0.08 / 0.3	0.64
0.6%	Rye	0.03 / 0.12	0.58
0.5%	Milk: Sheep	0.03 / 0.04	0.54
0.4%	Sheep: Edible offals (other	0.15 / 0.57	0.39
0.3%	Poultry: Liver	0.05 / 0.07	0.33
0.3%	Poultry: Muscle	0.02 / 0.03	0.33

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
63%	Wine grapes / juice	0.8 / 1.44	83
1%	Wheat / milling (flour)	0.03 / 0.12	1.5
1%	Oat / boiled	0.07 / 0.28	1.0
1%	Barley / cooked	0.07 / 0.28	1.0
0.8%	Oat / milling (flakes)	0.07 / 0.28	0.84
0.7%	Wheat / milling (wholemeal)	0.03 / 0.12	0.67
0.5%	Barley / milling (flour)	0.07 / 0.28	0.51
0.4%	Rye / boiled	0.03 / 0.12	0.44
0.4%	Rye / milling (wholemeal)-bal	0.03 / 0.12	0.42
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
30%	Wine grapes / juice	0.8 / 1.44	30
14%	Wine grapes / wine	0.8 / 1.44	14
4%	Table grapes / raisins	0.8 / 3.38	4.1
2%	Barley / beer	0.07 / 0.06	2.0
0.5%	Wheat / bread/pizza	0.03 / 0.12	0.53
0.5%	Wheat / pasta	0.03 / 0.12	0.46
0.4%	Oat / boiled	0.07 / 0.28	0.43
0.4%	Wheat / bread (wholemeal)	0.03 / 0.12	0.42
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!
#NUM!	#NUM!	#NUM!	#NUM!

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity.
A short term intake of residues of Spiroxamine is unlikely to present a public health risk.
For processed commodities, no exceedance of the ARfD/ADI was identified.

CA 6.10 Other studies

A summary of a published paper regarding potential stereoselective metabolism of spiroxamine is submitted for consideration.

Data Point:	KCA 6.10/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Stereoselective metabolism of the sterol biosynthesis inhibitor fungicides fenpropidin, fenpropimorph, and spiroxamine in grapes, sugar beets, and wheat
Report No:	M-689208-01-1
Document No:	M-689208-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The following summary presents a paper from open literature that describes the potential for stereoselective metabolism of three fungicides, one being spiroxamine, in a number of crops including wheat, grapes and sugar beet. Only information pertinent to metabolism of stereoisomers of spiroxamine will be discussed in this summary. This paper is included as supporting information only as the isomer ratio of spiroxamine for the crops under consideration in this Renewal of Approval dossier has been evaluated during the conduct of crop residue trials and the data is discussed in full in Section CA 6.7.1.

It has long been noted that metabolism of chiral pesticides in crops are typically studied using achiral analytical methods and consequently the stereoisomer composition of residues in edible commodities is often unknown. The authors of this paper developed an enantioselective GC-MS/MS method to quantify residues of three fungicides including spiroxamine in plant matrices. The fungicides were applied to wheat, grapes or sugar beet. The four stereoisomers of spiroxamine were found to metabolise at different rates, but selectivity was only found between diastereomers and not between enantiomers.

It was concluded that *cis*-spiroxamine was preferentially degraded in grapes and *trans*-spiroxamine in wheat*.

*Note to reviewer: it was concluded upon technical review of this paper that there may be an error in the naming and configuration of the diastereomers of spiroxamine where A = *trans* and B = *cis*. As a result, this summary has transposed the naming of the *cis* and *trans* isomers from those described in the original paper.

The product Input Xpro (250 g/L spiroxamine, 100 g/L prothioconazole and 50 g/L bixafen) was applied once to wheat at BBCH 31 at a rate of 375 g spiroxamine/ha. Samples of forage were taken at 2 hours, 14, 29 and 57 days after application. The product Prosper (500 g/L spiroxamine) was applied to grapevines at BBCH 77 at a rate of 400 g a.s./ha. Samples were collected at 3 hours, 8, 14, 30, 50 and 76 days after application. [Note: for grapes these application rates, timings and sampling intervals do

not correspond to the critical GAP for Renewal of Approval of spiroxamine as discussed in CA 6.3, the application rate used in this paper is 133% of the critical application GAP].

