Bayer CropScience

Document Title

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

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013

Document MCA

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

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Date

2016-03-28

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Version history

Date	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.

As published in Commission Directive 2008/44/EC of 04th April 2008 and with an Entry into Force (EIF) date of 01st August 2008, the fungicide fluoxastrobin was first included in Annex I to Commission Directive 91/414/EEC.

Now, with the aim to achieve European Re-Approval under Regulation 1107/2009, Bayer CropScience (BCS) provides this '**Supplementary Dossier**'. It contains only new data which were not submitted at the time of the Annex I inclusion of fluoxastrobin under Commission Directive 91/414/EEC and which were therefore not evaluated during the first European review.

In addition to submitting the above mentioned Supplementary Dossier, all studies relied upon under 91/414 and contained in the Draft Assessment Report and its Addenda are – for the convenience of the reviewers – included in what BCS calls '**Baseline Dossier**' (Document K level only).

In order to ease the reviewers' orientation on 'old' studies in the Baseline Dossier versus 'new' studies in the Supplementary Dossier, BCS has decided to apply the following basic principles:

1. Conversion of the Document K part of the old EU dossier structure into the new structure (acc. to Commission Regulations 283/2013 and 284/2013 and linking the old studies to the new structure according to the cross-walk tables provided in Guidance Document SANCO/10181/2013 – rev. 2.1 of 13th May 2013).
2. On a case-by-case basis and where useful for the reader, old studies from the Baseline Dossier are occasionally summarised on the Document M level of the Supplementary Dossier; the text of those summaries is formatted in grey font colour.
3. For any referenced old study, its bibliographic information (e.g. author, year, document number) is formatted in grey font colour.
4. For any new study, its bibliographic information and its free flow summary text and table content is formatted in standard black font colour.

Where applicable, the above formatting rules apply to all dossier elements (e.g. MCA, MCP, JCA etc.).

According to the guidance of EFSA on the "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" (EFSA Journal 2011; 9(2):2092), literature for the active substance and its metabolites needs to be presented, covering the last 10 years prior to the submission of this Annex I renewal dossier. In relation to this section 6 no adequate scientific peer-reviewed open literature was identified which would need to be reported. There were no findings in the scientific peer-reviewed open literature for the active substance fluoxastrobin and its metabolites which might have a possible impact on an end-point or the risk assessments.

The crops for which uses were supported in the initial Annex II dossier were wheat, rye and barley (seed dressing followed by two spray applications with an FS 080 and EC 100, respectively). The evaluated representative uses as fungicide comprised foliar spraying to control a range of fungal diseases in wheat, rye and barley at an application rate of 2 ×200 g fluoxastrobin per hectare.

The scientific information evaluated in the EU peer review is summarised in the DAR (June 2005) including addenda and the EFSA Scientific Report (2007) 102, 1-84, Conclusion on the peer review of fluoxastrobin (rev 2007), finalized the 13th June 2007.

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Confirmatory data related to the residue section were evaluated by the RMS UK and the assessment was made available with Addendum 8 to the DAR (initially January 2011 and a revised and updated version, April 2012). After assessment of the confirmatory data, the revised review report (SANCO/3921/07 dated 28 September 2012) was issued.
Fluoxastrobin was evaluated in the frame of the review of the existing MRLs according to Art. 12 of Reg. (EC) 396/2005 (EFSA Reasoned opinion; 2012; 10(12):3012).

This Supplementary Dossier contains only detailed study summaries of additional studies which were not part of the dossier during inclusion in Annex I of Directive 91/414 and were, therefore, not evaluated during the first EU review of this compound. The summaries on the relevant endpoints were taken from the EFSA-DAR and its Addenda, from the EFSA Conclusion and the EFSA Reasoned Opinion on existing MRLs and are supplemented with additional information (additional studies and further comments).

MRL application
An MRL application for barley, oat, wheat and rye will be submitted along with and as part of this supplementary dossier. Since the crops concerned in the MRL application form (barley, oats, wheat and rye) and the crops selected for the representative uses are the same the procedure to combine the supplementary dossier and the MRL application is considered to provide more transparency and efficiency for the evaluation process. The process was agreed with the RMS UK.
All data relevant to the MRL application - including the summary forms of the supervised residue trials - are included in the supplementary dossier. The representative uses and the MRL application for the corresponding crops are supported by the same residue data.

Note: Denomination of the active substance and its isomers
In the original reports the active substance and the E-and Z-isomer are sometimes denominated differently. Initially the common name fluoxastrobin (chemical code HEC 5725) was assigned to both, the E-and Z-isomer as a sum and thus in some reports the active substance fluoxastrobin is used as a synonym for both isomers as a sum. During the EU peer review it was agreed to define the active substance fluoxastrobin as the E-isomer only and the Z-isomer was assigned as an impurity. The definition of the active substance is laid down in the EFSA Scientific Report (2007) 102, 1-84 and in the Inclusion Directive 2008/44/EC (4 April 2008).
Nevertheless, analytical results are always available for both isomers and the total residue is calculated as the sum of both isomers.
Throughout this dossier section the following denominations are used:

- Fluoxastrobin (HEC 5725 E-isomer), fluoxastrobin (E-isomer)
- HEC 5725 Z-isomer, Z-isomer of fluoxastrobin
- Total residue HEC 5725 (sum of HEC 5725 E-and Z-isomer, calculated)
- Parent compound: mixture or sum of fluoxastrobin and its Z-isomer (in the metabolism chapter).

The livestock metabolite HEC 5725-phenoxy-hydroxypyrimidine is also addressed as M55 or HEC 7154 in the study reports.

For substance codes, synonyms and abbreviations please refer to '*Document N3 - 'Substances and metabolites, structure, codes, synonyms – Fluoxastrobin*'.

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CA 6.1 Storage stability of residues

The storage stability of the residue for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer was examined in plant matrices. The results are given in detail in the DAR (2005) up to storage periods of 12 and 24 months (interim reports [redacted]; 2001; M-068950-01-1 and [redacted]; 2002; M-069819-01-1) and in Addendum 1 up to 30 months (final report [redacted]; 2003; M-085223-01-1). These data were peer reviewed during the Annex I inclusion process.
The results demonstrate that, under freezer conditions, residues of fluoxastrobin (HEC 5725 E-isomer and its Z-isomer were stable over a storage period of up to 30 months.

Following the Annex I inclusion additional data were generated in order to cover all relevant commodity categories as outlined in OECD guideline 506 or in order to address US data requirements for the processed commodities.
A brief summary of the data is presented in Table 6.1- 1.

Table 6.1- 1: Summary of storage stability data for fluoxastrobin in plant matrices

Compounds	Plant matrix	Stability	Storage conditions	Dossier reference	Reference
HEC 5725-E-isomer HEC 5725-Z-isomer	Wheat forage Wheat grain Wheat straw Potato tuber Tomato fruit Lettuce head	At least for 30 months	≤ -18°C	[redacted]; 2001; M-068950-01-1 (12 months) [redacted]; 2002; M-069819-01-1 (24 months) [redacted]X; 2003; M-085223-01-1 (30 months)	EFSA Scientific Report (2007), 102 List of endpoints
HEC 5725-E-isomer HEC 5725-Z-isomer	Rape seed Rape green material	At least for 24 months	≤ -18°C	[redacted]; 2005; M-261360-01-1	CA 6.1
HEC 5725-E-isomer HEC 5725-Z-isomer	processed products from barley grain (beer, brewers' malt, brewers' grains, and malt sprouts)	At least for 12 months	≤ -18°C	[redacted]; 2004; M-075659-01-1	CA 6.1
	Orange fruit Bean, dry seed	At least for 6 months (phase report)	≤ -18±2°C	[redacted]; [redacted]; 2015; M-531541-01-1	CA 6.1

In addition a short-term storage stability study is reported addressing stability of HEC 5725 E-and Z-isomers under conditions relevant to those which occurred during shipment of field samples from a residue study on onions. For some field samples the requested storage temperature of -18°C was exceeded during the transportation. The impact of this temperature deviation is addressed by a short-term storage stability experiment under conditions relevant to these samples.

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The supplementary information is summarized below.

Report:	KCA 6.1/04[redacted]S; 2005; M-261360-01-1
Title:	Storage stability of fluoxastrobin in/on rape seeds and green material during freezer storage for 24 months
Report No.:	MR-203/02
Document No.:	M-261360-01-1
Guideline(s):	EU Ref.: EU Council Directive 91/414/EEC US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data
Guideline deviation(s):	none
GLP/GEP:	**yes**

Storage stability of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer was examinened in/on rape seeds and green material during freezer storage for 24 months. The study was evaluated on national level in EU member states.

Test system:
The test conditions of the storage stability study on fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer were as follows:

Test substance:	HEC 5725, containing 90% E-isomer (fluoxastrobin) and 10% Z-isomer
Fortification level:	0.50 mg/kg HEC 5725 in/on rape green material, i.e. (nominal) 0.45 mg/kg E-isomer and 0.05 mg/kg Z-isomer 0.2 mg/kg fluoxastrobin in/on rape seed, i.e. (nominal) 0.18 mg/kg E-isomer and 0.02 mg/kg Z-isomer
Plant matrices:	rape green material and seed
Storage temperature:	≤ -18°C
Storage intervals:	nominally 0, 30, 90, (green material additionally 115), 180, 330, 540 and 720 days

Test Commodities
Control material was taken from residue studies and included rape green material and oil seed rape (seed).

Test Procedures
Untreated samples were prepared by shredding with dry ice in a cutter. The spiked samples for storage were prepared by spraying a large aliquot of each homogenised sample material in deep-frozen state in a cutter bowl with a suitable amount of a standard solution of fluoxastrobin and its Z-isomer resulting in a target fortification level of the 10fold LOQ for each plant material. After spraying, the fortified sample material in the cutter bowl was homogenised again with dry ice and small aliquots were transferred into polystyrene containers for storage. The used fortification procedure is only semi-quantitative, as losses of sample material and losses of spiking solution can occur when the spiking solution is sprayed into the open cutter bowl. The actual fortification level achieved was determined via the analysis of the day 0 samples. For rape seed, the actual fortification levels of the test substances were 83% for E-isomer, 91% for Z-isomer and 84% for the total residue of HEC 5725 relative to the target amount. For rape green material, actual fortification levels of 117% for E-isomer, 127% for Z-isomer and 118% for the total residue of HEC 5725 were achieved. The average recovery found in the spiked samples on day 0 was defined as 100% for each substance and each sample material and the recoveries from later storage intervals were calculated as relative recoveries from that value.

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Results and discussion

The residues of fluoxastrobin were determined according to method 00649/M001 ([redacted]; 2001; M-137093-01-1). The LOQ of the method is 0.045 mg/kg for green material and 0.018 mg/kg in seed for the HEC 5725 E-isomer and 0.005 mg/kg in green material and 0.002 mg/kg in seed for HEC 5725 Z-isomer (calculated LOQ for the total residue HEC 5725 is 0.05 and 0.02 mg/kg, respectively). A full set of validation data is reported in CA 4.1.2.

Method 00649/M001 was validated during the study by sets of pre-running and/or concurrent recovery experiments at each storage interval. These recovery experiments were performed by spiking control samples with HEC 5725 E- and Z-isomer. In the case of pre-running recovery experiments for method validation, control samples were fortified at a fortification level corresponding to the LOQ. In the case of concurrent recoveries, the fortification level corresponded to the 10 fold LOQ.

Pre-running recovery sets for method validation were conducted at the nominal storage intervals of 0, 180, 330, 540 and 720 days. For this purpose, stored control samples were freshly fortified with the test substances at the respective LOQ level. The freshly fortified samples were then extracted, cleaned up and analysed before or concurrently with the control and spiked samples of the corresponding nominal storage intervals. The obtained recovery data for method validation are presented in Table 6.1- 2.

Concurrent recoveries were conducted at the nominal storage intervals of 30, 90, 180, 330, 540 and 720 days, and, for green material only, also at the nominal storage interval 115 days. For this purpose, stored control samples were freshly fortified with the test substances at the respective 10 fold LOQ level. The freshly fortified samples were then extracted, cleaned up and analysed concurrently with the control and spiked samples of the corresponding nominal storage intervals.

The relative recovery values for HEC 5725 E-and Z-isomer and the calculated total residue at the different storage intervals for rape seed and green material in spiked samples are reported in Table 6.1- 3 and Table 6.1- 4 along with the obtained concurrent recovery data. The values presented in these tables were not corrected for the concurrent recoveries at the respective intervals. At each storage interval, control samples were analysed concurrently with the spiked samples. The blank values for all control samples analysed were below 10% of the respective LOQ values for HEC 5725 E-and Z-isomers.

The mean relative recoveries to day 0 at all storage intervals were between 93 and 115% for sample material of rape seeds and between 78 and 98% for rape green material for fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the calculated total residue (sum of E-and Z-isomer).

It can be concluded, that residues of fluoxastrobin (HEC 5725 E- isomer) and HEC 5725 Z-isomer feature good stability during deep-frozen storage for a period of (at least) 24 months in commodities of high oil content (rape seed) and in plant green material. There was no trend indicating that decomposition may occur in a time dependent mode after longer storage periods.

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Table 6.1- 2: Recovery data for method validation for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 – Z-isomer

Analyte	Sample Material	Fortification Level [mg/kg]	Date of Extraction	Nominal Storage Interval	Individual Recoveries [%]		Mean [%]	RSD [%]
Fluoxastrobin (HEC 5725 E-isomer)	Rape Seed	0.018	2002-11-18	0	90	91	91	-
			2003-05-21	180	89	92	91	-
			2003-10-20	330	89	92	91	-
			2004-05-05	540	88	85	87	-
			2004-11-08	720	86	86	86	-
			Overall Mean and RSD [%]				**89**	**2.8**
Fluoxastrobin (HEC 5725 E-isomer)	Rape Green Material	0.045	2002-11-18	0	97	98	98	-
			2003-05-21	180	91	92	92	-
			2003-10-20	330	94	94	94	-
			2004-05-05	540	95	98	97	-
			2004-11-08	720	88	89	89	-
			Overall Mean and RSD [%]				**94**	**4.1**
HEC 5725 Z-isomer	Rape Seed	0.002	2002-11-18	0	92	84	88	-
			2003-05-21	180	83	81	82	-
			2003-10-20	330	92	90	91	-
			2004-05-05	540	89	84	87	-
			2004-11-08	720	91	81	86	-
			Overall Mean and RSD [%]				**87**	**5.2**
HEC 5725 Z-isomer	Rape Green Material	0.005	2002-11-18	0	95	101	98	-
			2003-05-21	180	90	93	92	-
			2003-10-20	330	100	95	98	-
			2004-05-05	540	102	100	101	-
			2004-11-08	720	86	87	87	-
			Overall Mean and RSD [%]				**97**	**4.5**
Total residue HEC 5725	Rape Seed	0.02	2002-11-18	0	90	90	90	-
			2003-05-21	180	89	91	90	-
			2003-10-20	330	90	92	91	-
			2004-05-05	540	88	85	87	-
			2004-11-08	720	86	85	86	-
			Overall Mean and RSD [%]				**89**	**2.8**
Total residue HEC 5725	Rape Green Material	0.05	2002-11-18	0	97	99	98	-
			2003-05-21	180	91	92	92	-
			2003-10-20	330	95	94	95	-
			2004-05-05	540	96	98	97	-
			2004-11-08	720	88	89	89	-
			Overall Mean and RSD [%]				**94**	**4.0**

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Table 6.1- 3: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and total residue HEC 5725 in rape seed
Recovery day 0 = 100%; RSD: relative standard deviation

Nominal Interval	Day 0	Day 30	Day 90	Day 180	Day 330	Day 540	Day 720
Substance	**HEC 5725 Z-Isomer**						
Nominal Fortification Level [mg/kg]	**0.02**						
Stored Samples	99	93	99	104	104	104	93
Relative Recovery	99	93	99	104	110	121	93
[%]	104	93	104	99	99	121	93
	99						
	99						
Stored Samples	**100**	93	101	103	104	115	93
Rel. Mean [%]							
RSD [%]	2.5	0.0	3.1	3.1	5.3	8.2	0.0
Number of Values, n	5	3	3	3	3	3	3
Concurrent recoveries		86	90	95	93	86	81
[%]		77	84	88	85	87	81
Mean concurrent recovery [%]		82	87	92	89	87	81
Substance	**Fluoxastrobin (HEC 5725 E-Isomer)**						
Nominal Fortification Level [mg/kg]	**0.18**						
Stored Samples	99	95	103	102	109	101	105
Relative Recovery	98	96	103	105	109	112	105
[%]	101	95	102	105	101	110	101
	100						
	103						
Stored Samples	**100**	95	103	104	107	107	104
Rel. Mean [%]							
RSD [%]	1.7	0.8	0.7	1.9	4.4	5.7	2.2
Number of Values, n	5	3	3	3	3	3	3
Concurrent recoveries		87	91	92	90	85	86
[%]		82	92	91	92	86	86
Mean concurrent recovery [%]		85	92	92	91	86	86
Substance	**HEC 5725 Total Residue**						
Nominal Fortification Level [mg/kg]	**0.2**						
Stored Samples	99	94	103	102	109	101	104
Relative Recovery	98	95	102	105	109	113	104
[%]	101	94	101	105	100	111	100
	100						
	103						
Stored Samples	**100**	94	102	104	106	108	103
Rel. Mean [%]							
RSD [%]	1.8	0.6	0.9	1.7	4.7	5.9	2.0
Number of Values, n	5	3	3	3	3	3	3
Concurrent recoveries		86	91	92	90	98	89
[%]		82	91	90	91	95	92
Mean concurrent recoverys [%]		84	91	91	91	97	91

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Table 6.1- 4: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and total residue HEC 5725 in rape green material

Recovery day 0 = 100%; RSD: relative standard deviation

Nominal Interval	Day 0	Day 30	Day 90	Day 115	Day 180	Day 330	Day 540	Day 720
Substance	HEC 5725 Z-Isomer							
Nominal Fortification Level [mg/kg]	0.05							
Stored Samples	104	98	77	87	85	85	91	90
Relative Recovery [%]	95	98	85	82	92	90	98	80
	101	96	87	85	87	87	91	85
	101							
	99							
Stored Samples Rel. Mean [%]	**100**	97	83	85	88	87	94	85
RSD [%]	3.5	0.9	6.1	2.8	4.7	2.8	3.9	5.6
Number of Values, n	5	3	3	3	3	3	3	3
Concurrent recoveries [%]		91	101	93	95	100	99	88
		93	98	92	92	107	95	91
Mean concurrent recovery [%]		92	100	93	94	104	97	90
Substance	Fluoxastrobin (HEC 5725 E-Isomer)							
Nominal Fortification Level [mg/kg]	0.45							
Stored Samples	101	99	73	85	90	91	89	92
Relative Recovery [%]	96	98	78	84	93	91	86	86
	101	96	83	88	89	89	84	91
	101							
	101							
Stored Samples Rel. Mean [%]	**100**	98	78	86	91	90	86	90
RSD [%]	2.0	1.6	5.8	2.7	2.1	1.7	3.0	3.8
Number of Values, n	5	3	3	3	3	3	3	3
Concurrent recoveries [%]		93	94	92	94	98	98	89
		96	99	94	93	98	95	92
Mean concurrent recovery [%]		95	97	93	94	98	97	91
Substance	HEC 5725 Total Residue							
Nominal Fortification Level [mg/kg]	0.5							
Stored Samples	101	99	74	85	89	91	89	91
Relative Recovery [%]	96	98	79	84	92	91	87	85
	101	96	83	88	89	89	84	91
	101							
	101							
Stored Samples Rel. Mean [%]	**100**	98	79	86	90	90	87	89
RSD [%]	2.1	1.6	5.9	2.5	2.3	1.5	2.6	3.9
Number of Values, n	5	3	3	3	3	3	3	3
Concurrent recoveries [%]		92	95	92	94	98	98	89
		95	99	94	93	99	95	92
Mean concurrent recovery [%]		94	97	93	94	99	97	91

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Report:	KCA 6.1/05 [redacted]; 2004; M-075659-01-1
Title:	Storage stability of fluoxastrobin in/on processed products from barley grain (Beer, brewers' malt, brewers' grains, and malt sprouts) during freezer storage for 12 months
Report No.:	MR-555/00
Document No.:	M-075659-01-1
Guideline(s):	EU Ref.: EU Council Directive 91/414/EEC US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system:

The test conditions of the storage stability study on fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer were as follows:

Test substance:	HEC 5725, containing 90% E-isomer (fluoxastrobin) and 10% Z-isomer
Fortification level:	0.50 mg/kg HEC 5725 in /on brewer's malt, brewer's grain and malt sprouts, i.e. (nominal) 0.45 mg/kg E-isomer and 0.05 mg/kg Z-isomer; 0.125 mg/kg on beer, i.e. (nominal) 0.1125 mg/kg E-isomer and 0.0125 mg/kg Z-isomer
Processed commodities:	beer, brewer's malt, brewer's grain and malt sprouts
Storage temperature:	≤ -18°C
Storage intervals:	nominally 0, 30, 90, 180 and 360 days

Test Commodities

Control material was taken from a brewery (brewer's malt, brewer's grain, malt sprouts) and a retail store (beer).

Test Procedures

A deep-freezer storage stability study was conducted with HEC 5725 E-and Z-isomer in sample materials from beer processing. Samples of beer, brewer's malt, brewer's grain and malt sprouts were fortified at a level well above the limit of quantification (LOQ) for all matrices.

Untreated samples of brewer's malt, brewer's grain and malt sprouts were prepared by shredding with dry ice in a cutter. The spiked samples for storage were prepared by spraying a large aliquot of each solid sample material in deep-frozen state in a cutter bowl with a suitable amount of a standard solution of HEC 5725 E-and Z-isomer (ratio E/Z = 90/10) in dichloromethane. The target fortification level was approximately 0.5 mg/kg HEC 5725 for all sample materials. After spraying, the fortified sample material in the cutter bowl was homogenised again with dry ice and small aliquots were transferred into polystyrene containers for storage. The used fortification procedure for the solid sample materials is only semi-quantitative, as losses of sample material and losses of spiking solution can occur when the spiking solution is sprayed into the open cutter bowl. The actual fortification level achieved was determined by the analysis of the day 0 samples. The actual fortification levels of the test substances for brewer's malt were between 62% and 66%. For brewer's grain, the actual fortification levels ranged from 57% to 60% and for malt sprouts from 58% to 61%. Deviations between the fortification results for the different test substances can be attributed mainly to rounding effects and analytical variations. For beer, fortification was performed by weighing aliquots of 2.0 g of beer into individual brown-glass bottles with plastic caps and adding a small volume of a suitable spiking solution to each sample, resulting in a fortified amount of 0.125 mg/kg for the sum of both isomers. The liquid sample material beer was homogenised by shaking.

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The actual fortification levels obtained for HEC 5725 E-and Z-isomers and the calculated total residue at the nominal storage interval day 0 were used as the basis for the evaluation of the storage stability of residues of fluoxastrobin. The average recovery found in the spiked samples on day 0 was defined as 100% for each substance and each sample material, and the recoveries from later storage intervals were calculated as relative recoveries from that value ('relative recoveries to day 0').

Results and discussion

In the solid sample materials, HEC 5725 E-and Z-isomer were analysed according to method 00649 ([redacted]; 2001; M-137093-01-1) and for beer method 00604 ([redacted]; 2001; M-055517-01-1) was used. The LOQ for the total residue HEC 5725 was set at 0.05 mg/kg for residues in/on beer, brewer's malt, brewer's grain and malt sprouts. To validate the method at this LOQ level, samples were fortified with a reference substance containing 90% E-isomer and 10% Z-isomer. The validated LOQ for HEC 5725 E-isomer can therefore be calculated as 0.045 mg/kg for all sample materials included in this study. Accordingly, for HEC 5725 Z-isomer an LOQ of 0.005 mg/kg was validated for all sample materials. Both methods are reported in the initial Annex II dossier and were evaluated in the EU peer review.

The analytical methods 00649 and 00604 were validated during the study by sets of pre-running and/or concurrent recovery experiments at each storage interval. These recovery experiments were performed by spiking control samples with HEC 5725 E- and Z-isomer. In the case of pre-running recovery experiments for method validation, control samples were fortified at a fortification level corresponding to the LOQ. In the case of concurrent recoveries, the fortification level corresponded to approximately the actual fortification level of the spiked stored samples.

Pre-running recovery sets for method validation were conducted at the nominal storage intervals of 0, 180 and 360 days. For this purpose, stored control samples were freshly fortified with the test substances at the respective LOQ level. The freshly fortified samples were then extracted, cleaned up and analysed before or concurrently with the control and spiked samples of the corresponding nominal storage intervals. The obtained recovery data for method validation are presented in Table 6.1- 5.

Concurrent recoveries were conducted at the nominal storage intervals of 30, 90, 180, and 360 days. For this purpose, stored control samples were freshly fortified with the test substances at a fortification level corresponding to the fortification level of the spiked stored samples. The freshly fortified samples were then extracted, cleaned up and analysed concurrently with the control and spiked samples of the corresponding nominal storage intervals.

Low recoveries around 50 to 60% for HEC 5725 Z-isomer were obtained as concurrent recoveries for brewer's malt at the nominal storage interval of 30 days. As the corresponding results for fluoxastrobin (HEC 5725 E-isomer) and the calculated total residue were within the acceptable range, a repetition of the analytical set was not considered necessary. At the nominal storage intervals 90 and 180 days, several concurrent recoveries were out of range in the initial analyses of the sample sets, so that the whole sets were repeated, including stored samples and concurrent recoveries. It was observed, that these low recoveries were due to the fact that the dry residues after an evaporation step were not dissolved sufficiently. If extensive ultrasonic treatment was applied, all recoveries were well within the acceptable range (see for instance the results obtained at the nominal storage interval of 360 days).

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The relative recovery values for HEC 5725 E-and Z-isomer and the calculated total residue at the different storage intervals for beer, brewer's malt, brewer's grain and malt sprouts in spiked samples are reported in Table 6.1- 6 to Table 6.1- 9 along with the obtained concurrent recovery data. The values presented in these tables were not corrected for the concurrent recoveries at the respective intervals. At each storage interval, control samples were analysed concurrently with the spiked samples. The blank values for all control samples were below 10% of the respective LOQ values for HEC 5725 E-isomer, Z-isomer and the calculated total residue.

For beer, the mean relative recoveries to day 0 at all storage intervals were between 83% and 100% for fluoxastrobin Z-isomer, fluoxastrobin (E-isomer) and the calculated total residue of fluoxastrobin
For brewer's malt, the mean relative recovery values to day 0 at all storage intervals were between 81% and 101% for all three compounds, with the exception of the nominal storage interval day 30, where relative recoveries between 49 and 66% were obtained. However, this was not considered as an indication for a degradation of the residues of fluoxastrobin as the concurrent recoveries at that storage interval were also low, and as the relative recoveries at the following intervals were considerably higher again. The low residues obtained were probably due to problems which occurred during extraction of the stored samples.
For brewer's grain, the mean relative recovery values to day 0 at all storage intervals were between 69% and 106% and for malt sprouts they ranged between 82 and 111%.
It can be concluded, that the residues of fluoxastrobin (E-isomer) and HEC 5725 Z-isomer feature good stability during deep-frozen storage for a storage period of (at least) 12 months in beer, brewer's malt, brewer's grain and malt sprouts.

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Table 6.1- 5: Recovery data for method validation for fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and total residue HEC 5725;
RSD: relative standard deviation

Analyte	Sample Material	Fortification Level [mg/kg]	Date of Extraction	Nominal Storage Interval	Individual Recoveries [%]		Mean [%]	RSD [%]
Fluoxastrobin (HEC 5725 E-isomer)	Beer	0.045	2001-10-24	0	109	72	91	-
			2002-05-22	180	100	96	98	-
			2002-10-22	360	99	101	100	-
			Overall Mean and RSD [%]				**96**	**13.1**
	Brewer's Malt	0.045	2001-10-24	0	105	105	105	-
			2002-05-22	180	100	102	101	-
			2002-10-22	360	98	98	98	-
			Overall Mean and RSD [%]				**101**	**3.2**
	Brewer's Grain	0.045	2001-10-24	0	108	107	108	-
			2002-05-22	180	98	100	99	-
			2002-10-22	360	97	98	98	-
			Overall Mean and RSD [%]				**101**	**4.8**
	Malt Sprouts	0.045	2001-10-24	0	103	105	104	-
			2002-05-22	180	104	100	102	-
			2002-10-22	360	100	100	100	-
			Overall Mean and RSD [%]				**102**	**2.2**
HEC 5725 Z-isomer	Beer	0.005	2001-10-24	0	94	81	88	-
			2002-05-22	180	95	87	91	-
			2002-10-22	360	98	99	99	-
			Overall Mean and RSD [%]				**92**	**7.6**
	Brewer's Malt	0.005	2001-10-24	0	94	101	98	-
			2002-05-22	180	100	97	99	-
			2002-10-22	360	101	94	98	-
			Overall Mean and RSD [%]				**98**	**3.4**
	Brewer's Grain	0.005	2001-10-24	0	102	99	101	-
			2002-05-22	180	109	117	113	-
			2002-10-22	360	98	96	97	-
			Overall Mean and RSD [%]				**104**	**7.7**
	Malt Sprouts	0.005	2001-10-24	0	121	93	107	-
			2002-05-22	180	97	101	99	-
			2002-10-22	360	98	101	100	-
			Overall Mean and RSD [%]				**102**	**9.7**

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Analyte	Sample Material	Fortification Level [mg/kg]	Date of Extraction	Nominal Storage Interval	Individual Recoveries [%]	Mean [%]	RSD [%]
Total residue HEC 5725	Beer	0.05	2001-10-24	0	107 73	90	-
			2002-05-22	180	99 95	97	-
			2002-10-22	360	99 101	100	-
			Overall Mean and RSD [%]			**96**	**12.3**
	Brewer's Malt	0.05	2001-10-24	0	103 105	104	-
			2002-05-22	180	100 101	101	-
			2002-10-22	360	98 98	98	-
			Overall Mean and RSD [%]			**101**	**2.8**
	Brewer's Grain	0.05	2001-10-24	0	107 106	107	-
			2002-05-22	180	99 102	101	-
			2002-10-22	360	97 97	97	-
			Overall Mean and RSD [%]			**101**	**4.4**
	Malt Sprouts	0.05	2001-10-24	0	105 104	105	-
			2002-05-22	180	103 100	102	-
			2002-10-22	360	100 100	100	-
			Overall Mean and RSD [%]			**102**	**2.2**

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Table 6.1- 6: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and total residue HEC 5725 in <u>beer</u>
Recovery day 0 = 100%; RSD: relative standard deviation

Nominal Interval	Day 0	Day 30	Day 90	Day 180	Day 360
Substance	**HEC 5725 Z-Isomer**				
Nominal Fortification Level [mg/kg]	**0.0125**				
Stored Samples	108	92	100	92	92
Relative Recovery [%]	100	75	92	92	92
	100	83	100	83	83
	100				
	92				
Stored Samples Rel. Mean [%]	**100**	83	97	89	89
RSD [%]	5.9	10.0	4.9	5.4	5.4
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	94	110	110	97
		103	108	104	96
Mean Concurrent Recovery [%]	-	99	109	107	97
Substance	**Fluoxastrobin (HEC 5725 E-Isomer)**				
Nominal Fortification Level [mg/kg]	**0.1125**				
Stored Samples	105	101	103	93	96
Relative Recovery [%]	102	87	104	100	94
	93	91	95	86	91
	102				
	98				
Stored Samples Rel. Mean [%]	**100**	93	100	93	94
RSD [%]	4.5	7.6	4.5	7.5	3.0
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	95	105	111	94
	-	101	107	105	98
Mean Concurrent Recovery [%]	-	98	106	108	96
Substance	**HEC 5725 Total Residue**				
Nominal Fortification Level [mg/kg]	**0.125**				
Stored Samples	106	100	102	93	96
Relative Recovery [%]	101	86	102	100	93
	94	94	96	86	90
	101				
	98				
Stored Samples Rel. Mean [%]	**100**	92	100	93	93
RSD [%]	4.3	7.8	3.8	7.6	3.1
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	95	108	111	95
	-	102	108	105	98
Mean Concurrent Recovery [%]	-	99	108	108	97

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Table 6.1- 7: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and total residue HEC 5725 in <u>brewer's malt</u>
Recovery day 0 = 100%; RSD: relative standard deviation

Nominal Interval	Day 0	Day 30	Day 90	Day 180	Day 360
Substance	**HEC 5725 Z-Isomer**				
Fortification Level [mg/kg]	**Nominal 0.05** (Actual 0.03)				
Stored Samples	96	51	103	93	77
Relative Recovery [%]	103	54	106	93	87
	99	42	96	90	80
	103				
	99				
Stored Samples Rel. Mean [%]	**100**	49	101	92	81
RSD [%]	2.7	13.6	4.8	2.0	6.0
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	50	105	108	99
		62	101	106	104
Mean Concurrent Recovery [%]	-	56	103	107	102
Substance	**Fluoxastrobin (HEC 5725 E-Isomer)**				
Fortification Level [mg/kg]	**Nominal 0.45** (Actual 0.30)				
Stored Samples	100	68	100	86	81
Relative Recovery [%]	98	69	99	86	88
	100	62	98	80	86
	100				
	102				
Stored Samples Rel. Mean [%]	**100**	66	99	84	85
RSD [%]	1.6	6.3	0.9	3.7	4.2
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	68	104	103	103
		81	100	101	103
Mean Concurrent Recovery [%]	-	75	101	102	103
Substance	**HEC 5725 Total Residue**				
Fortification Level [mg/kg]	**Nominal 0.50** (Actual 0.33)				
Stored Samples	100	66	100	87	81
Relative Recovery [%]	98	68	99	87	88
	100	60	98	81	86
	100				
	102				
Stored Samples Rel. Mean [%]	**100**	65	99	85	85
RSD [%]	1.2	6.6	1.1	3.5	4.4
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	66	101	103	102
	-	79	100	101	103
Mean Concurrent Recovery [%]	-	73	101	102	103

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Table 6.1- 8: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and total residue HEC 5725 in <u>brewer's grain</u>
Recovery day 0 = 100%; RSD: relative standard deviation

Nominal Interval	Day 0	Day 30	Day 90	Day 180	Day 360
Substance	**HEC 5725 Z-Isomer**				
Fortification Level [mg/kg]	**Nominal 0.05** (Actual 0.03)				
Stored Samples	109	70	113	102	102
Relative Recovery [%]	106	74	102	106	95
	92	62	102	106	99
	102				
	92				
Stored Samples Rel. Mean [%]	**100**	69	106	104	99
RSD [%]	8.1	7.8	4.8	1.9	3.6
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	77	108	113	104
		73	105	104	106
Mean Concurrent Recovery [%]	-	76	107	108	105
Substance	**Fluoxastrobin (HEC 5725 E-Isomer)**				
Fortification Level [mg/kg]	**Nominal 0.45** (Actual 0.27)				
Stored Samples	101	81	102	94	96
Relative Recovery [%]	98	82	97	96	92
	99	75	98	93	93
	107				
	96				
Stored Samples Rel. Mean [%]	**100**	79	99	94	93
RSD [%]	4.4	5.1	2.3	1.8	2.3
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	83	104	106	100
		84	102	102	102
Mean Concurrent Recovery [%]	-	84	102	104	101
Substance	**HEC 5725 Total Residue**				
Fortification Level [mg/kg]	**Nominal 0.50** (Actual 0.30)				
Stored Samples	102	80	103	95	96
Relative Recovery [%]	99	82	98	97	92
	98	74	99	94	93
	107				
	95				
Stored Samples Rel. Mean [%]	**100**	78	100	95	94
RSD [%]	4.3	5.4	2.7	1.3	2.5
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	82	102	106	100
		83	102	102	103
Mean Concurrent Recovery [%]	-	83	102	104	102

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Table 6.1- 9: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and total residue HEC 5725 in malt sprouts
Recovery day 0 = 100%; RSD: relative standard deviation

Nominal Interval	Day 0	Day 30	Day 90	Day 180	Day 360
Substance	**HEC 5725 Z-Isomer**				
Fortification Level [mg/kg]	**Nominal 0.05** (Actual 0.03)				
Stored Samples	107	90	90	114	103
Relative Recovery [%]	97	76	93	107	97
	100	79	124	114	107
	97				
	100				
Stored Samples Rel. Mean [%]	**100**	85	102	111	102
RSD [%]	4.2	18.8	18.6	3.6	5.1
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	83	100	105	92
		89	93	92	96
Mean Concurrent Recovery [%]	-	85	97	99	94
Substance	**Fluoxastrobin (HEC 5725 E-Isomer)**				
Fortification Level [mg/kg]	**Nominal 0.45** (Actual 0.27)				
Stored Samples	104	98	93	109	104
Relative Recovery [%]	98	88	92	104	101
	99	91	119	103	105
	97				
	102				
Stored Samples Rel. Mean [%]	**100**	92	101	105	103
RSD [%]	2.6	5.3	14.7	2.8	1.8
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	89	93	103	95
		94	94	95	98
Mean Concurrent Recovery [%]	-	92	94	99	97
Substance	**HEC 5725 Total Residue**				
Fortification Level [mg/kg]	**Nominal 0.50** (Actual 0.30)				
Stored Samples	104	97	93	109	104
Relative Recovery [%]	98	87	92	104	101
	99	90	119	104	105
	97				
	102				
Stored Samples Rel. Mean [%]	**100**	91	101	106	103
RSD [%]	2.7	6.0	15.1	2.7	2.1
Number of Values, n	5	3	3	3	3
Concurrent Recoveries [%]	-	89	94	103	95
	-	93	94	95	98
Mean Concurrent Recovery [%]	-	91	94	99	97

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Report:	KCA 6.1/06[redacted],;[redacted]; 2015; M-531541-01-1
Title:	Storage stability of fluoxastrobin (HEC5725) in/on orange (fruit) and bean (dry seed) for 24 months
Report No.:	MR-15/121
Document No.:	M-531541-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals. Stability of Pesticide Residues in Stored Commodities. 506. 2007-10-16. US EPA OCSPP 860.1380, Storage Stability Data
Guideline deviation(s):	Yes, no impact, see original report
GLP/GEP:	**yes**

For description of deviation please refer to results and discussion on the following page.

The stability of fluoxastrobin (HEC5725 E-isomer) and HEC 5725 Z-isomer was investigated in the plant commodities orange fruit and dry bean (seed) representing commodities of high acid and high protein content for about 6 months (180 days) under frozen storage conditions. Results of the storage stability study are summarized up to 6 months in this **phase report**; the storage stability study is ongoing up to 24 months.

Test system:
The test conditions of the storage stability study on fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer were as follows:

Test substance:	HEC 5725 E-isomer (fluoxastrobin) and HEC 5725 Z-isomer
Fortification level:	0.09 mg/kg (10xLOQ) for HEC 5725 E-isomer; 0.01 mg/kg (10xLOQ) for HEC 5725 Z-isomer in both matrices
Plant matrices:	orange fruit and dry bean seed
Storage temperature:	≤ -18±2°C
Storage intervals:	nominally 0, 30, 90, and 180 days. The study is ongoing. Samples at 360, 540 and 720 days will be analysed in the future.

Test Commodities
Control material of orange fruit was taken from Bayer CropScience residue studies. Control material of dry bean seed was obtained from a local grocery.

Test Procedures
Untreated samples were shredded in a cutter with dry ice. 5-g aliquots of the homogenised control materials were weighed into glass bottles. Individual amber glass bottles were used as storage containers for each sample. This procedure allows extraction of the whole fortified sample in the bottle itself. HEC 5725 E-isomer and HEC 5725 Z-isomer were spiked separately to separate control material, resulting in fortification levels of 0.09 mg/kg for HEC 5725 E-isomer (10x LOQ) and 0.01 mg/kg for Z-isomer (10x LOQ) for both matrices. After fortification, the solvent was allowed to evaporate.
In addition, untreated samples of each sample material were prepared for control and recovery experiments. Subsequently the bottles were closed and stored deep frozen until analysis, except for the day 0 samples. On day 0 (zero time analyses) five spiked samples and one control sample were analysed. In addition, three recoveries spiked at the respective LOQ level and three recoveries spiked at the respective 10 fold LOQ level were performed for both isomers.
At each storage interval following day 0, three fortified and three control samples were removed from the deep-freezer and allowed to reach room temperature. Subsequently, two of the control samples of

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each sample material were freshly fortified with the test items to determine the concurrent recoveries. Fortification levels were at the same magnitude as the spiked storage samples. The samples were extracted and analysed concurrently with the control sample and the spiked storage samples.

The residues of fluoxastrobin were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). The LOQ of the method is 0.009 mg/kg for the HEC 5725 E-isomer and 0.001 mg/kg for HEC 5725 Z-isomer (calculated LOQ for the total residue HEC 5725 is 0.01 mg/kg). Orange (fruit) was extracted with acetone/water (3/1, v/v) using a blender. Bean (dry seed) was extracted with acetone/water (3/1, v/v) using duplicate microwave extraction. The extract was evaporated to dryness and the residues were re-dissolved in a standard solution containing isotopically labelled internal standards. HPLC-MS/MS in the positive ion mode was used for quantification.

Results and discussion

In order to assess the accuracy of the residue analyses, concurrent recoveries were determined by analyzing freshly fortified samples alongside with the stored fortified samples. At all storage intervals concurrent recoveries were determined at the 10-fold LOQ level (0.09 mg/kg for HEC 5725 E-isomer and 0.01 mg/kg for HEC 5725 Z-isomer). For day 0 analysis further concurrent recoveries were determined at the LOQ level (0.009 mg/kg for HEC 5725 E-isomer and 0.001 mg/kg for HEC 5725 Z-isomer). The mean concurrent recoveries determined from freshly fortified samples were in a range of 91% - 111%.

At each storage interval at least one control sample per matrix was analysed and the residues were always below 30% of the LOQ.

After a deep-freezer storage period of about 6 months, the mean recovery rates from the stored samples of orange fruit and dry bean seed were 99% and 107% for fluoxastrobin (HEC 5725 E-isomer) and 105% and 100% for the HEC 5725 Z-isomer, respectively.

Normalised to day 0, mean recoveries were 100% in both commodities for HEC 5725 E-isomer and 104% and 94% for HEC 5725 Z-isomer in orange fruit and dry bean seed, respectively after a storage period of 6 months.

Deviation to guideline: During the study conduct a deviation occurred. The targeted storage temperature of -18±2°C was exceeded due to a technical problem with the freezer on day 159 of storage. The threshold temperature was exceeded for about 6 hours and the storage temperature rose up to -1.5°C for a short time period. However, the average temperature during this event was still -16°C and the samples remained frozen during the complete occurrence. Also, any potential degradation of the analytes would have become evident at the next (6 months) storage interval. Since there was no indication of degradation after 6 months of storage the deviation is considered to have no negative impact on the quality of study.

Altogether, the study results demonstrate that the residues of HEC 5725 E-isomer and HEC 5725 Z-isomer are stable in commodities of high acid and high protein content for at least 6 months under deep-freezer storage conditions.

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Table 6.1- 10: Concurrent recoveries for HEC 5725 E-isomer and HEC 5725 Z-isomer in orange, fruit

Sample Material	Date of Extraction (yyyy-mm-dd)	Storage Interval [days]		Concurrent Recoveries [%]			
				Fortification level E-isomer: 0.009 mg/kg Z-isomer: 0.001 mg/kg		Fortification level E-isomer: 0.09 mg/kg Z-isomer: 0.01 mg/kg	
		nominal	actual	Single Values	Mean	Single Values	Mean
Orange, fruit HEC 5725 E-isomer	2014-11-03	0	0	93, 93, 96	95	97, 104, 102	101
	2014-12-01	30	28	--	--	101, 103	102
	2015-01-29	90	87	--	--	104, 102	103
	2015-05-13	180	191	--	--	101, 99	100
Orange, fruit HEC 5725 Z-isomer	2014-11-03	0	0	97, 91, 90	93	95, 102, 96	97
	2014-12-01	30	28	--	--	101, 100	101
	2015-01-29	90	87	--	--	105, 98	102
	2015-05-13	180	191	--	--	106, 103	105

Table 6.1- 11: Concurrent recoveries for HEC 5725 E-isomer and HEC 5725 Z-isomer in bean, dry seed

Sample Material	Date of Extraction (yyyy-mm-dd)	Storage Interval [days]		Concurrent Recoveries [%]			
				Fortification level E-isomer: 0.009 mg/kg Z-isomer: 0.001 mg/kg		Fortification level E-isomer: 0.09 mg/kg Z-isomer: 0.01 mg/kg	
		nominal	actual	Single Values	Mean	Single Values	Mean
Bean, dry seed HEC 5725 E-isomer	2014-11-04	0	0	100, 99, 101	100	105, 106, 106	106
	2014-12-03	30	29	--	--	112, 104	108
	2015-01-29	90	86	--	--	92, 95	94
	2015-05-13	180	190	--	--	110, 112	111*
Bean, dry seed HEC 5725 Z-isomer	2014-11-04	0	0	89, 93, 94	92	102, 104, 104	103
	2014-12-03	30	29	--	--	107, 103	105
	2015-01-29	90	86	--	--	89, 92	91
	2015-05-13	180	190	--	--	105, 111	108

*The mean value is slightly out of the range of 70%-110%. However, concurrent recoveries determined from freshly fortified samples were of about the same magnitude as the recoveries from stored samples. All other recoveries are well within the range of 70%-110% and therefore this mean recovery does not denote a deficit of the analytical method.

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Table 6.1- 12: Storage stability data and recovery data for HEC 5725 E-isomer and HEC 5725 Z-isomer in <u>orange fruit</u>

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Orange, fruit	**HEC 5725 E-isomer**						
	0	0.0869 0.0890 0.0891 0.0870 0.0888	97 99 99 97 99	98	100	98*	100
	28	0.0878 0.0884 0.0882	98 98 98	98	100	102	96
	87	0.0906 0.0942 0.0957	101 105 106	104	106	103	101
	191	0.0888 0.0873 0.0899	99 97 100	99	100	100	99
	HEC 5725 Z-isomer						
	0	0.0099 0.0099 0.0106 0.0100 0.0102	98 99 106 100 102	101	100	95*	107
	28	0.0107 0.0110 0.0107	107 110 107	108	107	101	107
	87	0.0105 0.0103 0.0104	105 103 104	104	103	102	102
	191	0.0106 0.0109 0.0101	106 109 101	105	104	105	101

[a] Normalized Recovery = (Average recovery / average recovery at day 0) X 100%
[b] Corrected percent recovery = (Average % recovery (stored)/ Average of fresh concurrent recoveries) X 100%
*Average of both fortification levels (E-Isomer: 0.009 mg/kg and 0.09 mg/kg; Z-Isomer: 0.001 mg/kg and 0.01 mg/kg) as given in Table 6.1-10.

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Table 6.1- 13: Storage stability data and recovery data for HEC 5725 E-isomer and HEC 5725 Z-isomer in bean, dry seed

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Bean, dry seed	**HEC 5725 E-isomer**						
	0	0.0935 0.0953 0.0975 0.0963 0.0975	104 106 108 107 108	107	100	103*	104
	29	0.082 0.085 0.084	92 95 93	93	88	108	86
	86	0.0815 0.0802 0.0837	91 89 93	91	85	94	97
	190	0.0954 0.0961 0.0974	106 107 108	107	100	111	96
	HEC 5725 Z-isomer						
	0	0.0100 0.0111 0.0112 0.0107 0.0105	100 111 111 107 105	107	100	98*	109
	29	0.009 0.010 0.010	94 99 104	99	93	105	94
	86	0.0092 0.0094 0.0090	92 94 90	92	86	91	102
	190	0.0098 0.0103 0.0100	98 103 100	100	94	108	93

[a] Normalized Recovery = (Average recovery / average recovery at day 0) X 100%
[b] Corrected percent recovery = (Average % recovery (stored)/ Average of fresh concurrent recoveries) X 100%
*Average of both fortification levels (E-Isomer: 0.009mg/kg and 0.09 mg/kg; Z-Isomer: 0.001 mg/kg and 0.01 mg/kg) as given in Table 6.1- 11.

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Report:	KCA 6.1/07[redacted],;[redacted]; 2015; M-480441-03-1
Title:	7 Days freezer storage stability study with different combinations of a total of 61 analytes (parent and metabolite molecules) and five matrix types (high water / acidic / starch / protein / oil) - 2nd Interim report
Report No.:	M-480441-03-1
Document No.:	M-480441-03-1
Guideline(s):	Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data OECD Test Guideline 506, adopted 16 October 2007
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The study was performed to address temperature deviations which occurred during shipment of field samples from residue studies. Storage stability of in total 61 analytes (active substances and metabolites) was investigated in tomate (fruit), wheat (green material), onion (bulbs), grape (bunches), wheat (grain), potato (tuber), peas (dry peas) and oilseed rape (seeds) covering the worst case conditions which occurred as temperature deviations during shipment of field samples (period of 8 hours at +1°C followed by 7 days at -7°C). Storage stability of HEC 5725 E-and Z-isomer was tested in commodities of high water content (among others in onion bulb) addressing temperature deviations during shipment from study 13-2139 (trial 01), reported in chapter CA 6.3.3. In this onion residue study, the maximum temperature during shipment was -10.14°C, thus exceeding the requested value of -18°C. The average temperature was -13.54°C for the period in which the required value was not met (29 hours).

In the present dossier only stability data for HEC 5725 E-and Z-isomer on onions are reported which are relevant to the issue at hand.

Test system:
The test conditions of the storage stability study on fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer were as follows:

Test substance:	HEC 5725 E-isomer (fluoxastrobin); HEC 5725 Z-isomer, fortified as a mixture
Fortification level:	1.0 mg/kg for both compounds
Plant matrices:	Onion bulb
Storage conditions:	+1°C for 8 hours followed by 7 days at -7°C
Storage intervals:	0 and 7 days

Test Commodities
Control materials used for fortification were purchased in local markets or taken from other GLP studies.

Test Procedures
For the storage stability experiment, aliquots of 5 g of onion bulbs were fortified with a mixture of the analytes at a fortification level of 1.0 mg/kg. The samples were stored in plastic containers (50 mL centrifuge tubes with screw caps) at +1°C for 8 hours and at -7°C for the following 7 days. Samples were analysed on day 0 and after a storage interval of 7 days.

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On day 0, five freshly fortified onion specimen fortified at 1.0 mg/kg were analysed together with a control sample. After a 7 day storage interval, five stored fortified and one stored control sample were analysed for fluoxastrobin (HEC 5725 E Isomer) and HEC 5725 Z Isomer. In addition two freshly fortified onion samples were prepared from stored control samples and analysed together with the stored fortified samples as procedural recoveries.
Prior to analysis of the stored samples, the method was validated at 1.0 mg/kg for both analytes. The method procedure and all validation data for HEC 5725 E-and Z-isomers are described within the storage stability report. The method is based on the QuEChERS method, however using a deviating ratio of the solvents (acetonitrile/water 4/1, v/v). After addition of a salt mixture (Mg_2SO_4/NaCl/Na-citrate 2 H_2O/Na_2H citrate 6 H_2O) (4/1/1/0.5, w/w/w/w) and centrifugation, an aliquot of the acetonitrile phase was diluted (1:100) with methanol / water (1/1, v/v) prior to the HPLC-MS/MS determination. Samples were quantified using matrix matched standards.

Results and discussion
The residues of fluoxastrobin were determined according to the method as described in the storage stability report. All validation data are included in the storage stability report. Detailed information on the method validation data are reported in section CA 4.1.2.
In the control samples of onion (bulbs), residues of the analytes fluoxastrobin (E Isomer) and HEC 5725 Z Isomer were below the LOQ (< 0.01 mg/kg).
Method validation was performed at 1.0 mg/kg for both analytes based on five individual samples. The mean recoveries were in the range of 70 – 110% with RSD < 20% and proved the method performance (cf. Table 6.1- 14, Table 22 in the study report).
For procedural recoveries, on day 0 and at the storage interval following day 0 recovery experiments were performed by fortifying stored control samples with a mixture of the analytes (1.0 mg/kg each). On day 0 five recovery experiments were performed and two freshly fortified samples were extracted on day 7 and analysed concurrently with the control and stored spiked samples. Procedural recoveries were all in the range of 70 – 110% (Table 6.1- 15, Table 30 in the report).
The recoveries of the stored samples showed that the residues of both analytes were stable in onion bulbs under the conditions investigated (8 hours at +1°C followed by 7 days at -7°C). For onion bulb, recoveries were 76 and 73% for the E-and Z-isomer, respectively. When normalised to the day 0 value recoveries were 80 and 79% for the E-and Z-isomer, respectively. Detailed data are compiled in Table 6.1- 16 (Table 103 and 104 in the report) below. It is concluded that the temperature deviations which occurred during shipment of the field samples had no negative impact on the quality of the residue study.

Table 6.1- 14: Recovery data for method validation for fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer on onion bulb

Analyte	Commodity	Mass Transition		Fortification Level [mg/kg]	Recovery in validation samples: Individual Values [%]					Mean [%]	RSD [%]
Fluoxastrobin (HEC 5725 E Isomer)	Onion (bulb)	459 / 188	Q	1.0	103	96	106	100	99	101	3.8
		459 / 427	C	1.0	98	99	107	113	84	100	11
HEC 5725 Z Isomer	Onion (bulb)	459 / 188	Q	1.0	101	95	104	101	98	100	3.4
		459 / 427	C	1.0	105	110	90	90	113	102	11

*Q: Quantification, C: Confirmation; RSD: relative standard deviation

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Table 6.1- 15 : Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in onion bulb

Analyte	Fortification Level [mg/kg]	Date of extraction	Storage interval [days]	Individual recoveries [%]					Mean [%]	RSD [%]	SD [%]
Fluoxastrobin (HEC 5725 E Isomer)	1.0	2014-04-14	0	97	92	96	96	95	95	2.0	1.9
	1.0	2014-04-22	7	89	88				88	-	-
	Overall mean, RSD and standard deviation [%]								93	3.9	3.6
HEC 5725 Z Isomer	1.0	2014-04-14	0	93	95	91	92	91	92	1.8	1.7
	1.0	2014-04-22	7	89	89				89	-	-
	Overall mean, RSD and standard deviation [%]								91	2.4	2.1

Table 6.1- 16: Storage stability data and concurrent recoveries of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in onion bulb

Analyte	Storage Period (days)	Residue Level in Stored Spiked Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Fluoxastrobin (HEC 5725 E Isomer)	0	0.966	97	95	100	NA	100
		0.923	92				
		0.956	96				
		0.964	96				
		0.954	95				
	7	0.740	74	76	80	89	86
		0.760	76				
		0.767	77				
		0.771	77				
		0.755	76				
HEC 5725 Z Isomer	0	0.928	93	92	100	NA	100
		0.952	95				
		0.913	91				
		0.925	92				
		0.915	91				
	7	0.746	75	73	79	89	82
		0.745	75				
		0.697	70				
		0.722	72				
		0.743	74				

[a]Normalized Recovery = (Average recovery / average recovery at day 0) x 100%
[b]Corrected percent recovery = (Average % recovery (stored spiked sample) / Average of fresh concurrent recoveries) x 100%
NA = Not applicable

Storage stability in animal commodities

Tissues and milk samples from the cow feeding study ([redacted]; 2001; M-090059-01-1) submitted with the Annex II dossier and evaluated in the DAR were stored frozen for less than 1 month before being analysed.

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Tissues and eggs of laying hen from the hen feeding study ([redacted]; 2015; M-536059-01-1) reported in the present dossier were also analysed within 30 days, except for few egg samples collected at the day of sacrifice, from control animals or collected during the pre-dosing period. Thus a storage stability study was not considered necessary.
However, storage stability investigations were performed in the livestock metabolism studies (see below).

Storage stability investigations in the livestock metabolism studies

In the livestock metabolism studies with radiolabelled fluoxastrobin (see introduction to chapter CA 6.2 and Table 6.2-3, chapters CA 6.2.2 and CA 6.2.3) all first extractions and analyses for quantification of parent compound and metabolites were performed within 6 months after collection of the samples. However, samples of commodities were extracted again in the course of the metabolism studies and the metabolic profiles were compared with the first profiles, thus generating data of storage at -18°C for periods of up to almost two years (see Table 6.1-17). Except for a minor degradation of a group of hydroxylated metabolites in the hen liver sample, no change of the radioactive residues due to storage was observed.

The radioactive residues include fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55). These three compounds are the analytes of the residue analytical methods (see document MCA section 4 chapters 4.1.2 and 4.2) and also the components of the residue definition for risk assessment and the proposed residue definition for enforcement (see chapter CA 6.7.1).

Fluoxastrobin (HEC 5725 E-isomer) was detected in all commodities of the four livestock metabolism studies, its Z-isomer (HEC 5725 Z-isomer) was detected as a trace or minor component in only some of the matrices. The metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55) was detected in all commodities of the goat and hen metabolism studies with [chlorophenyl-UL-^{14}C]fluoxastrobin, it was not detectable in the studies with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (see Table 6.1-17).

In egg and muscle, storage stability of fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55) at -18°C was demonstrated for at least 5 and 4 months in the hen metabolism study with [chlorophenyl-UL-^{14}C]fluoxastrobin, and in the hen metabolism study with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin stability of fluoxastrobin and its Z-isomer was shown for at least 7 months. In liver, storage stability of all three components was shown for at least 11 months, stability of fluoxastrobin for at least 22 months. In kidney, stability of fluoxastrobin was shown for at least 11 months and, considering the results in liver, similar stability can be assumed also for the Z-isomer and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55). In milk, stability of fluoxastrobin and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55) was shown for at least 10 months and, considering the results in the other matrices, similar stability can be assumed also for the Z-isomer of fluoxastrobin.

In the livestock metabolism studies, no experiments were made on storage stability of residues in fat. However, considering the results of the tested five diverse animal matrices, similar storage stability can be assumed also in fat.

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In summary, it is concluded that residues of fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55) are stable in livestock commodities for periods of at least 4 - 22 months.

Table 6.1-17 Experiments in livestock metabolism studies on storage stability of radioactive residues: commodities, covered components of the residue definitions and periods of demonstrated storage stability at -18°C

	milk	egg	muscle	fat	liver	kidney
Goat metabolism study with [chlorophenyl-UL-^{14}C]fluoxastrobin, [redacted]; 2002; M-034595-03-1	10 months, fluoxastrobin, M55	-	-	-	11 months, fluoxastrobin, its Z-isomer, M55	-
Hen metabolism study with [chlorophenyl-UL-^{14}C]fluoxastrobin, [redacted]; 2015; M-030690-02-1[#]	-	5 months, fluoxastrobin, its Z-isomer, M55	4 months, fluoxastrobin, its Z-isomer, M55	-	5 months, fluoxastrobin, M55	-
Goat metabolism study with [methoxyiminotolyl-ring-UL-^{14}C] fluoxastrobin, [redacted]; 2001; M-036881-02-1	-	-	-	-	22 months, fluoxastrobin	11 months, fluoxastrobin
Hen metabolism study with [methoxyiminotolyl-ring-UL-^{14}C] fluoxastrobin, [redacted]; 2002; M-059027-01-1	-	7 months*, fluoxastrobin, its Z-isomer	7 months*, fluoxastrobin, its Z-isomer	-	7 months*, fluoxastrobin	-

* minor degradation of a group of hydroxylated metabolites was observed

[#] This is a recently amended version of document M-030690-01-1, see chapter CA 6.2.2.

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CA 6.2 Metabolism, distribution and expression of residues

In the EU Annex II dossier of fluoxastrobin submitted in March 2002, plant and livestock metabolism were summarised in Section 4, Point 6. Wheat metabolism studies (three radiolabels), tomato metabolism studies (two radiolabels), confined rotational crops studies (three radiolabels), lactating goat metabolism studies (two radiolabels) and a laying hen metabolism study (one radiolabel) were submitted and summarised. However, the tomato metabolism studies were neither addressed in the DAR nor peer reviewed during the evaluation.

Excerpt from the EFSA Scientific Report (2007) 102, 1-84, Conclusion on the peer review of fluoxastrobin (rev 2007), finalized the 13th June 2007:
"Based on the primary plant metabolism and processing data submitted for wheat, residues in cereal crops should be defined as fluoxastrobin and Z-isomer for monitoring and risk assessment purposes. However, due to the fact, that the investigation of the metabolic behaviour of fluoxastrobin is limited to cereals only, a final residue definition for plants in general can not be proposed."

Additional plant metabolism studies in peanuts (two radiolabels) and oilseed rape (one radiolabel) as well as another laying hen metabolism study (another radiolabel) were conducted in 2002/2003. This laying hen metabolism study was submitted during the evaluation (November 2002), it was included in the DAR and peer reviewed. The peanut and oilseed rape metabolism studies were submitted in an addendum to the EU Annex II dossier to the United [redacted] and to The Netherlands for the registration of a plant protection product used in potatoes, but these studies were never peer reviewed at EU level. In the "Reasoned opinion on the review of the existing maximum residue levels (MRLs) for fluoxastrobin" EFSA annotated that "… a complete peer review of these metabolism studies at EU level is still desirable" [EFSA Journal 2012;10(12):3012].

Therefore in this dossier, the metabolism studies which have not yet been peer reviewed are summarised, i.e. the tomato, peanut and oilseed rape metabolism studies.

Table 6.2-1 gives an overview on the plant metabolism studies, Table 6.2-2 on the confined rotational crops studies and Table 6.2-3 on the livestock metabolism studies available for fluoxastrobin. The table includes information on the labelling positions, the presentation of the reports in the submissions and whether or not they have been peer reviewed at EU level.

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Table 6.2-1: Fluoxastrobin: Overview on plant metabolism studies

Crop	Application scenario	Label	Report	Submission		Peer reviewed during the EU evaluation
				EU dossier, Annex II, Section 4, Point 6 (submitted in March 2002) and baseline dossier	Presented in the addendum to the EU dossier*, Annex II, Section 4, Point 6 and in supplementary dossier	
wheat	seed treatment & 2 foliar applications	ring 3	[redacted]; 2001; M-090419-01-1	included	-	yes
		ring 1	[redacted]; 2001; M-091330-01-1	included	-	yes
		ring 2	[redacted]; 2001; M-091386-01-1	included	-	yes
	seed treatment	ring 3	[redacted]; 2001; M-091406-01-1	included	-	yes
tomatoes	3 foliar applications	ring 1	[redacted]; 2001; M-090608-01-1	included but not peer reviewed	Yes	no
		ring 3	[redacted]; 2001; M-090638-01-1	included but not peer reviewed	Yes	no
peanuts	3 foliar applications	ring 3	[redacted]; 2002; M-070947-01-1	-	Yes	no
		ring 2	[redacted]; 2002; M-074227-01-1	-	Yes	no
oilseed rape	seed treatment	ring 3	[redacted]; 2003; M-109459-01-1	-	Yes	no

*submitted to the United [redacted] and to The Netherlands for the registration of a plant protection product used in potatoes

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Table 6.2-2: Fluoxastrobin: Overview on confined rotational crops studies

Rotational crops	Application scenario	Label	Report	Submission EU dossier, Annex II, Section 4, Point 6 (submitted in March 2002) and baseline dossier	Peer reviewed during the EU evaluation
wheat, Swiss chard, turnips	1 spray application on bare soil	ring 3	[redacted]; 2001; M-090320-01-1	included	yes
		ring 1	[redacted]; 2002; M-091191-02-1	included	yes
		ring 2	[redacted] 2001; M-091162-01-1	included	yes

Table 6.2-3: Fluoxastrobin: Overview on livestock metabolism studies

Animal	Label	Report	Submission EU dossier, Annex II, Section 4, Point 6 (submitted in March 2002) and baseline dossier	Submission Submitted to RMS (UK PSD) during evaluation (November 2002), included in the DAR and in baseline dossier	Peer reviewed during the EU evaluation
laying hen	ring 3	[redacted]; 2001; M-030690-01-1*	included	-	yes
	ring 1	[redacted]; 2002; M-059027-01-1	-	Yes	yes
lactating goat	ring 3	[redacted]; 2001; M-036881-02-1	included	-	yes
	ring 1	[redacted]; 2002; M-034595-03-1	included	-	yes

*The study report was recently amended resulting in document M-030690-02-1, see chapter CA 6.2.2.

The metabolism of fluoxastrobin in the plant and in livestock was investigated using [chlorophenyl-UL-^{14}C]-, [pyrimidine-2-^{14}C]- and [methoxyiminotolyl-ring-UL-^{14}C]-labelled fluoxastrobin. Metabolism studies on a specific test material were generally done using all three labels. In those cases, where not all labels were tested in parallel, the [methoxyiminotolyl-ring-UL-^{14}C]-label was always included because this ring is considered to be the best description of the pharmacophore and is one of the two central rings. The chemical formula of fluoxastrobin and the position of the used ^{14}C-labels are presented below:

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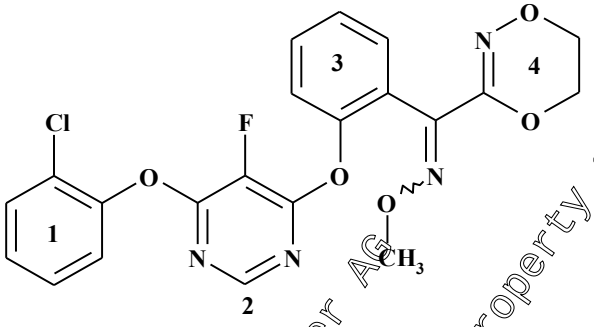

2

Nos. 1 – 3 indicate position of ^{14}C-label

1 = [chlorophenyl-UL-^{14}C]fluoxastrobin
(short form: "ring 1-label")

2 = [pyrimidine-2-^{14}C]fluoxastrobin
(short form: "ring 2-label")

3 = [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin
(short form: "ring 3-label")

4 = dioxazine ring
(short form: "ring 4")

The test compound used in the metabolism studies was a mixture of fluoxastrobin (HEC 5725 E-isomer) and its Z-isomer (HEC 5725 Z-isomer) at a ratio of about 98/2 to 97/3. Hence, in the studies and in the summaries presented in this chapter the mixture or the sum of fluoxastrobin and its Z-isomer is denoted as parent compound. However, the E-isomer only is defined as the active substance and the Z-isomer was declared as an impurity (see background information in chapter CA 6).

Categorisation, naming and presentation of metabolites

For an easier nomenclature and categorisation of the metabolites of fluoxastrobin, the rings of the molecule were numbered as 1, 2, 3 and 4, which is shown in the chemical formula above. The various metabolites of fluoxastrobin were grouped by the rings (or fragments of rings) they still contained, i.e. "ring 3,4 metabolites" still contain ring 3 (methoxyiminotolyl-ring) and ring 4 (dioxazine-ring). Furthermore, there are "ring 1,2,3,4 metabolites", "ring 2,3,4 metabolites", "ring 1,2 metabolites" and "ring 1 metabolites".

The names of the metabolites consist of the code number parent compound as prefix (HEC 5725-) followed by a unique suffix (e.g. HEC 5725-amide). The prefix (HEC 5725-) was preferably omitted when the name of the metabolite was identical with the chemical name of the compound (e.g. salicylic acid, 2-chlorophenol). Isomerisation of the oximether (methoxyimino) group was a common process. In several cases the stereochemical orientation of the chemical structures of the metabolites was determined and this was indicated in the report name by either inserting the letters "E" and "Z" or adding "E-isomer" or "Z-isomer". In other cases the structures of the metabolites which still contain the oximether were drawn as E-isomers since this is the most likely configuration, however, any indication in the report name was omitted. A list of metabolites contains the structures, various names, short forms and code numbers attributed to the metabolites and the matrices in which the metabolites were identified are also included in this list (see document N3).

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CA 6.2.1 Metabolism, distribution and expression of residues in plants

The behaviour and metabolism of fluoxastrobin was investigated in wheat, tomatoes, peanuts and oilseed rape under simulated field conditions. So far only the wheat metabolism studies were evaluated under peer review at EU level. Therefore the tomatoes, peanuts and oilseed rape metabolism studies are summarised in the following.

Metabolism in tomato (foliar spray application)

Metabolism studies in tomatoes were conducted with [chlorophenyl-UL-^{14}C]- and [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin:

Report:	KCA 6.2.1/07 [redacted]; 2001; M-090608-01-1
Title:	Metabolism of [chlorophenyl-UL-14C]HEC5725 in tomatoes
Report No.:	MR-128/01
Document No.:	M-090608-01-1
Guideline(s):	US EPA OPPTS 860.1300; Canada PMRA Ref. DACO 6.3; EC 91/414/EEC, amended by 96/68/EC
Guideline deviation(s):	none
GLP/GEP:	**yes**

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Executive Summary

The metabolism of [chlorophenyl-UL-^{14}C]fluoxastrobin (= [chlorophenyl-UL-^{14}C]HEC 5725) formulated as an SC 360 was investigated in tomatoes following three foliar spray applications. The first application was performed shortly after BBCH 64 (small tomatoes visible and a few flowers were still open). The second treatment was performed when the majority of the fruits had reached approx. 50% of the final size, corresponding to approx. at BBCH 72. The third treatment was three days before harvest, approx. at BBCH 83 (30% of the tomato fruits showed the typical ripe colour). The single application rate was approx. 144 g a.s./ha, the total application rate was approx. 432 g a.s./ha.

The total radioactive residue (TRR) in tomato fruits amounted to 0.418 mg/kg, expressed as active substance equivalents. Most of the radioactivity (91.5% of the TRR, 0.383 mg/kg) was extracted by surface wash with methanol. After homogenisation of the tomato fruits and extraction with acetonitrile/water the TRR was extracted almost quantitatively (99.8%). The radioactivity of the acetonitrile/water extract was partitioned mainly into the dichloromethane phase and only a small amount of radioactivity remained in the aqueous phase.

The major component of the TRR (94.8%, 0.396 mg/kg) was fluoxastrobin (HEC 5725-E-isomer). In total, parent compound (sum of fluoxastrobin and its Z-isomer) accounted for 98.0% of the TRR (0.410 mg/kg) in tomatoes. Three metabolites were also identified: HEC 5725-phenoxy-aminopyrimidine (M56), HEC 5725-amide (M38) and HEC 5725-ketone (M34), each below 0.5% of the TRR.

Based on the metabolites identified the following metabolic routes were deduced:

- cleavage of the molecule at the pyrimidine-methoxyiminotolyl ether group followed by amination,
- cleavage of the dioxazine ring and formation of an amide group and
- hydrolysis of the methoxyimino group and formation of a keto group.

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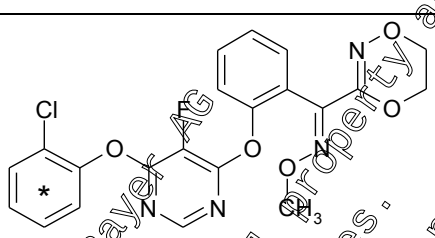
I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 only E-isomer displayed * position of the radiolabel
Radiolabelled test material	[chlorophenyl-UL-^{14}C]fluoxastrobin
Specific radioactivity	3.87 MBq/mg (104.5 µCi/mg)
Lot number	12712/1 and 12713/1
Ratio of fluoxastrobin (HEC 5725 E-isomer)/ Z-isomer (HEC 5725 Z-isomer)	97.8:2.2
Radiochemical purity	> 99% (HPLC) > 98% (TLC)

2. Soil: Standard Soil T

3. Plant: Tomato, variety "Bonset F1"

B. Study Design

1. Experimental conditions:

One tomato plant (variety: Bonset F1) planted in a 35 L pot filled with "Standard Soil T" was cultivated in the greenhouse of the test facility (controlled temperature, humidity and light conditions).

The tomato plant received three applications of the test compound [chlorophenyl-UL-^{14}C]fluoxastrobin. For each application an adequate amount of the test compound was mixed with a blank formulation in a test tube using a mini rotary mixer and two small steel balls to yield an SC 360 formulation. The radiolabelled formulation was suspended in 40 mL water and applied onto the tomato plant with a sprayer equipped with a flat fan nozzle. The first application was performed shortly after BBCH 64 (small tomatoes visible and a few flowers were still open). The second treatment was performed when the majority of the fruits had reached approx. 50% of the final size, corresponding to approx. at BBCH 72. The third treatment was three days before harvest, approx. at BBCH 83 (30% of the tomato fruits showed the typical ripe colour). The targeted total applied amount was approx. 17 mg a.s.. Based on a planting density of 25,000 plants/ha, the amount corresponded to a targeted total application rate of approx. 432 g a.s./ha. and to a single application rate of approx. 144 g a.s./ha.

2. Sampling:

The red and slightly red coloured tomato fruits were harvested three days after the last application when the plant had a growth stage of approx. BBCH 85 (approx. 50% of the tomatoes showed the typical ripe colour). The harvested fruits were divided into aliquots and either processed for analysis or stored frozen at –20°C or below.

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C. Analytical Procedures

1. Extraction:

An aliquot of the harvested tomato fruits was surface-washed with methanol, cut into pieces and extracted four times with acetonitrile/water (4:1, v:v) using a high-speed blender. After each extraction step, extracts and solids were separated by filtration. The acetonitrile/water extracts were combined and concentrated to the aqueous remainder using a rotary evaporator. The aqueous remainder was partitioned with dichloromethane.

The radioactivity in the surface wash, the combined acetonitrile/water extract, the dichloromethane phase and the aqueous phase was determined by liquid scintillation counting (LSC). The solids were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent / scintillation cocktail mixture and the radioactivity was measured by LSC. The actual TRR in the tomato fruits was calculated by summing up the radioactivity measured in the surface wash, the combined acetonitrile/water extract and remaining solids. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

2. Identification and characterisation:

The surface wash, dichloromethane phase and aqueous phase were analysed by HPLC with radiodetection (flow-through radiodetector equipped with a solid scintillator glass cell). Parent compound and metabolites were identified by HPLC co-chromatography using reference compounds. The identification was supported by LC-MS/MS analysis.

3. Storage stability:

All samples (fruit and surface wash solutions) were stored frozen at –20°C or below. The surface wash and the extraction of tomatoes for the metabolism study were conducted one day after sampling. The radioactivity was extracted almost completely and the first quantitation of the metabolite pattern was achieved within 3 weeks. Furthermore, based on other plant metabolism studies, no significant change was expected for the pattern of parent compound or metabolites during storage of samples or extracts. It was concluded that no special storage stability investigations were necessary.

II. Results and Discussion

The metabolism of [chlorophenyl-UL-^{14}C]fluoxastrobin (= [chlorophenyl-UL-^{14}C]HEC 5725) formulated as an SC 360 was investigated in tomatoes following three foliar spray applications, at a single rate of approx. 144 g a.s./ha and a total rate of approx. 432 g a.s./ha.

The total radioactive residue (TRR) in tomatoes harvested 3 days after the last application amounted to 0.418 mg/kg active substance equivalents (Table 6.2.1-1).

Table 6.2.1-1: TRR value in tomato fruits after foliar spray application of [chlorophenyl-UL-^{14}C]fluoxastrobin

Matrix	Timing and Application	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
tomato fruits	three foliar spray applications, at BBCH 64, BBCH 72 and BBCH 83; 3 x approx. 144 g a.s./ha	3	0.418

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Most of the radioactivity (91.5% of the TRR, 0.383 mg/kg) was extracted by surface wash with methanol (Table 6.2.1-2). The tomatoes were homogenised and further extracted using acetonitrile/water. The total extraction was very effective and amounted to 99.8% of the TRR. Following concentration of the radioactivity of the acetonitrile/water extracts the major portion was partitioned into the dichloromethane phase (7.3% of the TRR, 0.030 mg/kg) and a small amount remained in the aqueous phase (0.9% of the TRR, 0.004 mg/kg). The recovery of radioactivity indicated no significant losses.

Table 6.2.1-2: Distribution of radioactivity in the extracts of the tomato fruits after foliar spray application of [chlorophenyl-UL-^{14}C]fluoxastrobin

	Tomato fruits	
TRR [mg/kg] =	0.418	
	% of TRR	mg a.s. equiv./kg
Surface wash with methanol	91.5	0.383
Acetonitrile/water extracts	(8.2)	(0.034)
Dichloromethane phase	7.3	0.030
Aqueous phase	0.9	0.004
Total extracted	99.8	0.417
Solids (non-extractable residue)	0.2	0.001
Accountability	100.0	0.418

The surface wash, dichloromethane phase and aqueous phase were analysed by HPLC with radiodetection. Parent compound and metabolites were identified by co-chromatography with reference compounds, supported by LC-MS/MS analysis.

In the methanol surface wash solution (Table 6.2.1-3), fluoxastrobin (HEC 5725 E-isomer) accounted for 88.1% of the TRR (0.368 mg/kg), the amount of the Z-isomer of fluoxastrobin was significantly lower (2.9% of the TRR, 0.012 mg/kg). Three metabolites were detected in small amounts: HEC 5725-phenoxy-aminopyrimidine (M56), HEC 5725-amide (M38) and HEC 5725-ketone (M34) (0.1% – 0.3% of the TRR, 0.001 mg/kg each). In the dichloromethane phase fluoxastrobin (HEC 5725 E-isomer) accounted for 6.7% of the TRR (0.028 mg/kg). The Z-isomer of fluoxastrobin (0.4%, 0.002 mg/kg) and two minor metabolites were also detected: HEC 5725-phenoxy-aminopyrimidine (M56) and an unknown, each 0.1% of the TRR, <0.001 mg/kg. Four minor unknown metabolites were detected in the aqueous phase (0.1% - 0.6% of the TRR, <0.001 - 0.002 mg/kg).

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Table 6.2.1-3: Distribution of parent compound and metabolites in the extracts of tomato fruits after foliar spray application of [chlorophenyl-UL-^{14}C]fluoxastrobin

	Tomato fruits	
TRR [mg/kg] =	0.418	
Compound	% of TRR	mg a.s. equiv./kg
Surface wash with methanol		
parent compound, sum of	(90.9)	(0.380)
fluoxastrobin (HEC 5725 E-isomer)	88.1	0.368
and its Z-isomer (HEC 5725 Z-isomer)	2.9	0.012
HEC 5725-phenoxy-aminopyrimidine (M56)	0.3	0.001
HEC 5725-amide (M38)	0.1	<0.001
HEC 5725-ketone (M34)	0.2	0.001
Total in surface wash	91.5	0.383
Dichloromethane phase		
parent compound, sum of	(7.1)	(0.030)
fluoxastrobin (HEC 5725 E-isomer)	6.7	0.028
and its Z-isomer (HEC 5725 Z-isomer)	0.4	0.002
HEC 5725-phenoxy-aminopyrimidine (M56)	0.1	0.001
unknown	0.1	<0.001
Total in dichloromethane phase	7.3	0.030
Aqueous phase		
unknown	0.1	<0.001
unknown	0.1	<0.001
unknown	0.6	0.002
unknown	0.2	0.001
Total in aqueous phase	0.9	0.004
Total extracted	99.8	0.417
Solids (non-extractable residue)	0.2	0.001
Accountability	100.0	0.418

A total of 98.7% of the TRR (0.413 mg/kg) was identified (Table 6.2.1-4). The parent compound (sum of fluoxastrobin and its Z-isomer) accounted for 98.0% of the TRR (0.410 mg/kg) in tomato fruits. Fluoxastrobin (HEC 5725 E-isomer) was the predominant component (94.8% of the TRR, 0.396 mg/kg) whereas its Z-isomer (3.1% of the TRR, 0.014 mg/kg) was only minor. The ratio of the two isomers (96.6/3.4) was nearly unchanged compared to the ratio at the beginning of the study (97.8/2.2).

HEC 5725-phenoxy-aminopyrimidine (M56) was the main identified metabolite in tomato fruits, however amounting to only 0.4% of the TRR (0.002 mg/kg). Furthermore the metabolites HEC 5725-amide (M38) and HEC 5725-ketone (M34) were detected in traces of 0.1% and 0.2% of the TRR (each 0.001 mg/kg). Five minor metabolites remained unknown (each ≤0.6% of the TRR, ≤0.002 mg/kg).

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Table 6.2.1-4: Summary of characterisation and identification of radioactive residues in tomato fruits after foliar spray application of [chlorophenyl-UL-^{14}C]fluoxastrobin

	Tomato fruits	
TRR [mg/kg] =	0.418	
Compound	% of TRR	mg a.s. equiv./kg
parent compound, sum of	(98.0)	(0.410)
fluoxastrobin (HEC 5725 E-isomer)	94.8	0.396
and its Z-isomer (HEC 5725 Z-isomer)	3.3	0.014
HEC 5725-phenoxy-aminopyrimidine (M56)	0.4	0.002
HEC 5725-amide (M38)	0.1	<0.001
HEC 5725-ketone (M34)	0.2	0.001
Total identified	98.7	0.413
unknown	0.1	<0.001
unknown	0.1	<0.001
unknown	0.1	<0.001
unknown	0.6	0.002
unknown	0.2	0.001
Total characterised	1.0	0.004
Total extracted	99.8	0.417
Solids (non-extractable residue)	0.2	0.001
Accountability	100.0	0.418

III. Conclusions

After foliar spray application of [chlorophenyl-UL-^{14}C]fluoxastrobin, residues in tomato fruits consisted almost completely of parent compound accounting for 98.0% of the TRR. The ratio of fluoxastrobin and its Z-isomer remained nearly unchanged during the course of the study. Only a small number of metabolites were detected in low amounts.

Based on the identified metabolites the following metabolic routes were deduced:

- cleavage of the molecule at the pyrimidine-methoxyiminotolyl ether group followed by amination,
- cleavage of the dioxazine ring and formation of an amide group and
- hydrolysis of the methoxyimino group and formation of a keto group.

The metabolic pathway is proposed in Figure 6.2.1-1.

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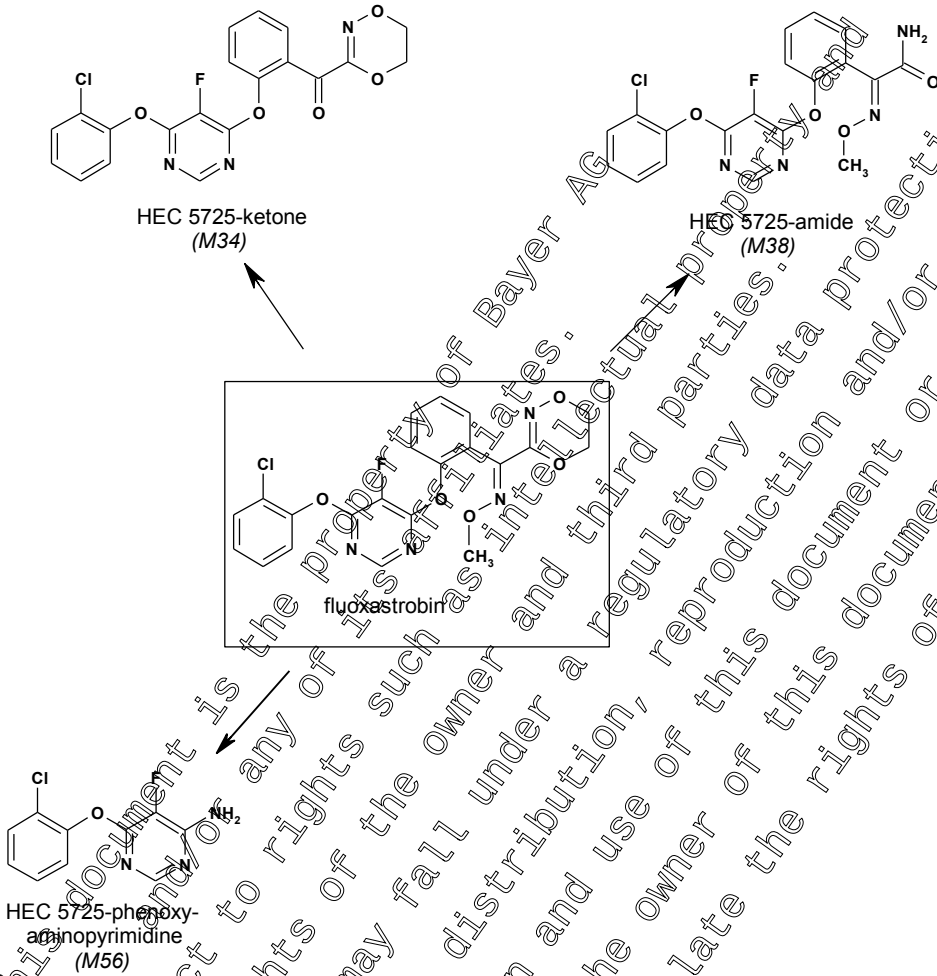
Figure 6.2.1-1: Proposed metabolic pathway of [chlorophenyl-UL-^{14}C]fluoxastrobin in tomatoes



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Report: KCA 6.2.1/08 [redacted]; 2001; M-090638-01-1
Title: Metabolism of [methoxyiminotolyl-ring-UL-14C]HEC5725 in tomatoes
Report No.: MR-129/01
Document No.: M-090638-01-1
Guideline(s): US EPA OPPTS 860.1300; Canada PMRA Ref.: DACO 6.3; EU 91/414/EEC amended by 96/68/EC
Guideline deviation(s): none
GLP/GEP: yes

Executive Summary

The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (= [methoxyiminotolyl-ring-UL-^{14}C]HEC 5725) formulated as an SC 360 was investigated in tomatoes following three foliar spray applications. The first application was performed shortly after BBCH 64 (small tomatoes visible and a few flowers were still open). The second treatment was performed when the majority of the fruits had reached approx. 50% of the final size, corresponding to approx. at BBCH 72. The third treatment was three days before harvest, approx. at BBCH 83 (30% of the tomato fruits showed the typical ripe colour). The single application rate was approx. 144 g a.s./ha, the total application rate was approx. 432 g a.s./ha.

The total radioactive residue (TRR) in tomatoes fruits amounted to 0.635 mg/kg, expressed as active substance equivalents. Most of the radioactivity (91.1% of the TRR, 0.578 mg/kg) was extracted by surface wash with methanol. After homogenisation of the tomato fruits and extraction with acetonitrile/water the TRR was extracted almost quantitatively (99.8%). The radioactivity of the acetonitrile/water extract was partitioned mainly into the dichloromethane phase and only a small amount of radioactivity remained in the aqueous phase.

The major component of the TRR (94.5%, 0.600 mg/kg) was fluoxastrobin (HEC 5725 E-isomer). In total, parent compound (sum of fluoxastrobin and its Z-isomer) accounted for 98.0% of the TRR (0.622 mg/kg) in tomatoes. Three metabolites were also identified: HEC 5725-dioxazinyl-phenylketone (M78), HEC 5725-amide (M38) and HEC 5725-ketone (M34), each below 0.5% of the TRR.

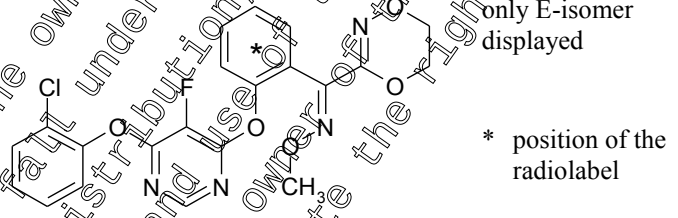
Based on the metabolites identified the following metabolic routes were deduced:

- cleavage of the molecule at the pyrimidine-methoxyiminotolyl ether group,
- cleavage of the dioxazine ring and formation of an amide group and
- hydrolysis of the methoxyimino group and formation of a keto group.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 only E-isomer displayed * position of the radiolabel
Radiolabelled test material	[methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin
Specific radioactivity	3.70 MBq/mg (100 µCi/mg)
Lot number	12250/1 and 12253/1
Ratio of fluoxastrobin (HEC 5725 E-isomer) / Z-isomer (HEC 5725 Z-isomer)	97.7:2.3
Radiochemical purity	> 99% (HPLC) > 98% (TLC)

2. Soil: Standard Soil T

3. Plant: Tomato, variety "Bonset F1"

B. Study Design

1. Experimental conditions:

One tomato plant (variety: Bonset F1) planted in a 35 L pot filled with "Standard Soil T" was cultivated in the greenhouse of the test facility (controlled temperature, humidity and light conditions).

The tomato plant received three applications of the test compound [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin. For each application an adequate amount of the test compound was mixed with a blank formulation in a test tube using a mini rotary mixer and two small steel balls to yield an SC 360

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formulation. The radiolabelled formulation was suspended in 40 mL water and applied onto the tomato plant with a sprayer equipped with a flat fan nozzle. The first application was performed shortly after BBCH 64 (small tomatoes visible and a few flowers were still open). The second treatment was performed when the majority of the fruits had reached approx. 50% of the final size, corresponding to approx. at BBCH 72. The third treatment was three days before harvest, approx. at BBCH 83 (30% of the tomato fruits showed the typical ripe colour). The targeted total applied amount was approx. 17 mg a.s.. Based on a planting density of 25.000 plants/ha, the amount corresponded to a targeted total application rate of approx. 432 g a.s./ha and to a single application rate of approx. 144 g a.s./ha.

2. Sampling:
The red and slightly red coloured tomato fruits were harvested three days after the last application when the plant had a growth stage of approx. BBCH 85 (approx. 50% of the tomatoes showed the typical ripe colour). The harvested fruits were divided into aliquots and either processed for analysis or stored frozen at –20°C or below.

C. Analytical Procedures
1. Extraction:
An aliquot of the harvested tomato fruits was surface-washed with methanol, cut into pieces and extracted four times with acetonitrile/water (4:1, v:v) using a high-speed blender. After each extraction step, extracts and solids were separated by filtration. The acetonitrile/water extracts were combined and concentrated to the aqueous remainder using a rotary evaporator. The aqueous remainder was partitioned with dichloromethane.

The radioactivity in the surface wash, the combined acetonitrile/water extract, the dichloromethane phase and the aqueous phase was determined by liquid scintillation counting (LSC). The solids were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent / scintillation cocktail mixture and the radioactivity was measured by LSC. The actual TRR in the tomato fruits was calculated by summing up the radioactivity measured in the surface wash, the combined acetonitrile/water extract and the remaining solids. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

2. Identification and characterisation:
The surface wash, dichloromethane phase and aqueous phase were analysed by HPLC with radiodetection (flow-through radiodetector equipped with a solid scintillator glass cell). Parent compound and metabolites were identified by HPLC co-chromatography using reference compounds. The identification was supported by LC-MS/MS analysis.

3. Storage stability:
All samples (fruit and surface wash solutions) were stored frozen at –20°C or below. The surface wash of tomatoes for the metabolism study was conducted on the same day as sampling (harvest). The extraction was continued within the first week and the radioactivity was extracted almost completely. The first quantitation of the metabolite pattern was achieved within 3 weeks. Furthermore, based on other plant metabolism studies, no significant change was expected for the pattern of parent compound or metabolites during storage of samples or extracts. It was concluded that no special storage stability investigations were necessary.

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II. Results and Discussion

The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (= [methoxyiminotolyl-ring-UL-^{14}C]HEC 5725) formulated as an SC 360 was investigated in tomatoes following three foliar spray applications, at a single rate of approx. 144 g a.s./ha and a total rate of approx. 432 g a.s./ha.

The total radioactive residue (TRR) in tomatoes harvested 3 days after the last application amounted to 0.635 mg/kg active substance equivalents (Table 6.2.1-5).

Table 6.2.1-5: TRR value in tomato fruits after foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin

Matrix	Timing and Application	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
tomato fruits	three foliar spray applications at BBCH 64, BBCH 72 and BBCH 83; 3 x approx. 144 g a.s./ha	3	0.635

Most of the radioactivity (91.1%, 0.578 mg/kg of the TRR) was extracted by surface wash with methanol (Table 6.2.1-6). The tomatoes were homogenised and further extracted using acetonitrile/water. The total extraction was very effective and amounted to 99.8% of the TRR. Following concentration of the radioactivity of the acetonitrile/water extracts, the major portion was partitioned into the dichloromethane phase (7.9% of the TRR, 0.050 mg/kg) and a small amount remained in the aqueous phase (0.8% of the TRR, 0.005 mg/kg). The recovery of radioactivity indicated no significant losses.

Table 6.2.1-6: Distribution of radioactivity in the extracts of the tomato fruits after foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin

	Tomato fruits	
TRR [mg/kg] =	0.635	
	% of TRR	mg a.s. equiv./kg
Surface wash with methanol	91.1	0.578
Acetonitrile/water extracts	(8.7)	(0.055)
Dichloromethane phase	7.9	0.050
Aqueous phase	0.8	0.005
Total extracted	99.8	0.634
Solids (non-extractable residue)	0.2	0.001
Accountability	100.0	0.635

The surface wash, dichloromethane phase and aqueous phase were analysed by HPLC with radiodetection. Parent compound and metabolites were identified by co-chromatography with reference compounds, supported by LC-MS/MS analysis.

In the methanol surface wash solution (Table 6.2.1-7), fluoxastrobin (HEC 5725 E-isomer) accounted for 87.3% of the TRR (0.554 mg/kg), the amount of the Z-isomer of fluoxastrobin was significantly lower (3.0% of the TRR, 0.019 mg/kg). Four metabolites HEC 5725-dioxazinyl-phenylketone (M78), HEC 5725-amide (M38), HEC 5725-ketone (M34) and an unknown were detected in small amounts (0.1% - 0.3% of the TRR, 0.001 - 0.002 mg/kg each). In the dichloromethane phase fluoxastrobin (HEC 5725 E-isomer) accounted for 7.3% of the TRR (0.046 mg/kg). The Z-isomer of fluoxastrobin

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(0.4%, 0.003 mg/kg) and three minor unknown metabolites were also detected (<0.1% - 0.1% of the TRR, <0.001 – 0.001 mg/kg). Six minor unknown metabolites were detected in the aqueous phase (<0.1% – 0.3% of the TRR, <0.001 - 0.002 mg/kg).

Table 6.2.1-7: Distribution of parent compound and metabolites in the extracts of tomato fruits after foliar spray application of [methoxyiminotolyl-ring-U1-^{14}C]fluoxastrobin

	Tomato fruits	
TRR [mg/kg] =	0.635	
Compound	% of TRR	mg a.s. equiv./kg
Surface wash with methanol		
parent compound, sum of	(90.3)	(0.573)
fluoxastrobin (HEC 5725 E-isomer)	87.3	0.554
and its Z-isomer (HEC 5725 Z-isomer)	3.0	0.019
HEC 5725-dioxazinyl-phenylketone (M78)	0.3	0.002
HEC 5725-amide (M38)	0.1	0.001
HEC 5725-ketone (M34)	0.3	0.002
unknown	0.1	0.001
Total in surface wash	91.1	0.578
Dichloromethane phase		
parent compound, sum of	(7.7)	(0.049)
fluoxastrobin (HEC 5725 E-isomer)	7.3	0.046
and its Z-isomer (HEC 5725 Z-isomer)	0.4	0.003
unknown	0.1	0.001
unknown	<0.1	<0.001
unknown	0.1	<0.001
Total in dichloromethane phase	7.9	0.050
Aqueous phase		
unknown	0.1	0.001
unknown	0.2	0.001
unknown	0.3	0.002
unknown	<0.1	<0.001
unknown	0.2	0.001
unknown	0.1	<0.001
Total in aqueous phase	0.8	0.005
Total extracted	99.8	0.634
Solids (non-extractable residue)	0.2	0.001
Accountability	100.0	0.635

A total of 98.7% of the TRR (0.627 mg/kg) was identified (Table 6.2.1-8). The parent compound (sum of fluoxastrobin and its Z-isomer) accounted for 98.0% of the TRR (0.622 mg/kg) in tomato fruits. Fluoxastrobin (HEC 5725 E-isomer) was the predominant component (94.5% of the TRR, 0.600 mg/kg) whereas its Z-isomer (3.4% of the TRR, 0.022 mg/kg) was only minor. The ratio of the two isomers (96.5/3.5) was nearly unchanged compared to the ratio at the beginning of the study (97.7/2.3).

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HEC 5725-dioxazinyl-phenylketone (M78) and HEC 5725-ketone (M34) were the main identified metabolites in tomato fruits, however amounting to only 0.3% of the TRR (0.002 mg/kg) each. Furthermore the metabolite HEC 5725-amide (M38) was detected in traces (0.1% of the TRR, 0.001 mg/kg). Ten minor metabolites remained unknown (each ≤0.3% of the TRR, ≤0.002 mg/kg).

Table 6.2.1-8: Summary of characterisation and identification of radioactive residues in tomato fruits after foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin

	Tomato fruits	
TRR [mg/kg] =	0.635	
Compound	% of TRR	mg a.s. equiv./kg
parent compound, sum of	(98.0)	(0.622)
fluoxastrobin (HEC 5725 E-isomer)	94.5	0.600
and its Z-isomer (HEC 5725 Z-isomer)	3.4	0.022
HEC 5725-dioxazinyl-phenylketone (M78)	0.3	0.002
HEC 5725-amide (M38)	0.1	0.001
HEC 5725-ketone (M34)	0.3	0.002
Total identified	98.7	0.627
unknown	0.1	0.001
unknown	0.1	0.001
unknown	<0.1	<0.001
unknown	0.1	<0.001
unknown	0.1	0.001
unknown	0.2	0.001
unknown	0.3	0.002
unknown	<0.1	<0.001
unknown	0.2	0.001
unknown	0.1	0.001
Total characterised	1.1	0.007
Total extracted	99.8	0.634
Solids (non-extractable residue)	0.2	0.001
Accountability	100.0	0.635

III. Conclusions

After foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin, residues in tomato fruits consisted almost completely of parent compound accounting for 98.0% of the TRR. The ratio of fluoxastrobin and its Z-isomer remained nearly unchanged during the course of the study. Metabolites were detected in low amounts.

Based on the identified metabolites the following metabolic routes were deduced:

- cleavage of the molecule at the pyrimidine-methoxyiminotolyl ether group,
- cleavage of the dioxazine ring and formation of an amide group and
- hydrolysis of the methoxyimino group and formation of a keto group.

The metabolic pathway is proposed in Figure 6.2.1-2.

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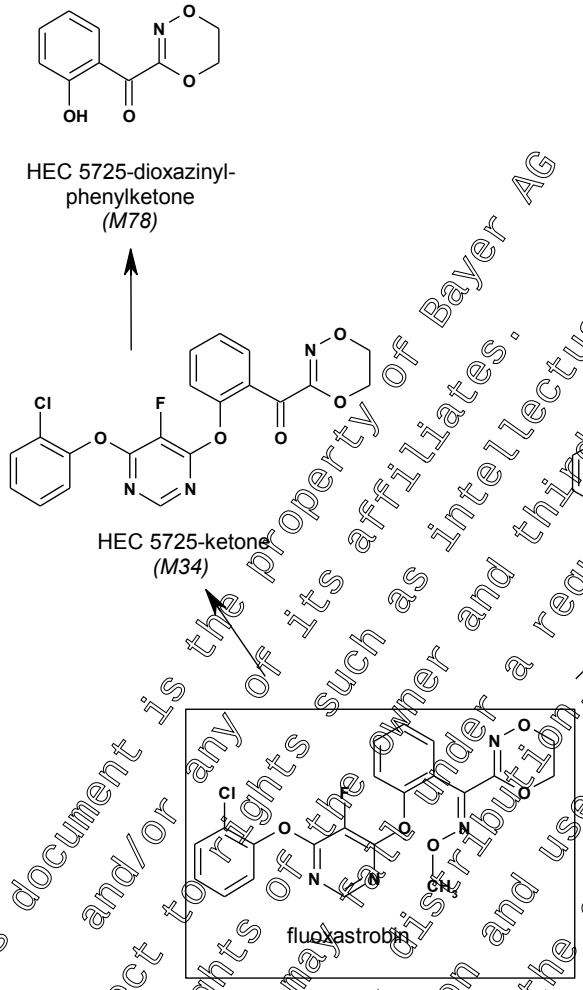
Figure 6.2.1-2: Proposed metabolic pathway of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin in tomatoes



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Metabolism in peanuts (foliar spray application)

Metabolism studies in peanuts were conducted with [pyrimidine-2-^{14}C]- and [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin.

Report:	KCA 6.2.1/09 [redacted] V; 2002; M-070947-01-1
Title:	Metabolism of [methoxyiminotolyl-ring-UL-14C]HEC5725 in peanuts
Report No.:	MR-531/01
Document No.:	M-070947-01-1
Guideline(s):	US-EPA OPPTS 860.1300; Canada PMRA Ref.: DACO 6.3; EU 91/414/EEC amended by 96/68/EC
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (= [methoxyiminotolyl-ring-UL-^{14}C]HEC 5725) formulated as an EC 100 was investigated in peanuts following three foliar spray applications. The applications were performed at BBCH 66, BBCH 79 and BBCH 88. The single application rate was in the range of 234 - 275 g a.s./ha, the total application rate was 781 g a.s./ha.

The total radioactive residue (TRR) in peanut hay amounted to 141.82 mg/kg. Compared to hay, the TRR in nutmeat was very low and amounted to just 0.055 mg/kg. From hay most of the radioactivity (95.2% of the TRR, 135.03 mg/kg) was extracted with methanol/water and was subsequently partitioned mainly into the dichloromethane phase with only a small amount of radioactivity remaining in the aqueous phase. From nutmeat, a portion of 51.1% of the TRR (0.028 mg/kg) was extracted with hexane and a further portion of 24.5% of the TRR (0.013 mg/kg) was extracted with methanol/water. The radioactivity of the methanol/water extract was partitioned with dichloromethane and remained mainly in the aqueous phase.

In hay, the radioactive residues mainly consisted of parent compound (sum of fluoxastrobin and its Z-isomer), amounting to 83.1% of the TRR. The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin in hay showed a complex pattern and a total of 17 metabolites were identified. However, no metabolite exceeded 2.7% of the TRR.

In nutmeat, 68.2% of the TRR was identified as natural products. The largest portion (51.1% of the TRR) was identified as fatty acids after alkaline saponification, hence originally representing fat. Smaller portions represented carbohydrates and proteins. All these ^{14}C labelled natural products probably resulted from an intensive mineralisation of the methoxyiminotolyl ring of fluoxastrobin residues in soil, subsequent assimilation of $^{14}CO_2$ released from soil by the peanut plants and deposition of assimilates in nutmeat. Only a few minor components (each < 10% of the TRR and ≤ 0.005 mg/kg) were characterised as metabolites of fluoxastrobin in nutmeat.

Neglecting the formation of natural products after assimilation of $^{14}CO_2$ and based on the identified metabolites, the following metabolic routes were deduced:

- isomerisation of the oximether with the formation of the Z-isomer,
- oxidative ring opening and degradation of the dioxazine ring,
- cleavage of the oximether,
- nucleophilic substitution of the chlorophenol ring by glutathione, followed by a stepwise degradation of the glutathione moiety,
- cleavage of the parent molecule mainly at the pyrimidine-methoxyiminotolyl ether group and to a minor extent at the chlorophenyl-pyrimidine ether group,
- conjugation of hydroxyl and thiol groups to glucosyl and glucosyl-malonyl conjugates and
- to a very minor extent hydroxylation of the chlorophenyl ring.

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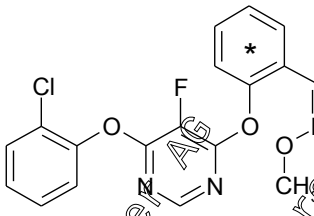
I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 only E-isomer displayed * position of the radiolabel
Radiolabelled test material	[methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin
Specific radioactivity	3.70 MBq/mg (100 µCi/mg)
Ratio of fluoxastrobin (HEC 5725 E-isomer)/ Z-isomer (HEC 5725 Z-isomer)	98:2 (normal dose and overdose experiment) 97.7:2.3 (translocation experiment)
Radiochemical purity	> 99% (HPLC) > 99% (TLC)

2. Soil: "[redacted]3" (sandy loam from Germany), pH ($CaCl_2$) = 6.3, 57.4% sand, 33.0% silt and 9.7% clay, 1.98% organic carbon, cation exchange capacity (CEC) of 10 meq/100 g

3. Plant: Peanut, variety: "Georgia Green"

B. Study Design

1. Experimental conditions:

Three individual experiments were conducted.

Normal dose experiment: This experiment simulated agricultural practice and was the main experiment within the present study. Eighteen peanut plants were grown in a plant container (surface area 1 m²) and a depth of 60 cm filled with a sandy loam soil. Only half of the container (0.5 m²) was used for the experiment. Hence, nine plants were sprayed with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin formulated as an EC 100. The remaining nine plants were not part of the experiment. The envisaged use pattern at initiation of the study included up to four spray applications, each 203 g a.s./ha, resulting in a maximum annual field rate of 812 g a.s./ha. The first field application was proposed after pegging to beginning of pod development (growth stage BBCH 66 – 75) and the last application 14 days before harvest. The metabolism studies simulated the envisaged use pattern and were based on the maximum proposed application rate. For simplification, only three applications were performed, each at increased actual rates of 234 g a.s./ha (BBCH 66), 272 g a.s./ha (BBCH 79), and 275 g a.s./ha (BBCH 88). This resulted in at total applied rate of 781 g a.s./ha, which was slightly less as compared to the envisaged total application rate of 812 g a.s./ha. The time intervals between the single applications were 42 days between the first and the second and 20 days between the second and the third application. The third application took place 14 days before harvest.

Overdose experiment: This experiment was set up for metabolite isolation and identification, if necessary. Two plants were grown in 7.5 L pots (1 plant/pot) filled with a sandy loam soil. Approximately the same amounts of the EC 100 formulation, which were applied onto nine peanut plants in the normal dose experiment, were applied onto 2 peanut plants in the overdose experiment. Hence, an approx. 5X application rate was achieved (20.02 mg a.s./plant in three applications) as

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compared to the normal dose experiment. The first application was performed at BBCH growth stage 67, the second at BBCH 79 and the third at BBCH 88. The time intervals between the single applications were 42 days between the first and the second and 20 days between the second and the third application. The third application took place 14 days before harvest.

Translocation experiment: This experiment was set up to obtain information on the translocation of radioactivity after seed treatment. Two plants (seeds) were grown in 7.5 L pots (1 plant/pot) filled with a sandy loam soil. The application of this experiment was performed with non-formulated [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin. The amount for seed treatment was calculated from a weight of ca. 0.5 g for a peanut seed and assuming an application rate of 25 g a.s./100 kg seeds. Hence, 0.13 mg a.s. per seed should be applied. Two peanut seeds were treated with 0.17 mg a.s. each, which was an exaggeration of approx. 30%.

All experiments were performed in the greenhouse with a day/night rhythm of 14/10 hours and an average temperature of 24/23°C (day) and 17/16°C (night) and a relative humidity of 60%.

2. Sampling
In all experiments peanut plants were removed from soil at BBCH growth stage 97 and dried for 4 days. After four days of drying in the greenhouse, all plants were separated into hay, fruits and roots. The fruits were manually separated into seeds (= nutmeat) and shells. Hay and nutmeat were the raw agricultural commodities (RACs) of interest. Roots and shells were not investigated. All samples were weighed, homogenised in liquid nitrogen and stored at – 20°C or below until analysed.

C. Analytical Procedures
Hay and nutmeat of the normal dose experiment were extracted and the extracts were analysed by HPLC and TLC. From the RACs of the other experiments (overdose and translocation), only the TRR was determined (by combustion). Due to the low TRR in the RACs of the translocation experiment, no extraction was performed. However, nutmeat of the overdose experiment was used for method development, but the RACs of the overdose experiment were not needed for isolation and identification of metabolites.

1. Extraction and fractionation:
The homogenised hay of the normal dose experiment was soaked in methanol/water (1:1, v/v) before being extracted using a high speed blender. The extraction was repeated using methanol/water (1:1, v/v) and methanol. The suspensions were vacuum filtered and combined, yielding the methanol/water extract (combined filtrates) and solids. The methanol/water extract was evaporated to the aqueous remainder. The aqueous remainder was partitioned against dichloromethane leaving the aqueous phase and the dichloromethane phase. The solids of hay were not further investigated since they were accidentally lost. However, an exhaustive microwave extraction was performed with the analogous solids 1 of a repeated extraction (third extraction of hay, see below). A second extraction of hay was performed analogously to the first one in order to have sufficient amounts of aqueous phase of hay for semi-preparative isolation of metabolites. Finally, a third extraction of hay was performed analogously to the first one and analysed to prove the storage stability of residues in hay. An aliquot of solids 1 from this third extraction was subjected to an additional exhaustive extraction step with methanol/water (1:1, v/v) using a microwave assistance. After extraction, the suspension was filtered, yielding solids 2 (non-extractable residues) and the microwave extract.

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The homogenised nutmeat of the normal dose experiment was twice macerated with n-hexane. The suspensions were vacuum filtered and combined, yielding the n-hexane extract (combined filtrates). Subsequently, the solid residue was soaked in deionised water and macerated after adding methanol. The extraction was repeated twice with methanol/water (1:1, v/v) and methanol. The suspensions were vacuum filtered and combined, yielding the methanol/water extract (combined filtrates) and solids 1. The methanol/water extract was evaporated to the aqueous remainder. The aqueous remainder was partitioned against dichloromethane leaving the aqueous phase. The dichloromethane solution was evaporated yielding the dichloromethane phase. The n-hexane extract was submitted to alkaline saponification. An aliquot of the n-hexane extract was rotary evaporated to the oily residue. The oily residue was dissolved with alkaline ethanol (10 g KOH in 100 mL ethanol/water 95:5, v/v) and refluxed for 10 h. The hydrolysate was partitioned against n-hexane, yielding the n hexane phase 1 and (alkaline) water/ethanol phase 1. After acidification with conc. HCl, the hydrolysate (i.e. water/ethanol phase 1) was again partitioned against n-hexane, yielding the n-hexane phase 2 and (acidic) water/ethanol phase 2. The aqueous phase of the nutmeat extraction was submitted to further investigation. The aqueous phase was evaporated to dryness. The residues were refluxed in 6N HCl and subsequently partitioned against ethyl acetate, leaving the H_2O phase 2. The solids 1 of nutmeat were exhaustively extracted following a modified cell wall fractionation procedure, i.e. omitting steps for dissolving pectin, lignin and hemicellulose. An aliquot of solids 1 was first washed with buffer solution and then successively treated with three different enzymes. During reaction, the batches were stirred with a magnetic stirrer. After reaction, the remaining solids were separated from the enzyme solution by centrifugation.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

For the normal dose experiment, the total radioactive residue (TRR) was determined by summation of the radioactivity of the combined extract(s) and of the remaining solids. For the translocation and overdose experiments, the TRR was determined by combustion of aliquots. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

2. Identification and characterisation:
For elucidation of metabolism, extracts and phases were analysed by HPLC and/or TLC with radiodetection. Metabolites were either identified by LC/MS/MS of isolated peaks (in some cases supported by NMR) or by co-chromatography with authentic reference compounds using two independent chromatographic methods with different selectivity (e.g. HPLC and automated multiple development TLC). ^{14}C-labelled natural products were identified or tentatively identified by their chemical/biochemical behaviour and/or TLC co-chromatography.

3. Storage stability:
Storage stability investigations proved that the metabolic profile of the dichloromethane and aqueous phases of all RACs did not change significantly during storage at -20°C for more than three years. However, some aglycon formation in the aqueous phase of hay was observed.

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II. Results and Discussion

The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (= [methoxyiminotolyl-ring-UL-^{14}C]HEC 5725) formulated as an EC 100 was investigated in peanuts following three foliar spray applications, at single rates of 234 - 275 g a.s./ha and a total rate of 781 g a.s./ha.

In the normal dose experiment the TRR in hay amounted to 141.82 mg/kg (Table 6.2.1-9). This high TRR can be explained by the growth conditions in the vegetation area where the plants, in contrast to a field situation, were protected from rain, and hence surface wash off was completely inhibited. It is very probable, that under field conditions the residue level would be much lower. Compared to hay, the TRR in nutmeat was very low amounting to only 0.055 mg/kg. In the overdose experiment (approx. 5X application rate) the TRR in hay and nutmeat amounted to 534.51 mg/kg and 0.086 mg/kg, respectively. In the translocation experiment (nominal application rate of 25 g a.s./ 100 kg seeds) the TRR in hay and nutmeat amounted to 0.138 mg/kg and 0.014 mg/kg, respectively.

Table 6.2.1-9: TRR values in peanut matrices after application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin

Matrix	Timing and Application	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
hay	normal dose experiment: three foliar spray applications, at BBCH 66, BBCH 79 and BBCH 88; 3 x 234 - 275 g a.s./ha = total 781 g a.s./ha	14	141.82
nutmeat			0.055
hay	overdose experiment (5X): three foliar spray applications, at BBCH 67, BBCH 79 and BBCH 88; approx. 5X of the rates in the normal dose experiment		534.51
nutmeat			0.086
hay	translocation experiment: seed treatment at a nominal rate of 25 g a.s./100 kg seeds	144	0.138
nutmeat			0.014

The peanut matrices of the normal dose experiment were extracted, the extracts were analysed by HPLC and TLC and parent compound and metabolites were identified. In the overdose and translocation experiments, only the TRRs of the matrices were determined. Nutmeat of the overdose experiment was extracted and used for method development. However, the matrices of the overdose experiment were not needed for isolation and identification of metabolites.

From peanut hay, a portion of 95.2% of the TRR (135.03 mg/kg) was extractable with methanol/water and methanol (Table 6.2.1-10). After partitioning, the dichloromethane phase contained 89.4% of the TRR (126.79 mg/kg) of the TRR and the aqueous phase contained 5.8% of the TRR (8.24 mg/kg). Microwave extraction of the remaining solids solubilised 2.9% of the TRR (4.16 mg/kg). In total, 98.1% of the TRR (139.19 mg/kg) was extracted/solubilised and only 1.9% of the TRR (2.63 mg/kg) remained non-extractable in the solids (solids 2).

From nutmeat, a portion of 51.1% of the TRR (0.028 mg/kg) was extractable with n-hexane. Methanol/water and methanol extracted further 24.5% of the TRR (0.013 mg/kg). After partitioning of the aqueous remainder of the methanol/water extract against dichloromethane, the dichloromethane phase contained 1.2% of the TRR (0.001 mg/kg) and the aqueous phase contained 23.4% of the TRR (0.013 mg/kg). However, 24.3% of the TRR (0.013 mg/kg) remained in the solids (solids 1). The radioactivity in these solids was almost completely released/solubilised by treatment with buffer

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solution (4.9% of TRR, 0.003 mg/kg) and enzymatic digestion with diastase (11.6% of TRR, 0.006 mg/kg), pronase (1.2% of TRR, 0.001 mg/kg) and cellulase (4.3% of TRR, 0.002 mg/kg). In total, 97.7% of the TRR (0.054 mg/kg) was extracted/solubilised and only 2.3% of the TRR (0.001 mg/kg) remained non-extractable in the solids (solids 2).

Table 6.2.1-10: Distribution of radioactivity in the extracts of the peanut matrices after foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (normal dose experiment)

	hay		nutmeat	
TRR [mg/kg] =	141.82		0.055	
	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
n-Hexane extract	-	-	51.1	0.028
Methanol/water extract	(95.2)	(135.03)	(24.5)	(0.013)
Dichloromethane phase	89.4	126.79	1.2	0.001
Aqueous phase	5.8	8.24	23.4	0.013
Solids 1	(4.8)	(6.79)	(24.3)	(0.013)
Microwave extract	2.9	4.16	-	-
Buffer soluble	-	-	4.9	0.003
Diastase extract	-	-	11.6	0.006
Pronase extract	-	-	1.2	0.001
Cellulase extract	-	-	4.3	0.002
Total extracted/solubilised	98.1	139.19	97.7	0.054
Solids 2 (non-extractable residue)	1.9	2.63	2.3	0.001
Accountability	100.0	141.82	100.0	0.055

For elucidation of metabolism, the extracts or phases were analysed by HPLC and/or TLC with radiodetection. Metabolites were either identified by LC/MS/MS of isolated peaks (in some cases supported by NMR) or HPLC co-chromatography with authentic reference compounds using two independent chromatographic methods with different selectivity. ^{14}C-labelled natural products were identified or tentatively identified by their chemical/biochemical behaviour and/or TLC co-chromatography.

In the dichloromethane phase of hay (Table 6.2.1-11), parent compound (sum of fluoxastrobin and its Z-isomer) was by far the main compound representing 81.9% of the TRR (116.16 mg/kg); metabolites were only minor, each representing ≤ 2.6% of the TRR. In the aqueous phase and in the microwave extract, parent compound and several metabolites were detected in trace amounts (each ≤ 1.0% of TRR).

The complete radioactivity detected in the hexane extract of nutmeat (51.1% of the TRR, 0.028 mg/kg) was identified as fatty acids after alkaline saponification, hence originally representing fat (e.g. triglicerides, peanut oil). The dichloromethane phase contained only 1.2% of the TRR (0.001 mg/kg) and was not analysed. In the aqueous phase at least five components were detected at low levels (each < 10% of the TRR and ≤ 0.005 mg/kg); approx. 50% of the radioactivity was characterised as metabolites of fluoxastrobin after acidic hydrolysis. After treatment of the remaining solids with buffer, diastase, pronase and cellulase, 4.9% of the TRR (0.003 mg/kg) was characterised as buffer soluble, 11.6% of the TRR (0.006 mg/kg) was identified as starch, 1.2% of the TRR (0.001 mg/kg) was identified as proteins and 4.3% of the TRR (0.002 mg/kg) was identified as cellulose.