Residues of spiroxamine in grape trials decreased from 19 mg/kg fresh weight in leaves after application to 0.59 mg/kg at harvest in leaves and 0.03 mg/kg in mature grapes. No significant change of the enantiomer fractions of cis- and trans-spiroxamine was observed, with enantiomer fraction (EF) values of 0.45−0.51 in leaves and grapes. However, the diastereomer composition of spiroxamine changed with time. In the leaves, the fraction of cis-spiroxamine decreased from 0.43 to 0.29 at harvest. The same extract from day 50, analysed with the achiral BGB-5 column showed the fraction of cis-spiroxamine was 0.30.

In mature grapes, the fraction of cis-spiroxamine was lower and amounted to 0.14. Hydrolysis experiments at pH 4 and 50 °C showed that the diastereomers do not interconvert but confirmed the faster degradation of cis-spiroxamine, with half-lives of 2.8 and 7.5 days for cis- and trans-spiroxamine, respectively.

In wheat, residues were up to 3 orders of magnitude lower on day 57 than directly after application. Cis- and trans-spiroxamine were metabolised without any enantioselectivity, however, selective with regard to its diastereomers. The fraction of cis-spiroxamine continuously increased from 0.43 immediately after application to 0.67 on day 57 (0.46 was measured in the applied product).

The four stereoisomers of spiroxamine were metabolised at different rates, but selectivity was only found between diastereomers and not between enantiomers. Cis-spiroxamine was preferentially degraded in grapes and trans-spiroxamine in wheat.

I. Materials and methods

The field phase for grape and wheat field trials was conducted at the research institute of Agroscope in Wädenswil, Switzerland between March 2014 and September 2014. No dates were reported for the analytical phase of the study.

Spiroxamine was applied to both grapevines and wheat and crops were cultivated according to good agricultural practice.

The grapevines (variety Riesling-Silvaner) were planted in 1998 in rows and were Guyot cane pruned. On July 15, 2014, Prosper (EC 500 g/L spiroxamine) was applied to 19 grapevines, at growth stage BBCH 77 (bunch closure). One row was left untreated for blank (control) samples. The application rate was 0.8 L/ha, corresponding to 400 g spiroxamine/ha. Leaf samples were taken three hours, 8, 14, 30, 50, and 76 days after application, and were stored in a freezer at -20°C before they were homogenized and extracted. Young leaves, which developed after fungicide application, were not sampled. Mature grapes were harvested on September 29 (day 76 after application).

Spring wheat (variety Forina) was sown on March 17, 2014 at a density of approximately 520 seeds/m^2 on a 6.6×30 m plot at Agroscope in Wädenswil. On May 05, Input Xpro (250 g/L spiroxamine, 100 g/L prothioconazole, and 50 g/L bixafen), was applied, at growth stage BBCH 31. A subplot was left untreated. The application rate of Input Xpro was 1.5 L/ha, corresponding to 375 g spiroxamine/ha. Samples of wheat forage were taken two hours, 14, 29, and 57 days after application, and were stored at -20°C before they were homogenized and extracted. Mature wheat was harvested on July 23, 79 days after application, but was not analysed as low residues were observed in the previous timepoint sample.

Plant samples from field trials were stored at -20°C for no more than 5 months before they were homogenised and extracted. This storage interval is therefore supported by the frozen storage stability studies detailed in CA 6.1.

Frozen plant samples were further cooled in liquid nitrogen before being homogenised using a knife mill. Homogenised plant samples were transferred to PETG wide-mouther bottles and closed. All samples were stored frozen (-20°C) until extraction approximately 24 hours later.

Extraction of samples was based on the QuEChERS multi-residue method, with some commonly used modifications. 10 g of homogenised plant material (or 5 g of wheat) was weighed into an extraction tube containing 6 g of magnesium sulphate and 1.5 g of sodium acetate. After addition of approximately 100 µg of the appropriate internal standard, dissolved in methanol, the samples were extracted with ethyl acetate by vigorous shaking for 5 minutes. The tubes were centrifuged and an aliquot of the extract was transferred into a clean-up tube containing 150 mg of magnesium sulphate and 50 mg of PSA sorbent (the primary secondary amine sorbent removes various polar, organic compounds such as acids, pigments, and sugars). After 1 minute of shaking, the tubes were centrifuged and the supernatant was transferred into an autosampler vial. Extracts were stored at 4°C, and GC-MS/MS analysis was performed within the next days.