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Table 6.2.1-11: Distribution of parent compound and metabolites in the extracts of peanut matrices after foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (normal dose experiment)

	hay		nutmeat	
TRR [mg/kg] =	141.82		0.055	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
Hexane extract				
fats / fatty acids	-	-	51.1	0.028
Total in hexane extract	-	-	51	0.028
Dichloromethane phase				
parent compound, sum of	(81.9)	(116.16)	-	-
fluoxastrobin (HEC 5725 E-isomer)	59.0	83.73	-	-
and its Z-isomer (HEC 5725 Z-isomer)	22.9	32.43	-	-
Ring 1,2,3,4 metabolites[a)]	*(7)*	*(10.45)*	-	-
HEC 5725-hydroxyphenyl metabolites	(traces)	(traces)	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(6.4)	(9.13)	-	
HEC 5725-dioxazine-OH (M19)	0.05	0.07	-	-
HEC 5725-CA-glycol ester (M39)	2.2	3.13	-	-
HEC 5725-E-amide (M38)	2.6	3.70	-	-
HEC 5725-Z-amide (M38)	1.2	1.73	-	-
HEC 5725-carboxylic acid (M40)	0.36	0.50	-	-
HEC 5725 oximether cleavage metabolites:	(0.9)	(1.32)	-	-
HEC 5725-ketone (M34)	0.9	1.32	-	-
Sum of 2 unknowns	0.1	0.18	-	-
Total in dichloromethane phase	89.4	126.79	1.2	0.001
Aqueous phase				
parent compound, sum of	(0.3)	(0.36)	-	-
fluoxastrobin (HEC 5725 E-isomer)	0.2	0.29	-	-
and its Z-isomer (HEC 5725 Z-isomer)	0.1	0.07	-	-
Ring 1,2,3,4 metabolites[a)]	*(0.2)*	*(0.27)*	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(0.2)	(0.27)	-	-
HEC 5725-OH-CA-Glc (M42)	0.2	0.27	-	-
Ring 2,3,4 metabolites[a)]	*(0.5)*	*(0.70)*	-	-
HEC 5725-des-chlorophenyl degradates:	(0.1)	(0.18)	-	-
HEC 5725-des-chlorophenyl-Glc (M48a)	0.1	0.18	-	-
HEC 5725-des-chlorophenyl-S-conjugates:	(0.4)	(0.52)	-	-
HEC 5725-des-chlorophenyl-S-Glc (M50)	0.2	0.23	-	-
HEC 5725-des-chlorophenyl-S-Glc-MA (M51)	0.1	0.19	-	-
HEC 5725-des-chlorophenyl-cys (M48b)	0.1	0.10	-	-
Ring 3,4 metabolites[a)]	*(1.4)*	*(2.06)*	-	-
HEC 5725-des-pyrimidine metabolites:	(0.4)	(0.62)	-	-
HEC 5725-E-des-pyrimidine-Glc (M75a)	0.3	0.48	-	-
HEC 5725-Z-des-pyrimidine-Glc (M75a)	0.1	0.14	-	-
HEC 5725-des-pyrimidine oximether cleavage metabolites:	(0.9)	(1.23)	-	-
HEC 5725-dioxazine-alcohol-Glc (M80a)	0.9	1.23	-	-
HEC 5725-des-pyrimidine-dioxazine-OH and ring 4 degr.:	(0.1)	(0.21)	-	-
HEC 5725-phenyl-glyoxylic acid (M90)	0.1	0.21	-	-
Sum of unknowns	3.4[b)]	4.83[b)]	23.4[c)]	0.013[c)]
Total in aqueous phase	5.8	8.24	23.4	0.013
Microwave extract				
parent compound, sum of	(1.0)	(1.40)	-	-
fluoxastrobin (HEC 5725 E-isomer)	0.8	1.10	-	-
and its Z-isomer (HEC 5725 Z-isomer)	0.21	0.30	-	-
Ring 1,2,3,4 metabolites[a)]	*(1.0)*	*(1.41)*	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(0.9)	(1.29)	-	-
HEC 5725-CA-glycol ester (M39)	0.2	0.35	-	-

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	hay		nutmeat	
TRR [mg/kg] =	141.82		0.055	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
HEC 5725-E-amide (M38)	0.1	0.19	-	-
HEC 5725-carboxylic acid (M40)	0.4	0.51	-	-
HEC 5725-des-dioxazine-nitrile (M38a)	0.17	0.24	-	-
HEC 5725 oximether cleavage metabolites:	(0.1)	(0.12)	-	-
HEC 5725-ketone (M34)	0.1	0.12	-	-
Ring 3,4 metabolites[a)]	*(0.4)*	*(0.58)*	-	-
HEC 5725-des-pyrimidine-dioxazine-OH and ring 4 degr.	(0.4)	(0.58)	-	-
HEC 5725-phenyl-glyoxylic acid (M90)	0.2	0.28	-	-
salicylic acid (M91)	0.2	0.30	-	-
Sum of ≥6 unknowns	0.5	0.77	-	-
Total in microwave extract	2.9	4.16	-	-
Buffer soluble				
Total buffer soluble	-	-	4.9	0.003
Diastase extract				
starch (tentatively)	-		11.6	0.006
Total diastase extract	-	-	11.6	0.006
Pronase extract				
proteins (tentatively)	-		1.2	0.001
Total pronase extract	-	-	1.2	0.001
Cellulase extract				
cellulose (tentatively)	-		4.3	0.002
Total cellulase extract	-		4.3	0.002
Total extracted/solubilised	98.1	139.19	97.7	0.054
Solids 2 (non-extractable residue)	1.9	2.63	2.3	0.001
Accountability	100.0	141.82	100.0	0.055

a) ring 1 = chlorophenyl, ring 2 = pyrimidine, ring 3 = methoxyiminotolyl, ring 4 = dioxazine (see also page 34);
b) ≥24 compounds, each <1.0% of TRR; c) ≥5 compounds, each <10% of TRR and ≤ 0.005 mg/kg

The radioactive residues in hay mainly consisted of parent compound (sum of fluoxastrobin and its Z-isomer), amounting to 83.1% of the TRR (Table 6.2.1-12). Isomerisation of the oximether group was the main reaction and was assisted by light. Fluoxastrobin (HEC 5725 E-isomer) was found at 60.0% of the TRR and its Z-isomer at 23.1% of the TRR. This corresponds to an E:Z ratio of approx. 70:30, changed from an initial ratio of 98:2 in the applied formulation. The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin in hay showed a complex pattern and a total of 17 metabolites were identified. However, no metabolite exceeded 2.7% of the TRR. Numerous minor metabolites (each < 1% of the TRR) were characterised by the extraction procedure, partitioning behaviour and retention time. Most of the metabolites identified in hay contained all four rings or at least three rings and fragments of the dioxazine ring (abbreviated as ring 1,2,3,4 metabolites). This metabolite group accounted for 8.6% of the TRR (12.14 mg/kg). The prevailing metabolite group in hay was the HEC 5725-dioxazine-OH and ring 4 degradates representing 7.5% of the TRR (10.71 mg/kg), of which the HEC 5725-E-amide (M38) was predominant (2.7% of the TRR, 3.89 mg/kg).

In nutmeat, 68.2% of the TRR (0.037 mg/kg) were ^{14}C-labelled natural products. The largest portion (51.1% of the TRR, 0.028 mg/kg) represented fat (e.g. triglicerides, peanut oil). Smaller portions represented carbohydrates (15.9% of the TRR, 0.008 mg/kg) and proteins (1.2% of the TRR, 0.001 mg/kg). All these ^{14}C-labelled natural products probably resulted from an intensive mineralisation of the methoxyiminotolyl ring of fluoxastrobin residues in soil, subsequent assimilation of $^{14}CO_2$ released from soil by the peanut plants and deposition of assimilates in nutmeat. Only a few

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minor components (each < 10% of the TRR and ≤ 0.005 mg/kg) were characterised as metabolites of fluoxastrobin in nutmeat.

Table 6.2.1-12: Summary of characterisation and identification of radioactive residues in peanut matrices after foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (normal dose experiment)

	hay		nutmeat	
TRR [mg/kg] =	141.82		0.055	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
parent compound, sum of	(83.1)	(117.92)	-	-
fluoxastrobin (HEC 5725 E-isomer)	60.0	85.12	-	-
and its Z-isomer (HEC 5725 Z-isomer)	23.1	32.80	-	-
Ring 1,2,3,4 metabolites[a)]	*(8.6)*	*(12.14)*	-	-
HEC 5725-hydroxyphenyl metabolites	(traces)	(traces)	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(7.5)	(10.71)	-	-
HEC 5725-dioxazine-OH (M19)	0.1	0.07	-	-
HEC 5725-CA-glycol ester (M39)	2.5	3.48	-	-
HEC 5725-E-amide (M38)	2.7	3.89	-	-
HEC 5725-Z-amide (M38)	1.2	1.73	-	-
HEC 5725-carboxylic acid (M40)	0.7	1.01	-	-
HEC 5725-OH-CA-Glc (M42)	0.2	0.27	-	-
HEC 5725-des-dioxazine-nitrile (M38a)	0.2	0.24	-	-
HEC 5725 oximether cleavage metabolites:	(1.0)	(1.44)	-	-
HEC 5725-ketone (M34)	1.0	1.44	-	-
Ring 2,3,4 metabolites[a)]	*(0.5)*	*(0.71)*	-	-
HEC 5725-des-chlorophenyl degradates:	(0.1)	(0.18)	-	-
HEC 5725-des-chlorophenyl-Glc (M48a)	0.1	0.18	-	-
HEC 5725-des-chlorophenyl-S-conjugates:	(0.4)	(0.52)	-	-
HEC 5725-des-chlorophenyl-S-Glc (M50)	0.2	0.23	-	-
HEC 5725-des-chlorophenyl-S-Glc-MA (M51)	0.1	0.19	-	-
HEC 5725-des-chlorophenyl-cys (M48b)	0.1	0.10	-	-
Ring 3,4 metabolites[a)]	*(1.9)*	*(2.64)*	-	-
HEC 5725-des-pyrimidine metabolites:	(0.4)	(0.62)	-	-
HEC 5725-E-des-pyrimidine-Glc (M75a)	0.3	0.48	-	-
HEC 5725-Z-des-pyrimidine-Glc (M75a)	0.1	0.14	-	-
HEC 5725-des-pyrimidine oximether cleavage metabolites:	(0.9)	(1.23)	-	-
HEC 5725-dioxazine-alcohol-Glc (M80a)	0.9	1.23	-	-
HEC 5725-des-pyrimidine-dioxazine-OH and ring 4 degr.:	(0.6)	(0.80)	-	-
HEC 5725-phenyl-glyoxylic acid (M90)	0.3	0.49	-	-
salicylic acid (M91)	0.2	0.30	-	-
[^{14}C]natural products	-	-	*(68.2)*	*(0.037)*
fats / fatty acids	-	-	51.1	0.028
carbohydrates (starch & cellulose, tentatively)	-	-	15.9	0.008
proteins (tentatively)	-	-	1.2	0.001
Total identified and tentatively identified	94.0	133.41	68.2	0.037
Characterised metabolites				
dichloromethane phase, subtotal	0.1	0.18	1.2	0.001
aqueous phase, subtotal	3.4[b)]	4.83[b)]	23.4[c)]	0.013[c)]
microwave extract, subtotal	0.5	0.77	-	-
buffer soluble fraction of solids 1	-	-	4.9	0.003
Total characterised	4.0	5.78	29.4	0.017
Total extracted/solubilised	98.1	139.19	97.7	0.054
Solids 2 (non-extractable residue)	1.9	2.63	2.3	0.001
Accountability	100.0	141.82	100.0	0.055

a) ring 1 = chlorophenyl, ring 2 = pyrimidine, ring 3 = methoxyiminotolyl, ring 4 = dioxazine (see also page 34);
b) >24 compounds, each <1.0% of TRR; c) >5 compounds, each <10% of TRR and ≤ 0.005 mg/kg

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III. Conclusions

After foliar spray application of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin, the residues in peanut hay mainly consisted of parent compound, amounting to 83.1% of the TRR. The ratio of fluoxastrobin and its Z-isomer changed from an initial ratio of 98:2 in the applied formulation to a ratio of approx. 70:30. A large number of metabolites were detected in low amounts. In nutmeat, the identified portion of the TRR (68.2%) was represented by natural products, i.e. fat, carbohydrates and proteins; only a few minor components were characterised as metabolites of fluoxastrobin.

Neglecting the formation of natural products after assimilation of $^{14}CO_2$ and based on the identified metabolites, the following metabolic routes were deduced:

- isomerisation of the oximether with the formation of the Z-isomer,
- oxidative ring opening and degradation of the dioxazine ring,
- cleavage of the oximether,
- nucleophilic substitution at the chlorophenol ring by glutathione, followed by a stepwise degradation of the glutathione moiety,
- cleavage of the parent molecule, mainly at the pyrimidine-methoxyiminotolyl ether group and to a minor extent at the chlorophenyl-pyrimidine ether group,
- conjugation of hydroxyl and thiol groups to glucosyl and glucosyl-malonyl conjugates and
- to a very minor extent hydroxylation of the chlorophenyl ring.

The metabolic pathway is proposed in Figure 6.2.1-2.

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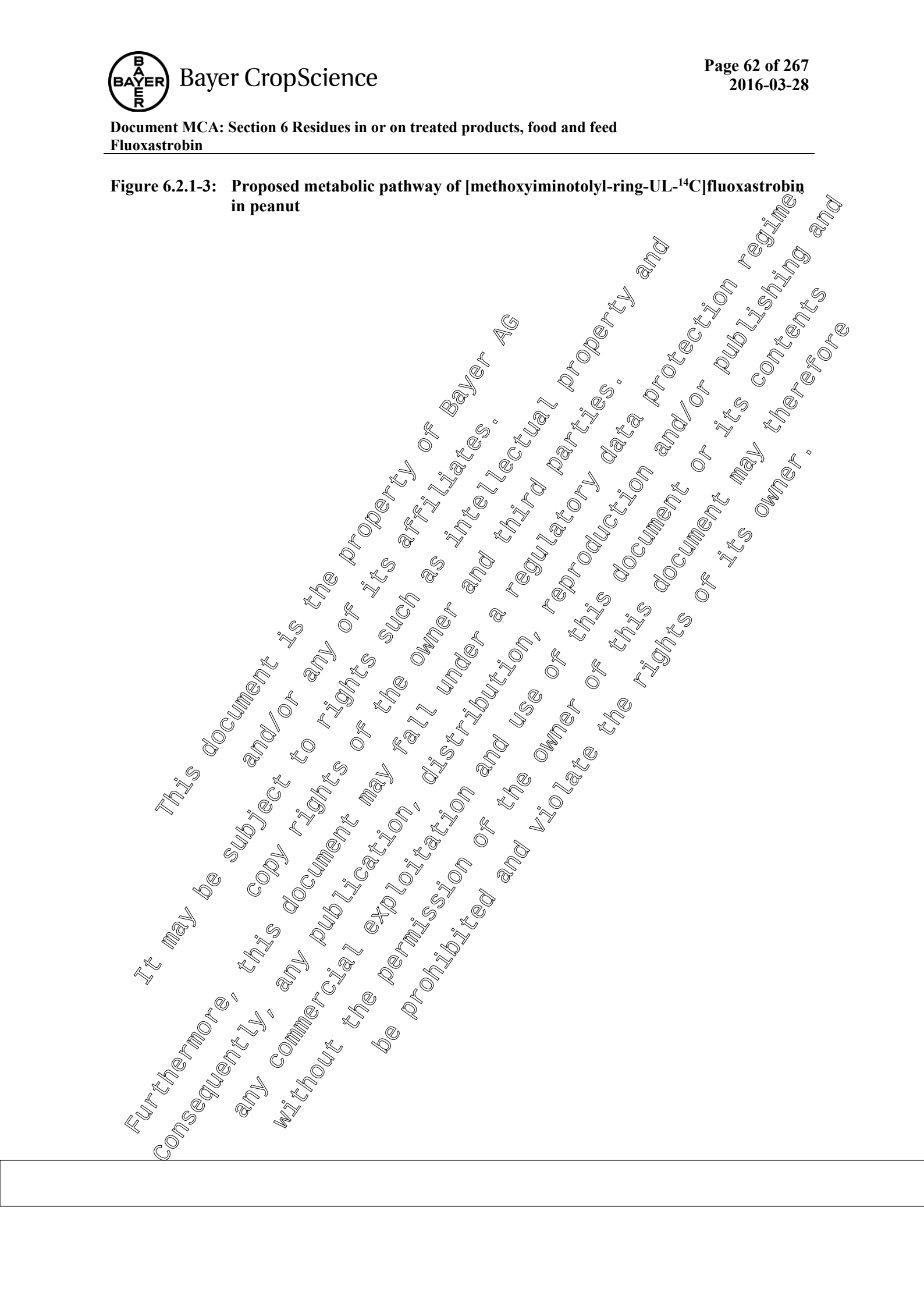
Figure 6.2.1-3: Proposed metabolic pathway of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin in peanut



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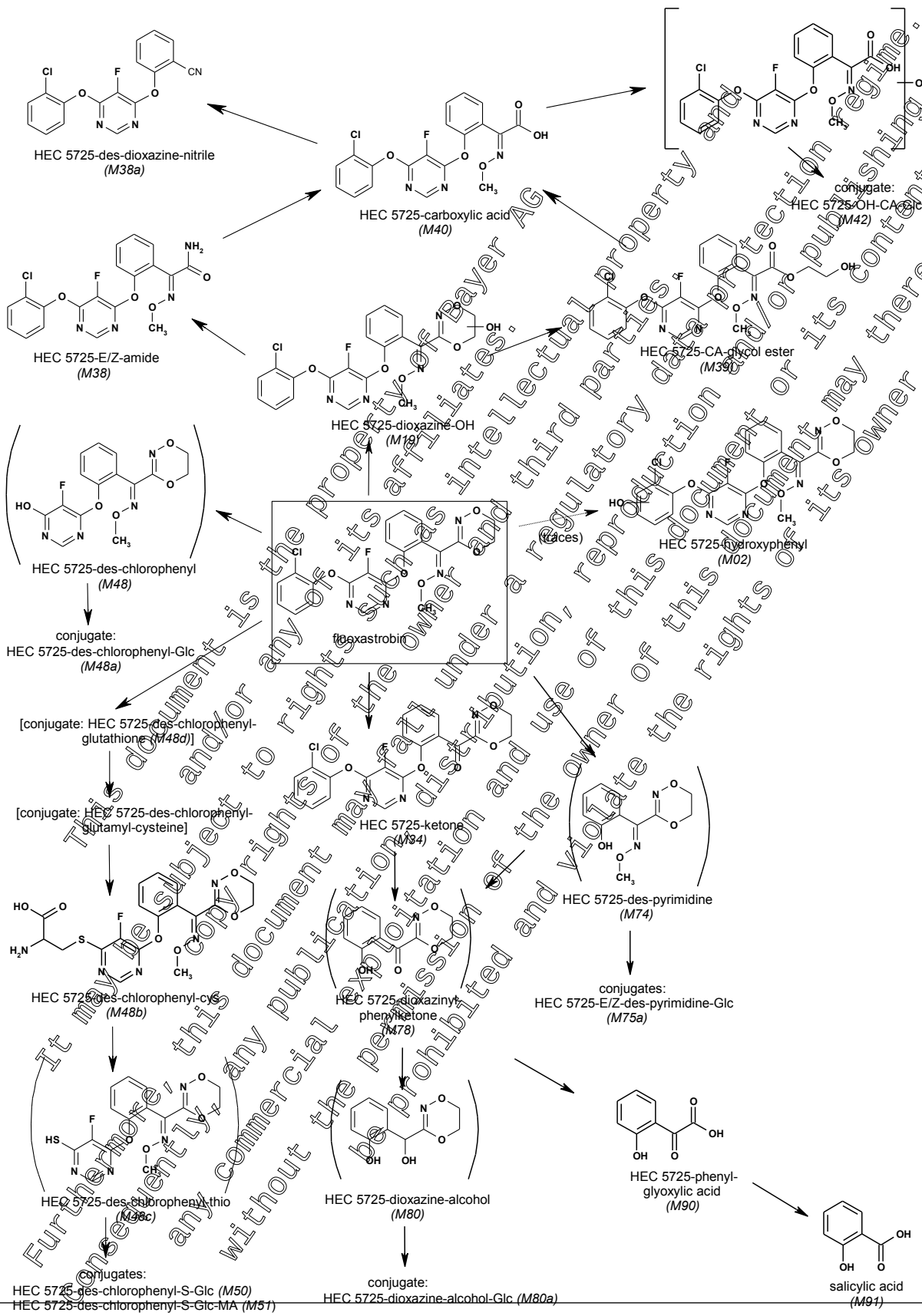

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Report:	KCA 6.2.1/10[redacted]Ä; 2002; M-074227-01-1
Title:	Metabolism of [pyrimidine-2-14C]HEC5725 in peanuts
Report No.:	MR-532/01
Document No.:	M-074227-01-1
Guideline(s):	US EPA OPPTS 860.1300; Canada PMRA Ref.: DACO 6.3; EU 91/414/EEC amended by 96/68/EC
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of [pyrimidine-2-^{14}C]fluoxastrobin (= [pyrimidine-2-^{14}C]HEC 5725) formulated as an EC 100 was investigated in peanuts following three foliar spray applications. The applications were performed at BBCH 66, BBCH 79 and BBCH 89. The single application rate was in the range of 261 - 274 g a.s./ha, the total application rate was 804 g a.s./ha.

The total radioactive residue (TRR) in peanut hay amounted to 129.86 mg/kg. Compared to hay, the TRR in nutmeat was very low and amounted to just 0.146 mg/kg. From hay most of the radioactivity (94.6% of the TRR, 122.90 mg/kg) was extracted with methanol/water and was subsequently partitioned mainly into the dichloromethane phase with only a small amount of radioactivity remaining in the aqueous phase. From nutmeat, a portion of 56.3% of the TRR (0.083 mg/kg) was extracted with hexane and a further portion of 21.4% of the TRR (0.031 mg/kg) was extracted with methanol/water. The radioactivity of the methanol/water extract was partitioned with dichloromethane and remained mainly in the aqueous phase.

In hay, the radioactive residues mainly consisted of parent compound (sum of fluoxastrobin and its Z-isomer), amounting to 85.5% of the TRR. The metabolism of [pyrimidine-2-^{14}C]fluoxastrobin in hay showed a complex pattern and a total of 16 metabolites were identified. However, no metabolite exceeded 2.2% of the TRR.

In nutmeat, 85.0% of the TRR was identified as natural products. The largest portion (56.5% of the TRR) was identified as fatty acids after alkaline saponification, hence originally representing fat. Smaller portions represented carbohydrates, lignin and proteins. All these ^{14}C labelled natural products probably resulted from an intensive mineralisation carbon atom at position 2 of the pyrimidine ring of fluoxastrobin residues in soil, subsequent assimilation of $^{14}CO_2$ released from soil by the peanut plants and deposition of assimilates in nutmeat.

Neglecting the formation of natural products after assimilation of $^{14}CO_2$ and based on the identified metabolites, the following metabolic routes were deduced:

- isomerisation of the oximether with the formation of the Z-isomer,
- oxidative ring opening and degradation of the dioxazine ring,
- cleavage of the oximether,
- nucleophilic substitution of the chlorophenol ring by glutathione, followed by a stepwise degradation of the glutathione moiety,
- cleavage of the parent molecule, mainly at the pyrimidine-methoxyiminotolyl ether group and to a minor extent at the chlorophenyl-pyrimidine ether group and
- conjugation of hydroxyl and thiol groups to glucosyl and glucosyl-malonyl conjugates.

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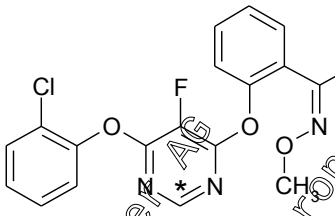
I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 only E-isomer displayed * position of the radiolabel
Radiolabelled test material	[pyrimidine-2-^{14}C]fluoxastrobin
Specific radioactivity	4.18 MBq/mg (113 µCi/mg)
Ratio of fluoxastrobin (HEC 5725 E-isomer)/ Z-isomer (HEC 5725 Z-isomer)	98.1:1.9 (normal dose and overdose experiment) 97.8:2.2 (translocation experiment)
Radiochemical purity	normal dose and overdose experiment: >99% (HPLC and TLC) translocation experiment: >99% (HPLC), >98% (TLC)

2. Soil: "[redacted]3" (sandy loam from Germany), pH ($CaCl_2$) = 6.3, 57.4% sand, 33.0% silt and 9.7% clay, 1.98% organic carbon, cation exchange capacity (CEC) of 10 meq/100 g

3. Plant: Peanut, variety: "Georgia Green"

B. Study Design

1. Experimental conditions:

Three individual experiments were conducted.

Normal dose experiment: This experiment simulated agricultural practice and was the main experiment within the present study. Eighteen peanut plants were grown in a plant container (surface area 1 m²) and a depth of 60 cm filled with a sandy loam soil. Only half of the container (0.5 m²) was used for the experiment. Hence, nine plants were sprayed with [pyrimidine-2-^{14}C]fluoxastrobin formulated as an EC 100. The remaining nine plants were not part of the experiment. The envisaged use pattern at initiation of the study included up to four spray applications, each 203 g a.s./ha, resulting in a maximum annual field rate of 812 g a.s./ha. The first field application was proposed after pegging to beginning of pod development (growth stage BBCH 66 – 75) and the last application 14 days before harvest. The metabolism studies simulated the envisaged use pattern and were based on the maximum proposed application rate. For simplification, only three applications were performed, each at increased actual rates of 264 g a.s./ha (BBCH 66), 269 g a.s./ha (BBCH 79), and 274 g a.s./ha (BBCH 89). This resulted in at total applied rate of 804 g a.s./ha, which was slightly less as compared to the envisaged total application rate of 812 g a.s./ha. The time intervals between the single applications were 42 days between the first and the second and 20 days between the second and the third application. The third application took place 14 days before harvest.

Overdose experiment: This experiment was set up for metabolite isolation and identification, if necessary. Two plants were grown in 7.5 L pots (1 plant/pot) filled with a sandy loam soil. Approximately the same amounts of the EC 100 formulation, which were applied onto nine peanut plants in the normal dose experiment, were applied onto 2 peanut plants in the overdose experiment.

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Hence, an approx. 4X application rate was achieved (18.96 mg a.s./plant in three applications) as compared to the normal dose experiment. The first application was performed at BBCH growth stage 67, the second at BBCH 79 and the third at BBCH 89. The time intervals between the single applications were 42 days between the first and the second and 20 days between the second and the third application. The third application took place 14 days before harvest.

Translocation experiment: This experiment was set up to obtain information on the translocation of radioactivity after seed treatment. Two plants (seeds) were grown in 7.5 L pots (1 plant/pot) filled with a sandy loam soil. The application of this experiment was performed with non-formulated [pyrimidine-2-^{14}C]fluoxastrobin. The amount for seed treatment was calculated from a weight of ca. 0.5 g for a peanut seed and assuming an application rate of 25 g a.s./100 kg seeds. Hence, 0.13 mg a.s. per seed should be applied. Two peanut seeds were treated with 0.12 mg a.s. each, which was slightly less (-8%) as compared to the envisaged amount.

All experiments were performed in the greenhouse with a day/night rhythm of 14/10 hours and an average temperature of 24/23°C (day) and 17/16C°C (night) and a relative humidity of 60%.

2. Sampling
In all experiments peanut plants were removed from soil at BBCH growth stage 97 and dried for 4 days. After four days of drying in the greenhouse, all plants were separated into hay, fruits and roots. The fruits were manually separated into seeds (= nutmeat) and shells. Hay and nutmeat were the raw agricultural commodities (RACs) of interest. Roots and shells were not investigated. All samples were weighed, homogenised in liquid nitrogen and stored at – 20°C or below until analysed.

C. Analytical Procedures
Hay and nutmeat of the normal dose experiment were extracted and the extracts were analysed by HPLC and TLC. From the RACs of the other experiments (overdose and translocation), only the TRR was determined (by combustion). Due to the low TRR in the RACs of the translocation experiment, no extraction was performed. However, nutmeat of the overdose experiment was used for method development, but the RACs of the overdose experiment were not needed for isolation and identification of metabolites.

1. Extraction and fractionation:
The homogenised hay of the normal dose experiment was soaked in methanol/water (1:1, v/v) before being extracted using a high speed blender. The extraction was repeated using methanol/water (1:1, v/v) and methanol. The suspensions were vacuum filtered and combined, yielding the methanol/water extract (combined filtrates) and solids. The methanol/water extract was evaporated to the aqueous remainder. The aqueous remainder was partitioned against dichloromethane leaving the aqueous phase and the dichloromethane phase. The solids of hay were not further investigated since they were accidentally lost. However, an exhaustive microwave extraction was performed with the analogous solids 1 of a repeated extraction (second extraction of hay, see below). A second extraction of hay was performed analogously to the first one in order to have sufficient amounts of aqueous phase of hay for semi-preparative isolation of metabolites and for storage stability investigations. An aliquot of solids 1 from this second extraction was subjected to an additional exhaustive extraction step with methanol/water (1:1, v/v) using a microwave assistance. After extraction, the suspension was filtered, yielding solids 2 (non-extractable residues) and the microwave extract.

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Nutmeat of the normal dose and overdose experiment was extracted. Purpose of the nutmeat extraction of the overdose experiment was method development, i.e. alkaline saponification of peanut oil and determination of fatty acids. After method development, the alkaline saponification and determination of fatty acids was not repeated for the n-hexane phase of the normal dose experiment, as TRRs, extraction efficiencies etc. were very similar to the overdose experiment and the nature of the radioactivity in the n-hexane phase was regarded to be the same.

The homogenised nutmeat of the normal dose and overdose experiment was twice macerated with n-hexane. The suspensions were vacuum filtered and combined, yielding the n-hexane extract (combined filtrates). Subsequently, the solid residue was soaked in deionised water and macerated after adding methanol. The extraction was repeated twice with methanol/water (1:1, v/v) and methanol. The suspensions were vacuum filtered and combined, yielding the methanol/water extract (combined filtrates) and solids 1. The methanol/water extract was evaporated to the aqueous remainder. The aqueous remainder was partitioned against dichloromethane leaving the aqueous phase. The dichloromethane solution was evaporated yielding the dichloromethane phase. The n-hexane extract of the overdose experiment was submitted to alkaline saponification. An aliquot of the n-hexane extract was rotary evaporated to the oily residue. The oily residue was dissolved with alkaline ethanol (10 g KOH in 100 mL ethanol/water 95:5, v/v) and refluxed for 10 h. The hydrolysate was partitioned against n-hexane, yielding the n-hexane phase 1 and (alkaline) water/ethanol phase 1. After acidification with conc. HCl, the hydrolysate (i.e. water/ethanol phase 1) was again partitioned against n-hexane, yielding the n-hexane phase 2 and (acidic) water/ethanol phase 2. The aqueous phase of the nutmeat extraction of the normal dose experiment was submitted to further investigation. An aliquot of the aqueous phase was evaporated to dryness. The residues were refluxed in 6N HCl and subsequently partitioned against ethyl acetate, leaving the H_2O phase 2. The H_2O phase 2 was further investigated by SPE. The H_2O phase 2 was given onto an SCX column, rinsed with 1% aqueous acetic acid/methanol (50/50 v:v) and eluted with 1% aqueous ammonia/methanol (50/50 v:v). The SCX rinse was adjusted to pH 9 using aqueous ammonia, given onto an SAX column, rinsed with 1% aqueous ammonia/methanol (50/50 v:v) and eluted with 1N HCl/methanol (50/50 v:v). The solids 1 of nutmeat of the normal dose experiment were exhaustively extracted following a modified cell wall fractionation procedure, i.e. omitting steps for dissolving pectin and hemicellulose. An aliquot of solids 1 was first washed with buffer solution and then successively treated with three different enzymes. During reaction, the batches were stirred with a magnetic stirrer. After reaction, the remaining solids were separated from the enzyme solution by centrifugation. As considerable amounts of radioactivity were not dissolved after the cellulase treatment, a lignin dissolving step with dioxane/HCl treatment (up to 70°C, 5h) was added.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

For the normal dose experiment, the total radioactive residue (TRR) was determined by summation of the radioactivity of the combined extract(s) and of the remaining solids. For the translocation and overdose experiments, the TRR was determined by combustion of aliquots. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

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2. Identification and characterisation:
For elucidation of metabolism, extracts and phases were analysed by HPLC and/or TLC with radiodetection. Metabolites were either identified by LC/MS/MS of isolated peaks (in some cases supported by NMR) or by co-chromatography with authentic reference compounds using two independent chromatographic methods with different selectivity (e.g. HPLC and automated multiple development TLC). ^{14}C-labelled natural products were identified or tentatively identified by their chemical/biochemical behaviour and/or TLC co-chromatography.

3. Storage stability:
Storage stability investigations proved that the metabolic profile of the dichloromethane and aqueous phases of all RACs did not change significantly during storage at -20°C for more than three years. However, some aglycon formation in the aqueous phase of hay was observed.

II. Results and Discussion

The metabolism of [pyrimidine-2-^{14}C]fluoxastrobin (= [pyrimidine-2-^{14}C]UEC 5725) formulated as an EC 100 was investigated in peanuts following three foliar spray applications at single rates of 261 - 274 g a.s./ha and a total rate of 804 g a.s./ha.

In the normal dose experiment the TRR in hay amounted to 129.86 mg/kg (Table 6.2.1-13). This high TRR can be explained by the growth conditions in the vegetation area where the plants, in contrast to a field situation, were protected from rain, and hence surface wash off was completely inhibited. It is very probable, that under field conditions the residue level would be much lower. Compared to hay, the TRR in nutmeat was very low amounting to only 0.146 mg/kg. In the overdose experiment (approx. 4X application rate) the TRR in hay and nutmeat amounted to 540.02 mg/kg and 0.193 mg/kg, respectively. In the translocation experiment (nominal application rate of 25 g a.s./ 100 kg seeds) the TRR in hay and nutmeat amounted to 0.116 mg/kg and 0.016 mg/kg, respectively.

Table 6.2.1-13: TRR values in peanut matrices after application of [pyrimidine-2-^{14}C]fluoxastrobin

Matrix	Timing and Application	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
hay	normal dose experiment: three foliar spray applications, at BBCH 66, BBCH 79 and BBCH 89; 3 x 261 – 274 g a.s./ha = total 804 g a.s./ha	14	129.86
nutmeat			0.146
hay	overdose experiment (4X): three foliar spray applications, at BBCH 67, BBCH 79 and BBCH 89; approx. 4X of the rates in the normal dose experiment		540.02
nutmeat			0.193
hay	translocation experiment: seed treatment at a nominal rate of 25 g a.s./100 kg seeds	144	0.116
nutmeat			0.016

The peanut matrices of the normal dose experiment were extracted, the extracts were analysed by HPLC and TLC and parent compound and metabolites were identified. In the overdose and translocation experiments, only the TRRs of the matrices were determined. Nutmeat of the overdose experiment was extracted and used for method development, it was also used for the identification of fatty acids. However, the matrices of the overdose experiment were not needed for further isolation and/or identification of metabolites.

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From peanut hay (Table 6.2.1-14), a portion of 94.6% of the TRR (122.90 mg/kg) was extractable with methanol/water and methanol. After partitioning, the dichloromethane phase contained 90.9% of the TRR (118.05 mg/kg) of the TRR and the aqueous phase contained 3.7% of the TRR (4.84 mg/kg). Microwave extraction of the remaining solids solubilised 3.5% of the TRR (4.58 mg/kg). In total, 98.2% of the TRR (127.48 mg/kg) was extracted/solubilised and only 1.8% of the TRR (2.39 mg/kg) remained non-extractable in the solids (solids 2).

From nutmeat, a portion of 56.5% of the TRR (0.083 mg/kg) was extractable with n-hexane. Methanol/water and methanol extracted further 21.4% of the TRR (0.031 mg/kg). After partitioning of the aqueous remainder of the methanol/water extract against dichloromethane, the dichloromethane phase contained 0.5% of the TRR (0.001 mg/kg) and the aqueous phase contained 20.8% of the TRR (0.030 mg/kg). However, 22.1% of the TRR (0.032 mg/kg) remained in the solids (solids 1). The radioactivity in these solids was almost completely released/solubilised by treatment with buffer solution (4.3% of TRR, 0.006 mg/kg) and enzymatic digestion with diastase (9.8% of TRR, 0.014 mg/kg), pronase (2.2% of TRR, 0.003 mg/kg) and cellulase (1.0% of TRR, 0.002 mg/kg) followed by treatment with dioxane/HCl (3.9% of TRR, 0.006 mg/kg). In total, 99.2% of the TRR (0.145 mg/kg) was extracted/solubilised and only 0.8% of the TRR (0.001 mg/kg) remained non-extractable in the solids (solids 2).

Table 6.2.1-14: Distribution of radioactivity in the extracts of the peanut matrices after foliar spray application of [pyrimidine-2-^{14}C]fluoxastrobin (normal dose experiment)

	hay		nutmeat	
TRR [mg/kg] =	129.86		0.146	
	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
n-Hexane extract	-	-	56.5	0.083
Methanol/water extract	(94.6)	(122.90)	(21.4)	(0.031)
Dichloromethane phase	90.9	118.05	0.5	0.001
Aqueous phase	3.7	4.84	20.8	0.030
Solids 1	(5.4)	(6.96)	(22.1)	(0.032)
Microwave extract	3.5	4.58	-	-
Buffer soluble	-	-	4.3	0.006
Diastase extract	-	-	9.8	0.014
Pronase extract		-	2.2	0.003
Cellulase extract		-	1.0	0.002
Dioxane/HCl extract	-	-	3.9	0.006
Total extracted/solubilised	98.2	127.48	99.2	0.145
Solids 2 (non-extractable residue)	1.8	2.39	0.8	0.001
Accountability	100.0	129.86	100.0	0.146

For elucidation of metabolism, the extracts or phases were analysed by HPLC and/or TLC with radiodetection. Metabolites were either identified by LC/MS/MS of isolated peaks (in some cases supported by NMR) or HPLC co-chromatography with authentic reference compounds using two independent chromatographic methods with different selectivity. ^{14}C-labelled natural products were identified or tentatively identified by their chemical/biochemical behaviour and/or TLC co-chromatography.

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In the dichloromethane phase of hay (Table 6.2.1-15), parent compound (sum of fluoxastrobin and its Z-isomer) was by far the main compound representing 83.7% of the TRR (108.64 mg/kg); metabolites were only minor, each representing ≤ 2.1% of the TRR. In the aqueous phase and in the microwave extract, several metabolites were detected in trace amounts (each < 0.5% of TRR). Parent compound was a minor component in the microwave extract (1.9% of TRR).

The complete radioactivity detected in the hexane extract of nutmeat (56.5% of the TRR, 0.083 mg/kg) was identified as fatty acids after alkaline saponification, hence originally representing fat (e.g. triglicerides, peanut oil). The dichloromethane phase contained only 0.5% of the TRR (0.001 mg/kg) and was not analysed. After acidic hydrolysis of the aqueous phase 7.3% of the TRR (0.011 mg/kg) was partitioned into ethyl acetate. The remaining aqueous phase was investigated by ion exchange SPE. Only 1.9% of the TRR (0.003 mg/kg) was found in the acidic SAX eluate. The main portion of radioactivity (11.6% of the TRR, 0.017 mg/kg) was found in the neutral fraction (SAX rinse), which was identified as glucose, probably originally representing starch. After treatment of the remaining solids with buffer, diastase, pronase, cellulase and dioxane/HCl, 4.3% of the TRR (0.006 mg/kg) was characterised as buffer soluble, 9.5% of the TRR (0.014 mg/kg) was identified as starch, 2.5% of the TRR (0.003 mg/kg) was identified as proteins, 1.0% of the TRR (0.002 mg/kg) was identified as cellulose and 3.9% of the TRR (0.006 mg/kg) was identified as lignin.

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Table 6.2.1-15: Distribution of parent compound and metabolites in the extracts of peanut matrices after foliar spray application of [pyrimidine-2-^{14}C]fluoxastrobin (normal dose experiment)

	hay		nutmeat	
TRR [mg/kg] =	129.86		0.146	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
Hexane extract				
fats / fatty acids	-	-	56.5	0.083
Total in hexane extract	-	-	56.5	0.083
Dichloromethane phase				
parent compound, sum of	(83.7)	(108.64)	-	-
fluoxastrobin (HEC 5725 E-isomer)	60.0	77.92	-	-
and its Z-isomer (HEC 5725 Z-isomer)	23.7	30.72	-	-
Ring 1,2,3,4 metabolites[a)]	*(6.2)*	*(8.07)*	-	-
HEC 5725-hydroxyphenyl metabolites	(-)	-	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(5.4)	(6.99)	-	-
HEC 5725-dioxazine-OH (M19)	0.05	0.07	-	-
HEC 5725-CA-glycol ester (M39)	1.5	2.00	-	-
HEC 5725-E-amide (M38)	2.1	2.74	-	-
HEC 5725-Z-amide (M38)	1.4	1.80	-	-
HEC 5725-carboxylic acid (M40)	0.29	0.38	-	-
HEC 5725 oximether cleavage metabolites:	(0.8)	(1.08)	-	-
HEC 5725-ketone (M34)	0.8	1.08	-	-
Ring 1,2 metabolites[a)]	*(0.7)*	*(0.97)*	-	-
HEC 5725-phenoxy-hydroxypyrimidine-metabolites:	(0.7)	(0.97)	-	-
HEC 5725-phenoxy-aminopyrimidine (M56)	0.7	0.97	-	-
Sum of 7 unknowns	0.3	0.38	-	-
Total in dichloromethane phase	90.9	118.05	0.5	0.001
Aqueous phase				
Ring 2,3,4 metabolites[a)]	*(0.3)*	*(0.41)*	-	-
HEC 5725-des-chlorophenyl degradates:	(<0.1)	(0.05)	-	-
HEC 5725-des-chlorophenyl-Glc (M48a)	<0.1	0.05	-	-
HEC 5725-des-chlorophenyl-S-conjugates:	(0.3)	(0.35)	-	-
HEC 5725-des-chlorophenyl-S-Glc (M50)	0.2	0.27	-	-
HEC 5725-des-chlorophenyl-S-Glc-MA (M51)	0.1	0.09	-	-
Ring 1,2 metabolites[a)]	*(0.4)*	*(0.61)*	-	-
HEC 5725-phenoxy-hydroxypyrimidine-metabolites:	(0.1)	(0.17)	-	-
HEC 5725-phenoxy-hydroxy-PMD-Glc (M55a)	0.1	0.17	-	-
HEC 5725-OH-phenoxy-hydroxypyrimidine-metabolites:	(0.3)	(0.44)	-	-
HEC 5725-OH-phenoxy-amino-PMD (M57)	0.2	0.21	-	-
HEC 5725-OH-phenoxy-amino-PMD-Glc (M57a)	0.1	0.18	-	-
HEC 5725-OH-phenoxy-amino-PMD-Glc-MA (M57b)	<0.1	0.05	-	-
Sum of unknowns	2.9[b)]	3.83[b)]	-	-
Ethyl acetate phase after acidic hydrolysis	-	-	7.3	0.011
Acidic SAX eluate after acidic hydrolysis	-	-	1.9	0.003
SAX rinse after acidic hydrolysis (starch)	-	-	11.6	0.017
Total in aqueous phase	3.7	4.84	20.8	0.030
Microware extract				
parent compound, sum of	(1.9)	(2.45)	-	-
fluoxastrobin (HEC 5725 E-isomer)	1.4	1.76	-	-
and its Z-isomer (HEC 5725 Z-isomer)	0.53	0.69	-	-
Ring 1,2,3,4 metabolites[a)]	*(0.6)*	*(0.88)*	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(0.6)	(0.88)	-	-
HEC 5725-CA-glycol ester (M39)	0.2	0.23	-	-
HEC 5725-E-amide (M38)	0.1	0.17	-	-
HEC 5725-carboxylic acid (M40)	0.2	0.32	-	-

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	hay		nutmeat	
TRR [mg/kg] =	129.86		0.146	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
HEC 5725-des-dioxazine-nitrile (M38a)	0.12	0.16	-	-
Ring 1,2 metabolites[a)]	*(0.6)*	*(0.70)*	-	-
HEC 5725-phenoxy-hydroxypyrimidine-metabolites:	(0.6)	(0.70)	-	-
HEC 5725-phenoxy-hydroxypyrimidine (M55)	0.4	0.46	-	-
HEC 5725-phenoxy-aminopyrimidine (M56)	0.2	0.24	-	-
Sum of 5 unknowns	0.4	0.53	-	-
Total in microwave extract	3.5	4.58	-	-
Buffer soluble				
Total buffer soluble	-	-	4.3	0.006
Diastase extract				
starch (tentatively)	-	-	9.5	0.014
Total diastase extract	-	-	9.5	0.014
Pronase extract				
proteins (tentatively)	-	-	2.5	0.003
Total pronase extract	-	-	2.5	0.003
Cellulase extract				
cellulose (tentatively)	-	-	1.0	0.002
Total cellulase extract	-	-	1.0	0.002
Dioxane/HCl extract				
lignin (tentatively)	-	-	3.9	0.006
Total dioxane/HCl extract	-	-	3.9	0.006
Total extracted/solubilised	98.2	127.48	99.2	0.145
Solids 2 (non-extractable residue)	1.8	2.39	0.8	0.001
Accountability	100.0	129.86	100.0	0.146

a) ring 1 = chlorophenyl, ring 2 = pyrimidine, ring 3 = methoxyiminotolyl, ring 4 = dioxazine (see also page 34);
b) ≥27 compounds, each <0.5% of TRR

The radioactive residues in hay mainly consisted of parent compound (sum of fluoxastrobin and its Z-isomer), amounting to 85.5% of the TRR (Table 6.2.1-16). Isomerisation of the oximether group was the main reaction and was assisted by light. Fluoxastrobin (HEC 5725 E-isomer) was found at 61.4% of the TRR and its Z-isomer at 24.2% of the TRR. This corresponds to an E:Z ratio of approx. 70:30, changed from an initial ratio of approx. 98:2 in the applied formulation. The metabolism of [pyrimidine-2-^{14}C]fluoxastrobin in hay showed a complex pattern and a total of 16 metabolites were identified. However, no metabolite exceeded 2.2% of the TRR. Numerous minor metabolites (each < 0.5% of the TRR) were characterised by the extraction procedure, partitioning behaviour and retention time. Most of the metabolites identified in hay contained all four rings or at least three rings and fragments of the dioxazine ring (abbreviated as ring 1,2,3,4 metabolites). This metabolite group accounted for 6.9% of the TRR (8.96 mg/kg). The prevailing metabolite group in hay was the HEC 5725-dioxazine-OH and ring 4 degradates representing 6.1% of the TRR (7.87 mg/kg), of which the HEC 5725-E-amide (M38) was predominant (2.2% of the TRR, 2.91 mg/kg).

In nutmeat, 85.0% of the TRR (0.125 mg/kg) were ^{14}C-labelled natural products. The largest portion (56.5% of the TRR, 0.083 mg/kg) represented fat (e.g. triglicerides, peanut oil). Smaller portions represented carbohydrates (22.1% of the TRR, 0.033 mg/kg), proteins (2.5% of the TRR, 0.003 mg/kg) and lignin (3.9% of the TRR, 0.006 mg/kg). All these ^{14}C-labelled natural products probably resulted from an intensive mineralisation of the carbon atom at position 2 of the pyrimidine ring of fluoxastrobin residues in soil, subsequent assimilation of $^{14}CO_2$ released from soil by the peanut plants and deposition of assimilates in nutmeat. No metabolites of fluoxastrobin were detected in nutmeat.

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Table 6.2.1-16: Summary of characterisation and identification of radioactive residues in peanut matrices after foliar spray application of [pyrimidine-2-^{14}C]fluoxastrobin (normal dose experiment)

	hay		nutmeat	
TRR [mg/kg] =	129.86		0.146	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
parent compound, sum of	(85.5)	(111.09)	-	-
fluoxastrobin (HEC 5725 E-isomer)	61.4	79.68	-	-
and its Z-isomer (HEC 5725 Z-isomer)	24.2	31.41	-	-
Ring 1,2,3,4 metabolites[a)]	*(6.9)*	*(8.96)*	-	-
HEC 5725-hydroxyphenyl metabolites	(-)	(-)	-	-
HEC 5725-dioxazine-OH and ring 4 degradates:	(6.1)	(7.87)	-	-
HEC 5725-dioxazine-OH (M19)	0.1	0.07	-	-
HEC 5725-CA-glycol ester (M39)	1.7	2.23	-	-
HEC 5725-E-amide (M38)	2.2	2.91	-	-
HEC 5725-Z-amide (M38)	1.4	1.80	-	-
HEC 5725-carboxylic acid (M40)	0.5	0.70	-	-
HEC 5725-des-dioxazine-nitrile (M38a)	0.1	0.16	-	-
HEC 5725 oximether cleavage metabolites:	(0.8)	(1.08)	-	-
HEC 5725-ketone (M34)	0.8	1.08	-	-
Ring 2,3,4 metabolites[a)]	*(0.3)*	*(0.41)*	-	-
HEC 5725-des-chlorophenyl degradates:	(<0.1)	(0.05)	-	-
HEC 5725-des-chlorophenyl-Glc (M48a)	<0.1	0.05	-	-
HEC 5725-des-chlorophenyl-S-conjugates:	(0.3)	(0.35)	-	-
HEC 5725-des-chlorophenyl-S-Glc (M50)	0.2	0.27	-	-
HEC 5725-des-chlorophenyl-S-Glc-MA (M51)	0.1	0.09	-	-
Ring 1,2 metabolites[a)]	*(1.8)*	*(2.29)*	-	-
HEC 5725-phenoxy-hydroxypyrimidine-metabolites:	(1.4)	(1.84)	-	-
HEC 5725-phenoxy-hydroxypyrimidine (M55)	0.4	0.46	-	-
HEC 5725-phenoxy-hydroxy-PMD-Glc (M55a)	0.1	0.17	-	-
HEC 5725-phenoxy-aminopyrimidine (M56)	0.9	1.21	-	-
HEC 5725-OH-phenoxy-hydroxypyrimidine-metabolites:	(0.3)	(0.44)	-	-
HEC 5725-OH-phenoxy-amino-PMD (M57)	0.2	0.21	-	-
HEC 5725-OH-phenoxy-amino-PMD-Glc (M57a)	0.1	0.18	-	-
HEC 5725-OH-phenoxy-amino-PMD-Glc-MA (M57b)	<0.1	0.05	-	-
[^{14}C]natural products	-	-	*(85.0)*	*(0.125)*
fats / fatty acids	-	-	56.5	0.083
carbohydrates (starch & cellulose, tentatively)	-	-	22.1	0.033
proteins (tentatively)	-	-	2.5	0.003
lignin (tentatively)			3.9	0.006
Total identified and tentatively identified	94.5	122.74	85.0	0.125
Characterised metabolites				
dichloromethane phase, subtotal	0.3	0.38	0.5	0.001
aqueous phase, subtotal	2.9[b)]	3.83[b)]	-	-
microwave extract, subtotal	0.4	0.53	-	-
ethyl acetate phase after acidic hydrolysis	-	-	7.3	0.011
acidic SAX eluate after acidic hydrolysis	-	-	1.9	0.003
buffer soluble fraction of solids 1	-	-	4.3	0.006
Total characterised	3.6	4.74	14.0	0.021
Total extracted/solubilised	98.2	127.48	99.2	0.145
Solids 2 (non-extractable residue)	1.8	2.39	0.8	0.001
Accountability	100.0	129.86	100.0	0.146

a) ring 1 = chlorophenyl, ring 2 = pyrimidine, ring 3 = methoxyiminotolyl, ring 4 = dioxazine (see also page 34);
b) ≥27 compounds, each <0.5% of TRR

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III. Conclusions

After foliar spray application of [pyrimidine-2-^{14}C]fluoxastrobin, the residues in peanut hay mainly consisted of parent compound, amounting to 85.5% of the TRR. The ratio of fluoxastrobin and its Z-isomer changed from an initial ratio of approx. 98:2 in the applied formulation to a ratio of approx. 70:30. A large number of metabolites were detected in low amounts. In nutmeat, the identified portion of the TRR (85.0%) was represented by natural products, i.e. fat, carbohydrates, proteins and lignin.

Neglecting the formation of natural products after assimilation of $^{14}CO_2$ and based on the identified metabolites, the following metabolic routes were deduced:

- isomerisation of the oximether with the formation of the Z-isomer,
- oxidative ring opening and degradation of the dioxazine ring,
- cleavage of the oximether,
- nucleophilic substitution at the chlorophenol ring by glutathione, followed by a stepwise degradation of the glutathione moiety,
- cleavage of the parent molecule, mainly at the pyrimidine-methoxyiminotolyl ether group and to a minor extent at the chlorophenyl-pyrimidine ether group and
- conjugation of hydroxyl and thiol groups to glucosyl and glucosyl-malonyl conjugates.

The metabolic pathway is proposed Figure 6.2.1-4.

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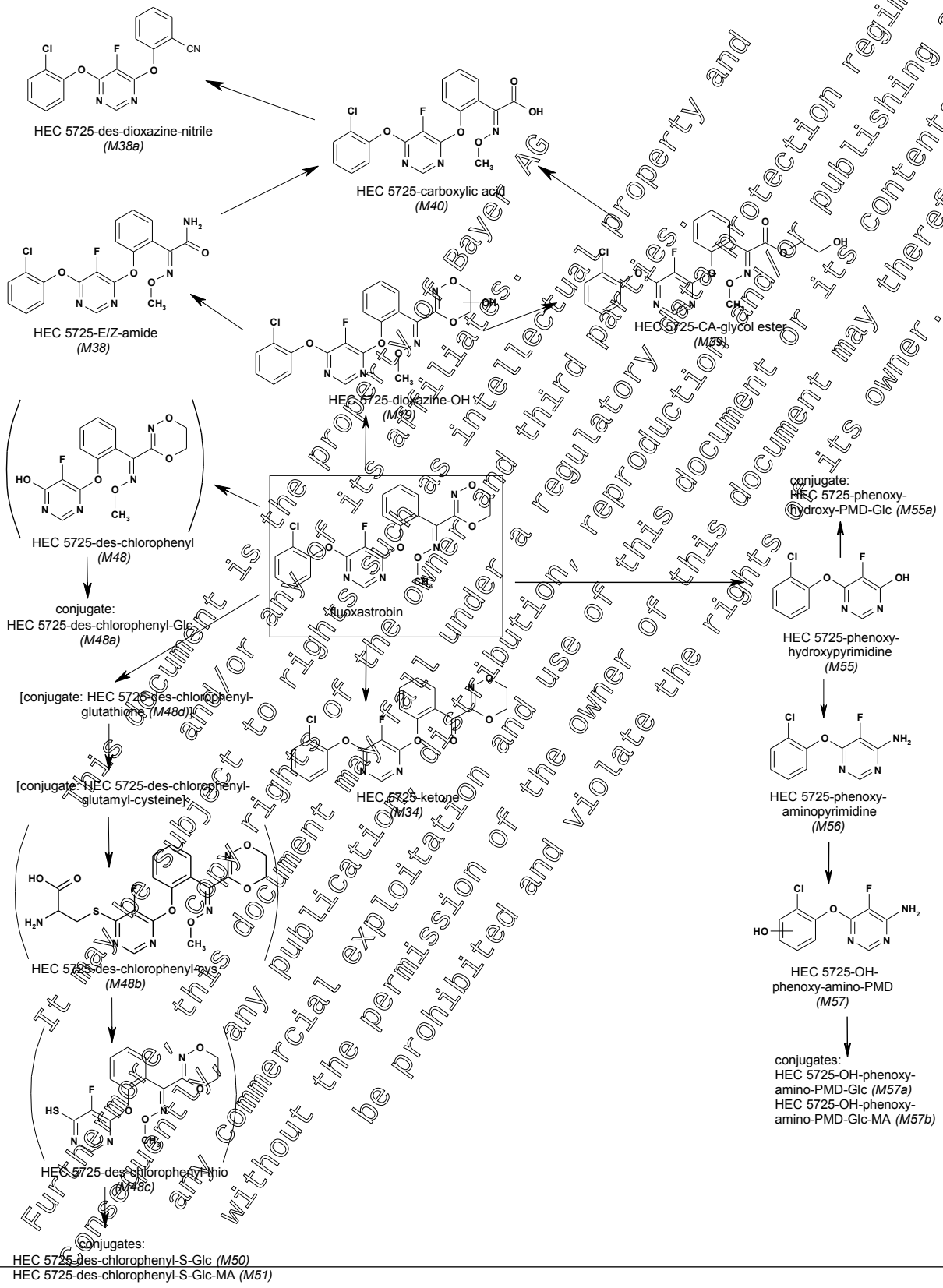
Figure 6.2.1-4: Proposed metabolic pathway of [pyrimidine-2-^{14}C]fluoxastrobin in peanut



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Metabolism in oilseed rape (seed treatment)

A metabolism study in oilseed rape was conducted with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin:

Report:	KCA 6.2.1/11 [redacted],; [redacted]; 2003; M-109459-01-1
Title:	Metabolism of [methoxyiminotolyl-ring-UL-14C]HEC5725 in oilseed rape after seed dressing
Report No.:	MEF-487/02
Document No.:	M-109459-01-1
Guideline(s):	US EPA OPPTS 860.1300; EU 91/414/EEC amended by 96/68/EC
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (= [methoxyiminotolyl-ring-UL-^{14}C]HEC 5725) formulated as an FS 140 was investigated in oilseed rape following seed treatment. The application rate was approx. 3.5 g a.s./ha corresponding to approx. 70 g a.i./100 kg seed. Additionally, a 10x overdose experiment was conducted.

The total radioactive residues (TRRs) in forage, seeds and straw were very low. The TRRs amounted to 0.001 mg/kg (expressed as active substance equivalents) in all matrices of the normal dose experiment. In the 10X overdose experiment, the TRRs were 0.002 mg/kg for forage, < 0.001 mg/kg for seeds and 0.005 mg/kg for straw. From forage, most of the radioactivity (85.5 – 94.1% of the TRR) was extracted with methanol/water and methanol; after partition with dichloromethane the major portion remained in the aqueous phase (56.1 – 85.5% of the TRR). From seeds, 7.8 – 23.5% of the TRR was extractable with hexane and up to 13.1% of the TRR with methanol/water and methanol. From straw, 54.1 – 71.2% of the TRR was extractable with methanol/water and methanol.

Due to the very low TRRs no identification and quantitation of parent compound and metabolites in forage, seeds and straw was performed. A low rate of uptake and translocation of fluoxastrobin and/or possible metabolites into the aerial parts of the plant was calculated (0.3% of the applied radioactivity).

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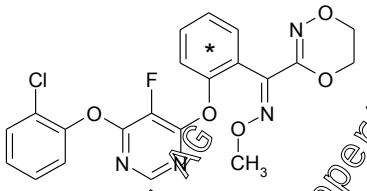
I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 only E-isomer displayed * position of the radiolabel
Radiolabelled test material	[methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin
Specific radioactivity	3.62 MBq/mg (97.8 µCi/mg)
Ratio of fluoxastrobin (HEC 5725 E-isomer)/ Z-isomer (HEC 5725 Z-isomer)	97.8:2.2
Radiochemical purity	> 99% (HPLC) > 98% (TLC)

2. Soil:

soil "[redacted]": loamy sand from Germany, pH ($CaCl_2$) = 5.9, 75.8% sand, 19.0% silt and 5.2% clay, 1.38% organic carbon, cation exchange capacity (CEC) of 5 meq/100 g

Standard Soil T: 85% white moor peat from Northern Germany, 15% clay, pH ($CaCl_2$) = ca. 5.8, 0.28% organic carbon, cation exchange capacity (CEC) of 50 meq/100 g

The treated seeds were germinated for 10 days in small pots containing a mixture of 75% of the soil "[redacted]" and 25% of Standard Soil T and then transplanted to 35 L planting buckets containing soil "[redacted]".

3. Plant: Summer Rape, variety "Lisonne"

B. Study Design

1. Experimental conditions:

Two individual experiments were conducted.

Normal dose experiment: This experiment simulated the envisaged seed treatment use pattern and was based on a maximum proposed application rate of 70 g a.i./100 kg seed resulting from a maximum of 500 mL FS 140/100 kg seeds. This rate corresponds to 3.5 g a.s./ha assuming a seed rate of 5 kg seeds/ha. Each seed was treated with 3.52 µg of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin formulated as an FS 140. This resulted in an actual application rate deviating by +0.6% from the maximum proposed application rate. A total of 110 seeds were sown in soil pots, 96 of which were transplanted after germination to eight 35 L planting-buckets (12 seedlings per bucket).

Overdose experiment (10X overdose): This experiment was set up for metabolite isolation and identification, if necessary. In this experiment [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin formulated as an FS 140 was applied at a rate of approx. 33 µg/seed. This resulted in an actual application rate deviating by -5.8% from the envisaged 10X rate. A total of 25 seeds were sown in soil pots, 23 of which were transplanted after germination to two 35 L planting-buckets.

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Both experiments were performed in the greenhouse with a day/night rhythm of 14/10 hours and an average temperature of 19/20°C (day) and 13/14C°C (night) and a relative humidity of 60%.

2. Sampling

Two plants per planting bucket were sampled at BBCH growth stage 34 (46 days after the application) for the forage samples. Aliquots of the forage samples were homogenised.

At maturity (BBCH growth stage 88 – 89, 160 days after the application), the aerial parts of the plants were harvested. Seeds were collected from the pods. The empty pods and the rest of the harvested plants were combined to yield the straw samples. Aliquots of the straw samples were homogenised.

All samples were stored at -20°C or below until analysed.