Enantioselective GC-MS/MS

In all samples the stereoisomer composition was determined. An enantioselective analytical method based on gas chromatography–tandem mass spectrometry (GC-MS/MS) was developed by the research group to quantify residues of spiroxamine in plant matrices.

Spiroxamine (98.5%, 8-tert-butyl-1,4-dioxaspiro[4,5]decan-2-ylmethyl(ethyl)-(propyl)amine)) was obtained from Sigma Aldrich, Germany. Fenpropidin (96.6%, 1-[(RS)-3-(4-*tert*-butylphenyl)-2-methylpropyl]-piperidine), also obtained from Sigma Aldrich was used as an internal standard. Small quantities of pure diastereomers of spiroxamine were isolated by HPLC. Fractions of the two diastereomers of spiroxamine were collected after separation on an achiral Luna phenyl-hexyl column (250×3.0 mm, 5 µm, Phenomenex) using 10 mM ammonium acetate buffer (adjusted to pH 8.3 with ammonia) and methanol as eluents, with the following gradient: 60% methanol to 99% methanol within 1 min, followed by 24 min of isocratic elution, then back to initial composition within 1 min, and 4 min equilibration time (flow rate, 0.15 mL/min; detection at 235 nm).

Cis- and trans-spiroxamine were separated on a chiral GC column coated with tert-butyldimethylsilyl-β-cyclodextrin (BSCD, 20% in 15% phenyl-, 85% methylpolysiloxane (BGB 172, 15 m, 0.25 mm i.d., 0.12 µm film, BGB, Boeckten, Switzerland). GC conditions were as follows: instrument, Agilent 6890N with a COMBI PAL autosampler; 1 µL split/splitless injection (220°C, initial 60 s splitless); temperature program, 70°C, 2 min isothermal, 10°C/min to 160°C, 1°C/min to 185°C, isothermal hold at 220°C; constant pressure, 100 kPa helium. The GC was coupled to a Quattro Micro triple quadrupole mass spectrometer, operated in electron impact ionization (70 eV, 180°C) and multiple-reaction-monitoring mode. Spiroxamine was quantified using the ion transition m/z 100→72 (100→58 for confirmatory purposes). Quantification was based on peak area ratios relative to the internal standards and in reference to suitable standard solutions of the racemic compounds in ethyl acetate.

Determination of the diastereomer composition of spiroxamine with achiral GC-MS/MS

Cis- and trans-spiroxamine were quantified using an achiral BGB 5 column (5% phenyl-, 95% methylpolysiloxane, 30 m, 0.32 mm i.d., 0.25 µm film). GC conditions were as follows: 1 µL split/splitless injection (260°C, initial 60 s splitless); temperature program, 70°C, 2 min isothermal, 25°C/min to 150°C, 10°C/min to 250°C, isothermal hold at 250°C; constant flow, 1.6 mL/min helium. MS conditions were identical to those with the enantioselective GC column. For quantification, matrix matched standard solutions were used. This achiral GC-MS/MS method allowed for a more accurate determination of the diastereomer composition because the two diastereomers eluted within less than 1 minute, whereas on the chiral column, the individual stereoisomers were separated by almost 9 minutes.

Spiroxamine was also analysed on a GC instrument equipped with a flame ionization detector (FID, Agilent 6890N, chromatographic parameters as described above). This method was used to determine

the diastereomer composition in the fungicide formulations Input Xpro and Prosper, and in the reference standard, assuming equal response of both diastereomers with this system.

II. Results and discussion

Residues of spiroxamine in grape trials decreased from 19 mg/kg fresh weight in leaves after application to 0.59 mg/kg in leaves at harvest and 0.03 mg/kg in mature grapes. The authors of this paper stated that is was known that spiroxamine was known to rapidly penetrate into plants (information taken from the original spiroxamine DAR) followed by acropetal translocation to the leaf tips and it was therefore concluded that metabolism was primarily responsible for the residue decline. No significant change of the enantiomer fractions of cis- and trans-spiroxamine was observed with EF values of 0.45−0.51 in leaves and grapes. An extract from day 50, measured with the chiral BSCD column, showed that the enantiomer composition of cis- and trans-spiroxamine was still racemic. However, the diastereomer composition of spiroxamine changed with time. In the leaves, the fraction of cis-spiroxamine decreased from 0.43 to 0.29 at harvest. The diastereomer composition of spiroxamine in the applied product was not exactly 1:1 (0.48 was measured in the applied product). The same extract from day 50, analysed with the achiral BGB-5 column showed the fraction of cis-spiroxamine was 0.30.

In mature grapes, the fraction of cis-spiroxamine was even lower and amounted to 0.14. Small amounts of the pure diastereomers were prepared to investigate whether the preferential hydrolysis of cis-spiroxamine in acidic water may also be the result of a conversion to trans-spiroxamine. However, this was not the case. Hydrolysis experiments at pH 4 and 50°C showed that the diastereomers do not interconvert but confirmed the faster degradation of cis-spiroxamine, with half-lives of 2.8 and 7.5 d for cis- and trans-spiroxamine, respectively. This preferential hydrolysis may, in part, explain the low fractions of cis-spiroxamine measured in grapes and grape leaves. Aqueous suspensions of homogenised plant material (ratio, 2.5:1) were quite acidic, with pH values of 3.7 and 4.0, respectively (76 d after application). Enzyme mediated metabolism was also expected to contribute to the observed stereoselectivity.

In wheat residue trials residues were up to 3 orders of magnitude lower on day 57 than directly after application. Although, metabolism still considerably contributed to the observed residue decline, an increase in plant biomass (approximately by a factor of 10) and heavy rainfall two days after application (which may have washed off a certain amount of fungicide) were also factors. Cis- and trans-spiroxamine were metabolised without any enantioselectivity. Metabolism of spiroxamine was, however, selective with regard to its diastereomers. The fraction of cis-spiroxamine continuously increased from 0.43 immediately after application to 0.67 on day 57 (0.46 was measured in the applied product). This trend was the opposite to that observed in grape leaves (and grapes). The preferential hydrolysis of cis-spiroxamine at low pH may, in part, explain the findings in grapes. In wheat forage, the pH was clearly higher (pH 6.2 in an aqueous suspension of homogenized wheat forage) and abiotic hydrolysis probably was insignificant. The preferential metabolism of trans-spiroxamine in wheat observed in this paper may therefore be attributed to diastereomer-selective enzymatic degradation.

The four stereoisomers of spiroxamine were also metabolised at different rates, but selectivity was only found between diastereomers and not between enantiomers. Cis-spiroxamine was preferentially degraded in grapes and trans-spiroxamine in wheat.

III. Conclusions:

This paper is included as supporting information only as the isomer ratio of spiroxamine for the crops under consideration in this Renewal of Approval dossier has been evaluated during the program of crop residue trials and the data is discussed in full in CA 6.7.1.

Input Xpro (250 g/L spiroxamine, 100 g/L prothioconazole and 50 g/L bixafen) was applied to wheat at BBCH 31 at a rate of 375 g spiroxamine/ha. Samples of forage were taken at 2 hours, 14, 29 and 57 days after application. Prosper (500 g/L spiroxamine) was applied to grapevines at BBCH 77 at a rate of 400 g a.s./ha. Samples were collected at 3 hours, 8, 14, 30, 50 and 76 days after application.

Residues of spiroxamine in grape trials decreased from 19 mg/kg fresh weight in leaves after application to 0.59 mg/kg at harvest and 0.03 mg/kg in mature grapes. No significant change of the enantiomer fractions of cis- and trans-spiroxamine was observed, with EF values of 0.45−0.51 in leaves and grapes. However, the diastereomer composition of spiroxamine changed with time. In the leaves, the fraction of cis-spiroxamine decreased from 0.43 to 0.29 at harvest. The same extract from day 50, analysed with the achiral BGB-5 column showed the fraction of cis-spiroxamine was 0.30.