C. Analytical Procedures

1. Extraction and fractionation:

The forage was extracted twice with methanol/water (1:1, v/v) and once with methanol using a high speed blender. The individual suspensions were vacuum filtered and the residue was washed with methanol. The filtrates were combined, yielding methanol/water extracts. The methanol/water extract was rotary evaporated (approx. 40°C) to the aqueous remainder which was partitioned against dichloromethane (3 times) yielding the aqueous phase and the dichloromethane phase. An aliquot of the dichloromethane phase was concentrated by rotary evaporation.

The seeds were macerated twice with hexane using a high speed blender. The suspensions were vacuum filtered and the filtrates combined, yielding the hexane extract. The solid residue was extracted twice with methanol/water (4/1, v/v) and once with methanol. The filtrates were combined yielding the methanol/water extract.

The straw was extracted twice with methanol/water (1/1, v/v) and once with methanol using a high speed blender. The suspensions were vacuum filtered and the filtrates combined, yielding the methanol/water extract.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent / scintillation cocktail mixture and the radioactivity was measured by LSC. The TRR was determined by summation of the radioactivity of the combined extract(s) and of the remaining solids. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

2. Identification and characterisation:

Due to the very low TRRs, the extracts were not analysed with chromatographic methods and no identification and quantitation of parent compound and metabolites in forage, seeds and straw was performed.

3. Storage stability:

All samples were stored frozen at –20°C or below. All sample materials (forage, seeds, straw) were extracted and the radioactivity partitioned within one week after sampling. From the experiences with the a.s. and metabolites made so far, alterations of the composition during storage affecting the residue levels or partitioning behaviour between organic and aqueous phases are not expected. The results can

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therefore be regarded as representative after seed dressing of oilseed rape with Fluoxastrobin FS 140 under the mentioned use pattern and growth conditions.

II. Results and Discussion

The metabolism of [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin (= [methoxyiminotolyl-ring-UL-^{14}C]HEC 5725) formulated as an FS 140 was investigated in oilseed rape following seed treatment. The application rate was approx. 3.5 g a.s./ha corresponding to approx. 70 g a.i./100 kg seed. Additionally, a 10x overdose experiment was conducted.

The total radioactive residues (TRRs) in forage, seeds and straw were very low (Table 6.2.1-17). The TRRs amounted to 0.001 mg/kg (expressed as active substance equivalents) in all matrices of the normal dose experiment. In the 10X overdose experiment, the TRRs were 0.002 mg/kg for forage, < 0.001 mg/kg for seeds and 0.005 mg/kg for straw.

Table 6.2.1-17: TRR values in oilseed rape matrices after seed treatment with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin

Matrix	Timing and Application	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
forage	normal dose experiment: seed treatment at a nominal rate of 500 mL FS 140/100 kg seeds corresponding to 70 g a.s./100 kg seeds or to 3.5 g a.s./ha	46	0.001
seeds		160	0.001
straw			0.001
forage	overdose experiment (10X): seed treatment at approx. 10X of the rate in the normal dose experiment	46	0.002
seeds		160	<0.001
straw			0.005

The results of the normal dose experiment are as follows:
From forage, 85.5% of the TRR (< 0.001 mg/kg) was extractable with methanol/water and methanol (Table 6.2.1-18). After partitioning, the dichloromethane phase contained < 0.1% of the TRR (< 0.001 mg/kg) and the aqueous phase contained 85.3% of the TRR (<0.001 mg/kg). Only 14.5% of the TRR (0.001 mg/kg) remained in the solids. From seeds, 7.8% of the TRR (0.001 mg/kg) was extractable with hexane. Methanol/water and methanol extracted further 13.1% of the TRR (< 0.001 mg/kg). However, 79.1% of the TRR (0.001 mg/kg) remained in the solids. From straw, 54.1% of the TRR (< 0.001 mg/kg) was extractable with methanol/water and methanol and 45.9% of the TRR (<0.001 mg/kg) remained in the solids.

The results of the overdose experiment (10X) are as follows:
From forage, 94.1% of the TRR (0.002 mg/kg) was extractable with methanol/water and methanol. After partitioning, the dichloromethane phase contained 42.5% of the TRR (0.001 mg/kg) and the aqueous phase contained 51.6% of the TRR (0.001 mg/kg). Only 5.9% of the TRR (<0.001 mg/kg) remained in the solids. From seeds, 23.5% of the TRR (<0.001 mg/kg) was extractable with hexane. Methanol/water and methanol did not extract further radioactivity. However, 76.5% of the TRR (<0.001 mg/kg) remained in the solids. From straw, 71.2% of the TRR (0.003 mg/kg) was extractable with methanol/water and methanol and 28.8% of the TRR (0.003 mg/kg) remained in the solids.

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Table 6.2.1-18: Distribution of radioactivity in the extracts of the oilseed rape matrices after seed treatment with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin

	forage		seeds		straw	
	normal dose experiment					
TRR [mg/kg] =	0.001		0.001		0.001	
	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
n-Hexane extract	-	-	7.8	<0.001	-	-
Methanol/water extract	(85.5)	(<0.001)	13.1	<0.001	54.1	<0.001
Dichloromethane phase	<0.1	<0.001	-	-	-	-
Aqueous phase	85.5	<0.001	-	-	-	-
Total extracted/solubilised	85.5	<0.001	20.9	<0.001	54.1	<0.001
Solids (non-extractable residue)	14.5	<0.001	79.1	0.001	45.9	<0.001
Accountability	100.0	0.001	100.0	0.001	100.0	0.001
	overdose experiment (10X)					
TRR [mg/kg] =	0.002		<0.001		0.005	
	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
n-Hexane extract	-	-	23.5	<0.001	-	-
Methanol/water extract	(94.1)	(0.002)	<0.1	<0.001	71.2	0.003
Dichloromethane phase	42.5	0.001	-	-	-	-
Aqueous phase	51.6	0.001	-	-	-	-
Total extracted/solubilised	94.1	0.002	23.5	<0.001	71.2	0.003
Solids (non-extractable residue)	5.9	0.002	76.5	<0.001	28.8	0.001
Accountability	100.0	0.002	100.0	<0.001	100.0	0.005

Due to the very low TRRs no identification and quantitation of parent compound and metabolites in forage, seeds and straw was performed in neither the normal dose nor the overdose experiment.

For the overdose experiment (10X), it was calculated that the amounts of radioactivity found in the forage at BBCH stage 34 and in the straw at harvest each corresponded to 0.3% of the applied radioactivity. This indicated a low rate of uptake and translocation of fluoxastrobin and/or possible metabolites into the aerial parts of the plants.

III. Conclusions

After seed treatment with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin at the envisaged use rate and at a 10X overdose rate, the residues in forage, seeds and straw of oilseed rape were very low. The TRRs did not exceed 0.001 mg/kg in seeds, 0.002 mg/kg in forage and 0.005 mg/kg in straw. No identification and quantitation of individual components of the TRR was performed. Fluoxastrobin and/or possible metabolites were taken up and translocated into the aerial parts of the plant only to a very small extent.

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Overall conclusions on plant metabolism

For the overall conclusion on the plant metabolism of fluoxastrobin, the results of all available plant metabolism studies and the confined rotational crops studies are briefly summarized in the following. Detailed summaries of the wheat metabolism and confined rotational crops studies, which were already peer reviewed at EU level, were presented in the Annex II dossier submitted in 2002 (see also Table 6.2-1 and Table 6.2-2). Detailed summaries of the tomato, peanut and oilseed rape metabolism studies, which have not yet been peer reviewed at EU level, are presented above (pages 36 to 80).

The metabolism of fluoxastrobin was investigated in primary crops and rotational crops from three different crop groups with different positions of radiolabel following foliar application (wheat, tomato, peanut) and soil application (wheat seed treatment, rotational crops). Due to very low TRRs, the nature of residues was not investigated in oilseed rape after seed treatment.

The parent compound (sum of fluoxastrobin and its Z-isomer) was mostly by far the major component of the residue in the primary crops wheat, tomato and peanut (see Table 6.2.1-19). After the foliar applications, parent compound occurred at 51.6 - 98.0% of the TRR in the plant matrices and the highest amount of an individual identified metabolite did not exceed 5.0% of the TRR. The highest concentrations in a food or feed commodity (excluding peanut hay) were found in wheat straw with 54.34 - 62.42 mg/kg for parent compound and 1.79 - 2.10 mg/kg for an individual identified metabolite, however, the highest amount of a metabolite did not exceed 2.1% of the TRR in straw.

Following seed treatment only, parent compound was predominantly the major component and occurred in all wheat matrices in a range of 17.3 - 34.9% of the TRR. In wheat forage, hay and straw the major identified metabolite was found at 4.5 - 20.5% of the TRR, the levels were low and did not exceed 0.08 mg a.s. equiv./kg. No metabolite was identified in wheat grain.

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Table 6.2.1-19: Plant metabolism studies: parent compound (sum of fluoxastrobin and its Z-isomer) and the major identified metabolite

Crop	Label	Type of application	Single application rate *(total appl. rate)* [g a.s./ha]	Sample material	Parent compound (sum of fluoxastrobin and its Z-isomer)		The major[d] identified metabolite	
					% of TRR	mg/kg	% of TRR	mg a.s. equiv./kg
Wheat	ring 1, 2 ,3	seed treatment + 2 foliar spray applications	53-64 281-317 *(651-696)*	forage[a]	22.3-27.0	<0.01-0.02	9.1-14.4	<0.01-0.01
				hay	79.1-88.0	8.54–46.38	1.2-2.2	0.22-0.84
				straw	72.3-79.9	34.34-62.42	2.4-2.7	1.79-2.10
				grain	51.6-86.0	0.30-0.45	2.4-3.0	0.01-0.04
	ring 3	seed treatment	54	forage	28.6	0.03	4.5	0.01
				hay	34.9	0.04	18.5	0.02
				straw	17.3	0.07	20.5	0.08
				grain	30.0	0.005	-	-
Tomato	ring 1, 3	3 foliar spray applications	144 *(432)*	fruit	98.0	0.410-0.622	0.3-0.4	0.002
Peanut	ring 2, 3	3 foliar spray applications	234-275 *(781-804)*	hay	83.1-85.5	111.09-117.92	2.2-2.7	2.91-3.89
				nutmeat	-[b]	-[b]	-[b]	-[b]
Rape	ring 3	seed treatment	3.5	forage, straw, seeds	[c]	[c]	[c]	[c]

a) Forage was sampled before the two foliar spray applications were conducted.
b) In nutmeat, the identified portion of the TRR (68.2 - 85.0%) was represented by natural products.
c) Due to the very low TRRs no identification and quantitation of parent compound and metabolites in forage, seeds and straw was performed.
d) metabolite with highest % of TRR value in the individual radiolabelled studies

After soil application and planting of the rotational crops, parent compound (sum of fluoxastrobin and its Z-isomer) was the predominant component of the residues in the plant matrices with up to 73.6% of the TRR in wheat, up to 32.6% of the TRR in Swiss chard and up to 50.3% of the TRR in turnips (Table 6.2.1-20). It was the major component in the plant matrices of the first rotation and in most matrices of the second and third rotation. In turnip leaves of the second and third rotation and in Swiss chard of the second rotation it occurred at amounts comparable to the major identified metabolite. In the individual plant matrices of the three rotations, the major identified metabolite represented up to 26.4% of the TRR. The residue levels of the metabolites were low and did not exceed 0.03 mg a.s. equiv./kg in the edible matrices wheat grain, leaves of Swiss chard and turnip roots.

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Table 6.2.1-20: Confined rotational crops studies: parent compound fluoxastrobin (E- and Z-isomer) and the major identified metabolite

Label	Application type and rate	Rotation, plant back interval	Rotational crops	Sample material	Fluoxastrobin (E- and Z-isomer)		The major[a)] identified metabolite	
					% of TRR	mg/kg	% of TRR	mg a.s. equiv./kg
ring 1, 2 ,3	1 spray application onto bare soil at 683 – 846 g a.s./ha	1st rotation, 30 days after application	wheat	forage	34.5-51.0	0.04-0.06	7.9-9.3	0.010
				hay	34.4-43.9	0.78-0.89	13.5-20.5	0.10-0.27
				straw	22.1-49.5	0.40-1.18	10.8-18.6	0.25-0.45
				grain	≤65.4	≤0.02	3.0-9.8	0.01
			Swiss chard	leaves	13.2-32.6	0.04-0.06	9.0-12.4	0.01-0.014
			turnips	leaves	11.6-35.6	0.004-0.02	3.0-12.4	<0.01
				roots	28.9-50.3	0.006-0.01	6.5-14.9	<0.01
		2nd rotation, 157-175 days after application	wheat	forage	43.8-67.6	0.05-0.09	4.9-11.9	0.01-0.02
				hay	32.2-37.9	0.10-0.38	9.0-24.0	0.08-0.10
				straw	26.0-35.6	0.34-0.45	11.2-16.0	0.15-0.21
				grain	≤73.6	≤0.02	2.1-8.1	0.01
			Swiss chard	leaves	≤30.6	≤0.05	11.5-25.3	0.01-0.03
			turnips	leaves	4.0-19.3	0.002-0.01	8.5-26.4	0.002-0.01
				roots	≤42.8	≤0.01	12.6-17.5	<0.01
		3rd rotation, 301-328 days after application	wheat	forage	6.9-65.9	0.01-0.04	4.0-17.6	<0.01-0.03
				hay	6.0-33.0	0.03-0.06	11.8-14.4	0.02-0.08
				straw	7.4-26.3	0.06-0.12	8.6-16.8	0.03-0.13
				grain	≤64.5	≤0.03	2.6-6.9	0.002
			Swiss chard	leaves	0.5-12.6	≤0.01	13.1-22.4	0.01-0.030
			turnips	leaves	≤10.0	≤0.002	6.9-13.5	<0.01
				roots[b)]	-	-	-	-

a) metabolite with highest % of TRR value in the individual radiolabelled studies
b) Due to the very low TRRs no identification and quantitation of parent compound and metabolites in turnip roots of the 3rd rotation was performed.

The following metabolic routes of fluoxastrobin in the plant were observed:

- cleavage and degradation of the dioxazine ring (wheat, tomato, peanut, rotational crops),
- hydroxylation of the chlorophenyl ring (wheat, peanut, rotational crops),
- cleavage of the parent molecule at the pyrimidine-methoxyiminotolyl ether group (wheat, tomato, peanut, rotational crops),
- cleavage of the parent molecule at the chlorophenyl-pyrimidine ether group (wheat, peanut, rotational crops),
- cleavage of the oximether (wheat, tomato, peanut),
- nucleophilic substitution at the chlorophenol ring by glutathione, followed by a stepwise degradation of the glutathione moiety (wheat, peanut, rotational crops),
- conjugation of hydroxyl and thiol groups to glucosyl and glucosyl-malonyl conjugates (wheat, peanut, rotational crops) and
- isomerisation of the oximether with the formation of the Z-isomer (wheat, peanut, partly rotational crops).

The metabolic pathway of fluoxastrobin in the plant is proposed in Figure 6.2.1-5.

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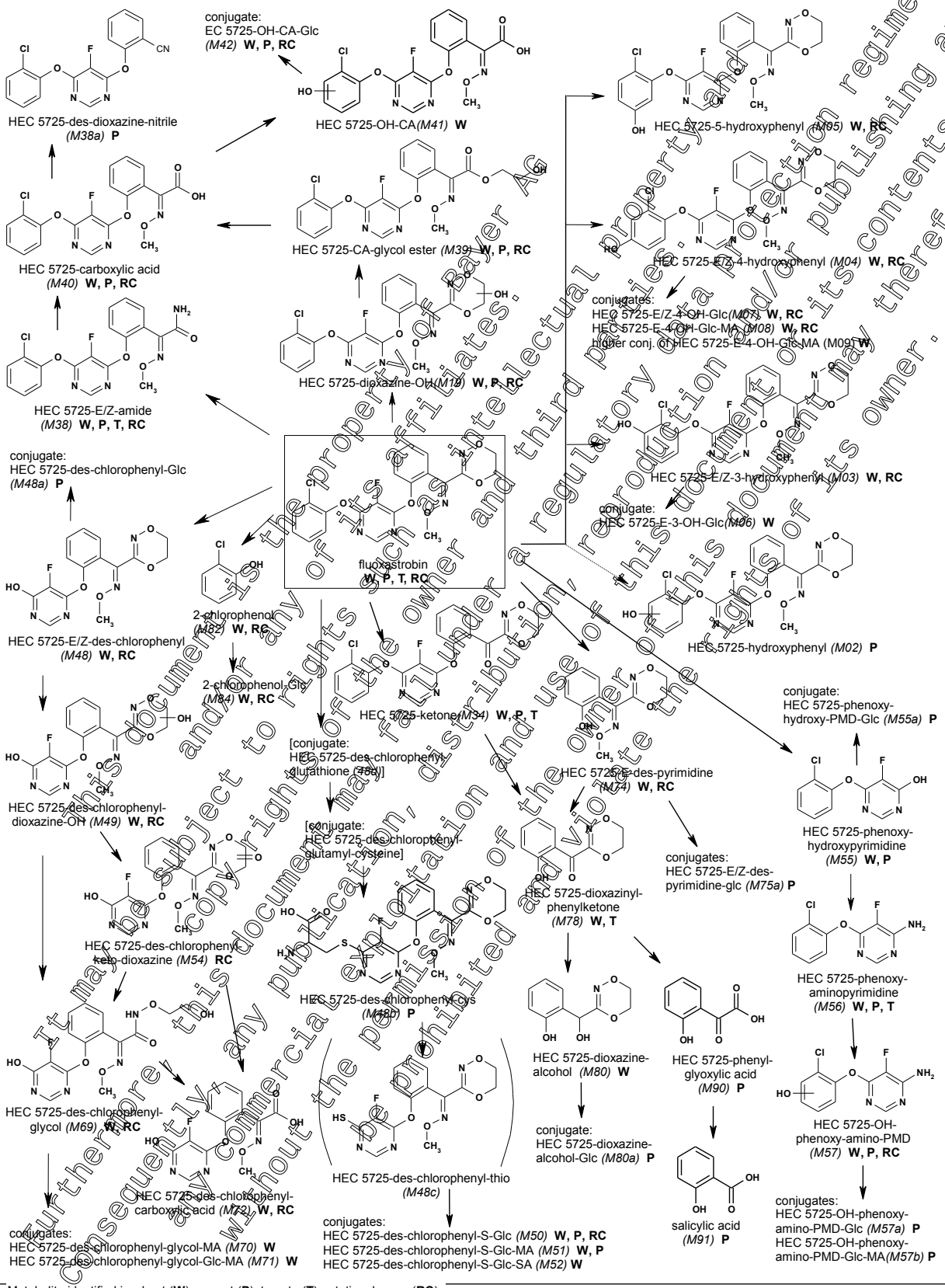
Figure 6.2.1-5: Proposed metabolic pathway of fluoxastrobin in the plant



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Metabolite identified in wheat (**W**), peanut (**P**), tomato (**T**), rotational crops (**RC**)

The metabolic routes observed in the tomato and peanut studies were also observed in the wheat metabolism studies. The metabolic routes found in three rotational crops were also found in the primary crops. Overall no metabolic route unique for any of the investigated crops was observed, and the metabolic routes in all studies are similar. The primary crops represent three different crop categories (cereals, fruit and pulses & oilseeds) covering foliar and soil application. Therefore it is concluded that the nature of residues in the plant after application of fluoxastrobin is sufficiently understood and that no further studies are needed.

Overall, parent compound, i.e. the sum fluoxastrobin and its Z-isomer, was observed as the predominant portion of the residues. Metabolites were only minor components after foliar applications (≤ 5% of the TRR) and not exceeding 0.08 mg a.s. equiv./kg after seed treatment or 0.03 mg a.s. equiv./kg in edible commodities of rotational crops. Therefore, it is concluded that fluoxastrobin and its Z-isomer are the appropriate components of the residue definition for risk assessment and for enforcement purposes in all plant commodities.

Conclusions on the metabolism in potatoes after soil application

For supplementing the conclusion on a residue definition for all crop groups (see pages 81 - 85), a rationale on the metabolism in potatoes after soil appliction is provided here. Such a rationale was provided by Bayer CropScience to the member states in the course of the evaluation of a dossier for a registration of a plant protection product used for in-furrow application in potatoes, submitted to The United [redacted] and The Netherlands. The rationale showed that the data from the available plant metabolism studies cover an in-furrow treatment of potato tubers at a rate of 390 g fluoxastrobin/ha and that the nature of the residue following this use is sufficiently known. In the "Reasoned opinion on the review of the existing maximum residue levels (MRLs) for fluoxastrobin" EFSA points out that "… metabolism in potatoes was not investigated but in this case assumed to be covered by the available studies …" and that "… a complete peer review of these metabolism studies at EU level is still desirable" [EFSA Journal 2012;10(12):3012]. Hence, the rationale is provided in the following.

According to the OECD guideline 501 "Metabolism in Crops", an in-furrow application (or seed treatment) is considered as a soil application. From the data of the confined rotational crops studies in roots of turnips sown 30 days after soil application, the nature of the residues in potatoes after in-furrow application or seed treatment of fluoxastrobin is derived. The data are recalculated to compensate for the different application rates of the confined rotational studies and the potato use pattern considering the dissipation of fluoxastrobin during the 30 days plant back interval

This calculation is based on the results of the eight field dissipation trials reported by [redacted]; 2001; M-136670-01-1. The first order DT50 values of fluoxastrobin in soil found in this study were in the range of 16.2 to 119 days with an arithmetic mean of 82 days. Based on these DT50 values the percentage of fluoxastrobin remaining in soil 30 days after application can be calculated. The amounts calculated for that date are in the range of 27.7 - 84.0% (mean 77.6%). The following Table 6.2.1-21 shows the percentages related to the individual trials.

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Table 6.2.1-21: Fluoxastrobin: Estimated amounts at day 30 based on the results of the field dissipation study (in percent)

Trial	Country	DT50 (days)	Percent of fluoxastrobin remaining in soil at day 30
R812390	Germany	16.2	27.7
R812404	Great Britain	119	84.0
R812412	France (North)	85.4	78.4
R812420	Great Britain	105	82.0
R812439	France (North)	107	82.3
R812447	Italy	97.3	80.8
R812455	France (South)	77	76.3
R814202	Germany	46.2	63.8
arithmetic mean:		*82*	*67.6*

The percentages of fluoxastrobin present in the soil 30 days after the soil application were translated into the amounts remaining in soil at the time of sowing turnips in the confined rotational crops studies by multiplying them with the three different application rates used (846 g a.s./ha, 841.3 g a.s./ha and 683 g a.s./ha, depending on the labelling position). The estimated amounts of fluoxastrobin at the time of sowing turnips of the first rotation (30 days after the soil application) were in the range of 189 - 710 g a.s./ha. Using the data from seven of the eight trials where degradation was slowest (and in line with the behaviour of fluoxastrobin in the environment) the estimates range from 436 – 710 g a.s./ha. The calculations described above are presented in Table 6.2.1-22.

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Table 6.2.1-22: Fluoxastrobin: Estimated amounts at the start of the first rotation (day 30) of the confined rotational crops studies in g a.s./ha

Field dissipation study			Confined rotational crops studies		
Trial	Country	Percent fluoxastrobin remaining in soil at day 30	Label ring	Application rate (g a.s./ha)	Estimated amounts of fluoxastrobin at day 30 (g a.s./ha)
R812390	Germany	27.7	1	846	234.4
			2	841.5	233.1
			3	683	189.2
R812404	Great Britain	84.0	1	846	710.4
			2	841.5	706.6
			3	683	573.5
R812412	France (North)	78.4	1	846	663.2
			2	841.5	659.6
			3	683	535.4
R812420	Great Britain	82.0	1	846	694.0
			2	841.5	690.3
			3	683	560.3
R812439	France (North)	82.3	1	846	696.6
			2	841.5	692.9
			3	683	562.4
R812447	Italy	80.8	1	846	683.2
			2	841.5	679.6
			3	683	551.6
R812455	France (South)	76.3	1	846	645.8
			2	841.5	642.4
			3	683	521.4
R814202	Germany	63.8	1	846	539.4
			2	841.5	536.5
			3	683	435.5
arithmetic mean		*77.6*	*1*	*846*	*656.5*
			2	*841.5*	*653.0*
			3	*683*	*530.0*
					mean: 613.2

The factors representing the overdose of fluoxastrobin expected to remain in the soil after 30 days compared to the highest supported field rate of the potato use (390 g fluoxastrobin/ha) are compiled in Table Table 6.2.1-23 The estimated amounts of fluoxastrobin available at sowing / planting the root / tuber crop for the first rotation are higher than the intended application rate of the potato use by a mean factor of 1.57. The data from nearly all field dissipation trials suggest an overdose (factors > 1) in the confined rotational crops study, the only exception was one field dissipation trial (conducted in Germany, R812390) resulting in factors of 0.49 to 0.6 based on the lowest DT50 value of 16.2 days.

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Table 6.2.1-23: Comparison of the estimated amounts of fluoxastrobin (g a.s./ha) at the first rotation (30 days after soil application) in the confined rotational crops (CRC) studies and the intended application rate of fluoxastrobin for the potato use

Trial	Country	Label ring	Estimated amounts of fluoxastrobin at day 30 (g a.s./ha)	Factor for amounts of fluoxastrobin in CRC studies at day 30 versus application rate of fluoxastrobin for the potato use (390 g fluoxastrobin/ha)
R812390	Germany	1	234.4	0.60
		2	233.1	0.60
		3	189.2	0.49
R812404	Great Britain	1	710.4	1.82
		2	706.6	1.81
		3	573.5	1.47
R812412	France (North)	1	663.2	1.70
		2	659.6	1.69
		3	535.4	1.37
R812420	Great Britain	1	694.0	1.78
		2	690.3	1.77
		3	560.3	1.44
R812439	France (North)	1	696.6	1.79
		2	692.9	1.78
		3	562.4	1.44
R812447	Italy	1	683.2	1.75
		2	679.6	1.74
		3	551.6	1.41
R812455	France (South)	1	645.8	1.66
		2	642.4	1.65
		3	521.4	1.34
R814202	Germany	1	539.4	1.38
		2	536.5	1.38
		3	435.5	1.12
arithmetic mean		*1*	*656.5*	*1.68*
		2	*653.0*	*1.67*
		3	*530.0*	*1.36*
				mean: 1.57

The results of the confined rotational crops studies show that the amount of TRR found in turnip roots sown 30 days after soil application with fluoxastrobin was low and ranged from 0.012 to 0.034 mg/kg. Table 6.2.1-24 shows the TRRs for the different labels.

Table 6.2.1-24: Total radioactive residues (TRRs) in roots of turnips sown 30 days after soil application with fluoxastrobin

Report	Label ring	TRR (mg a.s. equiv./kg)
MR-392/00[a)]	1	0.032[b)]
MR-124/01[a)]	2	0.034
MR-086/01[a)]	3	0.012

a) = presented in: EU-Dossier, March 2002; IIA, 6.6
b) = recalculation of TRR with 3 digits (0.032 mg/kg), data from report MR-392/00, Appendix 15, page 112

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Even though the TRRs were extremely low, a number of compounds were identified. The highest residue compound identified in turnip roots was the parent compound with a maximum of 0.012 mg/kg. Several metabolites at even lower levels were identified in the different labels. None of the identified metabolites and none of the characterised compounds exceeded 0.01 mg/kg. The highest level of any metabolite observed was HEC 5725-E-4-OH-Glc-MA at 0.005 mg/kg. The amounts of parent compound and metabolites in the roots of turnips sown 30 days after soil application with fluoxastrobin are summarised in Table 6.2.1-25.

Table 6.2.1-25: Summary of characterisation and identification of radioactive residues in turnip roots from the first rotation of the CRC studies

Compound	Label Ring 1		Label Ring 2		Label Ring 3	
	% of TRR	mg a.s. equiv./kg[a)]	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
parent compound, sum of	(38.5)	(0.012)	(28.9)	(0.010)	(50.3)	(0.005)
fluoxastrobin (HEC 5725 E-isomer)	36.0	0.012	26.8	0.009	47.5	0.005
and its Z-isomer (HEC 5725 Z-isomer)	2.6	0.001	2.1	0.001	2.8	0.001
HEC 5725-E-3-hydroxyphenyl (M03)	n.d.	n.d.	0.7	< 0.001	n.d.	n.d.
HEC 5725-E-4-hydroxyphenyl (M04)	3.2	0.001	2.4	0.001	n.d.	n.d.
HEC 5725-5-hydroxyphenyl (M05)	n.d.	n.d.	1.4	< 0.001	3.9	< 0.001
HEC 5725-E-4-OH-Glc (M07)	1.9	0.001	n.d.	n.d.	n.d.	n.d.
HEC 5725-E-4-OH-Glc-MA (M08)	14.9	**0.005**	n.d.	n.d.	n.d.	n.d.
HEC 5725-dioxazine-OH (M19)	1.0	< 0.001	n.d.	n.d.	n.d.	n.d.
HEC 5725-amide (M38)	0.7	< 0.001	n.d.	n.d.	n.d.	n.d.
HEC 5725-OH-CA-Glc (M42)	4.9	0.002				
HEC 5725-E-des-chlorophenyl (M48)			8.8	0.003	6.5	0.001
HEC 5725-2-chlorophenol-Glc (M84)	5.8	0.002				
Sum identified	71.0	0.023	42.2	0.014	60.7	0.007
Characterised[a)]	10.9	0.003	46.4	0.016	26.3	0.003
Subtotal identified and characterised	81.9	0.026	88.6	0.030	87.0	0.010
Non-extractable residue	18.1	0.006	11.4	0.004	13.0	0.002
Total	**100**	**0.032**	**100**	**0.034**	**100**	**0.012**

n.d. = not detected

a) = recalculation of TRR with 3 digits (0.032 mg/kg), data from report MR-392/00, Appendix 15, page 112

b) = Unassigned metabolites were characterised by extraction behaviour, phase partitioning and chromatographic behaviour. All characterised peaks amounted to < 0.004 mg/kg, each.

For the purpose of waiving a potato metabolism study, residues in potato tubers can be estimated based on the findings in turnip roots in the CRC studies, since turnip and potato belong to the same crop group according to OECD guideline 501.

Taking the worst case factor derived from the trial with the fastest degradation into account, the amount of fluoxastrobin in soil at the time of sowing of the turnips can be estimated to represent approx. 50 % of the potato use rate. Therefore the amounts of parent compound and metabolites found in turnip roots of the 1st rotation of the rotational crops studies are divided by a factor of 0.5 (see Table 6.2.1-26). This calculation indicates a presence of the metabolite HEC 5725-E-4-OH-Glc-MA at 0.01 mg/kg. However taking into account all other available data on the dissipation of fluoxastrobin it would be more realistic to divide residues by a mean factor of approx. 1.5, resulting in levels below 0.01 mg/kg for all metabolites. However, parent compound (sum of fluoxastrobin and its Z-isomer) would still be the highest residue compound.

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Table 6.2.1-26: Results of the confined rotational crops study in roots of turnips of the first rotation divided by a factor of 0.5 (expressed as mg a.s. equiv./kg)

Compound	Label		
	Ring 1	Ring 2	Ring 3
parent compound, sum of	**(0.025)**	(0.020)	(0.010)
fluoxastrobin (HEC 5725 E-isomer)	0.023	0.018	0.01
and its Z-isomer (HEC 5725 Z-isomer)	0.002	0.002	< 0.002
HEC 5725-E-3-hydroxyphenyl (M03)	n.d.	< 0.002	n.d.
HEC 5725-E-4-hydroxyphenyl (M04)	0.002	0.002	n.d.
HEC 5725-5-hydroxyphenyl (M05)	n.d.	< 0.002	< 0.002
HEC 5725-E-4-OH-Glc (M07)	0.002	n.d.	n.d.
HEC 5725-E-4-OH-Glc-MA (M08)	**0.010**	n.d.	n.d.
HEC 5725-dioxazine-OH (M19)	< 0.002	n.d.	n.d.
HEC 5725-amide (M38)	< 0.002	n.d.	n.d.
HEC 5725-OH-CA-Glc (M42)	0.003		
HEC 5725-E-des-chlorophenyl (M48)		0.006	0.002
HEC 5725-2-chlorophenol-Glc (M84)	0.004		

Although no specific metabolism study on potatoes after soil application was performed, sufficient information can be obtained from the available confined rotational crops studies to derive the nature of residues in potato after in furrow spray application. The residue levels of metabolites anticipated to be present in potato tubers at harvest after soil application are expected to be very low. The only compound that is anticipated to be detected at quantifiable levels is the parent compound. Only in one worst case scenario assuming an underdose in the confined rotational crops studies (overdose factor of 0.5) compared to the supported potato use rate of up 390 g a.s./ha, the highest concentration of only one metabolite was estimated to be at 0.01 mg/kg while parent compound was estimated to represent the highest residue compound. In a more realistic scenario with an overdose factor of approx. 1.5, all metabolites were estimated below 0.01 mg/kg. Therefore, it is considered unlikely that metabolites would need to be considered for risk assessment purpose and it is concluded that the definition of residue after in-furrow application of fluoxastrobin in/on potato should be the parent compound, i.e. the sum fluoxastrobin and its Z-isomer.

This proposal for the residue definition is perfectly in line with all other results from the metabolism studies with fluoxastrobin after spray application (wheat, peanut, tomato) and seed treatment (wheat, oil seed rape) and the results from the other crops (wheat, Swiss chard) in the confined rotational crop studies, and therefore with the conclusion on the residue definition for all plant commodities (see pages 81 - 85).

Since these conclusions can be drawn from confined rotational crops studies with a root crop, an additional potato metabolism study is not needed to supplement the already extensive metabolism database.

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CA 6.2.2 Poultry

Metabolism studies in the laying hen were conducted with [chlorophenyl-UL-^{14}C]fluoxastrobin ([redacted]; 2001; M-030690-01-1) and [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin ([redacted]; 2002; M-059027-01-1). These studies were peer reviewed at EU level (see also introduction to chapter CA 6.2 and Table 6.2-3). A short summary is given below.

Laying hens were dosed with radiolabelled fluoxastrobin at 10 mg/kg bw. The concentration in the feed was calculated to be 187 - 198 mg/kg, corresponding to approx. 425 - 454 times the exposure to poultry (based on the maximum dietary burden for poultry of 0.44 mg/kg, representing the 1N dose level, see chapter CA 6.4 and Table 6.4- 2). These studies demonstrated that the bulk of the radioactivity was excreted (72%) and therefore transfer of residues into eggs and the tissues was relatively low (approx. 2%). The TRRs accounted for up to 0.84 mg/kg in eggs, 0.31 - 0.66 mg/kg in muscle, 0.62 - 0.93 mg/kg in fat and 8.1 - 9.7 mg/kg in liver.

In eggs and tissue samples, two major components were identified as parent compound (sum of fluoxastrobin and its Z-isomer) and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55). They accounted together for up to 36% TRR in eggs, up to 84% TRR in muscle, up to 69% TRR in fat and up to 22% TRR in liver. Several other metabolites were identified and several unknowns were noted, which individually were present at levels of either not higher than approx. 10% of TRR (liver) or less than 0.07 mg/kg (eggs, muscle, fat).

As the studies were carried out at highly exaggerated doses compared to the expected exposure to poultry, it is unlikely that any of the compounds, except fluoxastrobin and its metabolite M55, would be present at levels greater than 0.01 mg/kg in studies with a 1N dose rate.

The report M-030690-01-1 of the laying hen study conducted in 2001 with [chlorophenyl-UL-^{14}C]fluoxastrobin and already evaluated under 91/414 was recently amended. The reason for the amendment was solely due to eperimental details on the procedure used for the extraction of eggs and resulted in document M-030690-02-1. These details are referred to in an analytical method developed for animal matrices (see document MCA section 4, chapters 4.1.2 and 4.2; [redacted]s; [redacted]; 2015; M-536049-01-1).

CA 6.2.3 Lactating ruminants

Metabolism studies in the lactating goat were conducted with [chlorophenyl-UL-^{14}C]fluoxastrobin ([redacted]; 2002; M-034595-03-1) and [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin ([redacted]; 2001; M-036881-02-1). These studies were peer reviewed at EU level (see also introduction to chapter CA 6.2 and Table 6.2-3). A short summary is given below.

Lactating goats were dosed with radiolabelled fluoxastrobin at 10 mg/kg bw. The concentration in the feed was calculated to be 180 - 265 mg/kg, corresponding to approx. 98 - 145 times the exposure to dairy and meat ruminants (based on the maximum dietary burden for ruminants of 1.83 mg/kg, representing the 1N dose level, see chapter CA 6.4 and Table 6.4- 2). These studies demonstrated that the bulk of the radioactivity was excreted (56 - 63%) and therefore transfer of residues into milk and the tissues was relatively low (1.3 – 1.8%). The TRRs accounted for up to 0.4 mg/kg in milk, 0.25 - 0.54 mg/kg in muscle, 0.36 - 0.65 mg/kg in fat, 8.3 - 18 mg/kg in liver and 2.6 - 3.9 mg/kg in kidney.

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In milk and tissue samples, two major components were identified as parent compound (sum of fluoxastrobin and its Z-isomer) and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55). They accounted together for up to 12% TRR in milk, up to 60% TRR in muscle, up to 75% TRR in fat, up to 16% TRR in liver and up to 29% TRR in kidney. Several other metabolites were identified and several unknowns were noted, which individually were present at levels of either not higher than approx. 15% of TRR (liver, kidney) or less than 0.05 mg/kg (milk, muscle, fat).

As the studies were carried out at highly exaggerated doses compared to the expected exposure to ruminants, it is unlikely that any of the compounds except fluoxastrobin and its metabolite M55 would be present at levels greater than 0.01 mg/kg, in studies with a 1N dose rate.

CA 6.2.4 Pigs

The general metabolic pathways in rodents and ruminants were found to be comparable; the findings in ruminants can therefore be extrapolated to pigs. This is also the conclusion in the reasoned opinion on the review of the existing maximum residue levels (MRLs) for fluoxastrobin according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012; 9(12):3012).

Metabolism studies on pigs are therefore not required and were not conducted.

CA 6.2.5 Fish

In March 2013 EU Commission Regulation 283/2013 was published setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market. This Regulation contains the new data requirement:

"6.2.5 Fish Metabolism
Metabolism studies on fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications."

These new data requirements do not include definitive triggers for when studies are required and unlike for ruminant and poultry there are currently no agreed test guidelines for the conduct of fish metabolism studies.

The procedure when no agreed test methods or guidance documents are available is described in the "*Guidance Document for applicant on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013"* (SANCO/10181/2013-rev 3). The document states:

"In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02."

In the summary report of the Standing Committee on Plants, Animals, Food and Feed (26 and 27 January 2015) the Commission recommended relative to the data requirements and acceptance of waivers / the implementation of document SANCO/10181/2013 *that Member States are invited to follow the procedures agreed when taking note of Guidance Document SANCO/10181/2013 in order*

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to harmonise the procedures, i.e. to accept as a general line the waiving for cases where no test guidelines are available. (A26).

At the time when the present document was prepared, no corresponding guidance document was published or listed addressing Reference 6.2.5 of the Annex to Regulation (EU) No 283/2013. Therefore no fish metabolism study was conducted.

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CA 6.3 Magnitude of residue trials in plants

Fluoxastrobin is a broad spectrum fungicide with mainly uses in cereals but also in some vegetables (potatoes, onions). The compound belongs to the chemical class of strobilurines (methoxyacrylates). The fungicidal activity is based on the inhibition of complex III in the mitochondrial respiratory chain. When applied as a foliar spray, the fungicide shows leaf-systemic action; when applied as seed dressing, the action is loco-systemic.

Fluoxastrobin is usually co-formulated with other fungicides such as prothioconazole and/or bixafen. The representative products for the renewal of the approval of fluoxastrobin are for uses in/on small grain cereals: 'Fluoxastrobin + Prothioconazole EC 200' (100+100 g/L) for the northern climatic zone and 'Bixafen+Fluoxastrobin+Prothioconazole EC 190' (40+50+100 g/L) for the southern climatic zone. As an additional representative use in/on vegetables the EC 200 is supported in the southern zone for use in/on onions.
A use on small grain cereals was already supported with the dossier for inclusion into Annex I of Directive EC 91/414. The representative product was an EC 100 straight formulation.

For the <u>northern climatic zone</u>, the critical GAPs for wheat, rye and barley evaluated in the EU peer review for Annex I inclusion based on the use of the straight product EC 100 will not be supported in the Post AIR process and thus the critical GAP will be replaced by the representative use for the product 'Fluoxastrobin + Prothioconazole EC 200'.
For the <u>southern climatic zone</u>, the GAPs on wheat, rye and barley evaluated in the EU peer review for Annex I inclusion for 'Fluoxastrobin EC 100' do no longer exist and are replaced by new critical GAPs which are considered to establish the risk envelope for the representative use of 'Bixafen + Fluoxastrobin + Prothioconazole EC 190'.

Thus complete sets of new data for the new critical GAPs are provided for both zones with the supplementary dossier. The new data for the southern zone are also meant to address the request in the EFSA Reasoned Opinion (2012) for additional residue trials complying with the southern cGAP for barley and oats.

In order to establish appropriate MRLs for the new critical GAPs an MRL application form is submitted along with the present dossier. All data relevant to the MRL application - including the summary forms of the supervised residue trials - are included in the supplementary dossier for renewal of approval of fluoxastrobin. The representative uses and the MRL application for the corresponding crops are supported by the same residue data.

The detailed tables (Tier 1 summary forms) of the supplementary trials and the processing trials are submitted in a separate document as additional information for the evaluator.
In this document, the residue data for barley and wheat evaluated in the EU peer review are also included for easy reference.

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Report:	KCA Section 6/02 [redacted]; 2015; M-542197-01-1
Title:	Tier 1 summary forms of the studies on the magnitude of residues in plants and the magnitude of residues in processed commodities for fluoxastrobin
Report No.:	M-542197-01-1
Document No.:	M-542197-01-1
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

CA 6.3.1 Barley and Oat

Representative uses for renewal of approval of fluoxastrobin

The representative uses supported for the renewal of approval for fluoxastrobin are summarised in Table 6.3.1- 1.

Table 6.3.1- 1: Summary of the GAPs of the representative uses supported for renewal of approval for fluoxastrobin

Crop	Region	Product	Maximum Number of Applications	Minimum Application Interval (days)	Growth stage (BBCH)	Maximum Rate fluoxastrobin per application (g a.s./ha)	Minimum PHI (days)
Barley, oat	EU-N	FXA+PTZ EC 200 (Fandango)	2	14-21	30-61	125	*
Barley	EU-S	BIX+FXA+PTZ EC 190 (Variano XPro)	2	14-21	30-61	75	*
Oat [a)]						87.5	

EU-N = northern Europe, EU-S = southern Europe
* The PHI is defined by the growth stage at the last application
a) in France, for oats the product is registered up to 1.75 L/ha (corresponding to 87.5 g fluoxastrobin/ha)
FXA+PTZ EC 200 containing 100 g fluoxastrobin/L + 100 g prothioconazole /L
BIX+FXA+PTZ EC 190 containing 40 g bixafen/L , 50 g fluoxastrobin/L , 100 g prothioconazole/L

For the northern zone, the cGAP for the active substance evaluated in the EU peer review for Annex I inclusion will not be further supported post AIR but will be replaced by a new cGAP examined with the supplementary data. For the northern region, the GAP of the representative use and the cGAP for the MRL application are the same (*GAP EU-N 2,*Table 6.3.1- 2) and pertaining to the same product (Fluoxastrobin + Prothioconazole EC 200).

For the southern zone, the supplementary data reported in the present dossier were generated to support the critical GAP in the southern region (*GAP EU-S 2* for the product 'Fluoxastrobin + Prothioconazole EC 150'). The GAP of the representative use of 'Bixafen + Fluoxastrobin + Prothioconazole EC 190' involves slightly lower individual application rates (*GAP EU-S 3*).
Table 6.3.1- 2 summarises the old and new critical GAPs for the compound and the GAPs of the representative uses.

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Table 6.3.1- 2: Summary of the previous and new critical GAPs and the GAP of the representative uses for fluoxastrobin in/on barley and oats

GAP no[a)]	Crop	Region *	Product	Maxim. Number of Applications	Minim. Application Interval (days)	Growth stage (BBCH)	Maximum Rate fluoxastrobin per application (g a.s./ha)	Minimum PHI (days)
Northern Europe: Critical GAP evaluated for Annex I inclusion in the EU peer review (will not be renewed)								
EU-N 1	Barley	EU-N	FXA EC 100	2	14 (refer to growth stage)	30-69	200	35
Northern Europe: GAP of the representative use = Critical GAP for fluoxastrobin Post AIR (GAP included in MRL application form)								
EU-N 2	Barley, oat	EU-N	FXA+PTZ EC 200	2	14-21	30-61	125	*
Southern Europe: Critical GAP evaluated for Annex I inclusion in the EU peer review (obsolete)								
EU-S 1	Barley	EU-S	FXA EC 100	2	(refer to growth stage)	30-69	200	35
Southern Europe: Critical GAP for fluoxastrobin (GAP included in MRL application form)								
EU-S 2	Barley	EU-S	FXA+PTZ EC 150	2	14-21	30-61	87.5	35*
	Oat					30-69	100	
Southern Europe: GAP of the representative use								
EU-S 3	Barley	EU-S	BIX+FXA + PTZ EC 190	2	14-21	30-61	75	*
	Oat						87.5	

EU-N = northern Europe, EU-S = southern Europe

[a)] for better reference in the text below numbers are assigned to the different GAPs

* As per growth stage (the PHI of 35 days was due to a former requirement in France but will not be applicable Post AIR)

FXA EC 100 : containing 100 g fluoxastrobin/L

FXA+PTZ EC 200 containing 100 g fluoxastrobin/L + 100 g prothioconazole /L

FXA+PTZ EC 150 containing 50 g fluoxastrobin/L + 100 g prothioconazole/L

BIX+FXA+PTZ EC 190 containing 40 g bixafen/L + 50 g fluoxastrobin/L + 100 g prothioconazole/L

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Summary of the residue data evaluated for Annex I inclusion (as reflected in the baseline dossier)

Table 6.3.1- 3 summarises the critical GAP evaluated in the EU peer review.

Table 6.3.1- 3: Summary of GAPs evaluated for Annex I inclusion and used for setting the EU MRL of fluoxastrobin (*GAPs EU-N 1 and EU-S 1*)

Crop	Region	Mode of application	Maximum Number of Appli-cations	Min. interval between applications	Growth stage	Maximum Rate (g a.s./ha) per application	Minimum PHI (days)	Reference
Barley	EU-N EU-S	Overall Spray	2	14 (refer to growth stage)	start 26 up to (BBCH 69	200	35	EFSA Scientific Report (2009) 102, 1-84

EU-N = northern Europe. EU-S = southern Europe

Summary of the trials evaluated in the EU peer review:

With the Annex II dossier residue data on the following critical GAP were submitted:
Seed treatment of barley grain (5 g fluoxastrobin/dt seed) was followed by 2 spray applications at application rates of 200 g as/ha up to growth stage BBCH 69. The representative formulation for the spray application was an EC 100 formulation containing 100 g fluoxastrobin/L. In the Monograph only the spray use was evaluated. However, the trials involving both application types – seed treatment and spray application – were considered suitable since it was evident from trials which were conducted with seed treatment alone that this application had no impact on the residue levels at harvest. The foliar use was evaluated as the representative use and formed the basis for the established MRL in barley.

For easy reference the residue trials on barley that have already been evaluated during the EU review of fluoxastrobin are summarised in Table 6.3.1- 4. Since these residue reports have been previously evaluated by the RMS UK they are only presented for reference purposes. All reports are included in the baseline dossier (KCA 6.3.1) and the tier 1 summary tables may be found in document [redacted] 2015; M-542197-01-1.

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Table 6.3.1- 4: Overview of European residue trials conducted in barley per geographical region and evaluated in the EU peer review (*GAPs EU-N 1 and EU-S 1*)

Region	GAP (appl. rate for fluoxastrobin)	Crop	Formu-lation	Number of Trials				Report-No. / Reference
				Vegetation period			Total	
				1998-1999 (winter barley)	1999 (spring barley)	1999-2000 (winter barley)		
EU-N	ST 5 g a.s./dt and SPI 2 x 200 g a.s./ha	Barley	FS 110 and EC 100	-	4	4	8	RA-2024/99 [redacted]; 2001; M-089498-01-1 RA-2025/99 [redacted]; 2001; M-087238-01-1
EU-S	SPI 2 x 200 g a.s./ha	Barley	EC 100	2		2	9	RA-2026/99 [redacted]; 2001; M-089494-01-1
	ST 5 g a.s./dt and SPI 2 x 200 g a.s./ha	Barley	FS 110 and EC 100	-	2	3		RA-2027/99 [redacted]; 2001; M-089816-01-1

ST: seed treatment SPI: spray
EU-S: Southern Europe EU-N: Northern Europe
FS 110: flowable concentrate containing 100 g fluoxastrobin/L and 10 g prothioconazole/L
EC 100: emulsifiable concentrate containing 100 g fluoxastrobin/L

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Table 6.3.1- 5: Overall summary of residue data for the total residues of HEC 5725 (sum of E- and Z- isomers) in barley trials evaluated in the EU peer review (*GAPs EU-N 1 and EU-S 1*)

Application Rate	Region	Formu-lation	Sample material	n	Residue level (mg/kg)			Reference
					Min.	Max.	STMR	
Seed treatment (5 g a.s./dt seed) followed by 2 spray applications at 200 g a.s./ha	EU-N	FS 110 and EC 100	Grain	8	0.02	0.07	0.03*	EFSA Scientific Report (2007) 102 and EFSA Reasoned Opinion 2012;10 (12):3012
			Straw		0.14	2.8	0.44	
Seed treatment (5 g a.s./dt seed) followed by 2 spray applications at 200 g a.s./ha or 2 spray applications at 200 g a.s./ha alone	EU-S	FS 110 and EC 100 or EC 100	Grain	(9)** 5	0.02	0.21	0.05	
			Straw		0.2	11	2.6***	

EU-N: northern Europe EU-S: southern Europe

* In the EFSA Scientific Report an STMR value is provided for barley grain in the Northern region. The value is taken from the Monograph.

** 5 trials out of 9 were selected for MRL setting

*** This value is derived from the EFSA conclusion. In the EFSA RAC (2012) the STMR was estimated to be 1.25 mg/kg since more trials and also at lower rates were included in the evaluation.

Evaluation in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012)

Northern Europe: The trials evaluated in the EU peer review for AI inclusion were found to be compliant with the old critical GAP (fluoxastrobin EC 100; *GAP EU-N 1)*.

Southern Europe: The data package evaluated in the EU peer review (for fluoxastrobin EC 100; *GAP EU-S 1)* was found to be not compliant with the critical GAP since the trials were considered to be overdosed. The current critical GAP (*GAP EU-S 2*) is attributed to the mixture Fluoxastrobin + Prothioconazole EC 150. The information in the EFSA document on the registered product is erroneous. Therefore, a data gap was identified in the Reasoned Opinion relative to barley in southern Europe since the available residue trials were supporting a more critical GAP (exceeding the 25% deviation). Tentative MRLs were derived from these data but 8 trials complying with the current southern cGAP are still required.

The supplementary data submitted with the present dossier are also meant to fill this data gap.

Re-approval process / new studies

Northern Europe: A set of new residue data is reported supporting the critical GAP for barley and oats in northern Europe post Annex I renewal for 'Fluoxastrobin + Prothioconazole EC 200' *(GAP EU-N 2, cf* Table 6.3.1- 2*)*. This GAP is considered in the MRL application form jointed to the dossier. Since the cGAP and is also the GAP for the representative use in the northern region all residue data supporting the cGAP also support the representative use.

This GAP involves 2 spray applications at 125 g fluoxastrobin/ha.

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Southern Europe: A complete data package of supplementary trials was generated supporting the critical GAP for barley (Fluoxastrobin + Prothioconazole EC 150, *GAP EU-S 2)*. The cGAP for Fluoxastrobin + Prothioconazole EC 150 is considered in the MRL application form jointed to the dossier. The cGAP can be used to establish the risk envelope for the GAP of the representative use for'Bixafen + Fluoxastrobin + Prothioconazole EC 190' *(GAP EU-S 3)*. The representative use involves a slightly lower individual application rate compared to the cGAP (75 g/ha vs. 87.5 g as/ha for barley and 87.5 g/ha vs. 100 g/ha for oat; cf Table 6.3.1- 2).

According to the EU guidance document SANCO 7525/VI/95-rev.9 of March 2011 ('Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs') the data obtained from trials conducted on barley can be extrapolated to oat.

Trials reported in support of the cGAPs / representative uses in the northern and southern climatic zone are summarised in Table 6.3.1- 6.

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Table 6.3.1- 6: Supplementary residue trials conducted per geographical region and vegetation period

Year	GAP rate last appl.	Formulation	N° of trials	Study number	Reference
Barley foliar spray residue trials – northern EU					
2000	2 x 125 g a.s./ha BBCH61-69	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	4	RA-2013/00	[redacted]; 2001; M-083920-01-1
2000	2 x 150 g a.s./ha BBCH61-69	EC 150 (75 g/L fluoxastrobin, 75 g/L tebuconazole)	3 (4*)	RA-2062/00	[redacted]; 2003; M-074486-01-1
2013	2 x 125 g a.s./ha BBCH61	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	2	13-2157	[redacted]; 2015; M-501711-03-1
2013	2 x 125-135 g a.s./ha BBCH61	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	2	13-2158	[redacted]; 2014; M-501503-01-1
TOTAL northern EU region			**11 (12*)**		
Barley foliar spray residue trials – southern EU					
2003	2 x 75 g a.s./ha BBCH61-65	EC 300 (75 g fluoxastrobin, 150 g/L prothioconazole, 75 g/L trifloxystrobin)	4	RA-2017/03	[redacted]; 2015; M-062669-03-1
2010	2 x 75 g a.s./ha BBCH61	EC 190 (40 g/L bixafen, 50 g fluoxastrobin/L, 100 g/L prothioconazole)	2	10-2206	[redacted]; 2011; M-414709-01-1
2010	2 x 87.5 g a.s./ha BBCH61-65	EC 150 (50 g/L fluoxastrobin, 100 g/L prothioconazole)	4 (5*)	10-2157	[redacted]; 2011; M-403199-02-1
2011	2 x 87.5 g a.s./ha BBCH 61-69	EC 150 (50 g/L fluoxastrobin, 100 g/L prothioconazole)	5	11-2111	[redacted]; 2013; M-434980-04-1
TOTAL southern EU region			**15 (16**)**		

* In one trial the last application was carried out at a later growth stage (BBCH 83); the trial is disregarded in the summary
** In one trial the interval between applications was only 3 days; the trial is disregarded in the summary

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Supplementary field trials – northern Europe:

Report:	KCA 6.3.1/05[redacted]; [redacted]; 2001; M-083920-01-1
Title:	Determination of residues of HEC5725 & JAU6476-Desthio on winter barley following spray application of HEC5725 & JAU6476 200 EC in [redacted], France, Great Britain and Germany
Report No.:	RA-2013/00
Document No.:	M-083920-01-1
Guideline(s):	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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

In the season 1999/2000, four residue trials were conducted on winter barley in northern Europe. The studies were located in [redacted], the north of France, the United [redacted] and Germany.

In each trial, barley was treated twice at a product rate of 1.25 L/ha 'Fluoxastrobin + Prothioconazole EC 200' corresponding to 0.125 kg a.s./ha fluoxastrobin. The water rate was 300 L/ha. The spray interval ranged from 10 - 22 days. The application dates were growth stage related: BBCH 37 – 39 for the 1st and BBCH 61 for the 2nd application. In one instance, the second treatment was carried out at a slightly later growth stage than originally intended, however still during flowering (BBCH 69 instead of BBCH 61 in trial no. 0154-00).

Samples were taken at the following intervals:
- prior to and immediately after the final application;
- on day 35 - 36 following the final treatment as well as at harvest maturity 47 – 71 days after the final treatment. On day 35 the crop had yet not reached a development stage which would allow for collection of harvestable grain and straw, therefore ear and rest of plant were collected instead at this sampling event. Finally grain and straw were sampled at full maturity of the crop.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 (sum of E-and Z-isomer) were determined according to method 00649. The Limit of Quantification (LOQ), for barley rest of plant and straw was 0.045 mg/kg for HEC 5725 E-isomer and 0.005 mg/kg for HEC 5725 Z-isomer (corresponding to a theoretical LOQ of 0.05 mg/kg for the calculated total residue of HEC 5725). The LOQ for grain / ear was set at 0.018 mg/kg for HEC 5725 E-isomer and at 0.002 mg/kg for HEC 5725 Z-isomer (corresponding to 0.02 mg/kg for the calculated total residue of HEC 5725).

Findings

- Method performance: Method 00649 was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples with HEC5725 for all matrices relevant to this study ([redacted]; 2001; M-137093-01-1). The method was submitted with the initial Annex II dossier and evaluated in the EU peer review. Overall mean values for procedural recoveries at fortification levels at the LOQ and tenfold LOQ levels for HEC 5725 E-isomer and HEC 5725 Z-isomer were within the range of 70-110 % with RSD <20%.

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Table 6.3.1- 7: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley
The LOQ is marked in bold

Study Trial No. / GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2013/00 / R 2000 0152/5 / R 2000 0153/3 / R 2000 0154/1 / R 2000 0156/8 / GLP: yes 2000	Barley winter	rest of plant	fluoxastrobin	3	**0.045**	97; 102; 103	97	103	101	3.2
				4	0.45	104; 104; 106; 103	103	106	104	1.2
				7	overall		97	106	103	2.7
			HEC 5725 Z-Isomer	3	**0.005**	98; 100; 97	97	100	98	1.6
				4	0.05	104; 101; 101; 100	100	104	102	1.7
				7	overall		97	104	100	2.3
			total residue HEC 5725 [a)]	3	**0.05**	97; 101; 102	97	102	100	2.6
				4	0.5	104; 104; 105; 103	103	105	104	0.8
				7	overall		97	105	102	2.6
		straw	fluoxastrobin	3	**0.045**	96; 97; 99	96	99	97	1.6
				4	0.45	106; 86; 100; 94	86	106	97	8.9
				7	overall		86	106	97	6.3
			HEC 5725 Z-Isomer	3	**0.005**	98; 92; 100	92	100	97	4.3
				4	0.05	101; 80; 98; 90	80	101	92	10.2
				7	overall		80	101	94	7.9
			total residue HEC 5725 [a)]	3	**0.05**	96; 97; 99	96	99	97	1.6
				4	0.5	105; 86; 100; 94	86	105	96	8.5
				7	overall		86	105	97	6.1
		grain/ear *	fluoxastrobin	5	**0.018**	98; 96; 99; 96; 92	92	99	96	2.8
				7	0.18	75; 77; 98; 99; 101; 95; 96	75	101	92	11.8
				12	overall		75	101	94	9.1
			HEC 5725 Z-Isomer	5	**0.002**	104; 105; 101; 100; 93	93	105	101	4.7
				7	0.02	77; 73; 95; 103; 109; 92; 95	73	109	92	14.1
				12	overall		73	109	96	11.5
			total residue HEC 5725 [a)]	5	**0.02**	98; 97; 99; 96; 92	92	99	96	2.8
				7	0.2	75; 77; 98; 99; 102; 95; 96	75	102	92	12.0
				12	overall		75	102	94	9.2

*Sample material ear is validated by recoveries for grain

[a)] Residues calculated as sum of residues of HEC 5725 E- and Z-isomer

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- Storage periods: The maximum storage period of deep-frozen treated samples was up to 310 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage periods investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
RA-2013/00	Grain	254
	Ear	310
	Rest of plant	308
	Straw	250

- Residue results: The findings indicate that residues of fluoxastrobin decline well with time. The impact of the first application, for instance, had obviously decreased significantly until the point in time when the second treatment was conducted. This becomes evident when comparing the results for samples, which were collected either just before or shortly after the second treatment took place. Residues caused by the second and final treatment declined well again.
At the time of harvest, residues in grain were at or below the LOQ for the calculated total residue HEC 5725 (<0.02 to 0.02 mg/kg) in grain and ranged from 0.14 to 0.44 mg/kg in straw.

- No residues above the respective LOQs of 0.045 mg/kg (E-isomer for rest of plant and straw), 0.018 mg/kg (E-isomer for grain / ear), 0.005 mg/kg (Z-isomer for rest of plant and straw) or 0.002 mg/kg (Z- isomer for grain / ear) were detected in any of the corresponding control samples.

Table 6.3.1- 8: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2013/00 R 2000 0152/5 0152-00 GLP: yes 2000	Barley, winter Jura	[redacted] Europe, North	200 EC	2	0.125	0.042	61	ear	0* 0 35	<0.018 6.6 0.03	0.005 0.14 0.01	0.02 6.7 0.04
								rest of plant	0* 0 35	0.53 2.5 0.39	0.17 0.18 0.21	0.70 2.6 0.60
								straw	47	0.26	0.15	0.41
								grain	47	<0.018	0.003	0.02
RA-2013/00 R 2000 0153/3 0153-00 GLP: yes 2000	Barley, winter Nickel	France F-[redacted] Europe, North	200 EC	2	0.125	0.042	61	ear	0* 0 35	0.05 5.4 0.02	0.007 0.07 <0.002	0.06 5.5 0.02
								rest of plant	0* 0 35	0.37 2.0 0.12	0.15 0.16 0.05	0.51 2.1 0.17
								straw	54	0.15	0.07	0.22
								grain	54	<0.018	<0.002	<0.02

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Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/h L (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2013/00 R 2000 0154/1 0154-00 GLP: yes 2000	Barley, winter Regina	United [redacted] GB-[redacted] Europe, North	200 EC	2	0.125	0.042	69	ear	0*	<0.018	<0.002	<0.02
									0	6.0	0.28	6.3
									36	0.02	0.01	0.03
								rest of plant	0*	0.12	0.05	0.16
									0	2.4	0.12	2.5
									36	0.18	0.10	0.28
								straw	56	0.27	0.17	0.44
								grain	56	<0.018	<0.003	<0.02
RA-2013/00 R 2000 0156/8 0156-00 GLP: yes 2000	Barley, winter Theresa	Germany D-[redacted] Europe, North	200 EC	2	0.125	0.042	71	ear	0*	0.02	0.007	0.03
									0	5.5	0.13	5.6
									35	0.01	0.003	0.02
								rest of plant	0*	0.40	0.15	0.55
									0	3.2	0.22	3.4
									35	0.21	0.10	0.31
								straw	71	0.09	0.05	0.14
								grain	71	<0.018	<0.002	<0.02

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer))
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation

Report: KCA 6.3.1/06 [redacted]; [redacted]; 2003; M-074486-01-1
Title: Determination of residues of HEC 5725 & tebuconazole on winter barley after spray application of HEC 5725 & HWG 1608 150 EC in the field in [redacted], Northern France, Great Britain and Germany
Report No.: RA-2062/00
Document No.: M-074486-01-1
Guideline(s): 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
Guideline deviation(s): none
GLP/GEP: **yes**

Test system

In the season 1999/2000, four residue trials were conducted in northern Europe. The studies were located in [redacted], the north of France, the United [redacted] and Germany.

In each trial, winter barley was treated twice at a product rate of 2.0 L/ha 'Fluoxastrobin + Tebuconazole EC 150' (75 g fluoxastrobin/L + 75 g tebuconazole/L). The application rate corresponded to 0.150 kg fluoxastrobin/ha. The employed water rate was about 300 L/ha. The spray

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interval was 21 / 22 days in three trials and 49 days in one trial. The application dates were growth stage related: BBCH 37 – 39 for the 1st and BBCH 61-69 for the 2nd application. In one instance the second treatment was carried out at a later stage as originally intended (BBCH 83-85) because erroneously the last application was performed 35 days prior to the expected date of harvest (trial R 2000 0281/5).

Samples were taken at the following intervals:
- Samples of ear and rest of plant were taken just prior to and immediately after the final application in all trials;
- Following the second and final application, samples of barley plants were collected on day 35 - 36 (initially intended pre-harvest interval) following the final treatment as well as at the commercial harvest date 40-47days after the final treatment. Since in two trials on day 35 the crop had not yet reached a development stage which allowed for collection of harvestable grain and straw, ear and rest of plant were collected instead at this sampling event. The later sampling interval was needed to ensure that samples of mature plants (grain and straw) were available.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 (sum of E-and Z-isomer) were determined according to method 00649 with LOQs for the different commodities and for both isomers as described above for study RA-2013/00.

Findings

- Method performance: Method 00649 was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples with HEC 5725 for all matrices relevant to this study ([redacted]; 2001; M-137093-01-1). Mean values for procedural recoveries at the LOQ and tenfold LOQ fortification levels for HEC 5725 E-isomer and HEC 5725 Z-isomer were within the acceptable range of 70-110 % with RSD ≤20%.

Table 6.3.1- 9: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2062/00 R 2000 0278/5 0278-00 R 2000 0279/3 0279-00 R 2000 0280/7	Barley winter	rest of plant	fluoxastrobin	4	**0.045**	104; 105; 105; 105	104	105	105	0.5
				6	0.45	75; 83; 93; 94; 95; 96	75	96	89	9.5
				10	overall		75	105	96	10.6
			HEC 5725 Z-Isomer	4	**0.005**	95; 102; 109; 110	95	110	104	6.7
				6	0.05	71; 84; 88; 90; 92; 96	71	96	87	10.1
				10	overall		71	110	94	12.5
			total residue HEC 5725 a)	4	**0.05**	104; 104; 105; 105	104	105	105	0.6

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0280-00 R 2000 0281/5 0281-00 GLP: yes 2000				6	0.5	74; 83; 93; 94; 94; 95	74	95	89	9.6
				10	overall		74	105	95	10.8
		straw	fluoxastrobin	5	**0.045**	79; 79; 81; 92; 93	79	93	85	8.4
				5	overall		79	93	85	8.4
			HEC 5725 Z-Isomer	5	**0.005**	94; 98; 105; 108; 117	94	117	104	8.6
				5	overall		94	117	104	8.6
			total residue HEC 5725 [a)]	5	**0.05**	81; 85; 83; 93; 93	81	93	87	6.8
				5	overall		81	93	87	6.8
		Grain/ear *	fluoxastrobin	4	**0.018**	93; 97; 100; 100	93	100	98	3.4
				5	0.45	90; 91; 96; 97; 101	90	101	95	4.8
				9	overall		90	101	96	4.2
			HEC 5725 Z-Isomer	4	**0.002**	87; 98; 101; 108	87	108	99	8.9
				5	0.05	92; 94; 95; 97; 98	92	98	95	2.5
				9	overall		87	108	97	6.1
			total residue HEC 5725 [a)]	4	**0.02**	94; 96; 100; 101	94	101	98	3.4
				5	0.5	91; 92; 96; 96; 101	91	101	95	4.2
				9	overall		91	101	96	3.8

*Sample material ear is validated by recoveries for grain
[a)] Residues calculated as sum of residues of HEC 5725 E- and Z-isomer

- Storage periods: The maximum storage period of deep-frozen samples was up to 476 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage period investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
RA-2062/00	Grain	441
	Ear	476
	Rest of plant	476
	Straw	441

- Residue results: The findings indicate that residues of fluoxastrobin on ear and rest of plant decline well with time. The impact of the first application had obviously decreased significantly until the point in time when the second treatment was conducted. Residues caused by the second and final treatment declined well again.

At the time of harvest, residues in grain ranged between 0.02 and 0.03 mg/kg for the calculated total residue HEC 5725 in the three trials were the last application was performed at the proper growth stage and were 0.3 mg/kg in the remaining trial were the 2nd application was delayed (application at BBCH 83-85). This result was not considered for MRL calculation.

Residues in straw were between 0.47 and 0.72 mg/kg in the trials with the regular treatments and at 1.3 mg/kg in the trial with the delayed application.

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- No residues above the respective LOQs of 0.045 mg/kg (E-isomer for rest of plant and straw), 0.018 mg/kg (E-isomer for grain / ear), 0.005 mg/kg (Z-isomer for rest of plant and straw) or 0.002 mg/kg (Z- isomer for grain / ear) were detected in any of the corresponding control samples.

Table 6.3.1- 10: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in/on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Tebuconazole EC 150) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2062/00 R 2000 0278/5 0278-00 GLP: yes 2000	Barley, winter Jura	S-[redacted] Europe, North	150 EC	2	0.150 - 0.159	0.050	61	ear	0* 0 35	<0.018 14 0.05	0.004 0.54 0.02	0.02 15 0.07
								rest of plant	0* 0 35	0.47 2.6 0.59	0.16 0.24 0.33	0.64 2.8 0.91
								straw	47	0.44	0.28	0.72
								grain	47	0.018	0.006	0.02
RA-2062/00 R 2000 0279/3 0279-00 GLP: yes 2000	Barley, winter Nickel	France F-[redacted] Europe, North	150 EC	2	0.150	0.050	69	ear	0* 0 35 43	0.03 6.5 0.40 0.39	0.003 0.08 0.16 0.19	0.03 6.6 0.57 0.58
								rest of plant	0* 0	0.33 2.2	0.12 0.15	0.45 2.4
								grain	35 43	0.02 <0.018	0.009 0.007	0.03 0.03
RA-2062/00 R 2000 0280/7 0280-00 GLP: yes 2000	Barley, winter Regina	United [redacted] GB-[redacted] Europe, North	150 EC	2	0.150	0.050	69	ear	0* 0 36	<0.018 7.3 0.05	<0.002 0.22 0.02	<0.02 7.5 0.06
								rest of plant	0* 0 36	0.22 2.9 0.25	0.08 0.17 0.13	0.30 3.1 0.38
								straw	56	0.30	0.17	0.47
								grain	56	<0.018	0.004	0.02
RA-2062/00 R 2000 0281/5 0281-00 GLP: yes 2000	Barley, winter Theres	[redacted] Europe, North	150 EC	2	0.150	0.050	83	ear	0* 0	<0.018 2.5	<0.002 0.03	<0.02 2.6
								rest of plant	0* 0	0.29 5.4	0.14 0.17	0.43 5.5
								straw	36 40	0.76 0.73	0.51 0.48	1.3 1.2
								grain	36 40	0.17 0.24	0.04 0.06	0.21 0.30

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer))

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Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation

Report:	KCA 6.3.1/07 [redacted]; 2015; M-501711-03-1
Title:	Determination of the residues of fluoxastrobin and prothioconazole in/on spring barley after spray application of fluoxastrobin & prothioconazole EC 200 in Germany
Report No.:	13-2137
Document No.:	M-501711-03-1
Guideline(s):	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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

In 2013, two trials were performed in northern Europe (Germany) on spring barley with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) containing 100 g fluoxastrobin/L. The product was applied twice at application rates of 0.125 kg fluoxastrobin/ha. The treatments were performed with intervals of 6-14 days. The last application was performed at BBCH 61.
Both trials were designed as decline series. Samples of green plant material were taken prior to the last application, immediately thereafter, and in addition on day 7, 14, 21 and 28 post treatment. In addition, samples of green plant material were collected at growth stage BBCH 83 (on day 35 and 42) which is considered appropriate for silage production. Samples of grain and straw were collected at harvest maturity (BBCH 89) after 68 or 69 days. In one trial additional straw and grain samples were taken 35 days after the last treatment.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). The analytical method was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples. The limit of quantitation was 0.009 mg/kg for fluoxastrobin (HEC 5725 E-isomer), 0.001 mg/kg for HEC 5725 Z-isomer and nominally 0.01 mg/kg for the calculated total residue for all commodities.

Findings

- Method performance: Validation recoveries for method 00649/M003 for the matrices not included in the method validation report (wheat and barley green material, straw) were generated within studies 10-2157 ([redacted]; 2011; M-403199-02-1) and 10-2156 ([redacted]; 2011; M-399682-02-1). The studies including validation recoveries are reported further below (please cf. Table 6.3.1-19 and Table 6.3.2- 19).

Mean values of procedural recoveries at fortification levels from 0.009 to 4.5 mg/kg for HEC 5725 E-isomer and 0.001 to 0.5 mg/kg for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%.

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Table 6.3.1- 11: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
13-2137 13-2137-01 13-2137-01-T and 13-2137-02 13-2137-02-T GLP: yes 2013	Barley, spring	green material	fluoxastrobin	2	**0.009**	90;106	90	106	98	
				1	0.9	90	90	90	90	
				1	4.5	94	94	94	94	
				4	overall		90	106	95	8.0
			HEC 5725 Z-Isomer	2	**0.001**	89;120	89	120	105	
				1	0.1	82	82	82	82	
				1	0.5	85	85	85	85	
				4	overall		82	120	94	18.7
		grain	fluoxastrobin	1	**0.009**	85	85	85	85	
				1	0.09	91	91	91	91	
				2	overall		85	91	88	
			HEC 5725 Z-Isomer	1	**0.001**	74	74	74	74	
				1	0.01	99	99	99	99	
				2	overall		74	99	87	
		straw	fluoxastrobin	1	**0.009**	85	85	85	85	
				1	0.90	89	89	89	89	
				1	1.8	87	87	87	87	
				3	overall		85	89	87	2.3
			HEC 5725 Z-Isomer	1	**0.001**	105	105	105	105	
				1	0.01	85	85	85	85	
				1	0.2	88	88	88	88	
				3	overall		85	105	93	11.6

- Storage periods: The maximum storage period of deep-frozen samples was up to 340 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage periods investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
13-2137	Grain	305
	Straw	305
	Green material	340

- Residue results: In the northern European field trials, at a growth stage representative for commercial harvest (BBCH 89), the residues in grain ranged from < 0.01 - 0.026 mg/kg and were 0.17 - 0.18 mg/kg in straw for the total residue of HEC 5725. Residues in green material declined well with

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time as shown with the findings prior to the second treatment and with the samples of the decline series taken thereafter.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were detected in any of the corresponding control samples except for HEC 5725 Z-isomer which was present at a level of 0.001 mg/kg in a single straw sample resulting in a total residue of 0.01 mg/kg in the control sample of straw due to summation with the LOQ of the E-isomer.

Table 6.3.1- 12: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
13-2137 13-2137-01 13-2137-01-T GLP: yes 2013	Barley, spring Conch-ita	Germany [redacted] Europe, North	200 EC	2	0.125	0.0417	61	green material	0*	0.23	0.079	0.30
									0	3.8	0.11	3.9
									7	0.52	0.14	0.66
									14	0.25	0.076	0.33
									21	0.11	0.035	0.15
									28	0.070	0.024	0.094
									35	0.070	0.026	0.096
								grain	35	<0.009	<0.001	<0.01
									69	<0.009	<0.001	<u>≤0.01</u>
								straw	35	0.12	0.051	0.17
									69	0.12	0.058	<u>0.18</u>
13-2137 13-2137-02 13-2137-02-T GLP: yes 2013	Barley, spring Grace	Germany [redacted] Europe, North	200 EC	2	0.125	0.0313-0.0417	61	green material	0*	0.48	0.082	0.56
									0	2.7	0.10	2.8
									7	0.29	0.047	0.34
									14	0.23	0.041	0.27
									21	0.13	0.026	0.15
									28	0.11	0.027	0.14
									42	0.11	0.031	0.14
								grain	68	0.020	0.005	<u>0.026</u>
								straw	68	0.13	0.044/ 0.001**	<u>0.17</u> /0.010**

* prior to last treatment ** residue in control

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer))

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

Underlined values are used for MRL calculation

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Report:	KCA 6.3.1/08[redacted]>;[redacted]; 2014; M-501503-01-1
Title:	Determination of the residues of fluoxastrobin and prothioconazole in/on barley and spring barley after spray application of Fluoxastrobin & Prothioconazole EC 200 in France (North)
Report No.:	13-2158
Document No.:	M-501503-01-1
Guideline(s):	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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

In 2013, two trials were performed in northern France on barley with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) containing 100 g fluoxastrobin/L. The product was applied twice at application rates of nominal 0.125 kg fluoxastrobin/ha. In one trial the 1st and 2nd treatment were overdosed at 6.3 or 8.3%, respectively, resulting in actual application rates of 0.133 and 0.135 kg a.s./ha. The last application was conducted at BBCH 61. The treatments were performed with intervals of 15 or 20 days.
Both trials were designed as decline series. Samples of green material were taken prior to the last application and immediately thereafter, and in addition on day 7, 14, 21 (and 28 in one trial). In addition, samples of green plant material were collected at growth stage BBCH 83 (on day 17 or 31 post treatment) which is considered appropriate for silage production. Samples of grain and straw were collected after 35 and/or 43 days.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1).

Findings

- Method performance: Aspects relevant to the analytical method were as described above for study 13-2137.
Mean values of procedural recoveries at fortification levels from 0.009 to 9 mg/kg for HEC 5725 E-isomer and from 0.001 to 1 mg/kg for HEC 5725 for the Z-isomer were within the range of 70-110 %, with RSD <20%.

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Table 6.3.1- 13: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley
The LOQ is marked in bold

Study Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
13-2158 13-2158-01 13-2158-02 GLP: yes 2013	Barley	green material	fluoxastrobin	1	**0.009**	93	93	93	93	
				1	1.8	86	86	86	86	
				1	4.5	96	96	96	96	
				3	overall		86	96	92	5.6
			HEC 5725 Z-Isomer	1	**0.001**	89	89	89	89	
				1	0.2	83	83	83	83	
				1	0.5	89	89	89	89	
				3	overall		83	89	87	4.0
		grain	fluoxastrobin	1	**0.009**	92	92	92	92	
				1	0.09	94	94	94	94	
				2	overall		92	94	93	
			HEC 5725 Z-Isomer	1	**0.001**	100	100	100	100	
				1	0.01	78	78	78	78	
				2	overall		78	100	89	
		straw	fluoxastrobin	1	**0.009**	89	89	89	89	
				1	1.8	81	81	81	81	
				1	9	83	83	83	83	
				3	overall		81	89	84	4.9
			HEC 5725 Z-Isomer	1	**0.001**	94	94	94	94	
				1	0.2	79	79	79	79	
				1	1	77	77	77	77	
				3	overall		77	94	83	11.1

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 360 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage period investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
13-2158	Grain	325
	Straw	325
	Green material	360

-Residue results: In the two northern European field trials the residues in grain ranged from 0.011 - 0.020 mg/kg and were 0.44 - 2.7 mg/kg in straw for the total residue of HEC 5725. Residues in green material declined well with time as shown with the findings prior to the second treatment and with the samples of the decline series taken thereafter.

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- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were detected in any of the corresponding control samples.

Table 6.3.1- 14: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
13-2158 13-2158-01 13-2158-01-T GLP: yes 2013	Barley Esterel	France [redacted] Europe, North	200 EC	2	0.125	0.0625	61	green material	0*	0.11	0.036	0.15
									0	3.4	0.088	3.5
									7	0.32	0.10	0.42
									14	0.16	0.051	0.21
									21	0.15	0.050	0.20
									28	0.11	0.048	0.16
									31	0.16	0.061	0.22
								grain	35	<0.009	0.002	0.011
									43	0.014	0.006	<u>0.020</u>
								straw	35	0.092	0.042	0.13
									43	0.30	0.14	<u>0.44</u>
13-2158 13-2158-02 13-2158-02-T GLP: yes 2013	Barley, spring Sébastian	France [redacted] Europe, North	200 EC	2 - 2	0.133 - 0.135	0.0623 - 0.0625	61	green material	0*	0.30	0.099	0.40
									0	4.1	0.13	4.2
									7	0.50	0.16	0.66
									14	0.42	0.17	0.59
									21	0.28	0.12	0.39
									17	0.30	0.12	0.41
								grain	35	<0.009	0.002	<u>0.011</u>
								straw	35	1.8	0.92	<u>2.7</u>

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

Supplementary trials – southern Europe

Report: KCA 6.3.1/09[redacted]; 2015; M-062669-03-1
Title: Amendment No. 2 to report no: RA-2017/03 - Determination of residues of fluoxastrobin (HEC 5725), prothioconazole (JAU6476) and trifloxystrobin (CGA279202 in/on barley following spray application of HEC 5725 & JAU 6476 & CGA279202 (300 EC) in France, Italy and Spain
Report No.: RA-2017/03
Document No.: M-062669-03-1
Guideline(s): 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
Guideline deviation(s): none
GLP/GEP: **yes**

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Test system

In 2003, four trials were performed in southern Europe (southern France, Italy (2) and Spain) on barley with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole + Trifloxystrobin EC 300) containing 75 g fluoxastrobin/L, 150 g prothioconazole/L and 75 g trifloxystrobin/L. The product was applied twice at application rates of nominal 0.075 kg fluoxastrobin/ha. The water rate was about 300 L/ha in all trials. In one trial the 2nd application was slightly overdosed by 6.3%.
The applications were growth stage related and performed at growth stages BBCH 37-41 and BBCH 61-65 corresponding to intervals between 13 and 18 days.
All trials were designed as harvest trials. Samples of 'rest of plant' and ear were taken prior to the last application and immediately thereafter (day 0 samples). Samples of grain and straw were collected at harvest maturity (BBCH 89) on day 35 - 69 after the last treatment. In two trials two sets of grain and straw samples were collected: at day 35 (which was initially the intended pre-harvest interval) and at a later date when full harvest maturity was reached.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649 ([redacted]; 2001; M-237098-01-1). The method was submitted with the initial Annex II dossier and evaluated in the EU peer review.
The LOQ for barley 'rest of plant' and straw was 0.045 mg/kg for HEC 5725 E-isomer and 0.005 mg/kg for HEC 5725 Z-isomer (corresponding to a theoretical LOQ of 0.05 mg/kg for the calculated total residue of HEC 5725). The LOQ for grain and ear was set at 0.018 mg/kg for HEC 5725 E-isomer and at 0.002 mg/kg for HEC 5725 Z-isomer (corresponding to 0.02 mg/kg for the calculated total residue of HEC 5725).

Findings

- Method performance: Method 00649 was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples with HEC5725 for all matrices relevant to this study.
Mean values for procedural recoveries at fortification levels between 0.045 - 4.5 mg/kg (rest of plant, straw) and 0.018 - 1.8 mg/kg (ear, grain) for HEC 5725 E-isomer and 0.005 – 0.5 mg/kg (rest of plant, straw) and 0.002 - 0.2 mg/kg (grain, ear) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%.

Table 6.3.1- 15: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley
The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2017-03 R 2003 0132/4 0132-03	Barley	rest of plant	fluoxastrobin	2	**0.045**	91; 94	91	94	93	
				2	1.8	87; 91	87	91	89	
				2	4.5	98; 100	98	100	99	
				6	overall		87	100	94	5.2
0253/3 0253-03			HEC 5725 Z-Isomer	2	**0.005**	87; 89	87	89	88	
				2	0.2	96; 97	96	97	97	

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Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
R 2003 0254/1 0254-03 R 2003 0256/8 0256-03 GLP: yes 2003				2	0.5	95; 111	95	111	103	
				6	overall		87	111	96	8.8
			total residue HEC 5725 [a)]	2	**0.05**	90; 93	90	93	92	
				2	2.0	88; 92	88	92	90	
				2	5.0	97; 101	97	101	99	
				6	overall		88	101	94	5.1
		ear	fluoxastrobin	2	**0.018**	98; 99	98	99	99	
				2	0.45	97; 99	97	99	98	
				2	1.8	98; 99	98	99	99	
				6	overall		97	99	98	0.8
			HEC 5725 Z-Isomer	2	**0.002**	94; 109	94	109	102	
				2	0.05	95; 96	95	96	96	
				2	0.2	95; 98	95	98	97	
				6	overall		94	109	98	5.8
			total residue HEC 5725 [a)]	2	**0.02**	98; 99	98	99	99	
				2	0.5	96; 98	96	98	97	
				2	2.0	98; 99	98	99	99	
				6	overall		96	99	98	1.1
		grain	fluoxastrobin	6	**0.018**	96; 98; 96; 97; 99; 96	96	99	97	1.3
				6	overall		96	99	97	1.3
			HEC 5725 Z-Isomer	6	**0.002**	92; 104; 92; 97; 98; 90	90	104	96	5.5
				6	overall		90	104	96	5.5
			total residue HEC 5725 [a)]	6	0.02	95; 99; 96; 97; 99; 95	95	99	97	1.9
				6	overall		95	99	97	1.9
		straw	fluoxastrobin	2	**0.045**	81; 90	81	90	86	
				2	0.9	94; 95	94	95	95	
				2	1.8	80; 81	80	81	81	
				6	overall		80	95	87	8.0
			HEC 5725 Z-Isomer	2	**0.005**	78; 89	78	89	84	
				2	0.1	97; 99	97	99	98	
				2	0.2	80; 88	80	88	84	
				6	overall		78	99	89	9.7
			total residue HEC 5725 [a)]	2	**0.05**	80; 90	80	90	85	
				2	1.0	94; 95	94	95	95	
				2	2.0	81; 81	81	81	81	
				6	overall		80	95	87	8.0

a) Residues calculated as sum of residues of HEC 5725 E- and Z-isomer

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- Storage periods: The maximum storage period of deep-frozen treated samples was up to 287 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage period investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
RA-2017/03	Grain	233
	Straw	240
	Ear	280
	Rest of plant	287

-Residue results: In the four southern European field trials, the residues in grain ranged from < 0.02 - 0.04 mg/kg and were 0.15 - 0.74 mg/kg in straw for the total residue of HEC 5725.

- No residues above the respective LOQs of 0.045 mg/kg (E-isomer for rest of plant and straw), 0.018 mg/kg (E-isomer for grain / ear), 0.005 mg/kg (Z-isomer for rest of plant and straw) or 0.002 mg/kg (Z- isomer for grain / ear) were detected in any of the corresponding control samples.

Table 6.3.1- 16: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole + Trifloxystrobin EC 300) in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FI	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2017/03 R 2003 0132/4 0132-03 GLP: yes 2003	Barley Print	France F-[redacted] Europe, South	300 EC	2	0.075	0.025	55-65	rest of plant	0* 0	0.32 3.2	0.17 0.22	0.49 3.4
								ear	0* 0	0.02 2.8	0.009 0.03	0.03 2.9
								grain	35	<0.018	<0.002	<0.02
								straw	35	0.48	0.27	0.74
RA-2017/03 R 2003 0253/3 0253-03 GLP: yes 2003	Barley Aliseo	Italy I-[redacted] Europe, South	300 EC	2	0.075	0.025	61	rest of plant	0* 0	0.07 0.78	0.04 0.05	0.10 0.83
								ear	0* 0	<0.018 4.2	<0.002 0.12	<0.02 4.4
								grain	35 45	<0.018 0.02	0.009 0.01	0.03 0.04
								straw	35 45	0.06 0.09	0.04 0.06	0.10 0.15
RA-2017/03 R 2003 0254/1 0254-03 GLP: yes 2002	Barley Hispanic	Spain E-[redacted] Europe, South	300 EC	2	0.075 - 0.079	0.025	61	rest of plant	0* 0 35	0.13 1.3 0.17	0.05 0.09 0.09	0.17 1.4 0.26
								ear	0* 0 35	<0.018 4.1 <0.018	0.003 0.13 0.008	0.02 4.2 0.03
								grain	69	<0.018	0.003	0.02
								straw	69	0.39	0.22	0.61

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Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2017/03 R 2003 0256/8 0256-03 GLP: yes 2003	Barley Sonora	Italy I-[redacted] Europe, South	300 EC	2	0.075	0.025	61	rest of plant	0*	0.29	0.18	0.46
									0	3.8	0.36	4.2
								ear	0*	<0.018	<0.002	<0.02
									0	4.6	0.35	5.0
								grain	35	<0.018	0.004	0.02
									40	<0.018	0.004	0.02
								straw	35	0.12	0.08	0.20
									40	0.14	0.09	0.23

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation

Report: KCA 6.3.1/10[redacted]C; [redacted]; [redacted]; 2011; M-414709-01-1
Title: Determination of the residues of BYF 00587, HEC 5725 and prothioconazole in/on barley after spray application of bixafen & fluoxastrobin & prothioconazole EC 190 in the field in France (south) and Italy
Report No.: 10-2206
Document No.: M-414709-01-1
Guideline(s): 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)
Guideline deviation(s): none
GLP/GEP: **yes**

Test system

Two residue trials were conducted in 2010 on barley with 'Bixafen + Fluoxastrobin + Prothioconazole EC 190' containing 40 g bixafen/L, 50 g fluoxastrobin/L and 100 g prothioconazole/L. The test locations were in southern France and Italy. The product was applied twice at the required rate of 1.5 L product/ha corresponding to 0.075 kg fluoxastrobin/ha. The treatments were carried out at the growth stages BBCH 37-51 and BBCH 61. Depending on the study, the spray interval was 14 or 15 days. The water rate ranged from 300 - 400 L/ha.

Samples of green material were taken just prior to and immediately after the final application in both trials. In one trial, first grain and straw samples were taken on day 34 (BBCH 87); and an additional set of samples was taken on day 47 (BBCH 89). In the trial from Italy grain and straw samples were collected only at BBCH 89 (day 52).

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Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). The total residue of HEC 5725 was calculated as the sum of both isomers. The LOQ was 0.009 mg/kg for the E-isomer and 0.001 mg/kg for the Z-isomer for all sample materials, resulting in a theoretical LOQ of 0.01 mg/kg for the total residue of HEC 5725.

Findings

- Method performance: Validation recoveries for method 00649/M003 for the matrices not included in the method validation report (wheat and barley green material, straw) were generated within studies 10-2157 ([redacted]; 2011; M-403199-02-1) and 10-2156 ([redacted]; 2011; M-399682-02-1). The studies including validation recoveries are reported further below (please cf. Table 6.3.1- 19 and Table 6.3.2- 19).

Additional validation recoveries for method 00649/M003 for all matrices relevant to this study (green material, straw and grain) were also reported within this study and obtained from studies 10-2206, 10-2207, (10-2204 and 10-2205 not reported in the present dossier). Individual and mean recoveries at fortification levels between 0.009 and 9.6 mg/kg for HEC 5725 E-isomer and 0.001 and 0.4 mg/kg for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD ≤20%. The control sample used for 2 recoveries of HEC 5725 E-isomer in green material (fortification level 0.009 mg/kg) and for HEC 5725 Z-isomer (fortification level 0.001 mg/kg) contained more than 30% of the LOQ and therefore the recoveries were background corrected for the signal present in the control sample. The reason was a fluoxastrobin containing product which was erroneously used for maintenance in another study (trial 10-2204-01, not relevant to the present dossier).

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Table 6.3.1- 17: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
Recoveries were generated during analysis of 4 studies (10-2204 and 10-2206 (barley) and 10-2205 and 10-2207 (wheat)) GLP: yes 2010	Barley, wheat	Green material	Fluoxastrobin	8	**0.009**	86;91;95;(136*);97;102;108;(144*);113;103	86	113	99	9.0
				4	0.09	93;98;105;104	93	105	101	5.6
				1	2.7	84	84	84	84	
				1	3.6	87	87	87	87	
				14	Overall		84	113	98	9.0
			HEC 5725 Z-Isomer	8	**0.001**	81;87;97;98;(227*);102;113;(242*);105;120	81	120	100	12.7
				4	0.01	88;115;88;97	88	115	97	13.1
				1	0.30	86	86	86	86	
				1	0.40	91	91	91	91	
				14	Overall		81	120	98	12.3
		Grain	Fluoxastrobin	6	**0.009**	78;87;94;91;96;98	78	98	91	8.1
				4	0.09	88;102;92;98	88	102	95	6.5
				10	Overall		78	102	92	7.5
			HEC 5725 Z-Isomer	6	**0.001**	82;86;90;90;75;89	75	90	85	6.9
				4	0.01	88;91;93;93	88	93	91	2.6
				10	Overall		75	93	88	6.3
		Straw	Fluoxastrobin	6	**0.009**	87;88;86;93;103;92	86	103	92	6.9
				4	0.09	89;75;104;96	75	104	91	13.5
				1	2.7	84	84	84	84	
				1	3.6	82	82	82	82	
				12	Overall		75	104	90	9.3
			HEC 5725 Z-Isomer	6	**0.001**	69;76;89;109;116;98	69	116	93	19.8
				4	0.01	85;69;91;86	69	91	83	11.5
				1	0.30	85	85	85	85	
				1	0.40	85	85	85	85	
				12	Overall		69	116	88	16.2

*recovery before correction; the control sample used for 2 recoveries of HEC 5725 E-isomer (0.009 mg/kg) and for HEC 5725 Z-isomer (0.001 mg/kg) in green material contained more than 30% of the LOQ and therefore the recovery was background corrected for the signal present in the control sample. The control sample originated from study 10-2204 which is not relevant to the present dossier.

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- Storage periods: The maximum storage period of deep-frozen treated samples was up to 312 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage interval investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
10-2206	Grain	269
	Straw	269
	Green material	312

- Residue results: In the two southern European field trials, the total residue of HEC 5725 in grain ranged from 0.01 - 0.02 mg/kg and was 0.03 - 0.16 mg/kg in straw.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were detected in any of the corresponding control samples from this study.

Table 6.3.1- 18: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Bixafen + Fluoxastrobin + Prothioconazole EC 190) in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
10-2206 10-2206-01 GLP: yes 2010	Barley Ketos	France [redacted] Europe, South	190 EC	2	0.075	0.025	61	green material	0*	0.10	0.05	0.14
									0	1.2	0.16	1.4
								grain	34	0.01	0.006	<u>0.02</u>
									47	<0.009	0.004	0.01
								straw	34	0.08	0.04	0.12
									47	0.11	0.06	<u>0.16</u>
10-2206 10-2206-02 GLP: yes 2010	Barley Ketos	Italy [redacted] Europe, South	190 EC	2	0.075	0.019	61	green material	0*	0.07	0.04	0.11
									0	1.4	0.08	1.5
								grain	52	<0.009	0.001	<u>0.01</u>
								straw	52	0.02	0.01	<u>0.03</u>

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

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Report:	KCA 6.3.1/11 [redacted],; 2011; M-403199-02-1
Title:	Determination of the resiudes of fluoxastrobin and prothioconazole in/on barley and winter barley after spraying of fluoxastrobin & prothioconazole EC 150 in the field in France (South), Spain, Italy and Greece
Report No.:	10-2157
Document No.:	M-403199-02-1
Guideline(s):	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)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

Five residue trials were carried out in 2010 with Fluoxastrobin + Prothioconazole EC 150 on barley in France (2), Italy, Spain and Greece. 'Fluoxastrobin + Prothioconazole EC 150' was applied twice at the required rates of 1.75 L product/ha corresponding to 0.0875 kg fluoxastrobin/ha. The treatments were carried out at proper timing (BBCH 41-57 and BBCH 61-65). Depending on the study, the spray interval was 13 or 14 days except for trial 10-2157-02 where the interval was only 3 days due to unexpected fast crop development and in order to meet the requested growth stage for the 2nd application. The water rate was 300 or 400 L/ha in all trials.

Samples of green material were taken just prior to and immediately after the final application took place in all trials. Three trials were designed as decline series and in two trials samples were collected at harvest only. In the decline trials green material samples were collected on day 7, 14 and 28 at growth stages ranging from BBCH 67 - 87. Grain and straw samples were collected on day 35 and a later date (up to day 60 after the final treatment), in case the growth stage relevant for commercial harvest (BBCH 89) had not yet been reached at the first sampling event. If grain and straw could not be sampled 35 days post treatment the sample materials 'ear' and 'rest of plant' were sampled instead.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted] 2010; M-387385-01-1).

Findings

- Method performance: Validation recoveries for method 00649/M003 for matrices relevant to this study were generated. Recoveries for both analytes were obtained from green material, straw and grain samples. The sample materials chosen served to represent all relevant sample materials collected in these trials. Method performance was acceptable. Mean recoveries at fortification levels between 0.009 - 0.9 mg/kg (grain), 0.009 - 9 mg/kg (straw) and 0.009-20 mg/kg (green material) for HEC 5725 E-isomer and 0.001 - 0.1 mg/kg (grain), 0.001 - 1 mg/kg (straw) and 0.001 – 2 mg/kg (green material) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD < 20%.

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Table 6.3.1- 19: Recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
10-2157 10-2157-01 10-2157-02- 10-2157-03 10-2157-04 10-2157-05 GLP: yes 2010	Barley	green material*	Fluoxastrobin	4	**0.009**	95; 93; 93; 101	93	101	96	3.6
				4	0.09	96; 97; 96;94	94	97	96	1.3
				1	9	99	99	99	99	
				3	26	86; 86; 83	83	86	85	2.0
				12	overall		83	101	93	5.9
			HEC 5725 Z-Isomer	4	**0.001**	90; 107; 95; 101	90	107	98	8.5
				4	0.01	94; 95; 93;93	93	95	94	1.0
				1	1	97	97	97	97	
				3	1	78; 79; 77	77	79	78	1.3
				12	overall		77	107	92	10.1
		Grain**	Fluoxastrobin	4	**0.009**	93; 93; 90; 93	90	93	92	1.6
				4	0.09	90; 91; 91; 93	90	93	91	1.4
				1	0.9	79	79	79	79	
				9	overall		79	93	90	4.9
			HEC 5725 Z-Isomer	4	**0.001**	103; 93; 99; 104	93	104	100	5.0
				4	0.01	91; 88; 87; 84	84	91	88	3.3
				1	0.1	64	64	64	64	
				9	overall		64	104	90	13.4
		straw	Fluoxastrobin	6	**0.009**	83;85;86;88; 89;90	83	90	87	3.0
				3	0.09	74;90;90	74	90	85	10.9
				3	9	78; 71; 69	69	78	73	6.5
				12	overall		69	90	83	9.4
			HEC 5725 Z-Isomer	6	**0.001**	81; 82; 88; 88;119;119	81	119	96	18.6
				3	0.01	77;88;92	77	92	86	9.1
				3	1	69; 72; 69	69	72	70	2.5
				12	overall		69	119	87	19.3

*recoveries for green material also validate the sample material 'rest of plant'
** recoveries for grain also validate the sample material 'ear'

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- Storage periods: The maximum storage period of deep-frozen treated samples was up to 208 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage period investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
10-2157	Grain	167
	Straw	167
	Ear	173
	Rest of plant	173
	Green material	208

-Residue results: The findings from the decline series indicate that residues of fluoxastrobin decrease well with time. Residues due to the first application had decreased significantly until the point in time when the second treatment was conducted. Residues caused by the second and final treatment declined well again.
At the time of harvest, for the trials with proper spray intervals (*i.e.* excluding trial 10-2157-02) residues (total residue HEC 5725) in grain were 0.01 - 0.34 mg/kg in grain and ranged from 0.23 – 1.7 mg/kg in straw.
In trial 10-2157-04 initially ear samples were collected instead of grain, however ears were threshed in the laboratory for sample preparation and proper sample material was available for residue analysis. The elevated residue finding in grain from this trial might be considered as an outlier when compared to the residue levels found in other trials from the southern climatic zone. However, it appeared also within the data set for the previous critical GAP that in exceptional cases significantly elevated residue levels may be found in barley grain (cf. EFSA Conclusion, 2007, and Tier 1 summary forms reporting two trials with residues at 0.24 and 0.27 mg/kg). Although the application rate for the new critical GAP is lower, it cannot be excluded that this is a 'true' residue value and the value was considered for MRL calculation.
Residues levels from trial 10-2157-02 where the interval was only 3 days were not considered for MRL calculations although the residue findings were in an expected range.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were detected in the corresponding control samples except for one control grain sample from trial 10-2157-05 which contained residues of HEC 5725 Z-isomer at the LOQ level (0.001 mg/kg). This sample was not used for procedural recoveries.

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Table 6.3.1- 20: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 150) in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
10-2157 10-2157-01 10-2157-01-T GLP: yes 2010	Barley, winter Ketos	France F-[redacted] Europe, South	150 EC	2	0.0875	0.029	61	green material	0*	0.76	0.29	1.0
									0	3.5	0.32	3.8
									6	0.85	0.33	1.2
									14	0.75	0.28	1.0
									28	0.41	0.18	0.59
								ear	35	0.052	0.023	0.07
								rest of plant	35	0.62	0.27	0.89
								grain	50	0.012	0.006	0.02
								straw	50	0.63	0.34	0.97
10-2157 10-2157-02 10-2157-02-T GLP: yes 2010	Barley, winter Carpa-nil	France F-[redacted] Europe, South	150 EC	2	0.0875	0.029	61	green material	0*	1.2	0.22	1.4
									0	3.0	0.27	3.3
								ear	35	0.017	0.006	0.03
								rest of plant	35	0.046	0.015	0.06
								grain	60	<0.009	0.002	0.01
								straw	60	0.038	0.016	0.05
10-2157 10-2157-03 10-2157-03-T GLP: yes 2010	Barley, winter Gra-phic	Spain E-[redacted] Europe, South	150 EC	2	0.0875	0.029	65	green material	0*	0.16	0.065	0.23
									0	1.4	0.099	1.5
									7	0.24	0.084	0.32
									14	0.14	0.051	0.19
									28	0.10	0.037	0.14
								grain	35	<0.009	0.001	0.01
									52	<0.009	0.003	0.01
								straw	35	0.19	0.084	0.27
									52	0.22	0.096	0.31
10-2157 10-2157-04 10-2157-04-T GLP: yes 2010	Barley Baraka	Italy I-[redacted] Europe, South	150 EC	2	0.0875	0.022	61	green material	0*	0.028	0.015	0.04
									0	3.4	0.47	3.9
									7	2.9	1.4	4.2
									14	1.4	0.69	2.0
									28	0.67	0.40	1.1
								grain	35	0.24	0.11	0.34
								straw	35	1.0	0.66	1.7
10-2157 10-2157-05 10-2157-05-T GLP: yes 2010	Barley Thessa-loniki	Greece GR-[redacted] Europe, South	150 EC	2	0.0875	0.029	61	green material	0*	0.30	0.13	0.43
									0	2.3	0.14	2.4
									7	1.4	0.56	1.9
									14	0.82	0.34	1.2
								grain	35	<0.009	<0.001	<0.01
									28	<0.009	0.001/0.001**	0.01/0.01**
								straw	35	0.038	0.021	0.06
									28	0.15	0.078	0.23

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* prior to last treatment
** residue in control sample
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

Report:	KCA 6.3.1/12[redacted]; 2013; M-434980-04-1
Title:	Amendment no. 3 to report no: 11-2111 - Determination of the residues of fluoxastrobin and prothioconazole in/on winter barley after spray application of fluoxastrobin & prothioconazole EC 150 in southern France, Italy and Spain
Report No.:	11-2111
Document No.:	M-434980-04-1
Guideline(s):	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) US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

Five residue trials were carried out in 2011 with 'Fluoxastrobin + Prothioconazole EC 150' on barley in France (2), Italy (2) and Spain. The product was applied twice at the required rates of 1.75 L product/ha corresponding to 0.0875 kg fluoxastrobin/ha. The treatments were carried out at proper timing with the last application conducted during flowering (BBCH 37-43 and BBCH 61-69). Depending on the study, the spray interval was 13 -20 days. The water rate was 300 or 400 L/ha in all trials.

Samples of green material were taken just prior to and immediately after the final application took place in all trials. Three trials were designed as harvest trials and in two trials samples of green material were collected additionally on day 7, 14 and 28 after the final treatment. Grain and straw were sampled at harvest maturity (BBCH 89) 51 –63 days after the final application.
Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). Aspects relative to the analytical method were as described above for study 10-2206.

Findings

- Method performance: Validation recoveries for method 00649/M003 for the matrices not included in the method validation report were generated within studies 10-2157 ([redacted] 2011; M-403199-02-1) and 10-2156 ([redacted]; 2011; M-399682-02-1). Validation recoveries are reported in Table 6.3.1- 19 and Table 6.3.2- 19.

Additional validation recoveries for method 00649/M003 for all matrices relevant to this study (green material, straw and grain) were also reported within this study.
Procedural recoveries for both analytes were obtained from green material, straw, and grain samples. Method performance was acceptable. Mean and individual recoveries at fortification levels between 0.009 - 0.09 mg/kg (grain), 0.009 – 0.9 mg/kg (straw) and 0.009 - 3 mg/kg (green material) for HEC

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5725 E-isomer and 0.001 – 0.01 mg/kg (grain), 0.1 mg/kg (straw) and 0.001 – 0.3 mg/kg (green material) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%.

Table 6.3.1- 21: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on barley
The LOQ is marked in bold

Study Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
11-2111 11-2111-01 11-2111-02 11-2111-03 11-2111-04 11-2111-05	Barley, winter	green material	Fluoxastrobin	3	**0.009**	61;77;83	61	83	74	15.4
				3	0.09	99;99;99	99	99	99	0.0
				1	1.0	84	84	84	84	
				2	3.0	97;108	97	108	103	
				9	overall		61	108	90	16.3
GLP: yes 2011			HEC 5725 Z-Isomer	2	**0.001**	73;78	73	78	76	
				3	0.01	90;92;93	90	93	92	1.7
				1	0.10	78	78	78	78	
				2	0.30	91;98	91	98	95	
				8	overall		73	98	87	10.4
		grain	Fluoxastrobin	2	**0.009**	81;86	81	86	84	
				1	0.09	90	90	90	90	
				3	overall		81	90	86	5.3
			HEC 5725 Z-Isomer	2	**0.001**	90;95	90	95	93	
				1	0.01	91	91	91	91	
				3	overall		90	95	92	2.9
		straw	Fluoxastrobin	2	**0.009**	72;80	72	80	76	
				2	0.90	78;80	78	80	79	
				4	overall		72	80	78	4.9
			HEC 5725 Z-Isomer	2	0.10	70;80	70	80	75	
				2	overall		70	80	75	

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 228 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage period investigated in the stability studies.

Study number	Sample material	Maximum storage period (days)
11-2111	Grain	160
	Straw	160
	Green material	228

Findings

- Residue results: Residue levels of the green material samples taken from the decline series and collected prior to the final application show that residues decline well with time.

At the time of harvest, residues in grain were 0.01 - 0.03 mg/kg for the calculated total residue HEC 5725 in grain and ranged from 0.28 – 1.4 mg/kg in straw.

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- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were determined in any of the corresponding control samples except for one green material control sample from trial 11-2111-02 which contained residues of HEC 5725 E-isomer at 0.01 mg/kg and 0.006 mg/kg for HEC 5725 Z-isomer. This sample was not used for procedural recoveries.

Table 6.3.1- 22: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on barley treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 150) in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
11-2111 11-2111-01 11-2111-01-T GLP: yes 2011	Barley, winter Ketos	France [redacted] Europe, South	150 EC	2	0.0875	0.0292	61	green material	0* 0 7 14 28	0.25 2.0 0.32 0.20 0.11	0.075 0.071 0.11 0.071 0.047	0.33 2.0 0.42 0.27 0.16
								grain straw	51 51	0.018 0.47	0.011 0.26	<u>0.03</u> <u>0.73</u>
11-2111 11-2111-02 11-2111-02-T GLP: yes 2011	Barley, winter Cam-panil	France [redacted] Europe, South	150 EC	2	0.0875	0.029	61	green material	0 22	1.6 0.010* 0.097	0.059/ 0.006** 0.039	1.6/ 0.016** 0.14
								grain straw	55 55	<0.009 0.18	0.004 0.10	<u>0.013</u> <u>0.28</u>
11-2111 11-2111-03 11-2111-03-T GLP: yes 2011	Barley, winter Lutece	Italy [redacted] Europe, South	150 EC	2	0.0875	0.022	65	green material	0* 0 7 14 28	0.27 1.6 0.57 0.20 0.085	0.083 0.093 0.20 0.073 0.039	0.35 1.7 0.76 0.27 0.12
								grain straw	63 63	<0.009 0.18	0.006 0.11	<u>0.015</u> <u>0.29</u>
11-2111 11-2111-04 11-2111-04-T GLP: yes 2011	Barley, winter Ketos	Italy [redacted] Europe, South	150 EC	2	0.0875	0.029	69	green material	0 13	2.6 0.33	0.13 0.18	2.7 0.51
								grain straw	43 43	0.013 0.91	0.006 0.45	<u>0.019</u> <u>1.4</u>
11-2111 11-2111-05 11-2111-05-T GLP: yes 2011	Barley, winter Gra-phic	Spain [redacted] Europe, South	150 EC	2	0.0875	0.029	69	green material	0 10	2.2 0.31	0.15 0.11	2.3 0.43
								grain straw	49 49	<0.009 0.35	0.001 0.18	<u>0.010</u> <u>0.52</u>

* prior to last treatment
** residue in control sample

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer))

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

Underlined values are used for MRL calculation.

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Overall conclusion on barley

Northern Europe:

The GAP evaluated for Annex I inclusion for Fluoxastrobin EC 100 will not be supported following the renewal of approval of the active substance. Therefore, a set of new residue data is reported supporting the critical GAP for barley and oats in northern Europe post Annex I renewal for 'Fluoxastrobin + Prothioconazole EC 200' *(GAP EU-N 2; cf* Table 6.3.1- 2*)*. This GAP is considered in the MRL application form. Since the cGAP and is also the GAP for the representative use in the northern region all residue data supporting the cGAP also support the representative use.

In order to support the cGAP/representative use of fluoxastrobin with the product 'Fluoxastrobin + Prothioconazole EC 200' on barley and oats 11 supplementary trials are reported using different EC formulations containing fluoxastrobin (EC 200 or EC 250 with prothioconazole or tebuconazole as mixing partners). The trials were performed in 2000 or 2013 and according to GLP principles. In all trials fluoxastrobin was applied twice with the final application made at growth stage BBCH 61 (or 69 in 2 trials). The application rates ranged from 0.125 – 0.150 kg fluoxastrobin/ha.
For some trials two sets of grain and straw samples were collected (*e.g.* at a 35 d PHI and at a later date). For the summary and MRL calculations always the highest residue level has been considered.

Eleven trials are considered adequate to support the critical GAP/ the representative use and are used for MRL calculations.

Table 6.3.1- 23: Summary of residue data from barley trials with fluoxastrobin: Sum of HEC 5725 E- and Z-isomer

Commodity	Region	Use pattern	No of trials	Total residues of HEC 5725 (sum of E-and Z-isomer)		
				Individual residue levels (mg/kg)	HR (mg/kg)	STMR (mg/kg)
Supplementary data						
Barley grain	northern Europe	2 applications at about 0.125 kg/ha (0.125-0.150 kg as/ha)	11	<0.01; 0.01; <0.02; <0.02; 0.02; 0.02; 0.02; 0.020; 0.026; 0.03	0.03	0.02
Barley straw				0.14; 0.17; 0.18; 0.22; 0.41; 0.44; 0.44; 0.47; 0.58; 0.72; 2.7	2.7	0.44

Southern Europe:

The critical GAP of the product 'Fluoxastrobin + Prothioconazole EC 150'(*GAP EU-S 2)*is supported by a set of new residue data. This cGAP involves 2 applications at 0.0875 kg fluoxastrobin/ha with the last application be made at growth stage BBCH 61 for barley. The cGAP for Fluoxastrobin + Prothioconazole EC 150' is considered in the MRL application jointed to the dossier. The cGAP can be used to establish the risk envelope for the GAP of the representative use.

The representative use supported for the re-approval (Bixafen + Fluoxastrobin + Prothioconazole EC 190) involves a slightly lower application rate compared to the critical GAP (*i.e*. 0.075 kg as/ha for barley) (*GAP EU-S 3*; cf Table 6.3.1- 2).

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In total 15 trials are reported in the present dossier which are considered appropriate to support the critical GAP. The trials were performed in 2003, 2010 and 2011 with different EC formulations containing bixafen, prothioconazole and trifloxystrobin as mixing partners. All trials were performed according to GLP principles.
In all trials fluoxastrobin was applied twice with the final application carried out at growth stage BBCH 61-69. The application rate of fluoxastrobin was either 0.075 kg as/ha (6 trials) or 0.0875 kg as/ha (9 trials). The application rates used in the trials adequately support the cGAPs for barley and oat since the rates did not exceed the 25% deviation. Since the difference between the application rates of the cGAP and the GAP of the representative use is small the application rates used in the supporting residue trials also fall into the ± 25% range relative to the GAP of the representative use.

As for the northern zone, in some trials two sets of grain and straw samples were collected (*e.g.* at day 35 and a later date). In the summary table below and for MRL calculations always the highest value has been used. As explained above for trial 10-2157-04, the elevated residue finding in grain is included in the data set although the Dixon's Q-test highlights this value as an outlier and the experimental conditions showed deviations to the usual sampling procedure (threshing in the laboratory) which may serve as an explanation. The decision to nevertheless consider this result is based on the experience with the data from the previous critical GAP where it also appears that in individual cases significantly elevated residue levels were found in barley grain (cf. EFSA conclusion, 2007). Therefore the information from the outlier testing is handled with caution because it cannot be ruled out that this is a 'true' residue value and the value was not excluded from the data set.

Table 6.3.1- 24: Summary of residue data from barley trials with fluoxastrobin: Sum of HEC 5725 E-and Z-isomer

Commodity	Region	Use pattern	No of trials	Total residues of HEC 5725 (sum of E-and Z-isomer)		
				Individual residue levels (mg/kg)	HR (mg/kg)	STMR (mg/kg)
Barley grain	southern Europe	2 applications at about 0.0875 kg as/ha (0.075 - 0.0875 kg/ha)	15	0.01; 0.01; 0.01; 0.010; 0.013; 0.015; 0.019; <0.02; 0.02; 0.02; 0.02; 0.03; 0.04; 0.34	0.34	(< 0.02)
Barley straw				0.03; 0.13; 0.16; 0.23; 0.23; 0.28; 0.29 ;0.31; 0.52; 0.61; 0.73; 0.74; 0.97; 1.4; 1.7	1.7	0.31

Relevance for MRL setting
All supplementary residue data from the northern and southern region were well below the existing MRLs of 0.5 mg/kg for barley and oats as set out with Regulation (EC) 839/2008 or by the (tentative) MRL proposals EFSA made in their Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012) which was 0.7 mg/kg for both crops.
However, the tentative MRLs were proposed since EFSA found that the residue data evaluated for Annex I inclusion were overdosed relative to the exsting GAP in the southern region and a set of residue trials corresponding to the new cGAP was requested.

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Important note:
For the northern region, the GAP of the representative use and the cGAP for the MRL application are the same (GAP EU-N 2) and pertaining to the same product (FXA+PTZ EC 200). Thus, all supplementary trials (reference KCA 6.3.1/05; /06, /07, /08) are considered appropriate for MRL setting.
For the southern region, the cGAP (GAP EU-S 2) and the GAP of the representative use (GAP EU-S 3) are different. The cGAP is related to the product FXA+PTZ EC 150, while the representative use is for BIX+FXA+PTZ EC 190. All supplementary trials (reference KCA 6.3.1/09, /10, /11, /12) are considered to adequately support the cGAP and therefore are appropriate for the MRL calculation.
The set of new residue data reported in the present dossier supporting the new critical GAP for fluoxastrobin in southern Europe in barley and oats shall address the deficiency identified in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012).
An MRL application for barley and oats (M-543078-01-1) will be submitted along with and as part of this supplementary dossier in order to obtain final MRLs for the two crops.

MRL calculations for the supplementary data are provided in chapter CA 6.7.2.

CA 6.3.2 Wheat and Rye

Representative uses for renewal of approval of fluoxastrobin

The representative uses supported for the renewal of approval for fluoxastrobin are summarised in Table 6.3.2- 1.

Table 6.3.2- 1: Summary of the GAP of the representative uses supported for renewal of approval for fluoxastrobin

Crop	Region	Product	Maxim. Number of Appli-cations	Minimum Application Interval (days)	Growth stage (BBCH)	Maximum Rate fluoxastrobin per application (g a.s./ha)	Minimum PHI (days)
Wheat (incl. triticale), rye	EU-N	FXA+PTZ EC 200	2	14-21	30-69	150	*
Wheat (incl. triticale), rye	EU-S	BIX+FXA+PTZ EC 190	2	14-21	30-69	87.5	*

EU-N = northern Europe. EU-S = southern Europe
* The PHI is defined by the growth stage at the last application
FXA+PTZ EC 200 containing 100 g fluoxastrobin/L + 100 g prothioconazole /L
BIX+FXA+PTZ EC 190 containing 40 g bixafen/L, 50 g fluoxastrobin/L , 100 g prothioconazole/L

For the northern zone, the cGAP for the active substance evaluated in the EU peer review will not be further supported post AIR but will be replaced by a new cGAP examined with the supplementary data. For the northern region, the GAP of the representative use and the cGAP for the MRL application are the same (*GAP EU-N 2*) and pertaining to the same product (FXA+PTZ EC 200).

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For the southern zone, the supplementary data reported in the present dossier were generated to support the critical GAP in the southern region (*GAP EU-S 2*) for the product 'Fluoxastrobin + Prothioconazole EC 150'). The GAP of the representative use of 'Bixafen + Fluoxastrobin + Prothioconazole EC 190' involves slightly lower individual application rates (*GAP EU-S 3*). Table 6.3.2- 2 summarises the old and new critical GAPs for the compound and the GAPs of the representative uses.

Table 6.3.2- 2: Summary of the previous and new critical GAPs and the GAP of the representative uses for fluoxastrobin in/on wheat and rye

GAP no[a)]	Crop	Region	Product	Maxim. Number of Applications	Minim. Application Interval (days)	Growth stage (BBCH)	Maxim. Rate fluoxastrobin per application (g a.s./ha)	Minim. PHI (days)
Northern Europe: Critical GAP evaluated for Annex I inclusion in the EU peer review (will not be re-newed)								
EU-N 1	Wheat Rye	EU-N	FXA EC 100	2	14 (reference growth stage)	20-69	200	35
Northern Europe: GAP of the representative use = Critical GAP for fluoxastrobin Post AIR (GAP included in MRL application form)								
EU-N 2	Wheat Rye	EU-N	FXA+PTZ EC 200	2	14-21	30-69	150	*
Southern Europe: Critical GAP evaluated for Annex I inclusion in the EU peer review (obsolete)								
EU-S 1	Wheat Rye	EU-S	FXA EC 100	2	14 (reference growth stage)	20-69	200	35
Southern Europe: Critical GAP for fluoxastrobin (GAP included in MRL application form)								
EU-S 2	Wheat Rye	EU-S	FXA+PTZ EC 150	2	14-21	30-69	100	35*
Southern Europe: GAP of the representative use								
EU-S 3	Wheat (incl. Triticale) Rye	EU-S	BIX+FXA+ PTZ EC 190	2	14-21	30-69	87.5	*

EU-N = northern Europe, EU-S = southern Europe

[a)] for better reference in the text below numbers are assigned to the different GAPs

* As per growth stage (the PHI of 35 days was due to a former requirement in France but will not be applicable Post AIR)

FXA EC 100 : containing 100 g fluoxastrobin/L

FXA+PTZ EC 200 containing 100 g fluoxastrobin/L + 100 g prothioconazole /L

FXA+PTZ EC 150 containing 50 g fluoxastrobin/L + 100 g prothioconazole/L

BIX+FXA+PTZ EC 190 containing 40 g bixafen/L + 50 g fluoxastrobin/L + 100 g prothioconazole/L

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Representative use evaluated for Annex I inclusion (as reflected in the baseline dossier)

Table 6.3.2- 3 summarises the critical GAP evaluated in the EU peer review.