In mature grapes, the fraction of cis-spiroxamine was lower and amounted to 0.14. Hydrolysis experiments at pH 4 and 50°C showed that the diastereomers do not interconvert but confirmed the faster degradation of cis-spiroxamine, with half-lives of 2.8 and 7.5 d for cis- and trans-spiroxamine, respectively.

In wheat residue trials, residues were up to 3 orders of magnitude lower on day 57 than directly after application. Cis- and trans-spiroxamine were metabolised without any enantioselectivity, however, selective with regard to its diastereomers. The fraction of cis-spiroxamine continuously increased from 0.43 immediately after application to 0.67 on day 57 (0.46 was measured in the applied product).

The four stereoisomers of spiroxamine were metabolised at different rates, but selectivity was only found between diastereomers and not between enantiomers. Cis-Spiroxamine was preferentially degraded in grapes and trans-spiroxamine in wheat.

Assessment and conclusion by applicant:

Acceptable paper as supporting information only. Paper not previously submitted.

Note to reviewer: it was concluded upon technical review of this paper that there may be an error in the naming and configuration of the diastereomers of spiroxamine where A = trans and B = cis. As a result, this summary has transposed the naming of the cis and trans isomers from those described in the original paper.

The four stereoisomers of spiroxamine were metabolised at different rates, but selectivity was only found between diastereomers and not between enantiomers. Cis-Spiroxamine was preferentially degraded in grapes and trans-spiroxamine in wheat.

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of these studies is to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

New guidance was published in 2018[3] and was implemented from January 2020.

The GAP of the intended uses for renewal of approval of spiroxamine indicates only grapes are considered melliferous. Although not a typical rotated field crop, in order to cover an assumed 100% overspray situation at field margins, a study has been conducted to determine the residues of spiroxamine in honey after application to a surrogate melliferous crop at the maximum seasonal intended use rate for the active substance during flowering of the crop.

[3] Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey. SANTE/11956/2016 rev. 9 (14 September 2018)

Data Point:	KCA 6.10.1/01
Report Author:	[redacted]
Report Year:	2021
Report Title:	Determination of residues of spiroxamine in honey after two applications of spiroxamine EC 500 in Phacelia tanacetifolia at 4 sites in Northern and Southern Europe in 2020
Report No:	S20-00497
Document No:	M-763138-01-1
Guideline(s) followed in study:	OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) Commission Regulation (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 (Oct. 2009) Commission Regulation (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 (Oct. 2009)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and methods

A total of 4 trials conducted during growing season 2020 are available to evaluate the residues of spiroxamine in honey after two foliar spray applications of Spiroxamine EC 500 to *Phacelia tanacetifolia*. Applications were performed at BBCH 61 to BBCH 65, at a nominal rate of 0.75 L product/ha (375 g a.s./ha). The last application was conducted 5 to 10 days before sampling of the honey.

A broad geographical distribution of trials was achieved, with two supervised field trials being established in northern Europe in Germany and two in southern Europe in Spain. There were no reported significant weather events or deviations to the typical climate in northern and southern Europe. The trial parameters and residue results are summarised in Table CA 6.10.1-1.

The field sites in Germany were located in Stutensee, Baden-Württemberg (trial S20-00497-01) and Heelbronn-Bockingen, Baden-Württemberg (S20-00497-02) and those in Spain at Avora, Valencia (S20-00497-03) and Xative, Valencia (S20-00497-04). The distance between the German trials was approximately 49 km with 47 km between the Spanish trials.

In all trials, two foliar spray applications of spiroxamine were made to the phacelia crop during flowering, the first at BBCH 61 to BBCH 63 and the second at BBCH 63 to BBCH 65. The application rate was nominally 375 g a.s./ha (0.75 L product/ha) with actual values ranging from 357 to 387 g a.s./ha. The use pattern of the study is representative of the total seasonal rate for the supported critical GAP on cereals in order to cover an assumed (theoretical) worst case 100% overspray situation at field margins. Spray volumes for all trials ranged from 191 to 207 L/ha.