Table 6.3.2- 3: Summary of critical GAPs evaluated for Annex I inclusion and used for setting the EU MRLs of fluoxastrobin(GAPs EU-N 1 and EU-S 1)

Crop	Region	Mode of application	Maximum Number of Applications	Min. interval between applications	Growth stage	Maximum Rate (g a.s./ha) per application	Minimum PHI (days)	Reference
Wheat Rye	EU-N EU-S	Overall Spray	2	14 (refer to growth stage)	start 26 up to BBCH 69	200	35	EFSA Scientific Report (2007) 102, 1-84

EU-N = northern Europe EU-S = southern Europe

Summary of the trials evaluated in the EU peer review

With the Annex II dossier residue data on the following critical GAP were submitted:
Seed treatment of wheat grain (10 g fluoxastrobin/dt seed) was followed by 2 spray applications at application rates of 200 g a.s./ha up to growth stage BBCH 69. The representative formulation for the spray application was an EC 100 formulation containing 100 g fluoxastrobin/L. In the Monograph only the spray use was evaluated, however, the trials involving both applications – seed treatment and spray application – were considered suitable since it was evident from trials which were conducted with seed treatment alone that this treatment had no impact on the residue levels at harvest. The foliar use was evaluated as the representative use and formed the basis for the established MRL in wheat.

For easy reference, the residue trials on wheat that have already been evaluated during the EU review of fluoxastrobin are summarised in Table 6.3.2-4. Since these residue reports have been previously evaluated by the RMS UK they are only presented for reference purposes. All reports are included in the baseline dossier (KCA 6.3) and the tier 1 summary tables may be found in document [redacted] 2015; M-542197-01-1.

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Table 6.3.2- 4: Overview of European residue trials conducted in wheat per geographical region evaluated in the EU peer review *(GAP EU-N 1 and EU-S 1)*

Region	GAP (appl. rate for fluoxastrobin)	Crop	Formu-lation	Number of Trials			Report-No. Doc No.
				Vegetation period		Total	
				1999	1999-2000		
EU-N	ST 10 g a.s./dt and SPI 2 x 200 g a.s./ha	Wheat	FS 110 and EC 100	4	4	8	RA-2013/99 [redacted] M; 2001; M-086113-01-1 RA-2016/99 [redacted]; 2001; M-099802-01-1
EU-S	SPI 2 x 200 g a.s./ha	Wheat	EC 100	2	-		RA-2018/99 [redacted]; 2001; M-085052-01-1
	ST 10 g a.s./dt and SPI 2 x 200 g a.s./ha	Wheat	FS 110 and EC 100	4	4	8	RA-2018/99 [redacted] 2001; M-085052-01-1 RA-2021/99 [redacted]; 2001; M-089295-01-1

ST: seed treatment SPI: spray
EU-S: Southern Europe EU-N: Northern Europe
FS 110: flowable concentrate containing 100 g fluoxastrobin/L and 10 g cyproconazole/L
EC 100: emulsifiable concentrate containing 100 g fluoxastrobin/L

Table 6.3.2- 5: Overall summary of residue data for BEC 5225 (sum of E- and Z- isomers) in wheat trials evaluated in the EU peer review *(GAP EU-N 1 and EU-S 1)*

Application Rate	Region	Formu-lation	Crop	Sample material	n	Residue level (mg/kg)			Ref.
						Min.	Max.	STMR	
Seed treatment (10 g a.s./dt seed) followed by 2 spray applications at 200 g a.s./ha	EU-N	FS 110 and EC 100	Wheat	Grain	8	<0.02	<0.02	<0.02	EFSA Scientific Report (2007) 102 and EFSA Reasoned Opinion 2012;10 (12):3012
				Straw	8	0.14	0.97	0.63	
Seed treatment (10 g a.s./dt seed) followed by 2 spray applications at 200 g a.s./ha or 2 spray applications at 200 g a.s./ha alone	EU-S	FS 110 and EC 100 or EC 100	Wheat	Grain	8	<0.02	0.02	<0.02	
				Straw	8	0.50	6.0	0.76	

EU-N: northern Europe EU-S: southern Europe

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Evaluation in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012)

Northern Europe: The trials evaluated in the EU peer review for AI inclusion were found to be compliant with the old critical GAP (fluoxastrobin EC 100; *GAP EU-N 1)*.

Southern Europe: The use pattern evaluated in the EU peer review for the EC 100 straight formulation and also addressed in the EFSA Reasoned Opinion has been replaced by the new critical GAP for the mixture product 'Fluoxastrobin + Prothioconazole EC 150' which involves lower individual rates *(GAP EU-S 2*). However, no data GAP was identified for wheat / rye likely due to the fact that residues in grain remained always at or below the LOQ in both climatic regions when applied according to a more critical GAP.

Re-approval processs / new studies

Northern Europe: A set of new residue studies is reported supporting the critical GAP for wheat and rye in northern Europe post Annex I renewal for 'Fluoxastrobin + Prothioconazole EC 200' *(GAP EU-N 2)*. This GAP is considered in the MRL application form jointed to the dossier. Since the cGAP is also the GAP for the representative use in the northern region, all residue data supporting the cGAP also support the representative use.
This GAP involves 2 spray applications at 150 g fluoxastrobin/ha.

Southern Europe: A complete data package of supplementary trials was generated supporting the critical GAP for wheat (Fluoxastrobin + Prothioconazole EC 150, *GAP EU-S 2)* which is included in the MRL application form jointed to the dossier. The cGAP can be used to establish the risk envelope for the GAP of the representative use for 'Bixafen + Fluoxastrobin + Prothioconazole EC 190' (*GAP EU-S 3*). The representative use involves a slightly lower individual application rate compared to the cGAP (87.5 g a.s./ha vs. 100 g a.s./ha; cf Table 6.3.2-2).

According to the EU guidance document SANCO 7525/VI/95-rev.9 of March 2011 ("Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs") the data obtained from trials conducted on wheat can be extrapolated to rye.

Trials reported in support of the cGAPs / representative uses in the northern and southern climatic zone are summarised in Table 6.3.2-6.

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Table 6.3.2- 6: Supplementary residue trials conducted per geographical region and vegetation period

Year	GAP rate lasr appl.	Formulation	N° of trials	Study number	Reference
Wheat foliar spray residue trials – northern EU					
2000	2 x 150 g a.s./ha BBCH 69	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	2 (4*)	RA-3011/00*	[redacted]; 2002; M-091521-01-1
2000	2 x 150 g a.s./ha BBCH 69	EC 150 (75 g/L fluoxastrobin, 75 g/L tebuconazole)	1 (4*)	RA-2060/00*	[redacted]; 2004; M-104410-02-1
2013	2 x 150-158 g a.s./ha BBCH 69	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	3	13-2138	[redacted]; 2015; M-501083-02-1
2013	2 x 136-159 g a.s./ha BBCH 69	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	2	13-2159	[redacted]; 2014; M-501715-01-1
TOTAL northern EU region			**8 (13*)**		
Wheat foliar spray residue trials – southern EU					
2003	2 x 75 g a.s./ha BBCH 69	EC 300 (75 g fluoxastrobin, 150 g/L prothioconazole, 75 g/L trifloxystrobin)	2	RA-2019/03	[redacted]S; [redacted]; 2004; M-060549-02-1
2010	2 x 87.5 g a.s./ha BBCH 69	EC 190 (40 g/L bixafen, 50 g fluoxastrobin/L, 100 g/L prothioconazole)	2	10-2207	[redacted]B; [redacted]; 2011; M-414694-01-1
2010	2x 100 g a.s./ha BBCH 69	EC 150 (50 g/L fluoxastrobin,, 100 g/L prothioconazole)	7	10-2156	[redacted]; 2011; M-399682-02-1
TOTAL southern EU region			**11**		

*A set of 4 trials from study RA-2011/00 and RA-2060/00) was conducted side-by-side reducing the effective number of independent trials to 8 in the northern zone. One of these side-by-side trials (from each set) received the last application at BBCH 81 and is therefore also not considered. The trials were conducted at the same site but with different experimental conditions (formulations) and therefore the trials with the highest residue were selected and counted from the respective study.

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Supplementary field trials – northern Europe:

Report:	KCA 6.3.2/09[redacted],[redacted]; 2002; M-091521-01-1
Title:	Determination of residues of HEC 5725 & JAU 6476-desthio on winter wheat following spray application of HEC 5725 & JAU 6476 200 EC in [redacted], France, Great Britain and Germany
Report No.:	RA-2011/00
Document No.:	M-091521-01-1
Guideline(s):	Directive 91/414/EEC, residues in or on treated products, food and feed; July 15, 1991, Annex II, part A, point 6 and Annex III, part A, point 8, Residues in or on Treated Products, Food and Feed
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

In 2000, a set of 4 residue trials was conducted in northern Europe. The studies were located in [redacted], the north of France, the United [redacted] and Germany. The trials were performed side-by-side with trials from study RA-2060/00 (Fluoxastrobin + Tebuconazole EC 150) reported below.
In each trial, wheat was treated twice at a product rate of 1.5 L/ha 'Fluoxastrobin + Prothioconazole EC 200' (100 + 100 g/L) corresponding to 0.15 kg fluoxastrobin/ha. The water rate was 300 L/ha in all trials. The time of the first application was when the flag leaf sheath was opening (BBCH 47) in 3 trials and beginning of heading (BBCH 51) in one trial. The second treatment was performed at the end of flowering (BBCH 69) in 3 trials and at early dough stage (BBCH 81) in one trial. The spray interval was 19 days in 3 trials and 37 days in the trial where the final application was delayed.

Samples of ear and 'rest of plant' were taken prior to and immediately after the final application as well as on day 35 following the final application in three trials. Grain and straw samples were collected at harvest 42-61 days after the final treatment and additionally on day 35 in the trial with delayed application.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649 ([redacted], 2001; M-137093-01-1). The method was submitted with the initial Annex II dossier and evaluated in the EU peer review. The limit of quantitation was 0.045 mg/kg for fluoxastrobin (HEC 5725 E-isomer), 0.005 mg/kg for HEC 5725 Z-isomer and nominally 0.05 mg/kg for the calculated total residue for straw and 'rest of plant'. For ear and grain, the LOQ was 0.018 mg/kg (E-isomer), 0.002 mg/kg (Z-isomer) and 0.02 mg/kg for the calculated total residue.

Findings

- Method performance: Method 00649 was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples with HEC5725 for all matrices relevant to this study. Procedural recoveries for both analytes were obtained from 'rest of plant', straw and grain, ear. Mean and individual recoveries at fortification levels between 0.018 - 0.18 mg/kg (grain, ear), 0.045 – 0.45 mg/kg (straw, rest of plant) for HEC 5725 E-isomer and 0.002 – 0.02 mg/kg (grain, ear), 0.005-0.05 mg/kg (straw, rest of plant) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%. All results of the method validations were in accordance with the general requirements for residue analytical methods.

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Table 6.3.2- 7: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg) (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2011/00 R 2000 0144/4 0144-00	Wheat, winter	Rest of plant	Fluoxastrobin	6	**0.045**	88; 89; 91; 84; 85; 86	84	91	87	3.0
				4	0.45	95; 110; 102; 101	95	110	102	6.0
				10	overall		84	110	93	9.3
R 2000 0145/2 0145-00			HEC 5725 Z-Isomer	6	**0.005**	85; 85; 89; 76; 86; 80	76	89	84	5.6
				4	0.05	91; 94; 105; 98	91	105	97	6.2
R 2000 0146/0 0146-00				10	overall		76	105	89	9.6
			total residue HEC 5725	6	**0.05**	88; 89; 90; 83; 85; 86	83	90	87	3.0
R 2000 0147/9 0147-00				4	0.5	95; 109; 102; 101	95	109	102	5.6
				10	overall		83	109	93	9.3
GLP: yes 2000		Straw	Fluoxastrobin	6	**0.045**	82; 87; 86; 68; 78; 78	68	87	80	8.8
				6	0.45	72; 73; 71; 100; 73; 77	71	100	78	14.3
				12	overall		68	100	79	11.4
			HEC 5725 Z-Isomer	6	**0.005**	81; 86; 90; 70; 77; 76	70	90	80	9.0
				6	0.05	83; 78; 79; 100; 69; 80	69	100	82	12.5
				12	overall		69	100	81	10.5
			total residue HEC 5725	6	**0.05**	83; 87; 87; 68; 78; 78	68	87	80	9.0
				6	0.5	73; 73; 72; 100; 73; 77	72	100	78	14.0
				12	overall		68	100	79	11.2
		Grain*	Fluoxastrobin	6	**0.018**	100; 93; 96; 97; 97; 99	93	100	97	2.5
				8	0.18	91; 77; 93; 95; 97; 94; 92; 93	77	97	92	6.7

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Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg) (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
				14	overall		77	100	94	5.9
			HEC 5725 Z-Isomer	6	**0.002**	97; 101; 95; 104; 88; 92	88	104	96	6.1
				8	0.02	90; 77; 100; 94; 96; 93; 89; 100	77	100	92	8.0
				14	overall		77	104	94	7.3
			total residue HEC 5725	6	**0.02**	99; 94; 96; 97; 96; 99	94	99	97	2.0
				8	0.1	91; 77; 94; 95; 97; 94; 92; 94	77	97	92	6.8
				14	overall		77	99	94	5.7

*Sample Material ear is validated by recoveries for grain

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 250 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
RA-2011/00	Grain	209
	Straw	210
	Ear	250
	Rest of plant	247

-Residue results: The findings from the ear and 'rest of plant' samples taken prior to the final application or immediately thereafter show that residues declined well with time.
At harvest, residues in grain were always <0.02 mg/kg for the calculated total residue HEC 5725 and ranged from 0.31 – 0.86 mg/kg in straw from those trials with proper application date. In trial R 2000 0147/9 with delayed application at BBCH 81 straw and grain residues were not elevated, however, this trial may be disregarded for MRL calculation and the STMR estimate. Since all trials were conducted side-by-side with the trials from study RA-2060/00 below only the trials (0145/00 in France and 0146/00 in the UK) which show the highest value from a pair of trials performed at the same location are taken into account for calculation of the MRL and STMR estimate. The values considered for MRL calculation/STMR estimate are underlined in the table below.

- No residues above the respective LOQs were determined in any of the corresponding control samples of grain, ear, straw and rest of plant.

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Table 6.3.2- 8: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2011/00 R 2000 0144/4 0144-00 GLP: yes 2000	Wheat, winter Tarso	S-[redacted] Europe, North	200 EC	2	0.150	0.050	65	ear	-1	0.14	0.05	0.19
									0	4.2	0.27	4.5
									35	0.08	0.03	0.11
								rest of plant	-1	0.24	0.09	0.33
									0	1.8	0.12	1.9
									35	0.22	0.10	0.32
								straw	61	0.21	0.10	0.31
								grain	61	<0.018	<0.002	<0.02
RA-2011/00 R 2000 0145/2 0145-00 GLP: yes 2000	Wheat, winter Shango	France E-[redacted] Europe, North	200 EC	2	0.150	0.050	69	ear	0*	0.08	0.02	0.09
									0	2.1	0.07	2.2
									35	0.09	0.03	0.12
								rest of plant	0*	0.19	0.06	0.25
									0	2.7	0.09	2.8
									35	0.53	0.22	0.74
								straw	48	0.41	0.18	0.59
								grain	48	<0.018	<0.002	<0.02
RA-2011/00 R 2000 0146/0 0146-00 GLP: yes 2000	Wheat, winter Abbot	United GB-[redacted] Europe, North	200 EC	2	0.150	0.050	69	ear	0*	0.06	0.007	0.06
									0	2.1	0.10	2.2
									35	0.11	0.04	0.15
								rest of plant	0*	0.14	0.05	0.19
									0	2.4	0.11	2.5
									35	0.45	0.20	0.65
								straw	58	0.60	0.26	0.86
								grain	58	<0.018	<0.002	<0.02
RA-2011/00 R 2000 0147/9 0147-00 GLP: yes 2000	Wheat winter Flair	Germany D-[redacted]	200 EC	2	0.150	0.050	81	ear	0*	<0.018	<0.002	<0.02
									0	1.7	0.04	1.7
								rest of plant	0*	<0.045	0.005	0.05
									0	2.9	0.09	3.0

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Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	Total residue HEC 5725 (mg/kg)
		[redacted] Europe, North						straw	35	0.11	0.06	0.16
									42	0.08	0.04	0.11
								grain	35	<0.018	<0.002	<0.02
									42	<0.018	<0.002	<0.02

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

Report: KCA 6.3.2/10 [redacted]; [redacted]; 2004; M-104410-02-1
Title: Determination of residues of fluoxastrobin (HEC 5725) and tebuconazole (HWG 1608) in/on winter wheat after spray application of HEC 5725 & HWG 1608 150 EC in the field in [redacted], Northern France, Great Britain and Germany
Report No.: RA-2060/00
Document No.: M-104410-02-1
Guideline(s): Directive 91/414/EEC, residues in or on treated products, food and feed; July 15, 1991, Annex II, part A, point 6 and Annex III, part A, point 8, Residues in or on Treated Products, Food and Feed
Guideline deviation(s): none
GLP/GEP: **yes**

Test system

In 2000, a set of 4 residue trials was conducted in northern Europe ([redacted], the north of France, the United [redacted] and Germany), using 'Fluoxastrobin + Tebuconazole EC 150' (75 +75 g/L). The trials were performed side-by-side with trials from study RA-2011/00 (Fluoxastrobin + Prothioconazole EC 150) reported above.
In each trial, wheat was treated twice at a product rate of 2.0 L/ha 'Fluoxastrobin + Tebuconazole EC 150' corresponding to 0.15 kg fluoxastrobin/ha. The water rate was 300 L/ha in all trials. The spray applications were carried out at growth stages 47-51 and 65-69 except for trial R 2000 0273/4. In this trial, the growth stage at second application was delayed (BBCH 81) since the last treatment was conducted 35 days prior to the expected date of harvest. The spray interval was 19 days in 3 trials and 37 days in the trial where the final application was delayed.
Samples of ear and 'rest of plant' were taken from the treated plots before the last application, on day 0 after the last application and on day 35. At harvest time, grain and straw samples were taken between day 42 and 61 and on day 35 in addition in the trial with the late application.

Two additional grain samples were taken from the treated plots in studies R2000 0269/6 and R 2000 0273/4, each, for processing purposes. The processing studies are reported in chapter CA 6.5.3 (report no 3060/00).

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Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649 ([redacted]; 2001; M-137093-01-1). Aspects relative to the analytical method were as described above for study RA-2011/00.

Findings

- Method performance: Method 00649 was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples with HEC5725 for all matrices relevant to this study. Procedural recoveries for both analytes were obtained from 'rest of plant' straw and grain / ear. Mean and individual recoveries at fortification levels of 0.018 mg/kg (grain, ear), 0.045 mg/kg (straw, rest of plant) for HEC 5725 E-isomer and 0.002 mg/kg (grain, ear) and 0.005 mg/kg (straw, rest of plant) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%.

Table 6.3.2- 9: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
					(mg/kg)	Individual recoveries	Min	Max	Mean	RSD
RA-2060/00 R 2000 0269/6 0269-00	Wheat, winter	Rest of plant	Fluoxastrobin	10	**0.045**	85; 87; 88; 88; 88; 90; 92; 92; 96; 100	85	100	91	5.0
				10	overall		85	100	91	5.0
R 2000 0271/8 0271-00 R 2000 0272/6 0272-00 R 2000 0273/4 0273-00			HEC 5725 Z-Isomer	10	**0.005**	80; 85; 88; 87; 91; 91; 94; 96; 96; 96	80	96	90	6.0
				10	overall		80	96	90	6.0
			total residue HEC 5725	10	**0.05**	85; 87; 88; 88; 88; 91; 90; 94; 96; 100	85	100	91	5.1
				10	overall		85	100	91	5.1
GLP: yes 2000		Grain*	Fluoxastrobin	6	**0.018**	91; 92; 92; 95; 97; 103	91	103	95	4.8
				6	overall		91	103	95	4.8
			HEC 5725 Z-Isomer	6	**0.002**	90; 93; 86; 93; 96; 97	86	97	93	4.4
				6	overall		86	97	93	4.4
			total residue HEC 5725	6	**0.02**	91; 91; 93; 95; 96; 102	91	102	95	4.4
				6	overall		91	102	95	4.4

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Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg) (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
		Straw	Fluoxastrobin	5	**0.045**	68; 68; 69; 71; 72	68	72	70	2.6
				5	overall		68	72	70	2.6
			HEC 5725 Z-Isomer	5	**0.005**	64; 69; 70; 72; 74	64	74	70	5.4
				5	overall		64	74	70	5.4
			total residue HEC 5725	5	**0.05**	68; 69; 69; 71; 71	68	71	70	1.9
				5	overall		68	71	70	1.9

*Sample material ear is validated by recoveries for grain

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 419 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the storage interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
RA-2060/00	Grain	377
	Straw	377
	Ear	419
	Rest of plant	419

- Residue results: As for the previous study, the findings from ear and 'rest of plant' samples taken prior to the final application immediately thereafter and at day 35 post treatment show that residues declined well with time.
  At harvest, residues in grain were always < 0.02 mg/kg for the calculated total residue HEC 5725 and ranged from 0.16 – 0.64 mg/kg (day 48 - 61) in straw from those trials with proper application timing. In trial R 2000 0273/4 with delayed application at BBCH 81 grain and straw residues were not elevated, however, this trial may be disregarded for MRL calculation and the STMR estimate.
  Since all trials were conducted side-by-side with the trials from study RA-2011/00 above only the trial (0269/00 in [redacted]) that shows the highest value from a pair of trials performed at the same location is taken into account for calculation of the MRL and STMR estimate.
  The values considered for MRL calculation/STMR estimate are underlined in the table below.

- No residues above the respective LOQs were determined in any of the corresponding control samples of grain, ear, straw and 'rest of plant'.

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Table 6.3.2- 10: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Fluoxastrobin + Tebuconazole EC 150) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 E-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2060/00 R 2000 0269/6 0269-00 GLP: yes 2000	Wheat, winter Tarso	[redacted] Europe, North	150 EC	2	0.150	0.050	65	ear	-1 0 35	0.19 2.5 0.15	0.06 0.11 0.05	0.25 2.7 0.21
								rest of plant	-1 0 35	0.38 2.7 0.18	0.10 0.11 0.07	0.48 2.2 0.25
								grain	61	<0.018	<0.002	<u><0.02</u>
								straw	61	0.46	0.18	<u>0.64</u>
RA-2060/00 R 2000 0271/8 0271-00 GLP: yes 2000	Wheat, winter Shango	France F-[redacted] Europe, North	150 EC	2	0.150	0.050	69	ear	0* 0 35	0.05 1.2 0.09	0.01 0.06 0.03	0.06 1.3 0.11
								rest of plant	0* 0 35	0.14 1.4 0.23	0.04 0.07 0.10	0.18 1.4 0.33
								straw	48	0.22	0.09	0.31
								grain	48	<0.018	<0.002	<0.02
RA-2060/00 R 2000 0272/6 0272-00 GLP: yes 2000	Wheat, winter Abbot	United GB-[redacted] Europe, North	150 EC	2	0.150	0.050	69	ear	0* 0 35	0.07 1.7 0.10	0.008 0.08 0.04	0.08 1.8 0.14
								rest of plant	0* 0 35	0.22 2.1 0.14	0.07 0.11 0.07	0.29 2.2 0.21
								straw	58	0.11	0.05	0.16
								grain	58	<0.018	<0.002	<0.02
RA-2060/00 R 2000 0273/4 0273-00 GLP: yes 2000	Wheat, winter Flair	Germany D-[redacted] Europe,	150 EC	2	0.150	0.050	81	ear	0* 0	<0.018 1.8	<0.002 0.05	<0.02 1.8
								rest of plant	0* 0	0.14 3.4	0.06 0.13	0.19 3.5

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Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)		
		North						straw	26 42	<0.045 0.08	0.02 0.04	0.07 0.12
								grain	36 42	<0.018 0.018	<0.002 0.002	<0.02 0.02

* prior to last treatment
** residue in control
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

Report: KCA 6.3.2/11 [redacted]s; [redacted]; 2015; M-501083-02-1
Title: Determination of the residues of fluoxastrobin and prothioconazole in/on spring wheat after after spray application of fluoxastrobin & prothioconazole EC 200 in the field in Germany and United [redacted]
Report No.: 13-2038
Document No.: M-501083-02-1
Guideline(s): 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
Guideline deviation(s): none
GLP/GEP: **yes**

Test system

Three residue trials were carried out in 2013 with 'Fluoxastrobin + Prothioconazole EC 200'. The test locations were in Germany (2) and the United [redacted]. The product was applied twice at the required rates of 1.5 L/ha corresponding to 0.150 kg fluoxastrobin/ha. In trial 13-2138-03, the 1st application was slightly overdosed (+ 6%). The treatments were carried out at proper timing (BBCH 47-49 and BBCH 69). Depending on the study, the spray interval was 14 - 19 days. The water rate ranged between 200 and 400 L/ha.

Green material samples were collected on day 0 (prior to the final application and thereafter), 7, 14, 21 and 28 in order to demonstrate decline of the residues. In all trials samples of green plant material were collected at growth stage BBCH 83 which was between day 24 and 35 post treatment. The growth stage was considered representative for silage stage. Grain and straw were collected at harvest maturity (BBCH 89, day 35 – 57). In one trial an additional set of grain and straw was sampled 35 days after treatment, however grain was not fully ripe (BBCH 85) at that time.

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Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). The limit of quantitation was 0.009 mg/kg for fluoxastrobin (HEC 5725 E-isomer), 0.001 mg/kg for HEC 5725 Z-isomer and nominally 0.01 mg/kg for the calculated total residue for all commodities.

Findings

- Method performance: Validation recoveries for method 00649/M003 for the matrices not included in the method validation report (wheat and barley green material, straw) were generated within studies 10-2157 ([redacted]; 2011; M-403199-02-1) and 10-2156 ([redacted]; 2011; M-399682-02-1). Validation recoveries are reported in Table 6.3.1- 19 and Table 6.3.2- 19).

Procedural recoveries for both analytes were obtained from green material, straw and grain samples. Method performance was acceptable. Mean recoveries at fortification levels between 0.009 - 9 mg/kg (green material, straw) and 0.009 – 0.09 mg/kg (grain) for HEC 5725 E-isomer and 0.001 - 1 mg/kg (green material, straw) and 0.001 – 0.01 mg/kg (grain) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%. Lower recoveries (mean 65%) were obtained for HEC 5725 Z-isomer in green material at the highest fortification level of 1 mg/kg. However, this deviation was considered acceptable since this value is only slightly below the acceptable range and the recoveries at the other fortification levels of 0.001 – 0.5 mg/kg were acceptable (73 - 95%). The lower recovery obtained at 1 mg/kg is considered to be incidental and does not denote a deficit of the analytical method.

Table 6.3.2- 11: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
13-2138 13-2138-01	Wheat, spring	green material	Fluoxastrobin	3	**0.009**	93; 95; 98	93	98	95	2.6
13-2138-01-T				1	1.8	85	85	85	85	
				1	4.5	75	75	75	75	
13-2138-02				2	9	66;74	66	74	70	
13-2138-02-T				7	overall		66	98	84	14.7
13-2138-03			HEC 5725 Z-Isomer	3	**0.001**	92;96;98	92	98	95	3.2
13-2138-03-T				1	0.2	88	88	88	88	
				1	0.5	73	73	73	73	
GLP: yes 2013				2	1	63;67	63	67	65	
				7	overall		63	98	82	17.5
		grain	Fluoxastrobin	1	**0.009**	96	96	96	96	
				1	0.09	94	94	94	94	
				2	overall		94	96	95	
			HEC 5725 Z-Isomer	1	**0.001**	94	94	94	94	
				1	0.010	96	96	96	96	

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Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
				2	overall		94	96	95	
		straw	Fluoxastrobin	2	**0.009**	95;93	93	95	94	
				1	1.8	85	85	85	85	
				1	4.5	89	89	89	89	
				2	9.0	91;95	91	95	93	
				6	overall		85	95	91	4.2
			HEC 5725 Z-Isomer	2	**0.001**	102;98	98	102	100	
				1	0.2	86	86	86	86	
				1	0.5	89	89	89	89	
				2	1	90;99	90	99	95	
				6	overall		86	102	94	6.9

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 351 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by time period examined in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
13-2138	Grain	316
	Straw	316
	Green material	351

-Residue results: As shown with the three decline series fluoxastrobin derived residues decreased well with time in green material of wheat. Residues for the calculated total residue of HEC 5725 were at or below the LOQ (≤ 0.01 mg/kg) in grain and ranged from 0.10 to 2.3 mg/kg in straw at the time of commercial harvest (BBCH 89).

- No residues above the respective LOQs of 0.009 mg/kg (E-isomer) and 0.001 mg/kg (Z-isomer) were present in any of the corresponding control samples.

Table 6.3.2- 12: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)

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Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
13-2138 13-2138-01 13-2138-01-T GLP: yes 2013	Wheat, spring Taifun	Germany [redacted] Europe, North	200 EC	2	0.15	0.050	69	green material	0*	0.061	0.024	0.085
									0	3.9	0.060	3.9
									7	0.21	0.063	0.27
									14	0.093	0.037	0.13
									21	0.067	0.026	0.093
									28	0.044	0.018	0.062
									28	0.054	0.023	0.078
								grain	35	<0.009	0.001	<0.01
									57	<0.009	0.001	<0.01
								straw	35	0.054	0.028	0.082
									57	0.067	0.034	0.10
13-2138 13-2138-02 13-2138-02-T GLP: yes 2013	Wheat, spring Kadrilj	Germany [redacted] Europe, North	200 EC	2	0.15	0.038	69	green material	0*	0.20	0.051	0.25
									0	3.1	0.070	3.2
									7	1.0	0.34	1.3
									14	0.75	0.29	1.0
									21	0.64	0.25	0.89
									28	0.38	0.18	0.56
									35	0.49	0.24	0.73
								grain	54	<0.009	0.001	0.010
								straw	54	0.48	0.29	0.77
13-2138 13-2138-03 13-2138-03-T GLP: yes 2013	Wheat, spring Alderon (seed)	United [redacted] Europe, North	200 EC	2	0.15-0.158	0.075	69	green material	0*	0.57	0.18	0.75
									0	6.1	0.28	6.3
									7	2.9	0.95	3.9
									14	2.4	0.89	3.3
									21	0.55	0.22	0.77
									28	0.70	0.30	1.0
									24	0.67	0.28	0.96
								grain	35	<0.009	0.001	0.010
								straw	35	1.6	0.68	2.3

* prior to last treatment
Residues for total residue HEC 5725 determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation

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Report:	KCA 6.3.2/12[redacted],[redacted]; 2014; M-501715-01-1
Title:	Determination of the residues of fluoxastrobin and prothioconazole in/on wheat and spring wheat after spray application of fluoxastrobin & prothioconazole EC 200 in France (North)
Report No.:	13-2159
Document No.:	M-501715-01-1
Guideline(s):	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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

Two residue trials were conducted in 2013 with 'Fluoxastrobin + Prothioconazole EC 200' (100 + 100 g/L) in northern France. In trial 13-2159-01, the product was applied twice at the required rates of 1.5 L/ha corresponding to 0.150 kg fluoxastrobin/ha. In trial 13-2159-02, the rate for the 1st application was slightly less than requested (-9 %, 0.136 kg/ha) and slightly higher for the 2nd application (+6%, 0.159 kg/ha). The treatments were carried out at proper timing (BBCH 39 - 49 and BBCH 69). Depending on the study, the spray interval was 19 - 21 days. The water rate ranged between 181 and 200 L/ha.

Green material samples were collected on day 0 prior to the final application in one trial and thereafter in both trials. One trial was designed as decline series and samples of green material were collected additionally on day 7, 14, 21 and 28. In both trials samples of green material (whole plants without roots) were also collected at growth stage BBCH 83 on day 15 or 34 post treatment. The growth stage was considered representative for silage stage. Grain and straw were collected at harvest maturity (BBCH 89, day 35 or 49).

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). Aspects relevant to the analytical method are as described for study 13-2138 above.

Findings

- Method performance: Validation recoveries for method 00649/M003 for the matrices not included in the method validation report (barley and wheat green material, straw) were generated with study 10-2156 ([redacted]; 2011; M-399682-02-1) and 10-2157 ([redacted]; 2011; M-403199-02-1). Validation recoveries are reported in Table 6.3.1- 19 and Table 6.3.2- 19).

Procedural recoveries for both analytes were obtained from green material, straw and grain samples. Individual and mean recoveries at fortification levels between 0.009 – 9 mg/kg (green material, straw) and 0.009 – 0.09 mg/kg (grain) for HEC 5725 E-isomer and 0.001 – 1 mg/kg (green material, straw) and 0.001 – 0.01 mg/kg (grain) for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD ≤ 20% demonstrating acceptable method performance.

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Table 6.3.2- 13: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
13-2159 13-2159-01 13-2159-01-T and 13-2159-02 13-2159-02-T GLP: yes 2013	Wheat	green material	Fluoxastrobin	3	**0.009**	87;90;95	87	95	91	4.5
				1	1.8	70	70	70	70	
				1	9.0	91	91	91	91	
				5	overall		70	95	87	11.2
			HEC 5725 Z-Isomer	3	**0.001**	92;96;98	92	98	95	4.2
				1	0.2	69	69	69	69	
				1	1.0	93	93	93	93	
				5	overall		69	98	90	13.1
		grain	Fluoxastrobin	1	**0.009**	97	97	97	97	
				1	0.09	94	94	94	94	
				2	overall		94	97	96	
			HEC 5725 Z-Isomer	1	**0.001**	96	96	96	96	
				1	0.01	101	101	101	101	
				2	overall		96	101	99	
		straw	Fluoxastrobin	2	**0.009**	85;86	85	86	86	
				1	1.8	84	84	84	84	
				2	9	92;92	92	92	92	
				5	overall		84	92	88	4.4
			HEC 5725 Z-Isomer	2	**0.001**	95;102	95	102	99	
				1	0.2	86	86	86	86	
				2	1	95;96	95	96	96	
				5	overall		86	102	95	6.0

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 378 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
13-2159	Grain	329
	Straw	329
	Green material	378

-Residue results: Residues for the calculated total residue of HEC 5725 were below the LOQ (< 0.01 mg/kg) in grain in both trials and ranged from 0.58 to 1.5 mg/kg in straw.

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- No residues above the respective LOQs of 0.009 mg/kg (E-isomer) and 0.001 mg/kg (Z-isomer) were present in any of the corresponding control samples.

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Table 6.3.2- 14: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 200) in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
13-2159 13-2159-01 13-2159-01-T GLP: yes 2013	Wheat Siala	France [redacted] Europe, North	200 EC	2	0.15	0.075	69	green material	0*	0.11	0.040	0.14
									0	4.5	0.67	5.2
									7	0.82	0.43	1.2
									14	0.34	0.17	0.51
									21	0.28	0.15	0.43
									28	0.23	0.12	0.35
									34	0.24	0.15	0.39
								grain	49	0.009	0.001	<u><0.01</u>
								straw	49	0.37	0.22	<u>0.58</u>
13-2159 13-2159-02 13-2159-02-T GLP: yes 2013	Wheat, spring Val-bona	France [redacted] Europe, North	200 EC	2	0.136 - 0.159	0.075-0.075	69	green material	0	2.6	0.079	2.6
									15	0.41	0.17	0.57
								grain	35	0.009	0.001	<u><0.01</u>
								straw	35	1.0	0.45	<u>1.5</u>

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

Supplementary trials – southern Europe

Report: KCA 6.3.2/13[redacted]; [redacted]; 2004; M-060549-02-1
Title: Determination of residues of HEC 5725, JAU 6476 and trifloxystrobin in/on wheat following spray application of HEC 5725 & JAU 6476 & CGA 279202 300 EC in the field in Southern France and Spain
Report No.: RA-2019/03
Document No.: M-060549-02-1
Guideline(s): EC-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
Guideline deviation(s): none
GLP/GEP: **yes**

Test system

Two residue trials were conducted in 2003 in southern Europe (France and Spain) using the formulation 'Fluoxastrobin + Prothioconazole + Trifloxystrobin EC 300' (75 + 150 + 75 g/L).

Wheat was treated twice at a product rate of 1.0 L/ha corresponding to 0.075 kg fluoxastrobin/ha. In the Spanish trial the application rate was slightly less (-5.2%) than intended for both treatments. The

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water rate was 300 L/ha in both trials. The spray applications were carried out at growth stages 47-61 (1st application) and 69 (2nd application) with intervals of 10 and 15 days.

In the French trial samples of ear and 'rest of plant' were collected on day 0 before the 2nd application took place and immediately thereafter. In both trials, two sets of grain and straw samples were collected; the first one on day 34/35 post treatment which was initially the desired waiting period and at a later date at harvest maturity (day 41 and 46).
Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649 ([redacted], 2001; M-137093-01-1). Aspects relative to the analytical method were as described above for study RA-2011/00 and RA-2060/00.

Findings

- Method performance: Method 00649 was validated by recovery experiments prior to and concurrently with the residue analyses by spiking control samples with HEC5725 for all matrices relevant to this study. Procedural recoveries for both analytes were obtained from 'rest of plant', straw, grain and ear. Mean and individual recoveries at fortification levels of 0.018 mg/kg (grain), 0.018 – 0.45 mg/kg (ear), 0.045 – 4.5 mg/kg (straw, rest of plant) for HEC 5725 E-isomer and 0.002 mg/kg (grain), 0.002 – 0.05 mg/kg (ear) and 0.005 – 0.5 mg/kg (straw, rest of plant) for HEC 5725 Z-isomer were within the range of 70-110 % with RSD <20%.

Table 6.3.2- 15: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2019/03 R 2003 0134/0 0134-03 R 2003 0257/6 0257-03 GLP: yes 2003	Wheat	rest of plant	fluoxastrobin	2	**0.045**	91; 93	91	93	92	
				2	4.5	96; 99	96	99	98	
				4	overall		91	99	95	3.7
			HEC 5725 Z-Isomer	2	**0.005**	90; 91	90	91	91	
				2	0.5	104; 110	104	110	107	
				4	overall		90	110	99	10.0
			total residue HEC 5725	2	**0.05**	90; 93	90	93	92	
				2	5.0	97; 101	97	101	99	
				4	overall		90	101	95	5.0
		ear	fluoxastrobin	2	**0.018**	97; 98	97	98	98	
				2	0.45	99; 100	99	100	100	
				4	overall		97	100	99	1.3
			HEC 5725 Z-Isomer	2	**0.002**	85; 88	85	88	87	
				2	0.05	100; 102	100	102	101	
				4	overall		85	102	94	9.1

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Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
			total residue HEC 5725	2	**0.02**	96; 97	96	97	97	
				2	0.5	99; 100	99	100	100	
				4	overall		96	100	98	1.9
		grain	fluoxastrobin	5	**0.018**	96; 101; 100; 104; 100	96	104	100	2.9
				5	overall		96	104	100	2.9
			HEC 5725 Z-Isomer	5	**0.002**	93; 100; 97; 99; 108	93	108	99	5.5
				5	overall		93	108	99	5.5
			total residue HEC 5725	5	**0.02**	96; 101; 99; 103; 101	96	103	100	2.6
				5	overall		96	103	100	2.6
		straw	fluoxastrobin	2	**0.045**	91; 91	91	91	91	
				1	0.9	96	96	96	96	
				2	4.5	89; 95	89	95	92	
				5	overall		89	96	92	3.2
			HEC 5725 Z-Isomer	2	**0.005**	87; 91	87	91	89	
				1	0.1	93	93	93	93	
				2	0.5	90; 94	90	94	92	
				5	overall		87	94	91	3.0
			total residue HEC 5725	2	**0.05**	90; 91	90	91	91	
				1	1.0	95	95	95	95	
				2	5.0	90; 94	90	94	92	
				5	overall		90	95	92	2.5

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 246 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
RA-2019/05	Grain	217
	Straw	219
	Ear	244
	Rest of plant	246

-Residue results: In both southern European field trials, the calculated total residues of HEC 5725 in grain was at or below the LOQ (< 0.02 - 0.02 mg/kg) and 1.0 – 1.5 mg/kg in straw.
Relative to the critical GAP (*GAP EU-S 2*) of the southern zone the trials were under dosed by -25 or -29 % (75 or 71 *vs* 100 g as/ha). Although for the Spanish trial this application rate would nominally

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fall out of the range for comparability it is suggested to consider this trial for MRL setting since residues in wheat grain and straw were at the upper end of the range of residue data in the southern region.

- No residues above the respective LOQs of 0.045 mg/kg (E-isomer for rest of plant / straw), 0.018 mg/kg (E-isomer for grain / ear), 0.005 mg/kg (Z-isomer for rest of plant / straw) and 0.002 mg/kg (Z- isomer for grain / ear) were found in any of the corresponding control samples.

Table 6.3.2- 16: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole + Trifloxystrobin EC 300) in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
RA-2019/03 R 2003 0134/0 0134-03 GLP: yes 2003	Wheat Frelon	France, F-[redacted] Europe, South	300 EC	2	0.075	0.025	69	rest of plant	0* 0	0.31 2.2	0.14 0.31	0.44 2.5
								ear	0* 0	0.12 2.0	0.05 0.33	0.17 2.4
								grain	35 [a] 41	<0.018 <0.018	<0.002 <0.002	<0.02 <u>0.02</u>
								straw	35 [a] 41	0.69 0.65	0.34 0.36	1.0 <u>1.0</u>
RA-2019/03 R 2003 0257/6 0257-03 GLP: yes 2003	Wheat Yecora	Spain E-[redacted] Europe, South	300 EC	2	0.071	0.024	69	grain	34 46	<0.018 <0.018	0.003 0.005	0.02 <u>0.02</u>
								straw	34 46	0.86 0.95	0.43 0.51	1.3 <u>1.5</u>

* prior to last treatment

[a] During analysis of the samples of trial R 2003 0134/0 a sample mix-up occurred between two trials. The plots of trial R 2003 0167/7 (trifloxystrobin and tebuconazole, wheat, study RA-2028/03) were situated directly beside the plots of trial R 2003 0134/0. The corresponding grain and straw samples taken at day 35 in trial R 2003 0167/7 labelled with the above mentioned trial number and sample numbers were therefore analysed with regard to residues of fluoxastrobin (HEC5725) and these results are reported.

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

Underlined values are used for MRL calculation.

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Report:	KCA 6.3.2/14[redacted]C;[redacted];[redacted];[redacted]; 2011; M-414694-01-1
Title:	Determination of the residues of BYF 00587, HEC 5725 and prothioconazole in/on wheat after spray application of bixafen & fluoxastrobin & prothioconazole EC 190 in the field in France (South) and Portugal
Report No.:	10-2207
Document No.:	M-414694-01-1
Guideline(s):	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)
Guideline deviation(s):	none
GLP/GEP:	**yes**

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Test system

Two residue trials were conducted in 2010 with 'Bixafen + Fluoxastrobin + Prothioconazole EC 190' (40 + 50 + 150 g/L) on wheat. The test locations were in southern France and Portugal. 'Bixafen + Fluoxastrobin + Prothioconazole EC 190' was applied twice at the required rate of 1.75 L product/ha corresponding to 0.0875 kg fluoxastrobin/ha. The treatments were carried out at the requested growth stages BBCH 45 - 57 and BBCH 69. Depending on the study, the spray interval was 14 or 21 days. The water rate was 300 L/ha in both trials.

Samples of green material were taken just prior to and immediately after the final application in both trials in order to evaluate the impact of the final application. In one trial, first grain and straw samples were taken on day 35 (BBCH 87) and an additional set of samples was taken on day 38 at full maturity (BBCH 89). In the trial from Portugal grain and straw samples were collected only at BBCH 89 (day 63) since harvestable material was not yet available at the early sampling date.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). Aspects relevant to the analytical method are as described for study 13-2138 above.

Findings

Validation recoveries for method 00649/M003 for the matrices not included in the method validation report (wheat and barley green material, straw) were generated with study 10-2156 ([redacted]; 2011; M-399682-02-1) and 10-2157 ([redacted]; 2011; M-403199-02-1). Validation recoveries are reported in Table 6.3.1- 19 and Table 6.3.2- 19).

Validation recoveries for method 00649/M003 for all matrices relevant to this study (green material, straw and grain) were also reported within this study and obtained from studies 10-2206, 10-2207, (10-2204 and 10-2205 not reported in the present dossier). Individual and mean recoveries at fortification levels between 0.009 and 3.6 mg/kg for HEC 5725 E-isomer and 0.001 and 0.4 mg/kg for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%. The control sample used for 2 recoveries of HEC 5725 E-isomer in green material (fortification level 0.009 mg/kg) and for HEC 5725 Z-isomer (fortification level 0.001 mg/kg) contained more than 30% of the LOQ and therefore the recoveries were background corrected for the signal present in the control sample. The reason was a fluoxastrobin containing product which was erroneously used for maintenance in another study (trial 10-2204-01, not relevant to the present dossier).

Table 6.3.2- 17: Procedural recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
Recoveries were generated during analysis of 4 studies (10-2204 and 10-2206 (barley) and 10-2205 and 10-2207 (wheat)) GLP: yes 2010	Wheat	green material	Fluoxastrobin	8	**0.009**	86;91;95 (136*); 97;102; 108 (144*);*113*; 103	86	113	99	9.0
				4	0.09	93;98;*105*;104	93	105	100	5.6
				1	2.7	84	84	84	84	
				1	3.6	87	87	87	87	
				14	overall		84	113	98	9.0
			HEC 5725 Z-Isomer	8	**0.001**	81;87;97;98 (227*);102; 113;(342*); *105*; 120	81	120	100	12.7
				4	0.01	88;115;88; 97	88	115	97	13.1
				1	0.30	86	86	86	86	
				1	0.40	91	91	91	91	
				14	overall		81	120	98	12.3
		grain	Fluoxastrobin	6	**0.009**	78;87;94;91; 96;98	78	98	91	8.1
				4	0.09	88;*102*;92; 98	88	102	95	6.5
				10	overall		78	102	92	7.5
			HEC 5725 Z-Isomer	6	**0.001**	82;86;90;90; 75;89	75	90	85	6.9
				4	0.01	88;91;93;*93*	88	93	91	2.6
				10	overall		75	93	88	6.3
		straw	Fluoxastrobin	6	**0.009**	87;88;86;93; *103*;92	86	103	92	6.9
				4	0.09	89;75;*104*; 96	75	104	91	13.5
				1	2.7	84	84	84	84	
				1	3.6	82	82	82	82	
				12	overall		75	104	90	9.3
			HEC 5725 Z-Isomer	6	**0.001**	69;76;89;109; *116*; 98	69	116	93	19.8
				4	0.01	85;69;*91*;86	69	91	83	11.5
				1	0.30	85	85	85	85	
				1	0.40	85	85	85	85	
				12	overall		69	116	88	16.2

*recovery before correction; the control sample used for 2 recoveries of HEC E-isomer in green material (0.009 mg/kg) and for HEC 5725 Z-isomer in green material (0.001 mg/kg) contained more than 30% of the

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LOQ and therefore the recovery was background corrected for the signal present in the control sample. The control sample originates from study 10-2204.
Recoveries in italic were generated using control samples obtained from study 10-2207.

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 326 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
10-2207	Grain	263
	Straw	263
	Green material	326

-Residue results: The total residue of HEC 5725 in grain was < 0.01 mg/kg in both trials and ranged between 0.41 – 0.71 mg/kg in straw.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were determined in any of the corresponding control samples from this study.

Table 6.3.2- 18: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Bixafen + Fluoxastrobin + Prothioconazole EC 190) in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	PHI (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
10-2207 10-2207-01 GLP: yes 2010	Wheat Cezanne	France [redacted] Europe, South	190 EC	2	0.0875	0.0291	69	green material grain straw	0* 0 35 38 35 38	0.40 2.2 <0.009 <0.009 0.42 0.51	0.19 0.28 <0.001 <0.001 0.19 0.20	0.59 2.5 <0.01 <u><0.01</u> 0.61 <u>0.71</u>
10-2207 10-2207-02 GLP: yes 2010	Wheat Jordao	Portugal [redacted] Europe, South	190 EC	2	0.0875	0.0291	69	green material grain straw	0* 0 63 63	0.05 1.6 <0.009 0.28	0.02 0.08 <0.001 0.13	0.07 1.6 <u><0.01</u> <u>0.41</u>

* prior to last treatment
Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)
Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.
Underlined values are used for MRL calculation.

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Report:	KCA 6.3.2/15[redacted],[redacted]; 2011; M-399682-02-1
Title:	Determination of the residues of fluoxastrobin and prothioconazole in/on durum wheat and winter wheat after spraying of Fluoxastrobin & Prothioconazole EC 150 in the field in France (South), Spain, Italy, Portugal and Greece
Report No.:	10-2156
Document No.:	M-399682-02-1
Guideline(s):	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)
Guideline deviation(s):	yes, no impact; see report
GLP/GEP:	yes

The guideline deviation is described and evaluated in the summary below.

Test system

Seven residue trials were carried out in 2010 with Fluoxastrobin + Prothioconazole EC 150 (50 + 100 g/L) on wheat according to the critical GAP in southern Europe. The test locations were in France (2), Italy (2), Spain, Portugal and Greece. The product was applied twice at the required rates of 2.0 L/ha corresponding to 0.100 kg fluoxastrobin/ha. The treatments were carried out at proper timing (BBCH 45-61 and BBCH 69). Depending on the study, the spray interval was 14 - 19 days. The water rate was 300 or 400 L/ha in all trials.

Samples of green material were taken just prior to and immediately after the final application took place in all trials. Four trials were designed as decline series and in three trials samples were collected at harvest only. In the decline trials green material samples (whole plants without roots) were collected on day 7, 14 and 21 or 28 at growth stages ranging from BBCH 67 - 85 and thus also covering adequate growth stages for silage production. In most of the trials two sets of grain and straw samples were collected: the 1st set was collected on day 34/35 post treatment and the 2nd set on a later date at commercial harvest (day 41 – 53, BBCH 89) in case maturity of the crop was not yet reached at the first sampling event.

Deviation to guideline: In the Spanish trial, grain and straw samples were deep-frozen 31-32 hours after sampling and thus exceeding the requested time of 24 hours. Technical problems with the thresher allowed threshing only at the following day, however, a potential impact is considered to remain minor.

Residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725, were determined according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). Aspects relevant to the analytical method are as described for study 13-2138 above.

Findings

- Method performance: Validation recoveries for method 00649/M003 for all matrices relevant to this study were generated within this study. Recoveries for both analytes were obtained from green material, straw, and grain samples. The sample materials chosen served to represent all relevant sample materials collected in these trials. Mean recoveries at fortification levels between 0.009 - 0.9 mg/kg (grain), 0.009 – 4 mg/kg (straw) and 0.009 - 9 mg/kg (green material) for HEC 5725 E-isomer and 0.001 – 0.1 mg/kg (grain), 0.001 – 0.5 mg/kg (straw) and 0.001 – 1 mg/kg (green material) for

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HEC 5725 Z-isomer were within the acceptable range of 70-110 %, with RSD <20% demonstrating acceptable method performance.

Table 6.3.2- 19: Recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on wheat
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
10-2156 10-2156-01 to 10-2156-07 GLP: yes 2010	Wheat	green material*	Fluoxastrobin	3	**0.009**	82;88;98	82	98	89	9.0
				1	0.9	88	88	88	88	
				4	2	84;87;89;91	84	91	88	3.4
				2	9	68;76	68	76	72	
				10	overall		68	98	85	9.8
			HEC 5725 Z-Isomer	3	**0.001**	82;83;97	82	97	87	9.6
				1	0.1	80	80	80	80	
				4	0.2	82;85;88;91	82	91	87	4.5
				2	1	67;69	67	69	68	
				10	overall		67	97	82	11.1
		Grain**	Fluoxastrobin	4	**0.009**	83; 87;88;86	83	88	86	2.5
				3	0.09	85;88;92	85	92	88	4.0
				1	0.9	79	79	79	79	
				8	overall		79	92	86	4.5
			HEC 5725 Z-Isomer	4	**0.001**	90;90;93;73	73	93	87	10.5
				3	0.01	74;81;86	74	86	80	7.5
				1	0.1	63	63	63	63	
				8	overall		63	93	81	12.9
		straw	Fluoxastrobin	7	**0.009**	62;65;66;84;84;85;86	62	86	76	14.5
				5	0.09	69;84;86;91;92	69	92	84	10.9
				1	4	85	85	85	85	
				13	overall		62	92	80	13.0
			HEC 5725 Z-Isomer	7	**0.001**	60;69;75;82;83;87;87	60	87	78	13.1
				5	0.01	63;81;84;85;86	63	86	80	12.0
				1	0.5	91	91	91	91	
				13	overall		60	91	79	12.3

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*Validation data for the sample material 'green material' also validate the sample material 'rest of plant'.
**Validation data for the sample material 'grain' also validate the sample material 'ear'.

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 194 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
10-2156	Grain	159
	Straw	161
	Ear	159
	Rest of plant	159
	Green material	194

-Residue results: The findings from the decline series indicate that residues of fluoxastrobin in green plant material deceased well with time. Residues due to the first application had decreased significantly until the point in time when the second treatment was conducted. Residues caused by the second and final treatment declined well again.
At the time of harvest, residues were < 0.01-0.02 mg/kg (BBCH 87- 89) for the calculated total residue HEC 5725 in grain and ranged from 0.21 – 3.7 mg/kg in straw for the same interval.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) was found in any of the corresponding control samples

Table 6.3.2- 20: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on wheat treated with a fluoxastrobin EC formulation (Fluoxastrobin + Prothioconazole EC 150) in the field in southern Europe

Study Trial No. Plot No GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hl (a.s.)	GS	Portion analysed	Residues DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	Total residue HEC 5725 (mg/kg)
10-2156 10-2156-01 GLP: yes 2010	Wheat Pesca-dou	France [redacted] Europe, South	150 EC	2	0.10	0.033	69	green material	0* 0 7 14 27	0.19 2.3 0.22 0.14 0.069	0.055 0.079 0.061 0.046 0.027	0.24 2.4 0.28 0.18 0.10
								grain	34 41	<0.009 <0.009	<0.001 <0.001	<0.01 <u>≤0.01</u>
								straw	34 41	0.32 0.53	0.16 0.26	0.48 <u>0.79</u>
10-2156 10-2156-02 GLP: yes 2010	Wheat Nogal	Spain [redacted] Europe, South	150 EC	2	0.10	0.025 - 0.033	69	green material	0* 0	0.069 1.5	0.018 0.086	0.09 1.5
								grain	35 43	<0.009 <0.009	<0.001 <0.001	<0.01 <u>≤0.01</u>
								straw	35 43	0.60 0.93	0.23 0.41	0.83 <u>1.4</u>

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Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	Total residue HEC 5725 (mg/kg)
10-2156 10-2156-03 GLP: yes 2010	Wheat, durum Perseo	Italy [redacted] Europe, South	150 EC	2	0.10	0.033	69	green material	0*	0.21	0.072	0.28
									0	3.3	0.096	3.4
									7	1.2	0.45	1.7
									14	0.60	0.25	0.85
									28	0.39	0.17	0.57
								grain	35	<0.009	<0.001	<0.01
									42	<0.009	<0.001	<0.01
								straw	35	0.64	0.26	0.90
									42	0.87	0.39	1.3
10-2156 10-2156-04 GLP: yes 2010	Wheat, durum Saragolla	Italy [redacted] Europe, South	150 EC	2	0.10	0.033	69	green material	0*	0.29	0.11	0.40
									0	2.6	0.20	2.8
								grain	35	<0.009	0.002	0.01
									44	<0.009	<0.001	<0.01
								straw	35	0.68	0.33	1.0
									44	0.48	0.22	0.70
10-2156 10-2156-05 GLP: yes 2010	Wheat, winter Aubusson	France [redacted] Europe, South	150 EC	2	0.10	0.033	69	green material	0*	0.11	0.034	0.14
									0	1.4	0.057	1.5
								grain	34	<0.009	0.002	0.01
									53	<0.009	<0.001	<0.01
								straw	34	0.15	0.062	0.21
									53	0.32	0.15	0.47
10-2156 10-2156-06 GLP: yes 2010	Wheat, winter Poison	Portugal [redacted] Europe, South	150 EC	2	0.10	0.025	69	green material	0*	0.28	0.083	0.37
									0	1.4	0.058	1.5
									7	1.1	0.29	1.4
									14	0.87	0.27	1.1
									21	0.68	0.17	0.85
								ear	28	0.34	0.14	0.48
								rest of plant	28	0.39	0.18	0.57
								grain	48	0.015	0.005	0.02
								straw	48	1.9	0.86	2.7
10-2156 10-2156-07 GLP: yes 2010	Wheat, winter Yecora	Greece [redacted] Europe, South	150 EC	2	0.10	0.033	69	green material	0*	0.97	0.24	1.2
									0	2.9	0.22	3.1
									7	1.5	0.44	1.9
									14	0.70	0.22	0.92
									28	0.45	0.18	0.62
								grain	35	0.020	0.004	0.02
								straw	35	2.4	1.3	3.7

* prior to last treatment

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer).

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

Underlined values are used for MRL calculation.

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Overall conclusion on wheat

Northern Europe:

The GAP evaluated for Annex I inclusion will not be supported following the renewal of approval of the active substance. Therefore, a set of new residue data is reported supporting the representative use (Fluoxastrobin + Prothioconazole EC 200) which will become the critical GAP for wheat and rye in northern Europe post Annex I renewal (*GAP EU-N 2*); cf. Table 6.3.2- 2. This GAP is considered in the MRL application form jointed to the present dossier. Since the cGAP and is also the GAP for the representative use in the northern region all residue data supporting the cGAP also support the representative use.

In order to support the cGAP/representative use of fluoxastrobin with the product 'Fluoxastrobin + Prothioconazole EC 200' on wheat and rye supplementary trials are reported using different fluoxastrobin containing EC formulations (EC 200 or SC 150 with prothioconazole or tebuconazole as mixing partners). The trials were performed in 2000 or 2013 and according to GLP principles. In all trials fluoxastrobin was applied twice at a rate of about 0.150 kg fluoxastrobin/ha (136 – 159 g as/ha) with the final application made at growth stage BBCH 69.

Two sets of trials, each consisting of 4 trials, were conducted side-by-side using two different formulations (study RA-2011/00 and RA-2060/00). The data are not considered as independent trials but have to be understood as different experimental conditions within the same experimental site reducing the effective number of trials in the zone. From a pair of data points the highest residue was selected for MRL calculation and STMR estimate as proposed in EFSA's document on 'Residues trials and MRL calculations, proposals for a harmonised approach for the selection of the trials and data used for the estimation of the MRL' (September 2015, issued on the DG SANTE website).
Two trials from these side-by-side sets (one from each study) received the last application late at BBCH 81 and are therefore also not considered for calculations. However, the number of trials does not fall below the minimum number (8) required.
For easy reference the individual values of the site-by-side trials are compared in the following table:
(values in bold were used for STMR estimates and MRL calculations)

Table 6.3.2- 21: Comparison of residue data from trials conducted side-by-side in / on wheat with fluoxastrobin containing EC formulations in northern Europe

Trial no Location	Study RA-2011/00		Trial no Location	Study RA-2060/00	
	Grain (mg/kg)	Straw (mg/kg)		Grain (mg/kg)	Straw (mg/kg)
0144/00 [redacted]	0.02	0.31	0269/00 [redacted]	**< 0.02**	**0.64**
0145/00 France (north)	**<0.02**	**0.59**	0271/00 France (north)	<0.02	0.31
0146/00 UK	**< 0.02**	**0.86**	0272/00 UK	< 0.02	0.16
0147/00 Germany	Appl. at BBCH 81 (not considered)		0273/00 Germany	Appl. at BBCH 81 (not considered)	

In case that two sets of grain and straw samples were collected (*e.g.* at day 35 and a later date), the highest value was selected for the summary table below and calculations.

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The following individual residue values were identified *(the values in parentheses give the lower values originating from side-by-side trials):*

Table 6.3.2- 22 Summary of residue data from wheat trials with fluoxastrobin : Sum of HEC 5725 E-and Z-isomer

Commodity	Region	Use pattern	No of trials	Total residues of HEC 5725 (sum of E-and Z-isomer)		
				Individual residue levels (mg/kg)	HR (mg/kg)	STMR (mg/kg)
Supplementary data						
Wheat grain	EU-N	2 applications at about 0.150 kg/ha (0.136-0.159 kg as/ha)	8 (11*)	<0.01;<0.01;<0.01; 0.010; 0.010; <0.02; <0.02; <0.02; *(<0.02); (<0.02);<0.02);*	(< 0.02) *(< 0.02*)*	0.010 *(< 0.02*)*
Wheat straw				0.10; *(0.16)*; *(0.31)*; *(0.31)*; 0.58; 0.59; 0.64; 0.77; 0.86; 1.5; 2.3	2.3 *(2.3*)*	0.71 *(0.59*)*

EU-N northern Europe
*A set of trials was conducted side-by-side and therefore not all trials are counted individually. HR and STMR are derived from 8 independent trials. From an individual pair of trials the highest values were considered. HR and STMR values as obtained from all data are given in parentheses.

Southern Europe:

The critical GAP of the product 'Fluoxastrobin + Prothioconazole EC 150'*(GAP EU-S 2)* is supported by a set of new residue data. This cGAP involves 2 applications at 0.100 kg fluoxastrobin/ha with the last application be made at growth stage BBCH 69. The cGAP for Fluoxastrobin + Prothioconazole EC 150'is considered in the MRL application jointed to the dossier. The cGAP can be used to establish the risk envelope for the GAP of the representative use.

The representative use supported for the re-approval (Bixafen + Fluoxastrobin + Prothioconazole EC 190) involves a slightly lower individual application rate compared to the critical GAP (*i.e*. 0.0875 kg as/ha for wheat and rye; *GAP EU-S3)*.

In total 11 trials are reported in the present dossier which are considered appropriate to support the critical GAP. The trials were performed in 2003 and 2010 with different EC formulations containing bixafen, prothioconazole and trifloxystrobin as mixing partners. All trials were performed according to GLP principles.

In all trials fluoxastrobin was applied twice with the final application carried out at growth stage BBCH 69. Depending on the study, the application rates ranged from 0.075 (0.071)– 0.100 kg a.s./ha. Relative to the critical GAP, the amount of the active substance applied did not exceed the 25% deviation except for an individual trial where the target rate of 0.075 mg a.s./kg was erroneously under dosed (0.071 kg a.s./ha). However, in this trial the residue findings were at the upper end of the range of all residue data from the data set and thus all 11 trials are considered for the MRL calculation and the STMR estimate.
Since the difference between the application rates of the cGAP and the GAP of the representative use is small the application rates used in the supporting residue trials also fall into the ± 25% range relative to the GAP of the representative use.

As for the northern zone, in some trials two sets of grain and straw samples were collected. In the summary table below and for MRL calculations always the highest value is used. The residue levels in grain and straw are summarised inTable 6.3.2- 23 below.

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Table 6.3.2- 23: Summary of residue data from wheat trials with fluoxastrobin : Sum of HEC 5725 E-and Z-isomer

Commodity	Region	Use pattern	No of trials	Total residues of HEC 5725 (sum of E-and Z-isomer)		
				Individual residue levels (mg/kg)	HR (mg/kg)	STMR (mg/kg)
Wheat grain	EU-S	2 applications 0.075 (0.071) - 0.100 kg/ha	11	<0.01; <0.01; <0.01; <0.01; <0.01; 0.01; 0.01; <0.02; 0.02; 0.02; 0.02	0.02	0.01
Wheat straw				0.41; 0.47; 0.71; 0.79; 1.0; 1.0; 1.3; 1.4; 1.5; 2.7; 3.7	3.7	1.0

EU-S southern Europe

Relevance for MRL setting

All residue data from the northern and southern region were well below the existing MRLs of 0.05 mg/kg for wheat or 0.5 mg/kg for rye as set out with Regulation (EC) 839/2008.

Residue levels found in the supplementary trials are at or below the MRL proposals EFSA made in their Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012) which was 0.02 mg/kg for both crops.

Important note:

For the northern region, the GAP of the representative use and the cGAP for the MRL application are the same (GAP EU-N 2) and pertaining to the same product (FXA+PTZ EC 200). Thus, all supplementary trials (reference KCA 6.3.2/09; /10; /11; /12) are also considered appropriate for MRL setting.

For the southern region, the cGAP (GAP EU-S 2) and the GAP of the representative use (GAP EU-S 2) are different. The cGAP is related to the product FXA+PTZ EC 150, while the representative use is for BIX+FXA+PTZ EC 190. All supplementary trials (reference KCA 6.3.2/13, /14, /15) are considered to adequately support the cGAP and therefore are appropriate for the MRL calculation.

An MRL application for wheat and rye (M-543078-01-1) will be submitted along with and as part of this supplementary dossier in order to ensure that wheat and rye MRLs do not drop to 0.02 mg/kg.

MRL calculations for the supplementary data are provided in chapter CA 6.7.2.

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CA 6.3.3 Onion

Representative uses for renewal of approval of fluoxastrobin

The GAP for the representative use on onions in the southern climatic zone is summarised in Table 6.3.3- 1. The GAP for the representative use also forms the critical GAP in the southern zone. The use in/on onions was not evaluated for the first Annex I inclusion. The use was recently registered in Greece.

Table 6.3.3- 1: Summary of the GAP for the representative use supported for renewal of approval for fluoxastrobin

Crop	Region *	Product	F, G or I**	Maximum Number of Appli-cations	Minimum Application Interval (days)	Growth stage (BBCH)	Maximum Rate Fluoxastrobin per Application (g a.s./ha)	PHI (days)
Onion	EU-S	FXA+PTZ EC 200	F	2	10	15-47	125	21

* EU-S: southern Europe ** F Field; G Greenhouse; I Indoor.

Evaluation in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012

The use in northern Europe has been evaluated in the EFSA Reasoned Opinion on existing MRLs according to Art 12 of Regulation (EC) 396/2005 (EFSA Journal 2012;10(12):3012). The residue data were found to be compliant with the registered GAP. EFSA concluded that the available residue data on onion are considered sufficient to derive an adequate MRL proposal as well as risk assessment values.

At the time of harvest, the following residues were determined in onion bulbs:

Sum of fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer: 8 x < 0.02 mg; 1 x 0.03 mg/kg

The critical GAP established in the northern region is compiled in Table 6.3.3- 2.

Table 6.3.3- 2: Summary of the critical GAP of fluoxastrobin in/on onion (northern Europe)

Crop	Region *	Product	F, G or I**	Maximum Number of Appli-cations	Minimum Application Interval (days)	Growth stage (BBCH)	Maximum rate Fluoxastrobin per application (g a.s./ha)	PHI (days)
Onion	EU-N	FXA+PTZ EC 200	F	1-4	5	40-47	125	14

* EU-N: northern Europe ** F Field; G Greenhouse; I Indoor.

Comparing the critical GAPs of both climatic regions shows that the use pattern supported in the southern zone is less critical than the authorised GAP in northern Europe because it has less applications (2 instead of 4), longer intervals between applications (10 vs. 5 days) and a longer pre-harvest interval (21 *vs*. 14 days).

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Annex I renewal process / new studies

New residue data to support the representative use in the southern climatic zone are summarised in Table 6.3.3- 3.

Table 6.3.3- 3: Supplementary residue trials in/on onion conducted in the southern region

Year	GAP last appl.	Formulation	N° of trials	Study number	Reference
2012	2 x 114-124 g a.s./ha BBCH 47	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	4	12-F CL BY °P/A	[redacted]; 2014; M-475892-01-1
2013	2 x 125 g a.s./ha BBCH 47/48	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	4	13-2139	[redacted]; 2014; M-478312-01-1
2014	2 x 116-125 g a.s./ha BBCH 47	EC 200 (100 g/L fluoxastrobin, 100 g/L prothioconazole)	4	14-2175	[redacted]; 2015; M-518082-01-1

Report: KCA 6.3.3/01 [redacted]; 2014; M-475892-01-1
Title: Determination of the residues of fluoxastrobin and prothioconazole in/on onions after spraying of Fluoxastrobin & Prothioconazole EC 200 in the field in Spain - Season 2012
Report No.: 12 F CL BY P/A
Document No.: M-475892-01-1
Guideline(s): 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)
US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s): none
GLP/GEP: **yes**

Test system

In 2012, 4 residue trials were conducted in southern Europe (Spain) according to the use pattern of the representative use. In each trial, onions were treated with 2 applications. The targeted product rate was 1.25 L/ha 'Fluoxastrobin + Prothioconazole EC 200' (100 + 100 g/L) corresponding to 0.125 kg fluoxastrobin/ha. The actual rate was slightly less (0.114-0.124 kg fluoxastrobin/ha). The water rate ranged from 456 to 498 L/ha. The 1st treatment was conducted at BBCH 44 - 45, and the 2nd application was carried out at BBCH 47, 20 days before the anticipated commercial harvest. The interval between the two applications was 9-11 days.
Two trials were designed as decline series and two trials were set up to yield harvest values only. In the decline series, bulb samples were taken on day 0 prior to the last application and immediately thereafter. In addition bulb samples were collected at day 3, 7, 12-13, and 20 after the last application. In the harvest trials bulb samples were collected at day 20 only.

Residues of fluoxastrobin (E-isomer) and HEC 5725 Z-isomer were analysed using method 00649/M003 ([redacted] 2010; M-387385-01-1) by HPLC-MS/MS. The analytical method was designed to measure HEC 5725 E- and Z-isomer separately. The total residue of HEC 5725 was calculated as the sum of both isomers. The Limit of Quantification (LOQ), defined as the lowest

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validated fortification level, was 0.009 mg/kg for the E-isomer and 0.001 mg/kg for the Z-isomer, resulting in a theoretical LOQ of 0.01 mg/kg for the total residue of HEC 5725.

Findings

- Method performance: Recoveries for both analytes were performed prior to analysis of the field samples in order to validate the method and concurrently with the residue analysis of the study samples. Method performance was acceptable for onion bulb. Individual and mean recoveries at fortification levels between 0.009 and 0.9 mg/kg for HEC 5725 E-isomer and 0.001 and 0.1 mg/kg for HEC 5725 Z-isomer were within the range of 70-110 %, with RSD <20%. All results of the method validation were in accordance with the general requirements for residue analytical methods.

Table 6.3.3- 4: Recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on onion
The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Forti-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
12 F CL BY P/A	Onion	bulb	fluoxastrobin	5	**0.009**	91; 93; 89; 84; 91	84	93	90	3.8
12 F CL BY P01				3	0.090	101; 98; 86	86	101	95	8.4
12 F CL BY P02				2	0.90	83; 82	82	83	83	
12 F CL BY P04				10	overall		82	101	90	7.1
12 F CL BY P09			HEC 5726 Z-isomer	5	**0.001**	106; 93; 103; 99; 94	93	106	99	5.7
GLP: yes 2012				3	0.01	96; 91; 84	84	96	90	6.7
				2	0.10	80; 82	80	82	81	
				10	overall		80	106	93	9.4

- Storage periods: The maximum storage period of deep-frozen treated samples was up to 227 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
12F CL BY P/A	Onion bulb	227

-Residue results: At the sampling event on day 0 immediately after application residues of 0.022 and 0.011 mg/kg (total residue) were found in onion bulbs, however, all samples from the two decline series were free of residues on day 3, 7 and 13-14. In the four southern European field trials, no fluoxastrobin related residues (E-or Z-isomer) were determined in onion bulb at the envisaged PHI of 21 (actual 20) days. Residues were always less than the LOQ of the E-and Z-isomer (0.009 and 0.001 mg/kg, respectively), and thus the total residue HEC 5725 resulted in < 0.01 mg/kg.

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- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were determined in any of the corresponding control samples.

Table 6.3.3- 5: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on onion treated with 'Fluoxastrobin + Prothioconazole EC 200' in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
12 F CL BY P/A 12 F CL BY P01 GLP: yes 2012	Onion Civitatum	Spain [redacted] Europe, South	200 EC	2	0.114-0.116	0.0249-0.0251	47	bulb	0* 0 3 7 12 20	<0.009 0.022 <0.009 <0.009 <0.009 <0.009	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001	<0.01 0.022 <0.01 <0.01 <0.01 <u><0.01</u>
12 F CL BY P/A 12 F CL BY P02 GLP: yes 2012	Onion Ciclope	Spain [redacted] Europe, South	200 EC	2	0.116	0.0250-0.0251	47	bulb	0* 0 3 7 13 20	<0.009 0.011 <0.009 <0.009 <0.009 <0.009	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001	<0.01 0.011 <0.01 <0.01 <0.01 <u><0.01</u>
12 F CL BY P/A 12 F CL BY P04 GLP: yes 2012	Onion Pandero	Spain [redacted] Europe, South	200 EC	2	0.112-0.116	0.0250	47	bulb	20	<0.009	<0.001	<u><0.01</u>
12 F CL BY, P/A 12 F CL BY P09 GLP: yes 2012	Onion Valero	Spain [redacted] Europe, South	200 EC	2	0.114-0.124	0.0249-0.0250	47	bulb	20	<0.009	<0.001	<u><0.01</u>

* prior to last treatment

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

Underlined values are used for MRL calculation.

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Report:	KCA 6.3.3/02[redacted]; 2014; M-478312-01-1
Title:	Determination of the residues of fluoxastrobin and prothioconazole in/on onion after spray application of fluoxastrobin & prothioconazole EC 200 in Spain, Italy, southern France and Portugal
Report No.:	13-2139
Document No.:	M-478312-01-1
Guideline(s):	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
Guideline deviation(s):	yes, no impact; see report
GLP/GEP:	**yes**

A description and evaluation of the guideline deviation is given in the text below.

Test system

In 2013, a set of 4 residue trials was conducted in southern Europe (Spain, Italy, southern France and Portugal) according to the use pattern of the representative use. In each trial, onions were treated with 2 applications at the targeted product rate of 1.25 L/ha 'Fluoxastrobin + Prothioconazole EC 200' (100 + 100 g/L) corresponding to 0.125 kg fluoxastrobin/ha. The water rate was either 400 or 800 L/ha. The 1st treatment was carried out at BBCH 45 – 47 and the 2nd application was performed at BBCH 47/48 with a pre-harvest interval of 21 days. The interval between the two applications was 10-11 days.
Two trials were designed to generate decline series and two trials were set up to yield harvest values only. In the decline series, bulb samples were taken on day 0 prior to the last application and immediately thereafter. In addition samples were collected at day 3/4, 7, 14 and 21 after the last application. In the harvest trials bulb samples were collected at day 21 only.

Residues of fluoxastrobin (E-isomer) and HEC 5725 Z-isomer were analysed using method 00649/M003 ([redacted]2010; M-387385-01-1) by HPLC-MS/MS. The analytical method was validated by recovery experiments during the analysis of the samples by spiking control samples.

Deviation to guideline: For some samples from trial 13-2139-01 (Spain) the maximum temperature during shipment increased to -10.14°C, thus exceeding the requested value of -18°C. The average temperature for the period in which the required value was not met (29 hours) was -13.54°C. The impact of this temperature deviation is addressed in a short-term storage stability experiment under conditions relevant or more unfavourable to these samples (cf. CA 6.1/07; Table 6.1- 16 [redacted]; 2015; M-480441-03-1).

Findings

- Method performance: Recoveries for both isomers were performed prior to residue analysis in order to validate the method and concurrently with the analysis of the study samples. Mean recoveries at fortification levels between 0.009 - 0.9 mg/kg for HEC 5725 E-isomer and 0.001 - 0.1 mg/kg for HEC 5725 Z-isomer were within the range of 70-110 % with RSD <20%. All method validation data are in compliance with the guideline requirements for data collection methods.

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Table 6.3.3- 6: Recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on onion
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
13-2139 13-2139-01 13-2139-01-T to 13-2139-04-T GLP: yes 2013	Onion	bulb	fluoxastrobin	3	**0.009**	92;93;93	92	93	93	0.6
				3	0.09	91;93;93	91	93	92	1.3
				2	0.9	87;88	87	88	88	
				8	overall		87	93	91	2.7
			HEC 5725 Z-Isomer	3	**0.001**	89;98;99	89	99	95	5.8
				3	0.01	93;94;95	93	95	94	1.1
				2	0.1	79;79	79	79	79	
				8	overall		79	99	91	8.7

- storage periods: The maximum storage period of deep-frozen treated samples was up to 260 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the intervals investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
13-2139	Onion bulb	260

-Residue results: In the two decline series it was shown that residues decline well with time from initially 0.044 and 0.093 mg/kg on day 0 following the 2nd treatment to 0.010 and 0.011 mg/kg, respectively. Fluoxastrobin related residues (sum of E-and Z-isomer) in onion bulb ranged between < 0.01 and 0.021 at the envisaged PHI of 21 days.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were determined in any of the corresponding control samples.

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Table 6.3.3- 7: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on onion treated with 'Fluoxastrobin + Prothioconazole EC 200' in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxastrobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
13-2139 13-2139-01 13-2139-01-T GLP: yes 2013	Onion Beira	Spain [redacted] Europe, South	200 EC	2	0.125	0.031	47	bulb	0*	0.010	0.005	0.015
									0	0.077	0.016	0.093
									3	0.036	0.021	0.058
									7	<0.009	0.006	0.015
									14	<0.009	0.002	0.011
									21	<0.009	0.001	0.010
13-2139 13-2139-02 13-2139-02-T GLP: yes 2013	Onion Calabrese; Pink bulb onion	Italy [redacted] Europe, South	200 EC	2	0.125, 0.125	0.016	47	bulb	21	<0.009	0.001	<u>0.01</u>
13-2139 13-2139-03 13-2139-03-T GLP: yes 2013	Onion UX051	France [redacted] Europe, South	200 EC	2	0.125, 0.125	0.016	47	bulb	0*	<0.009	0.002	0.011
									0	0.040	0.003	0.044
									4	0.010	0.003	0.013
									7	<0.009	0.002	0.011
									14	<0.009	0.002	0.011
									21	<0.009	0.002	<u>0.011</u>
13-2139 13-2139-04 13-2139-04-T GLP: yes 2013	Onion King star; Onion Early	Portugal [redacted] Óbidos Europe, South	200 EC	2	0.125, 0.125	0.031	48	bulb	21	0.018	0.003	<u>0.021</u>

* prior to last treatment

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

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Report:	KCA 6.3.3/03 [redacted]>; [redacted]; 2015; M-518082-01-1
Title:	Determination of the residues of prothioconazole and fluoxastrobin in/on onion after spraying of fluoxastrobin & prothioconazole EC 200 in the field in Spain, Italy, France (South) and Greece
Report No.:	14-2175
Document No.:	M-518082-01-1
Guideline(s):	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 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Test system

A set of 4 residue trials was conducted in southern Europe (Spain, Italy, southern France and Portugal) in 2014 according to the supported use pattern. In each trial, onions were treated twice at the targeted product rate of 1.25 L/ha with 'Fluoxastrobin + Prothioconazole EC 200' (100 + 100 g/L) corresponding to 0.125 kg fluoxastrobin/ha. In one trial the actual application rate was slightly less (0.116-0.118 kg/ha) but within the acceptable range for comparability (max -8%). The water rate ranged between 500 and 800 L/ha. The 1st treatment was conducted at BBCH 45 and the 2nd application was carried out at BBCH 47 in all trials. The pre-harvest interval was 21 days and the interval between the two applications was 9-10 days.
Two trials were designed as decline series and two trials were set up to yield harvest values only. In the decline series, bulb samples were taken on day 0 prior to the last application and immediately thereafter. In addition samples were collected at day 3/4, 7, 13/14 and 21 after the last application. In both harvest trials bulb samples were collected at day 21 only.

Residues of fluoxastrobin (E-isomer) and HEC 5725 Z-isomer were analysed using method 00649/M003 ([redacted] 2010; M-387385-01-1) by HPLC-MS/MS.

Findings

- Method performance: The analytical method was validated by recovery experiments during the analysis of the samples by spiking control samples. Mean recoveries at fortification levels between 0.009 - 0.9 mg/kg for HEC 5725 E-isomer and 0.001 - 0.1 mg/kg for HEC 5725 Z-isomer were within the range of 70-110 % with RSD ≤ 20%. The method performance meets all guideline requirements for residue analytical methods.

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Table 6.3.3- 8: Recoveries for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer in/on onion

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
14-2175 14-2175-01 14-2175-01-T to 14-2175-04 14-2175-04-T GLP: yes 2014	Onion	bulb	fluoxastrobin	3	**0.009**	102;103; 106	102	106	104	2
				3	0.09	106;106; 107	106	107	106	0.5
				3	0.9	101;103; 106	101	106	103	2.4
				9	overall		101	107	104	2.1
			HEC 5725 Z-Isomer	3	**0.001**	101;102; 102	101	102	102	0.6
				3	0.01	103;105; 105	103	105	104	1.1
				3	0.1	99;101; 101	99	101	100	1.2
				9	overall		99	105	102	1.9

- storage periods: The maximum storage period of deep-frozen treated samples was up to 139 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer, and is covered by the interval investigated in the storage stability studies.

Study number	Sample material	Maximum storage period (days)
14-2175	Onion bulb	139

-Residue results: In the two decline series the total residues of HEC 5725 (sum of E-and Z-isomer) declined well with time from initially 0.025 and 0.032 mg/kg on day 0 to 0.010 and 0.017 mg/kg, respectively, on day 21. After the pre-harvest interval of 21 days the total residue HEC 5725 in onion bulbs ranged between < 0.01 - 0.017 mg/kg in the four southern European field trials.

- No residues above the LOQ of 0.009 mg/kg (E-isomer) or 0.001 mg/kg (Z-isomer) were determined in any of the corresponding control samples.

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Table 6.3.3- 9: Application data and residues of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the total residue HEC 5725 in / on onion treated with 'Fluoxastrobin + Prothioconazole EC 200' in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues				
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Fluoxa-strobin (mg/kg)	HEC 5725 Z-Isomer (mg/kg)	total residue HEC 5725 (mg/kg)
14-2175 14-2175-01 14-2175-01-T GLP: yes 2014	Onion Figueres, redish onion for dry harvest	Spain [redacted] Europe, South	200 EC	2	0.115 - 0.118	0.0208	47	bulb	0*	<0.009	0.001	0.010
									0	0.021	0.004	0.025
									3	0.014	0.007	0.021
									7	<0.009	0.002	0.011
									13	<0.009	0.001	0.010
									21	<0.009	0.001	0.010
14-2175 14-2175-02 14-2175-02-T GLP: yes 2014	Onion Dorata di Parma, Medium maturation	Italy [redacted] Europe, South	200 EC	2	0.125	0.0208	47	bulb	19	<0.009	<0.001	<0.01
14-2175 14-2175-03 14-2175-03-T GLP: yes 2014	Onion UX051, Production type	France [redacted] Europe, South	200 EC	2	0.125	0.0156	47	bulb	0*	<0.009	<0.001	<0.01
									0	0.030	0.002	0.032
									4	0.014	0.003	0.017
									7	<0.009	0.002	0.011
									14	<0.009	0.002	0.011
									21	<0.014	0.003	0.017
14-2175 14-2175-04 14-2175-04-T GLP: yes 2014	Onion Snow ball, white, Spherical bulb	Greece GR [redacted] Europe, South	200 EC	2	0.125	0.025	47	bulb	21	<0.009	<0.001	<0.01

* prior to last treatment

Residues for total residue HEC 5725 (determined as HEC 5725 E- and Z-isomer separately and calculated as HEC 5725 (sum of E- and Z-isomer)

Note: For the calculation of the total residue unrounded values were used, therefore minor deviations may occur when the values given in the table are used.

Underlined values are used for MRL calculation.

Overeall conclusion on onion

In total 12 trials on onion were conducted between 2012 and 2014 in the field in southern Europe using the product 'Fluoxastrobin + Prothioconazole EC 200'. The trials were designed to comply with the GAP of the representative use and were carried out according to GLP principles. 'Fluoxastrobin + Prothioconazole EC 200' was applied twice at rates of 1.25 L/ha corresponding to 0.125 kg fluoxastrobin/ha. In few trials the application rates were slightly less (up to -9%), however, the deviation to the target rate is well within the range of the EU's tolerance criteria for comparability (± 25%). The treatments were carried out at proper timing during the growth stages BBCH 44-47 and BBCH 47/48 with a pre-harvest interval of 19-21 days.

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Table 6.3.3- 10: Summary of residue data on onion with 'Fluoxastrobin + Prothioconazole EC 200' (sum of HEC 5725 E-and Z-isomer)

Commodity	Region	Use pattern	No of trials	Total residues of HEC 5725 (sum of E-and Z-isomer)		
				Individual residue levels (mg/kg)	HR (mg/kg)	STMR (mg/kg)
Onion bulb	EU-S	2 applications at about 0.125 kg/ha PHI 21 days	12	<0.01; <0.01; <0.01; <0.01; <0.01; <0.01; <0.01; 0.010; 0.011; 0.017; 0.021	0.021	(< 0.01)

EU-S: Southern Europe

The findings demonstrate that residues arising from the GAP supported in the southern climatic zone are well covered by the existing MRL of 0.05* mg/kg (Regulation 839/2008) and by the MRL proposed in the EFSA Reasoned Opinion (EFSA Journal 2012;10(12):3012) of 0.04 mg/kg. The Regulation following the review of existing MRLs is not yet published (SANCO/11739/2013). A modification of the established or proposed MRLs is not necessary.

An MRL calculation for the data set is provided in chapter CA 6.7.2.

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CA 6.4 Feeding studies

Evaluation for Annex I inclusion

A feeding study was conducted on dairy cattle and evaluated in the EU peer review. Fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 *Z*-isomer was fed to dairy cows in a ratio of 65% *E*-isomer, 35% *Z*-isomer in order to reflect the isomer ratio occurring in potential feed items.
Animals were dosed at 6, 30 and 100 mg/kg feed corresponding to 0.22, 1.09 and 3.64 mg/kg bw per day). Fluoxastrobin, the *Z*-isomer and metabolite M55 (HEC 5725 phenoxy-hydroxypyrimidine) was analysed, and positive results were found in milk and tissue samples. For milk residues above the LOQ (0.01 mg/kg for the sum of E-and Z-isomer and M55, each) were only found at the 100 mg/kg bw dose level and reached a plateau after 12 days. For muscle/meat, mean residues were <0.01, 0.04 and 0.08 mg/kg for the 6, 30 and 100 mg/kg dose levels, respectively. For fat, mean residues were 0.02, 0.10 and 0.20 mg/kg for the three dose levels. For liver, mean residues were 0.02, 0.09 and 0.25 mg/kg and for kidney 0.04, 0.17 and 0.41 mg/kg for the 6/30 and 100 mg/kg groups, respectively. The lowest dose level was considered to reflect the 2N rate for dairy cattle and the 1N rate for beef cattle based on the highest residue in the crops (DAR 2003). The study was considered suitable to propose MRLs for commodities of animal origin (EFSA Conclusion 2007).

Based on the cereal uses evaluated for Annex I inclusion a hen feeding study was not considered necessary due to predicted intakes being less than 0.1 mg/kg feed (EFSA Conclusion 2007). The hen metabolism study indicated that residues in poultry products would not be significant (<0.01 mg/kg) (DAR 2003).

Animal dietary burden calculation

Under the new data requirements (Regulation (EC) 283/2013), the animal dietary burdens have to be estimated considering the OECD feeding stuff tables and OECD approaches presented in the guidance document on residue in livestock No. 73. The estimated dietary burdens of total HEC 5725 residues (sum of HEC 2725 E-and Z-isomer) based on EU crop residue data and the European diet in the OECD feeding tables, are calculated below for the nine livestock species (cf. Table 6.4- 2).

For the dietary burden calculation input data (cf. Table 6.4- 1 below) are used as obtained from the supplementary residue trials on wheat and barley conducted according to the critical GAPs as described in the chapters above. Since for the northern region the cGAPs and the GAPs of the representative uses for wheat and barley are the same and in the southern region the cGAPs for wheat and barley establish the risk envelope for the representative uses there the results of the dietary burden calculation do not differ for the representative uses or the critical GAPs since the same input values are applicable for small grain cereals.

However, additional information is given *(in italics)* on input data for other crops for which fluoxastrobin uses are granted and which provide feed items. The reason for reporting this additional information is to help to understand the dietary burden calculation for all uses. In CA 6.4.1 (poultry) and CA 6.4.2 (ruminant) details on the dietary burden are presented for both scenarios:

i) The dietary burden arising from the critical GAPs/representative uses supported in the present dossier

ii) The dietary burden arising from all uses and which form the reference for the exaggerations in the (hen) feeding studies.

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In the OECD feedstuff tables, new feed items such as immature cereals (forage, hay, silage) have been introduced. Following the recommendation in the EFSA document 'Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin' (September 2015, issued on the DG SANTE website) uses on cereals are – by default – understood as 'uses on cereals for grain production' and therefore, only residues in grains and straw from cereals are considered for the animal dietary burden calculation below.
Residue levels are reported based on a residue definition that takes into account the sum of the E-and Z-isomer. This residue definition is anticipated to be in force when this dossier will be evaluated.

Table 6.4- 1: Input values for the dietary burden calculation for fluoxastrobin

Commodities	Relevant data set (cf summary tables: Table 6.3.1- 23 Table 6.3.1- 24 Table 6.3.2- 22 Table 6.3.2- 23)	Maximum dietary burden		Median dietary burden	
		Comment	Input value Residue level (mg/kg)	Comment	Input value Residue level (mg/kg)
Proposed enforcement and risk assessment residue definition: Sum of fluoxastrobin and its Z-isomer					
Wheat, triticale & rye grain	EU-N; EU-S	STMR	0.01	STMR	0.01
Barley and oat grain	EU-N; EU-S	STMR	0.02	STMR	0.02
Wheat, triticale & rye straw	EU-S	HR	3.7	STMR	1.0
Barley & oat straw	EU-N	HR	2.7	STMR	0.44
Brewer's grain (dried)	For derivation of PF: Processing studies RA-3024/99 and 13-3401	STMR × PF(1)	0.02	STMR × PF(1)	0.02
Wheat milled by-products	For derivation of PF: Processing study RA-3060/00 (bran)	STMR × PF(1.75)	0.02	STMR × PF(1.75)	0.02
Rape forage	*EU-N*	*HR*	*0.01*	*STMR*	*0.01*
Rape meal	*EU-N*	*STMR × PF(2)*	*0.02*	*STMR × PF(2)*	*0.02*
Potato tuber (culls)	*EU-N*	*HR*	*0.05*	*STMR*	*0.01*

EU-N northern Europe; EU-S southern Europe

In the following table the results of the dietary burden calculation which may arise from the representative uses are summarised following the guidance given in pesticides_mrl_guidelines_animal_intake_mrl_2015_en.pdf and using the official spreadsheet pesticides_mrl_guidelines_animal_model_2015_en.xls (September 2015). Both files can be downloaded from the DG SANTE site here ('Technical Guidance'/'Guidelines for residue data under Directive .../'New Guidelines 2015').

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Table 6.4- 2: Calculated dietary burden for fluoxastrobin residues in livestock arising from the representative uses according to OECD feeding tables (EU diet); RWCF approach (Reasonable worst case feed)

Animals	Median burden (mg/kg bw)	Maximum burden (mg/kg bw)	Above 0.004 mg /kg bw	Maximum burden (mg/kg DM)	Highest contributing commodities
Beef cattle	0.006	0.022	**Yes**	0.93	Barley straw
Dairy cattle	0.009	0.036	**Yes**	0.93	Barley straw
Ram/Ewe	0.016	0.061	**Yes**	1.83	Barley straw
Lamb	0.020	0.078	**Yes**	1.83	Barley straw
Pig (breeding)	0.001	0.001	No	0.02	Barley grain
Pig (finishing)	0.001	0.001	No	0.02	Barley grain
Poultry broiler	0.001	0.001	No	0.02	Barley grain
Poultry layer	0.009	0.030	**Yes**	0.44	Wheat straw
Turkey	0.001	0.001	No	0.02	Barley grain

As evident from the calculations above, the trigger value of 0.004 mg/kg bw/day is exceeded also for poultry (layer). Therefore, residues in eggs and poultry tissues were investigated in a feeding study on laying hen.

CA 6.4.1 Poultry

At the time when the study protocol for the poultry feeding study was set up there were uncertainties about the need to include immature cereals as feeding items into the poultry diet since such feeding items are included in the OECD feeding tables for the EU poultry diet (Guidance document on residues in livestock, series on pesticides no. 73 (ENV/JM/MONO(2013)8)), 10 July 2013). As discussed and agreed with the RMS in a pre-submission meeting the dose groups were determined taking into account both scenarios, *i.e.* with and without immature cereals as relevant feeding items. As a pragmatic solution the study was designed with 3 dose levels, *i.e.* approximately 1N reflecting a scenario without immature cereals, 1N reflecting the scenario with immature cereals and 5N relative to the second dose level thus covering the range of the dietary burden for both scenarios. The dietary burden and the dose levels for the feeding study were calculated taking into account not only the representative uses on cereals but all uses where feeding items can be derived.

Thus the following nominal target dose levels were calculated:

- 0.52 mg/kg feed approximating the 1N dose level for poultry layer without consideration of immature cereals (addressed as 0.2N in the study)
- 2.6 mg/kg feed approximating the 1N dose level taking into account feeding items from immature cereals. For calculation of the dietary burden residues were adjusted to the dry matter content from freshly sampled green plant material. The dose level is addressed as 1N dose level in the feeding study.
- 13.0 mg/kg feed as an intermediate 5N dose level between 3N and 10N dose level relative to the 2.6 mg/kg dose.

The test substance used in the study should be representative of the residue in the feeding items. In the case of fluoxastrobin, by far the major part of the residue in plants is formed by parent compound, whereby HEC 5725 E-and Z-isomer were found in varying ratios.

The main residues which might be taken up by poultry layer originate from cereal straw. Therefore, the test item was fed in a 70/30 ratio of HEC 5725 E/Z-isomer according to the occurrence in straw in

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the metabolism studies conducted with three different radiolabels. This approach was already followed for the conduct of the dairy cattle feeding study and considered reasonable in the EU peer review.

Table 6.4.1- 1 and Table 6.4.1- 2 below compile the dietary burdens arising from cereal commodities when treated according to the use pattern of the representative uses and when considering all EU uses, from which feeding items can be derived, i.e. cereals, rape and potatoes. This additional calculation is presented because it forms the reference for the exaggerations in the hen feeding study summarised below. (Spread sheet used as published on the DG SANTE website.)

Table 6.4.1- 1: Detailed results of the dietary burden for poultry layer according to OECD feeding tables (EU diet) arising from the representative uses on cereals; RWCF approach (Reasonable worst case feed)

Maximum Intake	Poultry											
	Broiler			1.7 kg : 0.12 kg		Layer			1.9 kg : 0.13 kg		Turkey			4 kg : 0.5 kg
(mg/kg bw/d)	**0.001**	mg/kg bw/d		%		**0.930**	mg/kg bw/d		%		**0.001**	mg/kg bw/d		%
Contributor 1	Barley	grain		70		Wheat	straw		10		Barley	grain		50
Contributor 2	Wheat	milled bypdts		20		Barley	grain		90		Wheat	milled bypdts		20
Contributor 3									0					
Contributor 4														
Median intake	0.001	mg/kg bw				0.000	mg/kg bw				0.001	mg/kg bw		

Intakes expressed on the dry mater basis (mg/kg DM)				
	Poultry			
	Broiler	Layer	Turkey	
Maximum	0.02	**0.44**	0.02	**Intake >0.1 mg/kg DM in red characters**
Median	0.02	0.13	0.02	

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Table 6.4.1- 2: Detailed results of the dietary burden for poultry layer according to OECD feeding tables (EU diet) arising from all EU uses; RWCF approach (Reasonable worst case feed)

Maximum Intake (mg/kg bw/d)	Poultry								
	Broiler	1.7 kg 0.12 kg		Layer	1.9 kg 0.13 kg		Turkey	7 kg 0.5 kg	
	0.003	mg/kg bw/d	%	0.032	mg/kg bw/d	%	0.005	mg/kg bw/d	%
Contributor 1	Potato	culls	10	Wheat	straw	10	Potato	culls	20
Contributor 2	Barley	grain	70	Potato	culls	10	Barley	grain	50
Contributor 3	Wheat	milled bypdts	20	Barley	grain	80	Wheat	milled bypdts	20
Contributor 4						0			
Median intake	0.002	mg/kg bw		0.009	mg/kg bw		0.002	mg/kg bw	

Intakes expressed on the dry mater basis (mg/kg DM)				
	Poultry			
	Broiler	Layer	Turkey	Intake >0.1 mg/kg DM in red characters
Maximum	0.04	0.46	0.07	
Median	0.02	0.14	0.03	

Report: KCA 6.4.1/01 [redacted]; 2015; M-536059-01-1
Title: Fluoxastrobin (HEC 5725 e-isomer) and HEC 5725 z-isomer - Magnitude of the residue in laying hen
Report No.: IF-14/03211784
Document No.: M-536059-01-1
Guideline(s): OECD Guidelines for the testing of chemicals, Number 505 Residues in Livestock, 2007-01-08
US: EPA Residue Chemistry Test Guidelines OPPTS 860.1000 „Background“ OPPTS 860.1480 Meat, milk, poultry, eggs
Guideline deviation(s): none
GLP/GEP: yes

Materials and Methods

Test system, dosing

Sixty mature laying hens (*Gallus gallus domesticus*) were dosed orally, via capsule, for 28 consecutive days with fluoxastrobin (HEC 5725 E-and Z-isomer in a 70/30 ratio) at nominal dose rates of 0 mg/kg feed/day (control; 9 hens, 2 subgroups), 0.52 mg/kg feed/day (low dose group B; 12 hens, 3 subgroups), 2.6 mg/kg feed/day (mid dose group C; 12 hens, 3 subgroups), 13.0 mg/kg feed/day (high dose group D; 12 hens, 3 subgroups). In order to investigate the depuration an additional group of hens (dose group E, 15 hens, 3 subgroups) were dosed at the highest rate and subsequently held untreated for 1, 2 and 3 weeks until sacrifice.
Dose rates used in this study were calculated according to Annex 1 (feedstuff tables) of the OECD Guidance Document on residues in livestock, series on pesticides no. 73 (ENV/JM/MONO [2013]8. The dose levels were calculated taking into account all possible feeding items that originate from EU uses (cereal commodities and potatoes). The dose levels in the study were initially derived from the calculated dietary burden of 2.6 mg/kg feed (defined as 1X and including immature cereals as hen

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feeding items, see frame in bold in the table below). The other levels were approximately 0.2X and 5X the anticipated maximum dietary burden arising from all uses of fluoxastrobin in Europe.
During the reporting stage of the study it was confirmed that immature cereals would not need to be considered for the EU livestock diets and thus the 1N rate was calculated based on all EU uses but without immature cereals (0.46 mg/kg feed; cf. Table 6.4.1- 2). Therefore the the lowest target dose level in the study (0.52 mg/kg feed) corresponds to 1.1N. Considering a scenario without immature cereals as relevant poultry feeding items the target dose levels reflect 1.1X, 5.5X and 28X of the anticipated dietary burden.

The target and actual dose levels employed in the study are summarized below in Table 6.4.1- 3. The dose rates were adjusted weekly, based on the actual weekly feed consumption by the hens in each dose group during the previous week. The table includes the calculated mean values for the whole dosing period.

Table 6.4.1- 3: Summary of target and actual HEC 5725 E and Z-isomer dose administration

Dose group	Number of hens	Dose group	Dose group	Dose levels		
		Calculated based on EU relevant residue data[1] (without consideration of immature cereals)	Calculated based on EU relevant residue data[2] (reported in study report RAHEX096)	In feed		**Per animal**
				Target in study [3] (mg/kg feed)	Actual in study [4] (mg/kg feed)	**Actual[5] (mg a.s./kg b.w./day)**
A	9	control		0	0	**0**
B	12	1.1X dose	0.2X dose	0.52	0.60	**0.032**
C	12	5.5X dose	1X dose	2.6	2.9	**0.17**
D	12	28X dose	5X dose	13.0	14.5	**0.83**
E	15	28 X dose (depuration)	5X dose (depuration)	13.0	14.4	**0.82**

Footnotes:
1: EU dose rate exaggerations are based on EU dietary burden of **0.46 mg a.s./kg feed** (all EU uses, i.e. cereals + potatoes) without consideration of immature cereals as relevant poultry feeding items (target dose level); Table 6.4.1- 2.
2. EU dose rate exaggerations are based on EU dietary burden of **2.61 mg a.s./kg feed** (all EU uses, i.e. cereals + potatoes) taking into account immature cereals (forage, hay, silage) as relevant poultry feeding items. Report RAHEX096 mainly uses the exaggerations relative to the mid dose considered as 1N , dose levels reported there as 0.2X, 1X and 5X.
3: Target dose levels were calculated based on EU dietary burdens according to Annex 1 of the OECD Guidance document on residue in livestock No. 73 and derived from **2.61 mg a.s./kg feed** initially considered as 1N.
4: Actual dose based on average feed consumption data collected from the study and average amount (mg) test substance for each dose group over the entire dosing period
5: Actual dose based on average amount (mg) test substance and the average body weight for each dose group over the entire dosing period.

The hens were dosed orally once per day each morning after collection of eggs and feeding. The control animals received a placebo (empty capsule) concurrently with the treated animals.

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Sampling
Eggs were collected twice daily (afternoon and morning prior to the day's dosing). The eggs collected in the afternoon from each sub-group were combined with the eggs collected the following morning from the same sub-group. The egg contents were combined (shells discarded) by sub-group into a labeled container, weighed, and thoroughly mixed by vigorous shaking. Composite egg samples collected on days 0, 2, 4, 7, 10, 14, 17, 19, 21, 24, 27 (from all groups), on day 30 and 33 (from all depuration subgroups), on day 35, 37 and 40 (from 2 depuration subgroups) and on day 42, 44, 46 and 48 from the last depuration subgroup. The composite samples per subgroup were deep-frozen on the same day and shipped to the analytical laboratory for homogenization and subsequent analysis.

On day 28 of the study, twelve hens from the highest dose group (group D) and all hens in the groups receiving the low dose (group B) and the mid-dose (group C) along with 6 control hens were sacrificed within 6 hours of the administration of the final dose. Liver (entire), muscle (leg and breast), and fat (abdominal and subcutaneous with overlaying skin) were collected, shipped to the analytical laboratory for homogenization in the presence of dry ice. After homogenization, the samples were shipped to the analytical test site on the same day.
Eighteen hens (3 from the control group and 15 from the highest dose group) entered into a 21-day depuration phase following the administration of the final dose. Egg samples were collected throughout the depuration phase and tissue samples were collected on study days 35, 42, and 49 for analysis.

Analysis
Fluoxastrobin (HEC 5725 E-isomer), its Z-isomer and its metabolite HEC 7154 (M55, HEC 5725 phenoxy-hydroxypyrimidine) were analytically determined using analytical method 00691/M002 ([redacted] 2015, M-536049-01-1), which was validated prior to and concurrently with the residue analysis of the samples. The LOQ was 0.009 mg/kg for HEC 5725 E-isomer, 0.001 mg/kg for HEC 5725 Z-isomer and 0.01 mg/kg for HEC 7154 (expressed in parent equivalents).

Findings

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 72-102%, with relative standard deviations less than 20%. Details of recovery data are shown in Table 6.4.1- 7 to Table 6.4.1- 10.

Feed consumption, body weights, and egg production were not adversely affected by treatment with HEC 5725 E-and Z-isomers. In fact, feed consumption remained stable during the dosing period. The dose levels (mg a.s./hen in a given dose group), which were calculated using the mean feed consumption from the previous 5 days, also remained stable during the 28-day dosing period, as shown in Table 6.4.1- 4. An exception occurred for the first week of dosing where the dose was not calculated based on the previous average feed consumption instead the animals from each dose group received an unadjusted amount of the test item resulting in about 150% of the nominal target dose.

In the groups representing the nominal worst-case EU dietary burden without consideration of immature cereals as feeding items (low dose group B, actual 0.60 mg/kg feed, target 1.1X, reported in the study as 0.2X), residues of HEC 5725 E-and Z-isomer and HEC 7154 (HEC 5725 phenoxy-hydroxy-pyrimidine) were measured at sacrifice in poultry tissues and were always less than the LOQ (0.009 mg/kg for HEC 5725 E-isomer, 0.001 mg/kg for HEC 5725 Z-isomer and 0.01 mg/kg for HEC

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7154) in muscle, fat with overlaying skin and in liver. In eggs taken throughout the study duration, fluoxastrobin derived residues in the group receiving the lowest rate were also less than the LOQ for all analytes.

In tissues from hens belonging to the mid dose group C (actual 2.9 mg/kg feed, target 5.5X, reported as 1X in the study report), no residues above the respective LOQs of HEC 5725 E-and Z-isomer and HEC 7154 were found at sacrifice. In eggs taken throughout the study duration, fluoxastrobin derived residues in the group receiving the mid dose were also less than the individual LOQs for all analytes.

In tissues from hens belonging to the high dose group D (actual 14.5 mg/kg feed, target 28X, reported as 5X in the study report), no residues above the respective LOQs of HEC 5725 E-and Z-isomer were found at sacrifice. No residues of HEC 7154 above the LOQ of 0.01 mg/kg were found in muscle from the group receiving the highest dose. The liver samples were found to contain residues of HEC 7154 in the range of 0.017 – 0.025 mg/kg (mean of 0.021 mg/kg, expressed as parent equivalent). Residues slightly above the LOQ were found in some samples of skin with fat (< 0.01 – 0.011, mean <0.01 mg/kg) from the highest dose group.
In eggs, residues of HEC 7154 in the range of <0.01 – 0.011 mg/kg (expressed as parent equivalent) were determined only on day 7. The average from the 3 subgroups was 0.010 mg/kg. Following day 7, the residue levels dropped to values below the LOQ in all subgroups and remained at this level for the rest of the dosing period.

No residues of HEC 5725 E- and Z-isomer and HEC 7154 were found in the tissues of the depuration group E (actual 14.4 mg/kg feed, target 28X, reported as 5X in the study report).
Depuration occurred quickly. No residues of HEC 7154 were found in liver or any other tissue of hens from the depuration group sacrificed already one week after cessation of the dosing.

The residues found in the eggs and tissues collected from laying hens during dosing, at the end of the dosing period, and during the depuration phase are summarised in Table 6.4.1- 5 and Table 6.4.1- 6

Conclusions

A feeding study was conducted with fluoxastrobin (mixture of HEC 5725 E-and Z-isomer in a 70/30 ratio) on poultry in order to elucidate the levels of relevant residues in poultry tissues and in eggs.

Fluoxastrobin and its Z-isomer was administered orally (via capsule) to laying hens for 28 consecutive days at nominal average dose rates of 0.52 mg/kg feed (approximating the 1X [0.46 ppm] dose not considering immature cereals as poultry feeding items), 2.6 mg/kg feed (1X dose taking into account immature cereals as feed items) and 13.0 mg/kg feed. Feed consumption, body weights, and egg production were not adversely affected by compound administration.
Based on the actual feed intakes of the birds from the individual dose groups and the amount of test item (HEC 5725 E-and Z-isomer) received via the capsules the actual dose levels for the three dose groups were calculated as follows:
0.60 mg/kg feed for the low dose, 2.9 mg/kg feed for the mid dose and 14.5 mg for the high dose group and 14.4 mg/kg for the depuration group. The dose rates were adjusted weekly according to the actual feed intake per dose subgroup.
The fact that the first dose was somewhat higher than the calculated target dose (about 150%) for a very limited period of time is not considered to adversely affect the quality of the study since the

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overdose occurred at the very beginning of the study and residue levels were very low in eggs and tissues from all dose groups.
Based on the actual bodyweights of the hens the following dose rates in term of mg/kg bw/day are derived: 0.032 (low dose group), 0.17 (mid dose group), 0.83(high dose group) and 0.82 mg/kg bw/d for the depuration group. The dietary burden expressed as mg/kg bw per day administered to the lowest dose group is very well in line with the calculated dietary burden for the 1N dose level for both scenarios, the representative uses on cereals only and when considering all EU uses (please cf. Table 6.4.1- 1 and Table 6.4.1- 2).

After the final dose, the animals were sacrificed and the key edible tissues were analysed for the relevant residues of fluoxastrobin. Data were generated for three analytes (HEC 5725 E-and Z-isomer, HEC 7154) in the study itself corresponding to the proposed residue definition in the EFSA documents for enforcement and risk assessment (EFSA conclusion (2007) and EFSA Reasoned Opinion [Art. 12 review, 2012]). The combined residues from the three individual analytes were calculated for eggs and tissues. A theoretical LOQ of 0.02 mg/kg can be derived for the three components by summing up the individual LOQ levels. The calculation of the combined residue follows the 'traditional' methodology considering all residues below the LOQ being at the LOQ.
Residue levels in eggs:
In the course of the study, no residues of HEC 5725 E- and Z-isomer above the LOQ of 0.009 and 0.001 mg/kg, respectively, were found in the eggs from any of the dose groups.
No residues of HEC 7154 above the LOQ of 0.01 mg/kg were found in eggs from all dose groups except for the highest dose level (group D), where residues in the range of < 0.01 – 0.011 g/kg (expressed as parent equivalent) were determined only on day 7. The average from the 3 subgroups was 0.010 mg/kg. Following day 7, the residue levels dropped to values below the LOQ and remained at this level for the rest of the dosing period.
As a consequence the combined residues were always < 0.02 mg/kg for the dose level representing the EU dietary burden (0.032 mg/kg bw/d for all EU fluoxastrobin uses)

No residues of HEC 5725 E- and Z-isomer and HEC 7154 above the respective LOQ were found in eggs or tissues collected from animals of the depuration groups.

Residue levels in tissues:
While no residues of both parent compound isomers were found in any of the hen tissues (muscle, fat with skin and liver) and at any dose level, low levels of the metabolite HEC 7154 were found in liver from the highest dose group (0.017 – 0.023 mg/kg; mean of 0.021 mg/kg, expressed as parent equivalent). Residues slightly above the LOQ were found in some samples of skin with fat (< 0.01 – 0.011; mean <0.01 mg/kg) from the highest dose group.
The combined residues of HEC 5725 E-and Z-isomer as well as HEC 7154 were < 0.02 mg/kg for the dose level group reflecting the EU dietary burden.

No residues of HEC 5725 E- and Z-isomer and HEC 7154 above the respective LOQ were found in eggs or tissues collected from animals of the depuration groups.

All tissue samples from hen were analysed within 30 days of collection and therefore the investigation of the storage stability was not required for hen's tissues. Also the eggs samples were usually analysed within 30 days of collection, except few egg samples either collected on the day of sacrifice, from the control group or during the pre-dosing period. For these egg samples the storage interval was up to 44 days. However, given the fact that the samples from the control group or collected during the pre-

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dosing did not contain residues and from treated animals the residue levels in eggs were very low (< LOQ) up to the highest dose group a freezer storage stability study on eggs was not considered necessary.

Table 6.4.1- 4: Dosing regime used in the poultry feeding study (dosing via capsules)

Dose Group (nominal)	Timing	Dose rate* (mg/kg feed)	Feed intake fresh (g/bird/d)	Feed intake dry (g/bird/d)	Administered dose (mg)	Average body weight** (kg)	Average HEC 5725 E- and Z-isomer (mg/kg bw)
Control	4 Weeks	0	106	93	0	1.698	0
1.1X (0.2X in study report)	Week 1	0.77	104	92	0.070	1.643	0.043
	Week 2	0.50	110	97	0.048	1.679	0.029
	Week 3	0.51	114	100	0.050	1.737	0.029
	Week 4	0.60	99	87	0.052	1.762	0.029
Overall Average		**0.60**	**107**	**94**	**0.055**	**1.706**	**0.032**
5.5X (1X in study report)	Week 1	3.5	110	97	0.34	1.644	0.21
	Week 2	2.5	115	101	0.25	1.684	0.15
	Week 3	2.5	119	104	0.26	1.740	0.15
	Week 4	3.1	101	89	0.27	1.772	0.16
Overall Average		**2.9**	**111**	**98**	**0.28**	**1.710**	**0.17**
28X (5X in study report)	Week 1	18.0	108	95	1.7	1.594	1.07
	Week 2	12.9	109	96	1.2	1.638	0.76
	Week 3	13.5	113	99	1.2	1.693	0.73
	Week 4	14.5	102	90	1.3	1.715	0.76
Overall Average		**14.5**	**108**	**95**	**1.35**	**1.660**	**0.83**
Depuration group 28X (5X in study report)	Week 1	18.6	105	92	1.7	1.615	1.06
	Week 2	12.6	110	97	1.2	1.659	0.73
	Week 3	13.1	111	98	1.3	1.700	0.75
	Week 4	13.2	112	99	1.3	1.737	0.75
Overall Average		**14.4**	**110**	**96**	**1.38**	**1.678**	**0.82**

* dose rate in feed calculated on a dry weight basis
** these weights reflect those determined at the end of the given study week

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Table 6.4.1- 5: Levels of the relevant residues of fluoxastrobin in eggs

Group	Group dose level in feed (nominal) / Dose group level actual (mg/kg bw/d)	sampling day	Residue levels of individual analytes (mg/kg)			Combined residue levels† (mg/kg)
			HEC 5725 E-isomer LOQ = 0.009	HEC 5725 Z-isomer LOQ = 0.001	HEC 7154 LOQ = 0.01 (expressed in parent compound equivalents)	Sum of HEC 5725 E- and Z-isomer + HEC 7154
B	1.1X (0.2X in study report) 0.032 mg/kg bw/d	0	< 0.009	< 0.001	< 0.01	<0.02
		1	< 0.009	< 0.001	< 0.01	<0.02
		3	< 0.009	< 0.001	< 0.01	<0.02
		5	< 0.009	< 0.001	< 0.01	<0.02
		7	< 0.009	< 0.001	< 0.01	<0.02
		10	< 0.009	< 0.001	< 0.01	<0.02
		14	< 0.009	< 0.001	< 0.01	<0.02
		17	< 0.009	< 0.001	< 0.01	<0.02
		19	< 0.009	< 0.001	< 0.01	<0.02
		21	< 0.009	< 0.001	< 0.01	<0.02
		24	< 0.009	< 0.001	< 0.01	<0.02
		27	< 0.009	< 0.001	< 0.01	<0.02
C	5.5X (1X in study report) 0.17 mg/kg bw/d	0	< 0.009	< 0.001	< 0.01	<0.02
		1	< 0.009	< 0.001	< 0.01	<0.02
		3	< 0.009	< 0.001	< 0.01	<0.02
		5	< 0.009	< 0.001	< 0.01	<0.02
		7	< 0.009	< 0.001	< 0.01	<0.02
		10	< 0.009	< 0.001	< 0.01	<0.02
		14	< 0.009	< 0.001	< 0.01	<0.02
		17	< 0.009	< 0.001	< 0.01	<0.02
		19	< 0.009	< 0.001	< 0.01	<0.02
		21	< 0.009	< 0.001	< 0.01	<0.02
		24	< 0.009	< 0.001	< 0.01	<0.02
		27	< 0.009	< 0.001	< 0.01	<0.02
D	28X (5X in study report) 0.83 mg/kg bw/d	0	< 0.009	< 0.001	< 0.01	<0.02
		1	< 0.009	< 0.001	< 0.01	<0.02
		3	< 0.009	< 0.001	< 0.01	<0.02
		5	< 0.009	< 0.001	< 0.01	<0.02
		7	< 0.009	< 0.001	0.10	0.02
		10	< 0.009	< 0.001	< 0.01	<0.02
		14	< 0.009	< 0.001	< 0.01	<0.02
		17	< 0.009	< 0.001	< 0.01	<0.02
		19	< 0.009	< 0.001	< 0.01	<0.02
		21	< 0.009	< 0.001	< 0.01	<0.02
		24	< 0.009	< 0.001	< 0.01	<0.02
		27	< 0.009	< 0.001	< 0.01	<0.02

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Table 6.4.1- 5(cont'd): Levels of the relevant residues of fluoxastrobin in eggs

Group	Group dose level per feed (nominal)	sampling day	Residue levels of individual analytes (mg/kg)			Combined residue levels† (mg/kg)
E1-E3	Dose group level actual (mg/kg bw/d)		HEC 5725 E-isomer LOQ = 0.009	HEC 5725 Z-isomer LOQ = 0.001	HEC 7154 LOQ = 0.01 (expressed in parent compound equivalents)	Sum of HEC 5725 E- and Z-isomer + HEC 7154
E1- E3**	28X (5X in report) 0.82 mg/kg bw/d	10‡	< 0.009	< 0.001	< 0.01	<0.02
		14	< 0.009	< 0.001	< 0.01	<0.02
		17	< 0.009	< 0.001	< 0.01	<0.02
		19	< 0.009	< 0.001	< 0.01	<0.02
		21	< 0.009	< 0.001	< 0.01	<0.02
		24	< 0.009	< 0.001	< 0.01	<0.02
		27	< 0.009	< 0.001	< 0.01	<0.02
		30*	< 0.009	< 0.001	< 0.01	<0.02
E2, E3**		33*	< 0.009	< 0.001	< 0.01	<0.02
		35*	< 0.009	< 0.001	< 0.01	<0.02
		37*	< 0.009	< 0.001	< 0.01	<0.02
E3**		40*	< 0.009	< 0.001	< 0.01	<0.02
		42*	< 0.009	< 0.001	< 0.01	<0.02
		44*	< 0.009	< 0.001	< 0.01	<0.02
		46*	< 0.009	< 0.001	< 0.01	<0.02
		48*	< 0.009	< 0.001	< 0.01	<0.02

* depuration phase, no dosing (sampling days 30-48)

** residue values from the depuration groups reflect the average of all subgroups until the day of sacrifice for a given subgroup

† this value reflects the proposed residue definition, and as such calculates each component at or above the respective LOQ

‡ Sample collection for the depuration subgroups started 10 days after start of dosing

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Table 6.4.1- 6: Levels of the relevant residues of fluoxastrobin in poultry tissues

Group	Group dose level in feed (nominal)	Dose group level per animal (actual) [mg/kg bw/d]	sampling day	Residue levels of individual analytes (mean of 3 subgroups) [mg/kg]			Combined residue levels† [mg/kg]
				HEC 5725 E-isomer LOQ = 0.009	HEC 5725 Z-isomer LOQ = 0.001	HEC 7154 LOQ = 0.01 (expressed in parent compound equivalents)	Sum of HEC 5725 E- and Z-isomer + HEC 7154
*POULTRY **FAT WITH OVERLAYING SKIN***							
B	1.1X	0.032	27	< 0.009	< 0.001	< 0.01	<0.02
C	5.5X	0.17	27	< 0.009	< 0.001	< 0.01	<0.02
D	28X	0.83	27	< 0.009	< 0.001	< 0.01[a)]	<0.02
E1	28X	0.82	35*	< 0.009	< 0.001	< 0.01	<0.02
E2	28X	0.82	42*	< 0.009	< 0.001	< 0.01	<0.02
E3	28X	0.82	48*	< 0.009	< 0.001	< 0.01	<0.02
*POULTRY **LIVER***							
B	1.1X	0.032	27	< 0.009	< 0.001	< 0.01	<0.02
C	5.5X	0.17	27	< 0.009	< 0.001	< 0.01	<0.02
D	28X	0.83	27	< 0.009	< 0.001	0.021[b)]	0.031
E1	28X	0.82	35*	< 0.009	< 0.001	< 0.01	<0.02
E2	28X	0.82	42*	< 0.009	< 0.001	< 0.01	<0.02
E3	28X	0.82	48*	< 0.009	< 0.001	< 0.01	<0.02
*POULTRY **MUSCLE***							
B	1.1X	0.032	27	< 0.009	< 0.001	< 0.01	<0.02
C	5.5X	0.17	27	< 0.009	< 0.001	< 0.01	<0.02
D	28X	0.83	27	< 0.009	< 0.001	< 0.01	<0.02
E1	28X	0.82	35*	< 0.009	< 0.001	< 0.01	<0.02
E2	28X	0.82	42*	< 0.009	< 0.001	< 0.01	<0.02
E3	28X	0.82	48*	< 0.009	< 0.001	< 0.01	<0.02

* depuration phase, no dosing (sampling days 30-48)

† this value reflects the proposed residue definition, and as such calculates each component at or above the respective LOQ

[a)] The individual values from the three subgroups were: < 0.01, < 0.01, 0.011 mg/kg (mean < 0.01 mg/kg)

[b)] The individual values from the three subgroups were: 0.017, 0.022, 0.025 mg/kg (mean 0.021 mg/kg)

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Table 6.4.1- 7: Procedural recovery data for the relevant residues of fluoxastrobin in poultry eggs

Sample Material	Analyte	FL [mg/kg]	Individual Values [%]					Mean Value [%]	RSD [%]	n	LOQ [mg/kg]
EGG	Fluoxastrobin (HEC 5725 E-Isomer)	0.009	85	81	91	85	90	84	6.6	26	0.009
			90	97	84	82	84				
			85	87	87	90	89				
			89	79	81	85	75				
			75	81	79	73	84				
			80								
		0.09	85	85	80	82	82	83	3.2	17	
			81	80	83	87	86				
			78	81	85	84	84				
			84	78							
	Overall Recovery for HEC 5725 E-Isomer:							84	5.6	43	
	HEC 5725 Z-Isomer	0.001	75	99	99	100	101	94	7.0	26	0.001
			84	91	98	99	99				
			89	92	101	89	88				
			88	86	93	91	89				
			94	98	98	104	99				
			94								
		0.01	90	90	90	87	84	88	7.5	17	
			80	86	88	85	87				
			95	87	87	78	95				
			86	108							
	Overall Recovery for HEC 5725 Z-Isomer:							92	7.7	43	
	HEC 7154*	0.01	90	105	105	97	101	99	5.2	26	0.01
			95	96	102	94	101				
			99	104	103	95	98				
			94	92	88	101	92				
			101	108	99	103	96				
			104								
		0.1	94	91	94	90	87	89	5.0	17	
			90	77	91	89	82				
			89	91	87	96	89				
			88	87							
	Overall Recovery for HEC 7154:							95	7.0	43	

*Final determination as: HEC 7154 Residues calculated as: HEC 5725 parent equivalent

FL: Fortification level; LOQ: limit of quantification;

RSD: Relative Standard Deviation; n: Number of individual values.