All trials were conducted using Spiroxamine EC 500 formulation (actual content 494.1 g/L spiroxamine). Honey was sampled from each tunnel at each trial site and was collected from initially empty combs which were introduced into each hive shortly before application. Honey was collected for residue analysis once mature, or if the water content was <20% or after comb closure (whichever came first) and was generally 5 to 10 days after the second application.

Analysis of spiroxamine residues in honey was performed according to validated chiral method 01480/M001, report reference M-763118-01-1 and as the common moiety method 00312/M004, report reference M-763119-01-1 **(see Doc MCA Section 4)**.

Spiroxamine parent enantiomers

The analytical method 01480/M001 was modified to determine the residues of spiroxamine in/on honey, pollen and nectar as sum of its four enantiomers A1, A2, B1 and B2 by HPLC-MS/MS detection. The samples (approximately 1.0 g) were diluted with a methanol/water mixture (3/1, v/v). After filtration of the raw extract, an aliquot was analysed by high performance liquid chromatography, chromatographed under chiral reverse phase column chromatography and detected by Tandem Mass Spectrometry with electrospray ionisation. Residues were quantified using solvent standards with an isotopic stable-labelled internal standard.

Concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples). The respective Limit of Quantification (LOQ) for the analytes, defined as the lowest validated fortification level, was 0.01 mg/kg (sum of four enantiomers) in honey. The corresponding respective Limit of Detection (LOD) was 0.003 mg/kg (sum of four enantiomers).

Total residue of spiroxamine

The analytical method 00312/M004 was modified to determine the total residue of spiroxamine as 4-t-butylcyclohexanone in/on honey by LC-MS/MS detection.

Samples (approximately 1.0 g) were diluted with a methanol/water mixture (1/3, v/v) and hydrolysed with hydrochloric acid under reflux conditions. After extraction with cyclohexane and concentration, the extracts were derivatised with 2,4-dinitrophenylhydrazine solution in sulphuric acid/methanol (1/4, v/v) to the corresponding hydrazone. The mixture was basified and filtered and an aliquot was analysed by high performance liquid chromatography, chromatographed under reversed-phase column chromatography and detected by Tandem Mass Spectrometry with electrospray ionisation. Residues of derivatised 4-TBCH were quantified using solvent standards with an isotopic stable-labelled internal standard.

Concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples). The respective Limit of Quantification (LOQ) for the analytes, defined as the lowest validated fortification level, was 0.01 mg/kg (expressed as spiroxamine) in honey. The corresponding respective Limit of Detection (LOD) was 0.003 mg/kg (expressed as spiroxamine).

The honey samples were stored deep frozen within 5 hours of sampling at ≤18°C. As shown in Table CA 6.10.1/01-4, samples being analysed by the chiral method were stored for a maximum of 123 days between sampling and extraction and between 160 to 174 days for the common moiety method. A storage stability testing is currently on-going, but no issues with stability on frozen storage are anticipated. These storage periods are covered by the available storage stability data for honey generated as part of the method validation studies which demonstrate stability of spiroxamine in honey samples for up to 6 months (refer to Point CA 6.1).

II. Results and discussion:

The recoveries from fortified samples analysed concurrently with treated samples were acceptable and mean results for each matrix were within the range of 70 to 110% (refer to Table CA 6.10.1/01-2 and Table 6.10.1/01-3). The limit of quantification (LOQ) for spiroxamine (sum of four enantiomers) and total spiroxamine (expressed as spiroxamine) in honey from phacelia was 0.01 mg/kg with a limit of detection (LOD) set at 0.003 mg/kg (30% of the LOQ) for both methods.

No residues above the LOQ (0.01 mg/kg) were detected in the control specimens.

For trials conducted under field conditions in NEU (Germany) on phacelia, spiroxamine residues (sum of 4 enantiomers) in mature honey sampled from treated crop were <0.01 and 0.0759 mg/kg. Corresponding reported total spiroxamine residues (via 4-TBCH) were 0.0167 and 0.102 mg/kg.

For trials conducted under field conditions in SEU (Spain) on phacelia, spiroxamine residues (sum of 4 enantiomers) in honey sampled from treated crop were <0.01 and 0.131 mg/kg. Corresponding reported total spiroxamine residues (via t-TBCH) were <0.01 and 0.167 mg/kg.

Table CA 6.10.1/01-1 Residue trials with Spiroxamine EC 500 on *Phacelia tanacetifolia* in Northern and Southern Europe – honey data

Doc. No. Trial Ref Location Crop Year	Application				Aerial crop part	DALA (days)	Reported residue spiroxamine enantiomers (mg/kg)					Reported total residue of spiroxamine (via 4-TBCH, mg/kg)
	No.	g a.s./ha	L/ha	Growth stage			A1 enantiomer	A2 enantiomer	B1 enantiomer	B2 enantiomer	Total residue of spiroxamine enantiomers	
CA 6.10.1/01 S20-00497-01 Germany, 76297, Stutensee, Baden-Württemberg Phacelia 2020	2	372 378	198 202	BBCH 61 BBCH 65	Honey	6	0.0206	0.0226	0.0170	0.0157	0.0759	0.102
CA 6.10.1/01 S20-00497-02 Germany, 74080, Heilbronn - Böckingen, Baden-Württemberg Phacelia 2020	2	373 374	199 199	BBCH 61 BBCH 63	Honey	7	<0.0027	<0.0027	<0.0023	<0.0023	<0.01	0.0167
CA 6.10.1/01 S20-00497-03 Spain, 46620, Ayora, Valencia Phacelia 2020	2	387 385	207 205	BBCH 61 BBCH 63-65	Honey	7	0.0338	0.0352	0.0314	0.0304	0.131	0.166, 0.169 [0.167[1]]
CA 6.10.1/01 S20-00497-02 Spain, 46800, Xativa, Valencia Phacelia 2020	2	381 357	203 191	BBCH 61 BBCH 63-65	Honey	10	<0.0027	<0.0027	<0.0023	<0.0023	<0.01	<0.01

1 – Mean residue of duplicate analysis

Table CA 6.10.1/01-2 Procedural recovery data for the determination of spiroxamine enantiomers

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.10.1/01	01480/M001	A1 enantiomer	Honey	0.0027 0.027 Overall	3 3 6	105; 102; 91 94; 107; 107 Mean: 101, RSD: 6.8
CA 6.10.1/01	01480/M001	A2 enantiomer	Honey	0.0027 0.027 Overall	3 3 6	101; 115; 99 110; 96; 103 Mean: 104, RSD: 6.9
CA 6.3.2/13	01480/M001	B1 enantiomer	Honey	0.0023 0.023 Overall	3 3 6	111; 109; 90 90; 110; 97 Mean: 101, RSD: 9.9
CA 6.3.2/13	01480/M001	B2 enantiomer	Honey	0.0023 0.023 Overall	3 3 6	106; 103; 88 90; 103; 93 Mean: 97, RSD: 8.0

Table CA 6.10.1/01-3 Procedural recovery data for the determination of total spiroxamine (via 4-t-butylcyclohexanone)

Document	Analytical method ref.	Analyte	Matrix	Fortification level (mg/kg)	Number of replicates (n)	Recovery rates (%)
CA 6.10.1/01	00312/M004	Total residue of spiroxamine	Honey	0.01 0.1 Overall	3 3 6	114; 108; 108 81; 85; 76 Mean: 95, RSD: 17.3

Table CA 6.10.1/01-4 Storage of honey samples before determination of spiroxamine residues

Document	Matrix	Minimum to maximum storage period from sampling to analysis [extraction] (days)
CA 6.10.1/01	Honey	12 to 123 (parent spiroxamine enantiomers) 20 and 174 (total residue of spiroxamine)

Isomers

Impact of isomers on risk assessment [EFSA Journal 2019;17(8):5804]

The following information evaluates the available information on the isomers of spiroxamine in honey as presented in the tables above and the impact on risk assessment.

As previously discussed in detail in CA 6.7, spiroxamine consists of two diastereomers, each with 1:1 mixture of enantiomers. From a chemistry perspective, there is no interconversion of diastereomers unless harsh chemical process conditions are applied which do not occur under environmental conditions post application to crops.

Interconversion of the enantiomers is considered to be even more unlikely and this is supported by the analytical data from residue trials.

Honey

Spiroxamine enantiomer ratio [A1:A2:B1:B2 or A:B] does not vary significantly in honey at harvest compared with the ratio in the test item (obtained from the Certificate of Analysis). In two of the trials, the data are inconclusive due to residues <LOQ.

When A (trans) and B (cis) isomers are considered as diastereomers and the EFSA guidance stereoisomer excess (se) values are determined, the difference in se values is <10% for honey.

Also, the % difference in A (trans) diastereomers in honey when compared to the test item is <10%. The A isomers are known to be the most fungicidally active.

Conclusion: parent spiroxamine isomers do not vary significantly in honey based on the data presented in the summary above. No impact on risk assessment and no isomer ratio uncertainty factor is required.

Honey isomer ratio data (from chiral analysis of spiroxamine)

Report: M-763138-01-1, 2021 'Determination of residues of spiroxamine in honey after two applications of spiroxamine EC 500 in phacelia tanacetifolia at 4 sites in northern and southern Europe in 2020'

Test item ratio (taken from CoA)

Test item isomer ratio					Test item (%)		Stereoisomer excess (se)
A1	A2	B1	B2		A	B	
0.262	0.262	0.238	0.238		52.44	47.56	4.88

Table CA 6.10.1/01-5 Trials 01 to 04 Summarised under CA 6.10.1/01

Honey (mature)	Isomer residue (mg/kg)				Isomer ratio fraction				% difference from test item			
Trial No.	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
S20-00487-01	0.0206	0.0226	0.017	0.0157	0.271	0.298	0.224	0.207	3.592	13.649	-5.891	-13.088
S20-00487-02	<0.00027	<0.00027	<0.0023	<0.0023	LOQ	LOQ	LOQ	LOQ	-	-	-	-
S20-00487-03	0.0338	0.0352	0.0314	0.0304	0.258	0.269	0.240	0.232	-1.370	2.715	0.866	-2.346
S20-00487-04	<0.00027	<0.00027	<0.0023	<0.0023	LOQ	LOQ	LOQ	LOQ	-	-	-	-

Honey (mature)	enantiomers sum		% diff from test item		stereoisomer excess (se) overview		
Trial No.	%A	%B	A	B	se (%A-%B)	Change of se from test item	% increase for A
S20-00487-01	56.92	43.08	8.54	-9.41	13.83	8.95	4.5
S20-00487-02	-	-	-	-	-	-	-
S20-00487-03	52.75	47.25	0.60	-0.66	5.50	0.62	0.3
S20-00487-04	-	-	-	-	-	-	-

III. Conclusions

A total of 4 trials were conducted in Germany and Spain on *Phacelia tanacetifolia* during growing season 2020 to evaluate the residues of spiroxamine (total isomers) and total spiroxamine (via 4-t-butylcyclohexanone) in honey after two foliar spray applications of Spiroxamine EC 500 at 375 g a.s./ha.

No residues at or above the limit of detection were found in any of the untreated samples. In the treated samples, spiroxamine residues (sum of 4 enantiomers) in honey sampled from treated plots ranged from <0.01 mg/kg to 0.131 mg/kg at 5 to 10 DALA. Corresponding reported total spiroxamine residues (via 4-TBCH) ranged from <0.01 to 0.167 mg/kg.

Based on the data from these four trials, a conservative MRL proposal for spiroxamine in honey of 0.2 mg/kg is proposed with no required conversion factors for risk assessment. The monitoring residue definition for honey is the same as for primary crops, **parent spiroxamine**.

Parent spiroxamine isomers do not vary significantly in honey based on the data presented in the summary above. No impact on risk assessment and no isomer ratio uncertainty factor is required.

Assessment and conclusion by applicant:

Acceptable study to address the data point. New study that has not been previously submitted. The study is considered compliant with OECD Guideline 509 – Crop Field Trials, September 2009 and also SANTE/11956/2016 rev. 9, EC (2018).

Based on the data from these four trials, a conservative MRL proposal for spiroxamine in honey of 0.2 mg/kg is proposed with no required conversion factors for risk assessment. The monitoring residue definition for honey is the same as for primary crops, **parent spiroxamine**.

Parent spiroxamine isomers do not vary significantly in honey based on the data presented in the summary above. No impact on risk assessment and no isomer ratio uncertainty factor is required.