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Table 6.4.1- 8: Procedural recovery data for the relevant residues of fluoxastrobin in poultry fat with overlaying skin

Sample Material	Analyte	FL [mg/kg]	Individual Values [%]		Mean Value [%]	RSD [%]	n	LOQ [mg/kg]
FAT	Fluoxastrobin (HEC 5725 E-Isomer)	0.009	73	80	76	-	2	0.009
		0.09	71	72	72	-	2	
	Overall Recovery:				74	5.7	4	
	HEC 5725 Z-Isomer	0.001	84	102	93	-	2	0.001
		0.01	79	87	83	-	2	
	Overall Recovery:				88	11.2	4	
	HEC 7154*	0.01	87	95	91	-	2	0.01
		0.1	98	88	93	-	2	
	Overall Recovery:				92	5.8	4	

*Final determination as: HEC 7154 Residues calculated as: HEC 5725 parent equivalent
FL: Fortification level; LOQ: limit of quantification;
RSD: Relative Standard Deviation; n: Number of single values

Table 6.4.1- 9: Procedural recovery data for the relevant residues of fluoxastrobin in poultry liver

Sample Material	Analyte	FL [mg/kg]	Individual Values [%]		Mean Value [%]	RSD [%]	n	LOQ [mg/kg]
LIVER	Fluoxastrobin (HEC 5725 E-Isomer)	0.009	89	95	92	-	2	0.009
		0.09	72	72	72	-	2	
	Overall Recovery:				82	14.4	4	
	HEC 5725 Z-Isomer	0.001	87	102	95	-	2	0.001
		0.01	76	86	81	-	2	
	Overall Recovery:				88	12.2	4	
	HEC 7154*	0.01	93	98	96	-	2	0.01
		0.1	90	98	94	-	2	
	Overall Recovery:				95	4.2	4	

*Final determination as: HEC 7154 Residues calculated as: HEC 5725 parent equivalent
FL: Fortification level; LOQ: limit of quantification;
RSD: Relative Standard Deviation; n: Number of single values

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Table 6.4.1- 10: Procedural recovery data for the relevant residues of fluoxastrobin in poultry meat

Sample Material	Analyte	FL [mg/kg]	Individual Values [%]			Mean Value [%]	RSD [%]	n	LOQ [mg/kg]
MUSCLE	Fluoxastrobin (HEC 5725 E-Isomer)	0.009	88	92	92	91	2.5	3	0.009
		0.09	80	80		80	-	2	
	Overall Recovery:					86	7.0	5	
	HEC 5725 Z-Isomer	0.001	88	88	85	87	2.0	3	0.001
		0.01	84	82		83	-	2	
	Overall Recovery:					85	3.1	5	
	HEC 7154*	0.01	101	103	101	102	1.1	3	0.01
		0.1	104	99		102	-	2	
	Overall Recovery:					102	1.9	5	

*Final determination as: HEC 7154 Residues calculated as: HEC 5725 parent equivalent
FL: Fortification level; LOQ: limit of quantification;
RSD: Relative Standard Deviation; n: Number of single values.

CA 6.4.2 Ruminants

The magnitude of fluoxastrobin residues in ruminants has been investigated in a feeding study with lactating cows. The study was evaluated in the EU peer review and was considered sufficient for deriving MRLs in ruminants and pigs. For details please see chapter CA 6.4. No further studies are provided.

The dietary burden was calculated using the input data arising from residue field trials supporting the representative uses/critical GAPs described above as well as for other uses for which registrations are granted and the feeding tables as provided in the OECD guidance document No 73 (Annex I). Immature cereals as feeding items are not considered as input data (Table 6.4- 1).

Table 6.4.2- 1 and Table 6.4.2- 2 below compile the dietary burdens arising from cereal commodities when treated according to the use pattern of the representative uses and when considering all EU uses, from which feeding items can be derived, i.e. cereals, rape and potatoes. However, the calculations result in very similar intake values. (Calculations were performed using the official spreadsheets *pesticides.mrl_guidelines_animal_model_2015_en.xls* (September 2015) downloaded from the DG SANTE website.)

In Table 6.4.2- 3 the dietary burden is compared to the dose levels of the dairy cow feeding study.

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Table 6.4.2- 1: Detailed results of the dietary burden for ruminants arising from the representative uses on cereals according to OECD feeding tables (EU diet); RWCF approach (Reasonable worst case feed)

Maximum Intake	Cattle					
	Beef	500 kg 12 kg		Dairy	650 kg 25 kg	
(mg/kg bw/d)	0.022	mg/kg bw/d	%	0.036	mg/kg bw/d	%
Contributor 1	Barley	straw	30	Barley	straw	30
Contributor 2	Barley	grain	70	Barley	grain	40
Contributor 3			0	Wheat	milled byprods	30
Contributor 4						
Median intake	0.0059	mg/kg bw/d		0.0093	mg/kg bw/d	

Maximum Intake	Sheep					
	Ram/Ewe	75 kg 2.5 kg		Lamb	40 kg 1.7 kg	
(mg/kg bw/d)	0.061	mg/kg bw/d	%	0.078	mg/kg bw/d	%
Contributor 1	Barley	straw	60	Barley	straw	60
Contributor 2	Barley	grain	40	Barley	grain	40
Contributor 3			0			0
Contributor 4						
Median intake	0.0156	mg/kg bw/d		0.0199	mg/kg bw/d	

Intakes expressed on the dry mater basis (mg/kg DM)				
mg/kg DM	Cattle		Sheep	
	Beef	Dairy	Ram/Ewe	Lamb
Maximum	0.93	0.93	1.83	1.83
Median	0.25	0.24	0.47	0.47

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Table 6.4.2- 2: Detailed results of the dietary burden for ruminants arising from all EU uses according to OECD feeding tables (EU diet); RWCF approach (Reasonable worst case feed)

Maximum Intake	Cattle							
	Beef		500 kg		Dairy		650 kg	
			12 kg				25 kg	
(mg/kg bw/d)	0.024	mg/kg bw/d	%		0.038	mg/kg bw/d	%	
Contributor 1	Barley	straw	30		Barley	straw	30	
Contributor 2	Potato	culls	30		Potato	culls	30	
Contributor 3	Barley	grain	40		Barley	grain	40	
Contributor 4			0				0	
Median intake	0.0061	mg/kg bw/d			0.0098	mg/kg bw/d		

Maximum Intake	Sheep							
	Ram/Ewe		75 kg		Lamb		40 kg	
			3 kg				1.7 kg	
(mg/kg bw/d)	0.063	mg/kg bw/d	%		0.080	mg/kg bw/d	%	
Contributor 1	Barley	straw	60		Barley	straw	60	
Contributor 2	Potato	culls	30		Potato	culls	20	
Contributor 3	Barley	grain	10		Barley	grain	20	
Contributor 4			0				0	
Median intake	0.0159	mg/kg bw/d			0.0201	mg/kg bw/d		

Maximum Intake	Swine							
	Breeding		260 kg		Finishing		100 kg	
			6 kg				3 kg	
(mg/kg bw/d)	0.003	mg/kg bw/d	%		0.004	mg/kg bw/d	%	
Contributor 1	Potato	culls	50		Potato	culls	50	
Contributor 2	Rape	forage	20		Barley	grain	50	
Contributor 3	Barley	grain	30				0	
Contributor 4			0					
Median intake	0.001	mg/kg bw/d			0.001	mg/kg bw/d		

Intakes expressed on the dry mater basis (mg/kg DM)						
mg/kg DM	Cattle		Sheep		Swine	
	Beef	Dairy	Ram/Ewe	Lamb	Breeding	Finishing
Maximum	0.99	0.99	1.90	1.87	0.14	0.14
Median	0.25	0.25	0.48	0.47	0.04	0.04
					Intake >0.1 mg/kg DM in red characters	

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Table 6.4.2- 3: Overview on the dietary burden of ruminants and residues obtained in the dairy cow feeding study

Commodity	Median EU Dietary Burden [a]	Max. EU Dietary Burden [a]	Results of livestock feeding study				Highest residue [d]	Median residue [e]	Remark
			Dose level [b]	No of animals	Results for enforcement = results for risk assessment [c] *Proposed residue definition: Fluoxastrobin + Z-isomer+ HEC 7154 (=M55)*				
		(mg/kg bw/d)	(mg/kg bw/d)		Mean (mg/kg)	Max (mg/kg)	(mg/kg)	(mg/kg)	
Cattle meat	(*0.006 for beef)* 0.009 for dairy	(*0.022 for beef)* 0.036 for dairy	0.22	3	0.02	0.02	0.02	0.02	Dietary burden from dairy cattle
Cattle fat				3	0.02	0.02	0.02	0.02	
Cattle liver				3	0.02	0.02	0.02	0.02	
Cattle kidney				3	0.04	0.05	0.02	0.02	
Cattle milk	0.009	0.036		30	0.02	0.02	0.02	0.02	
Sheep meat	0.020	0.078	0.22	3	0.02	0.02	0.02	0.02	Dietary burden from lamb
Sheep fat				3	0.02	0.02	0.02	0.02	
Sheep liver				3	0.02	0.02	0.02	0.02	
Sheep kidney				3	0.04	0.05	0.02	0.02	
Sheep milk	0.016	0.061	0.22	30	0.02	0.02	0.02	0.02	Dietary burden from ewe

[a] The dietary burden was calculated for the European livestock diet using the OECD feeding tables issued with the OECD guidance Document No 73 for the representative uses and without taking into account immature cereals (forage, hay, silage) as feeding items (cf. Table 6.4- 2). As a conservative approach the higher level calculated for dairy cattle is considered applicable also for beef cattle.

[b] The lowest dose level of ruminant feeding study was 0.22 mg/kg bw/d. Results from other dose levels are not listed here since the anticipated dietary burden is less than the lowest dose level of the feeding studies

[c] Data based on anticipated residue definition for enforcement and risk assessment involving fluoxastrobin (E-isomer) and its Z-isomer and HEC 5725-phenoxy-hydroxypyrimidine (HEC 7154; M55). Therefore, a conversion factor to adjust from the enforcement residue definition to the risk assessment residue definition is not required. Residues of individual components of the residue definition below the LOQ were calculated as being *at* the LOQ and summed up.

[d] Highest residue value (tissues) or mean residue value (milk) according to the enforcement residue definition, derived by transfer factor (OECD guidance document No 73) or interpolation/extrapolation of the maximum dietary burden between the relevant feeding groups of the study (FAO, 2009). No calculation is made here – except for liver and kidney (transfer factor) - since residues were < LOQ.

[e] Median residue value (tissues, milk) according to the enforcement residue definition, derived by transfer factor (OECD guidance document No 73) or interpolation/extrapolation of the median dietary burden between the relevant feeding groups of the study (FAO, 2009). No calculation is made here – except for kidney - since all residues were < LOQ.

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CA 6.4.3 Pigs

The general metabolic pathways in rodents and ruminants were found to be comparable and the findings in ruminants can therefore be extrapolated to pigs. This is also the conclusion in the 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for fluoxastrobin according to Article 12 of Regulation (EC) No 396/2005' [EFSA Journal 2012;10(12):3012].

The dietary burden for swine was calculated above (cf. Table 6.4- 2) using the input data arising from residue field trials described above and the feeding tables as provided in the OECD guidance document no 73 [Annex I]). The threshold value of 0.004 mg/kg bw/d was not exceeded for swine (breeding or finishing) taking into account the residue levels in cereals arising from the representative uses / critical GAPs.
Feeding studies on pigs are therefore not required and no supplementary study has been generated following the inclusion of the active substances in Annex I of Directive 91/414/EEC.

CA 6.4.4 Fish

In March 2013 EU Commission Regulation 283/2013 was published setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009. This Regulation states:

6.4.4 Fish Feeding
A fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation.

Unlike for ruminant and poultry, there are currently no agreed test guidelines for the conduct of fish feeding studies. The procedure when no agreed test methods or guidance documents are available is described in the "Guidance Document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 (SANCO/10181/2013-rev 3, 12 December 2014). The document states:

In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.

In the summary report of the Standing Committee on Plants, Animals, Food and Feed (26 and 27 January 2015) the Commission recommended relative to the data requirements and acceptance of waivers / the implementation of document SANCO/10181/2013 *that Member States are invited to follow the procedures agreed when taking note of Guidance Document SANCO/10181/2013 in order to harmonise the procedures, i.e. to accept as a general line the waiving for cases where no test guidelines are available. (A26).*

At the time when the present document was prepared, no corresponding guidance document was published or listed addressing reference 6.4.4 of the Annex to Regulation (EU) No 283/2013 and no fish feeding study was conducted.

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Bayer CropScience supports the need to assess the dietary risk arising from residues of plant protection products in fish commodities and where necessary to have appropriate MRLs established. However, in order to minimise vertebrate testing (cf. paragraph 40 of Regulation 1107/2009) the decision on the conduct of fish feeding studies shall be made once an agreed test guideline is in place.

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CA 6.5 Effects of processing

CA 6.5.1 Nature of the residue

A study on the stability of fluoxastrobin in aqueous solutions simulating representative conditions of processing was conducted with [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin ([redacted] 2001, M-032458-01-1). This study was peer reviewed at EU level. Fluoxastrobin was shown to be hydrolytically stable under conditions relevant to pasteurisation (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilisation (20 minutes at 120°C, pH 6). For all solutions, recovery of the total amount of applied radioactivity was greater than 95% (mean 99%).
It is concluded that fluoxastrobin does not degrade under conditions of industrial or domestic food processing. Thus, for processed commodities, the same residue definition as for the raw agricultural commodities is applicable.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

The distribution of the residue in peel and pulp is not relevant for the supported crops.

CA 6.5.3 Magnitude of residues in processed commodities

Processing of barley

Study evaluated for Annex I inclusion

In the DAR, a study comprising 2 processing trials on barley was evaluated. Barley grain was processed into bran and pearl barley. Residues of fluoxastrobin and HEC 5725 Z-isomer were determined in the end products as well as in several by-products. On analysis of the grain residues were 0.03 and 0.04 mg/kg in the raw agricultural commodity. On processing the grain samples, residues in the processed samples had not increased significantly with the exception of pearl barley rub off which had increased by a factor of 3. The study was considered acceptable in the DAR.
In the EFSA Conclusion (list of endpoints 2007) processing / transfer factors were set for barley rub off (3), malt sprout and brewer's malt (1.5) , brewer's grain (1.5) and for beer, pearl barley, hops draff, and brewer's yeast (1). Since the LOQ of the analytical method was higher for the processed fractions (0.05 mg/kg) than the residues present in the barley grain (0.03 / 0.04 mg/kg) the processing factor of 1 might be indicative.

Evaluation in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(12):3012)

EFSA concluded that the processing factors reported for barley in the List of Endpoints should be indicative as they are only supported by 2 studies. Nevertheless, additional processing studies were not required as they are not expected to affect the outcome of the risk assessment. However, EFSA concluded that for the purpose of derivation of more robust processing factors, additional studies would be considered necessary.

Two additional studies were conducted and are summarized below.
An overview on all data (old and new) from barley processing is provided following the summary of the new barley processing studies.

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Report:	KCA 6.5.3/02[redacted],[redacted]; 2015; M-513062-03-1
Title:	Determination of the residues of fluoxastrobin and prothioconazole in/on barley, spring and the processed fractions (malt sprouts; brewer's malt; brewer's grain; hops draff; brewer's yeast; beer; pearl barley rub off; pearl barley) after spraying of fluoxastrobin & prothioconazole EC 200 in the field in Germany and France (South)
Report No.:	13-3401
Document No.:	M-513062-03-1
Guideline(s):	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 OECD 508 adopted 3 October 2008, OECD Guideline for the testing of chemicals, magnitude of the pesticide residues in processed commodities US EPA OCSPP Guideline No. 860.1520 US EPA OCSPP Guideline No. 860.1500, Crop Field Trial
Guideline deviation(s):	none
GLP/GEP:	**yes**

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Materials and Methods

Two trials were conducted in the northern and southern European residue regions, one in Germany the other in France (south), in order to determine the total residues of HEC 5725 in unprocessed spring barley grain and then in the primary processed products beer and pearl barley, as well as in by-products, including malt.

'Fluoxastrobin + Prothioconazole EC 200' was sprayed twice in the field at application rates of 0.375 kg fluoxastrobin/ha and a water volume of 300 L/ha. The application rate reflects an overdosing (3N and 4.3N relative to the new critical GAPs in northern and southern Europe, respectively) in order to ensure that detectable residues would be found in the relevant raw commodities at harvest, thus allowing derivation of processing factors. The last application was done at BBCH 61 (beginning of flowering), the first application was done 14 days before the last application.

Spring barley (grain) samples to be processed were sampled at commercial harvest (BBCH 89), 50 or 69 days after the last application.

After processing (described below), residue analysis was performed according to method 00649/M003 ([redacted]; 2010; M-387385-01-1). The Limit of Quantification (LOQ), defined as the lowest validated fortification level, was 0.009 mg/kg for the HEC 5725 E-isomer and 0.001 mg/kg for the Z-isomer, resulting in a theoretical LOQ of 0.01 mg/kg for the total residue of HEC 5725 for all commodities.

Processing procedures:
The processing of barley grain into the processed fractions (malt sprouts; brewer's malt; brewer's grain; hops draff; brewer's yeast; beer; pearl barley rub off; pearl barley) was performed simulating the common industrial processes (cleaning, malting, brewing, pearl barley production).

Cleaning:

The field specimens for processing were cleaned using a "Windsichter", which allows the separation of soil particles and other contaminations from the grain in a steady air flow.

Malting:

Before malting was started, the grain was sieved (sieve mesh 2.5 mm). The steeping process was conducted as a combined wet and dry steeping. Sieved barley grain was transferred into a special steeping vessel. During steeping water is supplied to the interior of the kernel. As a result the enzymes become active and germination begins.

For proper performance the duration of germination, the mean temperature of wet air and the relative humidity of the air around the kernels was controlled. During the intensive respiration, the steeped good was turned over continuously. After germination, the life processes were terminated by kilning. Kiln-drying was conducted in a dry chamber. During kilning the water content of green malt is lowered down to < 10%. After kiln-drying, the germ (= malt sprouts) was removed mechanically by a trimmer. Brewer's malt and malt sprouts were sampled immediately after end of malting. Until brewing (approx. 4 weeks malt rest), the malt was stored at room temperature.

Brewing:

Before mashing, the brewer's malt was dry milled in a special malt mill. The crushed malt was mixed with brew water. Mashing was started in a heatable tun where the mash was heated up to 76°C.

After mashing, the wort was separated from the insoluble malt components (brewer's grain). The extract remaining in the brewer's grain was extracted by washing with hot water (first filter runnings). The wort separation was done using a refining vat. After separation, the brewer's grain was sampled.

Hop pellets were added and the separated wort was boiled (about 90 min at normal pressure). After boiling, the flocs (hops draff) were separated in a whirlpool, causing the sludge to deposit on the bottom in the shape of a cone. For cooling and ventilating the wort, an intra-plant circulation was used. By adding oxygen (intra-plant circulation), the conditions for the start of the fermentation were prepared. Hops draff was sampled.

In the pilot plant the classical primary fermentation (low fermentation) was carried out in bottom-fermentation containers. The fermentation temperature was 9°C. Fermentation heat was dissipated by means of room ventilation. As soon as the extract content of the fermented young beer was 2% higher than the final attenuation, the storing time began. Before maturation, the young beer was cooled down. During the main fermentation, the yeast deposited on the tank bottom and was sampled as brewer's yeast.

At the beginning of maturation, the young beer was stored at room temperature (warm maturation to break down the diacetyl) in casks. Then the young beer was stored under pressure (approx. 0.7-2.1 bar) at 2°C (cold maturation) for about 4 weeks. In this time, the remaining extract was fermented. Unwanted flavour and odorous substances were decomposed or expelled. Sludge particles and yeast settled at the bottom. The rack beer was filtered using a special filter combination. During filtration, all organisms harming the beer (bacteria and yeast) were removed and sludge particles were separated. The final product beer was sampled.

Pearl barley production:

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Before beginning pearl barley production, an optimal moisture content of barley grain of about 15% / 16% is required. Since the moisture content of the specimens was already nearly optimal (14.7-15.5%), it was not necessary to dry or moisture the grain.

The specimens of spring barley were hulled using a vertical hulling machine. Each sample was hulled until the stipulated abrasion for pearl barley of 30-35% was reached. The degree of abrasion (pearling dust/bran and flour) was determined by the proportion of pearl barley with respect to the total portion of cleaned grain used for hulling process. Pearl barley and pearl barley rub off were sampled.

The processes are illustrated in flow Diagram 6.5.3- 1, Diagram 6.5.3- 2 and Diagram 6.5.3- 3.

Findings

The validation of the sample materials beer and hops draff was conducted within the present study. The sample material hops draff is considered to be representative for sample material brewer's yeast. A full set of validation recoveries on cereal grain has been generated during method development of 00649/M003 ([redacted], 2010; M-387385-01-1). These recoveries for cereals grain can be considered to also validate the sample materials brewer's grain, brewer's malt, malt sprouts, pearl barley and stored grain. The limited number of procedural recoveries for brewer´s grain conducted during the study is therefore considered acceptable.

In beer, recovery samples for fluoxastrobin (HEC 5725 E-Isomer) were spiked at levels of 0.009 mg/kg and 0.09 mg/kg, in hops draff at 0.009 and 0.90 mg/kg, and in brewer´s grain recovery samples for fluoxastrobin were spiked at levels of 0.009, 0.09 and 0.90 mg/kg. Mean recoveries for all matrices were 88-99%, with RSDs in the larger validation sets (n > 2) of 0.6-4.5%.
For HEC 5725 Z-Isomer samples were spiked at levels of 0.001 mg/kg and 0.01 mg/kg in beer, at 0.001 mg/kg and 0.10 mg/kg in hops draff and at 0.001, 0.01 and 0.10 mg/kg in brewer´s grain. Mean recoveries for all matrices were 86-101%, with RSDs in the larger validation sets (n > 2) of 1.0-4.7%.

A tabular summary of the recovery values is presented below in Table 6.5.3- 6.

The mean (unrounded) residue values of harvested barley grain ("grain, stored") at BBCH growth stage 89 were used for the calculation of processing factors. In this summary the processing factors for the total residue HEC 5725 (sum of E-and Z-isomer) are reported according to the residue definition for enforcement and risk assessment proposed by EFSA (EFSA conclusion, 2007 and EFSA Reasoned Opinion on existing MRLs, 2012). Although the currently established residue definition for enforcement includes fluoxastrobin (HEC 5725 E-isomer) only, it is anticipated that the sum of both isomers will constitute the future residue definition also for monitoring. Combined residues of HEC 5725 E-and Z-isomer were calculated considering residue levels below the LOQ as being *at* the LOQ.

Raw agricultural commodities: barley grain
For brewing: In the two independent trials, residues in stored barley grain (mean of two individual samples) were < 0.009 and 0.01 mg/kg for fluoxastrobin (HEC 5725 E-isomer), 0.002 and 0.004 mg/kg for HEC 5725 Z-isomer and 0.011 and 0.014 mg/kg for the total residue of HEC 5725.
For pearl barley production: Residues in stored barley grain (mean of two individual samples) were < 0.009 and 0.011 mg/kg for fluoxastrobin (HEC 5725 E-isomer), 0.001 and 0.004 mg/kg for HEC 5725 Z-isomer and 0.010 and 0.015 mg/kg for the total residue of HEC 5725.

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● Malting:

Malt sprouts: The residues of fluoxastrobin (E-isomer) in malt sprouts were at or below LOQ (<0.009 – 0.009 mg/kg), residues of HEC 5725 Z-isomer were at 0.002 mg/kg. The levels of total residue HEC 5725 were at 0.011 – 0.012 mg/kg. The residue levels lead to a (mean) processing factor of 1.0 for total residue HEC 5725 (sum of E-and Z-isomer).

Brewer's malt: The residues of fluoxastrobin (HEC 5725 E-isomer) in brewer´s malt were below LOQ (<0.009 mg/kg), residues of HEC 5725 Z-isomer were at 0.001 and 0.003 mg/kg. The levels of total residue HEC 5725 were at 0.010 and 0.012 mg/kg. The residue levels lead to a (mean) processing factor of 1.0 for total residue HEC 5725.

The processing factors for malt sprouts and brewer's malt show the total residues of HEC 5725 remaining at the same level.

● Brewing:

Brewer's grain: In brewer's grain the residues of fluoxastrobin (HEC 5725 E-isomer) were below LOQ (<0.009 mg/kg), residues of HEC 5725 Z-isomer were at 0.001 and 0.003 mg/kg. The levels of total residue HEC 5725 were at 0.010 and 0.012 mg/kg for the two independent trials. The residue levels lead to a (mean) processing factor of 1.0 for total residue HEC 5725.

Hops draff: The levels of fluoxastrobin (HEC 5725 E-isomer) in hops draff were below LOQ (<0.009 mg/kg), levels of HEC 5725 Z-isomer were at 0.001 and 0.002 mg/kg. The levels of total residue HEC 5725 were at <0.01 and 0.011 mg/kg. The residue levels lead to a (mean) processing factor of <0.9 for the total residue HEC 5725.

Brewer's yeast and beer: In brewer´s yeast and beer, the levels of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and total residue HEC 5725 were below LOQ (<0.009, <0.001, and <0.01 mg/kg, respectively); leading to a (mean) processing factor of <0.9 for the total residue HEC 5725.

The results of the processing factors during the brewing process indicate a reduction in the total residue of HEC 5725 for the end product beer.

● Pearl barley production:

Pearl barley rub-off: For the two independent trials, the residues of fluoxastrobin (HEC 5725 E-isomer) in pearl barley rub-off were at 0.015 and 0.037 mg/kg, residues of HEC 5725 Z-isomer were at 0.006 and 0.015 mg/kg. The levels of total residue HEC 5725 were at 0.020 and 0.052 mg/kg. The residue levels lead to a mean processing factor of 2.8 for the total residue HEC 5725.

Pearl barley: In pearl barley the levels of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and total residues HEC 5725 were below LOQ (<0.009, <0.001, and <0.01 mg/kg, respectively); leading to a mean processing factor of <0.9 for the total residue HEC 5725.

These findings indicate that residues of the total residue HEC 5725 (sum of E-and Z-isomer) remain to a larger extent in pearl barley rub-off and can be removed from barley grain by cleaning and hulling, resulting in residues below LOQ in the end product pearl barley.

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The processing factors for the total residues of HEC 5725 are summarised below in Table 6.5.3- 1 and Table 6.5.3- 2. All trial data are summarised further below in Table 6.5.3- 4 and Table 6.5.3- 5 and in greater detail in the Tier 1 summary forms. Recovery data are reported in Table 6.5.3- 6.

Table 6.5.3- 1: Summary of the total residues of HEC 5725 (sum of E-and Z-isomer) in mg/kg and processing factors *(in italics and parentheses)* in barley RACs and processed products (processing into malt and beer)

Trial number	grain	malt sprouts	brewer's malt	brewer's grain	hops/draff	brewer's yeast	beer
13-3401-01	0.0105	0.011 *(1.0)*	0.010 *(1.0)*	0.010 *(1.0)*	<0.01 *(<1.0)*	<0.01 *(<1.0)*	0.01 *(<1.0)*
13-3401-02	0.014	0.012 *(0.9)*	0.012 *(0.9)*	0.012 *(0.9)*	0.011 *(0.8)*	<0.01 *(<0.7)*	<0.01 *(<0.7)*
Mean processing factors:		*(1.0)*	*(1.0)*	*(1.0)*	*(<0.9)*	*(<0.9)*	*(<0.9)*

< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodities are < LOQ, the processing factor is calculated as to be below the value calculated with the LOQ of the processed commodity

Table 6.5.3- 2: Summary of the total residues of HEC 5725 (sum of E-and Z-isomer) in mg/kg in barley RACs and processed products (processing into pearl barley) and processing factors *(in italics and parentheses)*

Trial number	grain	pearl barley rub-off	pearl barley
13-3401-01	0.010	0.020 *(2.0)*	<0.01 *(<1.0)*
13-3401-02	0.015	0.052 *(3.5)*	<0.01 *(<0.7)*
Mean processing factors:		*(2.8)*	*(<0.9)*

< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodities are < LOQ, the processing factor is calculated as to be below the value calculated with the LOQ of the processed commodity

Storage periods:

The sample material "grain, stored" served as RAC samples for the beer and pearl barley processing, which was done in fresh state. "Grain, stored" samples were taken in the field at the same time as the bulk samples for processing, stored and shipped under the same conditions as the samples for processing and deep frozen at ≤ -18°C at the very time when the processing started. As the processing processes proceeded aliquots of the individual processed fractions were deep-frozen on the same day they were generated during malting, brewing and pearl barley production. The maximum periods of deep-frozen storage are compiled in Table 6.5.3- 3. The storage periods are covered by the interval investigated in the storage stability studies.

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Table 6.5.3- 3: Maximum storage periods of barley grain (RAC) and processed commodities under deep-frozen conditions

Study number	Sample material	Maximum storage time [days]
13-3401	Grain, stored	270
	Beer	89
	Brewer's grain	122
	Brewer's malt	138
	Brewer's yeast	116
	Hops draff	124
	Malt sprouts	138
	Pearl barley	250
	Pearl barley rub off	250

Conclusions

In order to determine processing / transfer factors for the total residue HEC 5725 (sum of E-and Z-isomer) from barley grain in malt and beer as well as in pearl barley, two processing studies were conducted.

For malt sprouts and brewer's malt, mean processing factors were 1.0 for residues of HEC 5725 E-and Z-isomers.

The mean processing factors were 1.0 in brewer's grain and <0.9 in hops draff, brewer´s yeast and beer. These results indicate a reduction of residues for the end product beer during the brewing process.

The mean processing factor for pearl barley rub-off was 2.8 for residues of HEC 5725 E-and Z-isomers; the mean processing factor for pearl barley was <0.9. These findings indicate that residues of HEC 5725 remain to a larger extent in pearl barley rub-off and can be removed from barley grain by cleaning and hulling, resulting in residues below LOQ in the end product pearl barley.

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Diagram 6.5.3- 1: Industrial processing of barley grain to malt sprouts and brewer´s malt .

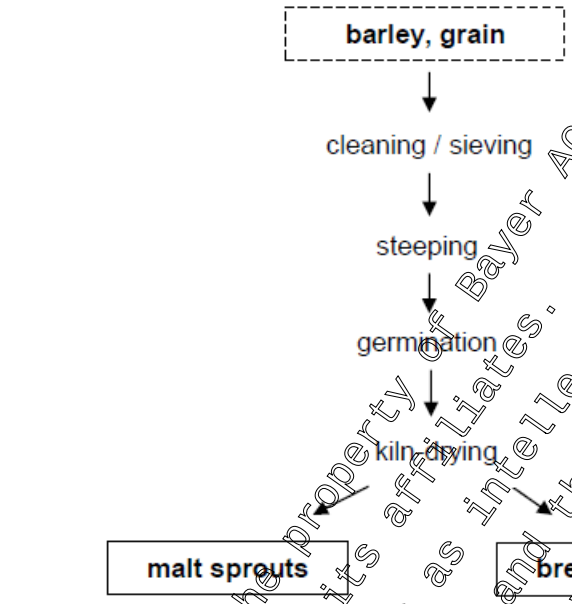

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Diagram 6.5.3- 2: Industrial processing of brewer´s malt into beer

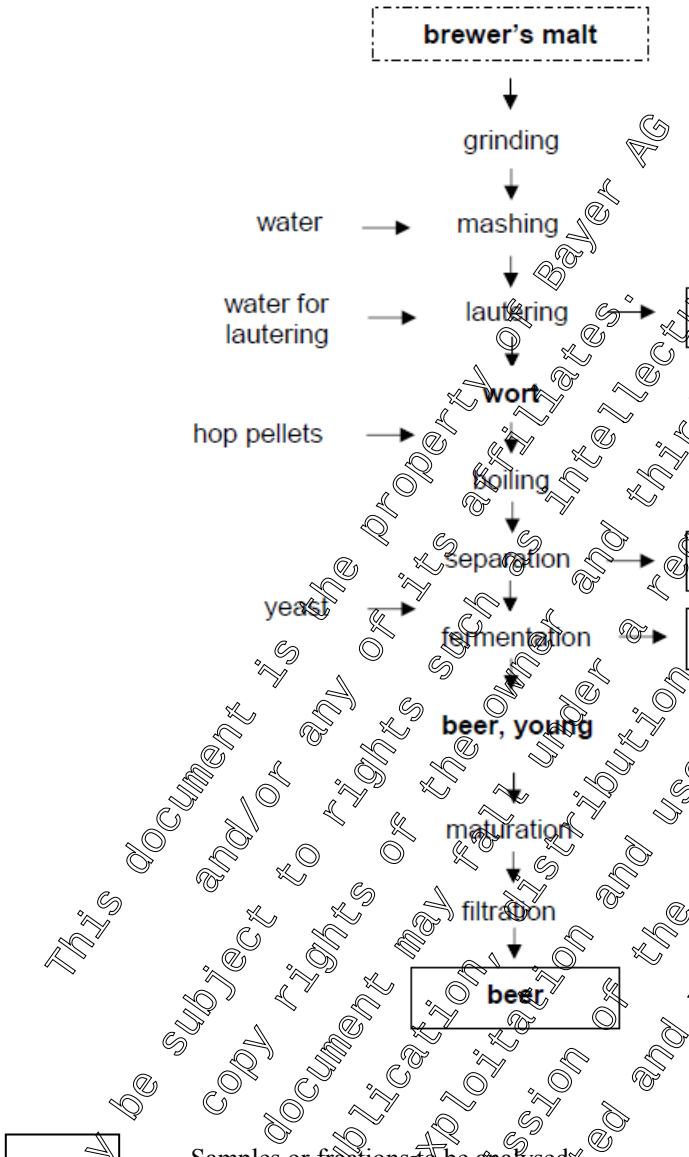

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Diagram 6.5.3- 3: Industrial processing of barley grain into pearl barley

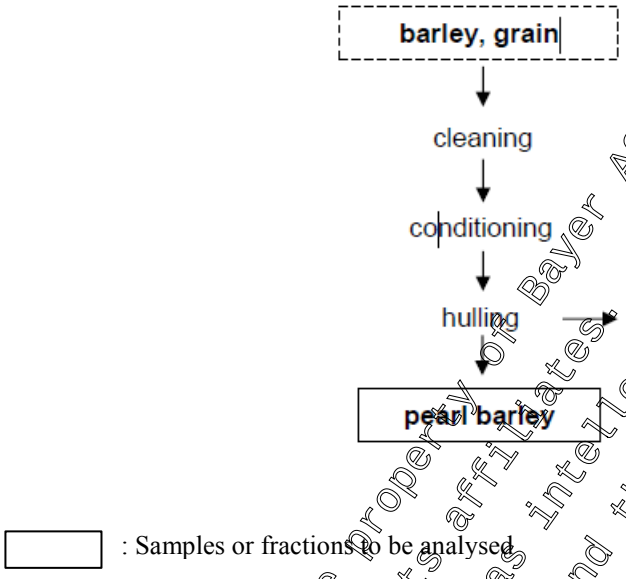

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Table 6.5.3- 4: Application scenario in residue processing trials conducted in/on barley after spraying with 'Fluoxastrobin + Prothioconazole EC 200' in northern and southern Europe

Study No. **(Trial No.)** **Country** **Location** **Region** **Year**	**Crop** **Variety**	**FL**	**Application**			
			No.	**kg/ha (a.s. fluoxastrobin)**	**kg/hl (a.s.)**	**GS**
13-3401 (13-3401-01) Germany D-[redacted] EU-N 2013	barley, spring Conchita	200 EC	2 1	0.375 0.375	0.125 0.125	32 61
13-3401 (13-3401-02) France F-[redacted] EU-S 2013	barley, spring Henley	200 EC	2 1	0.375 0.375	0.125 0.125	37 61

FL = formulation
GS = growth stage (BBCH-code) at treatment
EU-N = northern European residue region
EU-S = southern European residue region

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Table 6.5.3- 5: Results of residue processing trials conducted in/on barley after spraying with 'Fluoxastrobin + Prothioconazole EC 200' in Europe

Study No. (Trial No.) Country GLP	Portion analysed	DALT (days)	Residues (mg/kg) Fluoxastrobin (HEC 5725 E-Isomer)	HEC 5725 Z-Isomer	total residue of HEC 5725
13-3401 (13-3401-01) Germany GLP: yes	grain	69	<0.009	<0.001	<0.01
	grain, stored (RAC for beer production)	69	<0.009	0.001	0.010
		69	<0.009	0.002	0.011
		mean*	<0.009	0.002	0.0105
	malt processing				
	malt sprouts		<0.009	0.002	0.011
	brewer's malt		<0.009	0.001	0.010
	beer production				
	brewer's grain		<0.009	0.001	0.010
	hops draff		<0.009	0.001	<0.01 **
	brewer's yeast		<0.009	<0.001	<0.01
	beer		<0.009	<0.001	<0.01
	grain, stored (RAC for pearl barley)	69	<0.009	0.001	0.010
		69	<0.009	0.001	0.010
		mean*	<0.009	0.001	0.010
	pearl barley production				
	pearl barley rub-off		0.015	0.006	0.020 **
	pearl barley		<0.009	<0.001	<0.01
13-3401 (13-3401-02) France GLP: yes	grain	50	0.020	0.008	0.028
	grain, stored (RAC for beer production)	50	0.010	0.004	0.014
		50	0.010	0.003	0.014 **
		mean*	0.010	0.0035	0.014
	malt processing				
	malt sprouts		0.009	0.002	0.012 **
	brewer's malt		<0.009	0.003	0.012
	beer production				
	brewer's grain		<0.009	0.003	0.012
	hops draff		<0.009	0.002	0.011
	brewer's yeast		<0.009	<0.001	<0.01
	beer		<0.009	<0.001	<0.01
	grain, stored (RAC for pearl barley)	50	0.010	0.004	0.014
		50	0.012	0.004	0.016
		mean*	0.011	0.004	0.015
	pearl barley production				
	pearl barley rub-off		0.037	0.015	0.052
	pearl barley		<0.009	<0.001	<0.01

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RAC = raw agricultural commodity. The samples "grain, stored" served as RAC samples for the beer and pearl barley processing, which was done in fresh state. They are samples which were taken in the field at the same time as the samples for processing, stored and shipped under the same conditions as the samples for processing and deep frozen at ≤ -18°C at the very time when the processing started.

*mean = For the calculation of the processing factor the average of the residues in the two RAC samples was taken

** The total residue HEC 5725 was calculated by summing up the individual results (unrounded values) for HEC 5725 E- and Z-isomer. Therefore slight deviations may occur when the values as displayed in the table are used.

Table 6.5.3- 6: Recovery data for fluoxastrobin (HEC 5725 E- isomer) and HEC 5725 Z-isomer in barley grain and processed commodities
The LOQ is marked in bold

Study No. GLP Year	Crop	Portion analysed	Analyte	n	Fortification level (mg/kg)	Individual recoveries	Recovery (%) Min	Max	Mean	RSD
13-3401 GLP: yes 2013	barley	brewer´s grain*	fluoxastrobin (HEC 5725 E-Isomer)	2	**0.009**	87; 92	87	92	90	-
				2	0.09	87; 96	87	96	92	-
				2	0.90	88; 90	88	90	89	-
				6	overall		87	96	90	3.9
			HEC 5725 Z-Isomer	2	**0.001**	84; 88	84	88	86	-
				3	0.01	81; 92	81	92	87	-
				2	0.10	84; 90	84	90	87	-
				6	overall		81	92	87	4.8
		beer	fluoxastrobin (HEC 5725 E-Isomer)	3	**0.009**	96; 100; 101	96	101	99	2.7
				3	0.09	97; 97; 98	97	98	97	0.6
				6	overall		96	101	98	2.0
			HEC 5725 Z-Isomer	3	**0.001**	91; 92; 96	91	96	93	2.8
				3	0.01	100; 101; 102	100	102	101	1.0
				6	overall		91	102	97	4.9
		hops draff**	fluoxastrobin (HEC 5725 E-Isomer)	3	**0.009**	84; 88; 92	84	92	88	4.5
				3	0.90	87; 89; 89	87	89	88	1.3
				6	overall		84	92	88	3.0
			HEC 5725 Z-Isomer	3	**0.001**	85; 87; 93	85	93	88	4.7
				3	0.10	86; 87; 91	86	91	88	3.0
				6	overall		85	93	88	3.5

* A full set of validation recoveries on cereal grain has been generated during development of method 00649/M003. These recoveries can be considered to also validate the residue determination in the sample materials brewer's grain, brewer´s malt, malt sprouts, pearl barley and stored grain.

** The sample material hops draff is considered to be representative for sample material brewer´s yeast.

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Summary of residue data and processing factors from all barley processing studies

The following table compiles residue data and processing factors from study RA-3024/99 ([redacted]; 2001; M-089502-01-1), which was peer reviewed for Annex I inclusion, and the new study 13-3401. For easy reference the old and new data are both provided in the Tier I summary forms.

According to the OECD guideline 508 the median processing factor was calculated - in addition to the mean value - for commodities where more than 2 individual processing factors were derived.

It might be considered to disregard the processing factors derived from the two trials from study RA-3024/99 in those cases where residues in the processed commodities were less than the LOQ (highlighted in grey) because the LOQ was higher than the level of residues determined in the RAC and thus the processing factor is of limited value.

Table 6.5.3- 7: Summary of the total residues of HEC 5725 (sum of E- and Z-isomer) in mg/kg in barley RACs and processed products and processing factors *(in italics and parentheses)* (processing into malt and beer)

Trial number	Grain (stored)	malt sprouts	brewer's malt	brewer's grain	hops draff	brewer's yeast	beer
RA-3024/99 R 1999 0117/8	0.03	*<0.05/0.05* *(1.7)*	*<0.05/<0.05* *(1.7)*	*<0.05/0.05* *(1.7)*	*<0.05* *(1.7)*	*<0.05* *(<1.7)*	*<0.05* *(<1.7)*
RA-3024/99 R 1999 0118/6	0.04	*0.06* *(1.5)*	*0.05* *(1.3)*	*0.05* *(1.3)*	*<0.05* *(<1.3)*	*<0.05* *(<1.3)*	*<0.05* *(<1.3)*
13-3401-01	0.0105	0.011 *(1.0)*	0.010 *(1.0)*	0.010 *(1.0)*	0.01 *(1.0)*	<0.01 *(<1.0)*	<0.01 *(<1.0)*
13-3401-02	0.014	0.012 *(0.9)*	0.012 *(0.9)*	0.012 *(0.9)*	0.011 *(0.8)*	<0.01 *(<0.7)*	<0.01 *(<0.7)*
Mean processing factors: *Median processing factors:*		*(1.3)* *(1.3)*	*(1.3)* *(1.2)*	*(1.2)* *(1.2)*	*(1.2)* *(1.2)*	*(<1.2)* *(<1.2)*	*(<1.2)* *(<1.2)*
Mean processing factors: *Median processing factors:*		*n=3 (1.1)* *n=3 (1.0)*	*n=3 (1.1)* *n=3 (1.0)*	*n=3 (1.1)* *n=3 (1.0)*	*n=2 (0.9)*	*n=2 (<0.9)*	*n=2 (<0.9)*

< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodities are < LOQ, the processing factor is calculated as to be below the value calculated with the LOQ of the processed commodity

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Table 6.5.3- 8: Summary of the total residues of HEC 5725 (sum of E-and Z-isomers) in mg/kg and processing factors *(in italics and parentheses)* in barley RACs and processed products (processing into pearl barley)

Trial number	Grain (stored)	pearl barley rub-off	pearl barley
RA-3024/99 R 1999 0117/8	0.03	*0.085* *(2.8)*	*0.05* *(<1.7)*
RA-3024/99 R 1999 0118/6	0.04	*0.115* *(2.9)*	*<0.05* *(<1.3)*
13-3401-01	0.010	0.020 *(2.0)*	0.01 *(<1.0)*
13-3401-02	0.015	0.052 *(3.5)*	<0.01 *(<0.7)*
Mean processing factors: *Median processing factors:*		*(2.8)* *(2.9)*	*(<1.2)* *(<1.2)*
Mean processing factors:			*n=2 (<0.9)*

< value: in case the residue level in the RAC is < LOQ but residues in the processed commodities are < LOQ, the processing factor is calculated as to be below the value calculated with the LOQ of the processed commodity

Processing of wheat

Report: KCA 6.5.3/03 [redacted]; 2004; M-104410-02-1
Title: Determination of residues of fluoxastrobin (HEC 5725) and tebuconazole (HWG 1608) in/on winter wheat after spray application of HEC 5725 & HWG 1608 150 EC in the field in [redacted], Northern France, Great Britain and Germany
Report No.: RA-2060/00
Document No.: M-104410-02-1
Guideline(s): Directive 91/414/EEC, residues in or on treated products, food and feed; July 15, 1991, Annex II, part A, point 6 and Annex III, part A, point 8, Residues in or on Treated Products, Food and Feed
Guideline deviation(s): none
GLP/GEP: **yes**

Report: [redacted]b; [redacted]; 2004; M-104435-02-1
Title: Determination of residues of fluoxastrobin (HEC 5725) in processing products from winter wheat (grain, flour, bran, white bread, whole-meal flour, whole-meal bread, semolina, semolina bran, wheat germs) after spray application of HEC 5725..
Report No.: RA-3060/00
Document No.: M-104435-02-1
Guideline(s): 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
Guideline deviation(s): none
GLP/GEP: **yes**

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Materials and Methods

In order to determine the magnitude of the residues of fluoxastrobin in/on processed fractions of wheat, two trials were conducted in the northern European residue region, in [redacted] and Germany. Residues of fluoxastrobin (HEC 5725 E-Isomer), HEC 5725 Z-isomer, and total residue HEC 5725 were determined in unprocessed wheat grains and then in the processing products flour and bread (white and whole meal), semolina, germ, and by-products bran and semolina bran.

Fluoxastrobin + Tebuconazole EC 150 (called HEC 5725 & HWG 1608 in the report) was sprayed twice to winter wheat at application rates of 150 g fluoxastrobin/ha and a water volume of 300 L/ha. The last application was done at BBCH 65-69 or 81, the first application was done at BBCH 47. Wheat (grain) samples to be processed were sampled at harvest, 61 or 36 days after the last application. For each trial, the collected grain sample was divided into two portions which were processed independently.

After processing (described below), residue analysis was performed according to methods 00649 and 00604. Both methods were reported in the initial Annex II dossier and considered acceptable in the EU peer review (please cf. to baseline dossier).

Method 00649 ([redacted]; 2007; M-197093-01-1): Residues of HEC 5725 in wheat grain and the processing products flour, bran, semolina, semolina bran, whole-meal flour and germs were determined by HPLC-MS/MS according to method 00649 after extraction and clean-up by solid-phase-extraction on a Bond-Elut ENV-cartridge. The analytical method allows separate determination of HEC 5725 E- and Z-isomers. The total residue of HEC 5725 was calculated as the sum of both isomers. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, for wheat grain, (semolina) bran and flour was set at 0.018 mg/kg for HEC5725 E-isomer and 0.002 mg/kg for HEC 5725 Z-isomer (corresponding to 0.02 mg/kg for the calculated total residue of HEC 5725). The LOQ for wheat germs was set at 0.045 mg/kg for HEC5725 E-isomer and 0.005 mg/kg for HEC 5725 Z-isomer (corresponding to 0.05 mg/kg for the calculated total residue of HEC 5725).

Method 00604 ([redacted]; 2001; M-055515-01-1): Residues of HEC 5725 in white bread and whole-meal bread were determined by HPLC-MS/MS according to method 00604 after accelerated solvent extraction and clean-up by solid-phase-extraction on a Bond-Elut ENV-cartridge. The analytical method was designed to measure HEC 5725 E- and Z-isomer separately; in addition the total residue of HEC 5725 was calculated as the sum of both isomers. The Limit of Quantitation (LOQ) for white bread and whole-meal bread was 0.045 mg/kg for HEC 5725 E-isomer and 0.005 mg/kg for HEC 5725 Z-isomer (corresponding to a theoretical LOQ of 0.05 mg/kg for the calculated total residue of HEC 5725).

Wheat processing:

The processing of wheat samples into the processed fractions semolina, semolina bran, bran; white flour, white bread, whole meal, wholemeal bread, and wheat germ was performed to simulate industrial procedures at a laboratory scale

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Cleaning and conditioning of wheat grain:
Frozen field samples for processing were defrosted and cleaned with the "Labofix" purifier. After cleaning the grain was conditioned to a moisture content of approx. 15-16%. For reaching moisture content in this range, the wheat grains of trial R 2000 0269/6 were dried in a dry box at 40°C for 3 hours. The wheat grains of trial R 2000 0273/4 were moistened in a special box for 6 hours.

Milling of flour (type 550) and baking of white bread:
The grain was milled to flour and bran in a "Bühler Mahlautomat". Intermediate products were semolina and semolina bran. Samples of bran, flour, semolina and semolina bran were collected.

For baking of white bread, flour type 550, yeast (5%), salt (1.5%), and water were mixed. The resulting dough was kneaded for 1 min. After kneading the dough rested for 20 min. The dough was further moved for 10 min followed by a second rest of 50 minutes in a form. The temperature during the rest times was 31-33 °C. The baking was conducted at 230°C for 30 min. Afterwards, a sample of white bread was taken.

The process is illustrated in flowDiagram 6.5.3- 4.

Milling of whole meal and baking of whole-meal bread:
For the generation of whole meal and whole-meal bread, the whole grain was ground by crashing with baffle plates. After impact grinding (milling), whole meal flour was sampled.

For baking, whole meal flour, yeast (5%), salt (1.5%), and water were mixed. The resulting dough was kneaded for 1 minute. After kneading the dough rested for 20 min. The dough was further moved for 10 min. and a second rest of 50 minutes in a form followed. The temperature during the total rest time was 31-33 °C. The baking was conducted at 210 °C for 50 minutes. Afterwards, a sample of whole meal bread was taken.

The process is illustrated in flow Diagram 6.5.3- 5.

Production of wheat germ:
Wheat grain was broken to "bruised grain" in a special mill. The fraction 400-1000 µm was collected, the fraction above 1000 -1250 µm was broken once more. This milling/sieving process was performed a total of three times. The fraction obtained below 400 µm was completely excluded from further processing.

The fraction 400-1000 µm, a mixture of bran, semolina and germs, was put in a special separator ("Leichtgewichtsausleser"). Due to the different specific weights of the bran, semolina, and germ, the semolina/germ mixture was separated from most parts of the bran.

The semolina/germ mixture was milled to flour and small wheat germ discs in a mill with a pair of smooth rollers. The wheat germ with parts of bran was then manually sieved and sorted to remove the remaining parts of bran. A sample of wheat germ was taken.

The process is illustrated in flow Diagram 6.5.3- 6.

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The analytical methods 00649 and 00604 were validated prior to analysis by running a set of recoveries at the LOQ and the 10-fold LOQ (for bread within method 00604, for grain and wheat sprouts within method 00649). In addition, during analysis of the samples pre-validation and concurrent recovery experiments were performed by spiking control samples with HEC 5725 (E- and Z-Isomer).
In wheat grain, germ and bread, recovery samples were spiked at levels of the respective LOQ for HEC 5725 E- and Z-Isomer within the conduct of the study.
In bran / flour / semolina / semolina bran, recovery samples for fluoxastrobin (HEC 5725 E-Isomer) were spiked at levels of 0.018 mg/kg (=LOQ), 0.045 mg/kg and 0.45 mg/kg.
For HEC 5725 Z-Isomer samples were spiked at levels of 0.002 mg/kg (=LOQ), 0.005 mg/kg and 0.05 mg/kg in bran / flour / semolina / semolina bran.

A tabular summary of the recovery values is presented below in Table 6.5.3- 13.

The residue values of harvested wheat grain at BBCH growth stage 89/92 were used for the calculation of processing factors. In this summary the processing factors for the total residue HEC 5725 (sum of E-and Z-isomer) are reported. It is anticipated that both components will form the residue definition for enforcement and risk assessment in the future according to the residue definition proposed in EFSA documents (see above).

For most processing fractions no processing factors could be calculated, since residues in both the raw commodity and in the processed product were below LOQ. Only for bran and semolina bran processing factors could be calculated.

● Grain, semolina, white flour, whole meal flour:

The residue levels of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and total residue HEC 5725 were below LOQ (<0.018, <0.002, and <0.02 mg/kg, respectively); no processing factors were calculated.

● White bread, whole meal bread, germs:

The residue levels of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and total residue HEC 5725 were below LOQ (<0.045, <0.005, and <0.05 mg/kg, respectively); no processing factors were calculated.

● Bran and semolina bran:

The residues of fluoxastrobin (HEC 5725 E-isomer) in bran were at < 0.018 or 0.04 mg/kg, residues of HEC 5725 Z-isomer ranged between 0.002 and 0.007 mg/kg. The levels of total residue HEC 5725 were at 0.02 or 0.05 mg/kg. The residue levels lead to a mean processing factor of > 1.75 for total residue HEC 5725.

The residues of fluoxastrobin (HEC 5725 E-isomer) in semolina bran were at < 0.018 and 0.03 / 0.04 mg/kg, residues of HEC 5725 Z-isomer were at < 0.002 or 0.003 mg/kg. The levels of total residue HEC 5725 were at < 0.02 or 0.04 mg/kg. The residue levels lead to a mean processing factor of >2.0 for total residue HEC 5725.

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The processing factors for the total residues of HEC 5725 are summarised below in Table 6.5.3- 9. All trial data are summarised further below in Table 6.5.3- 11and Table 6.5.3- 12and in greater detail in the Tier 1 summary forms. Recovery data are reported in Table 6.5.3- 13.

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Table 6.5.3- 9: Summary of the total residues of HEC 5725 (sum of E-and Z-isomer) in mg/kg in wheat RACs and processed fractions and processing factors *(in italics and parentheses)* in wheat processed products

Trial number	wheat grain (RAC)	Sub sample	bran	semoli-na	semolina bran	flour	white bread	whole-meal flour	whole-meal bread	wheat germ
RA-3060/00 R 2000 0269/6 [redacted]	<0.02	A	0.02 *(>1.0)*	<0.02 *(n.c.)*	<0.02 *(n.c.)*	<0.02 *(n.c.)*	<0.05 *(n.c.)*	<0.02 *(n.c.))*	<0.05 *(n.c.)*	<0.05 *(n.c.)*
		B	0.02 *(>1.0)*	<0.02 *(n.c.)*	<0.02 *(n.c.)*	<0.02 *(n.c.)*	<0.05 *(n.c.)*	<0.02 *(n.c.))*	<0.05 *(n.c.)*	<0.05 *(n.c.)*
RA-3060/00 R 2000 0273/4 Germany	<0.02	A	0.05 *(>2.5)*	<0.02 *(n.c.)*	0.04 *(>2.0)*	<0.02 *(n.c.)*	<0.05 *(n.c.)*	<0.02 *(n.c.)*	<0.05 *(n.c.)*	<0.05 *(n.c.)*
		B	0.05 *(>2.5)*	<0.02 *(n.c.)*	0.04 *(>2.0)*	<0.02 *(n.c.)*	<0.05 *(n.c.)*	<0.02 *(n.c.)*	<0.05 *(n.c.)*	<0.05 *(n.c.)*
Mean processing factors:			*(>1.75)*	*(n.c.)*	*(>2.0)*	*(n.c.)*	*(n.c.)*	*(n.c.)*	*(n.c.)*	*(n.c.)*

n.c.: Not calculated . No processing factor can be calculated (residues < LOQ in RAC and in processed commodity)

> value: in case the residue level in the RAC is < LOQ but residues in the processed commodities are ≥ LOQ, the processing factor is calculated as to be above the value calculated with the LOQ of the RAC

Storage periods: The maximum storage periods of deep-frozen treated samples was up to 377 days for fluoxastrobin (HEC 5725 E-isomer) and HEC 5725 Z-isomer and is covered by the interval investigated in the storage stability studies.

Table 6.5.3- 10: Maximum storage periods of wheat grain and processed commodities

Study number	Sample material	Maximum storage time [days]
RA-3060/00	Grain	377
	Bran	272
	Whole meal bread	265
	Flour	272
	Semolina bran	272
	Whole meal flour	272
	Semolina	272
	White bread	265
	Wheat germs	266

Conclusions

In order to determine processing transfer factors for the total residue HEC 5725 (sum of E-and Z-isomer) from wheat grain to bran, semolina bran, semolina, white flour, white bread, whole-meal flour, whole-meal bread, and wheat germ two processing studies were conducted. Per study, two processing procedures were performed independently with two sub-samples.

In both trials, wheat was treated twice at 0.150 kg as/ha which corresponds to the application rate of the new critical GAP for the product 'Fluoxastrobin + Prothioconazole EC 200' in the northern climatic region.

For most processing fractions no processing factors could be calculated, since residues in both the raw commodity and in the processed product were below LOQ. Only for bran and semolina bran processing factors were calculated using the LOQ of the raw agricultural commodity.

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Indication of concentration was observed in bran and semolina bran, however at very low absolute residue levels.

Diagram 6.5.3- 4: Milling of white flour (type 550) and baking of white bread

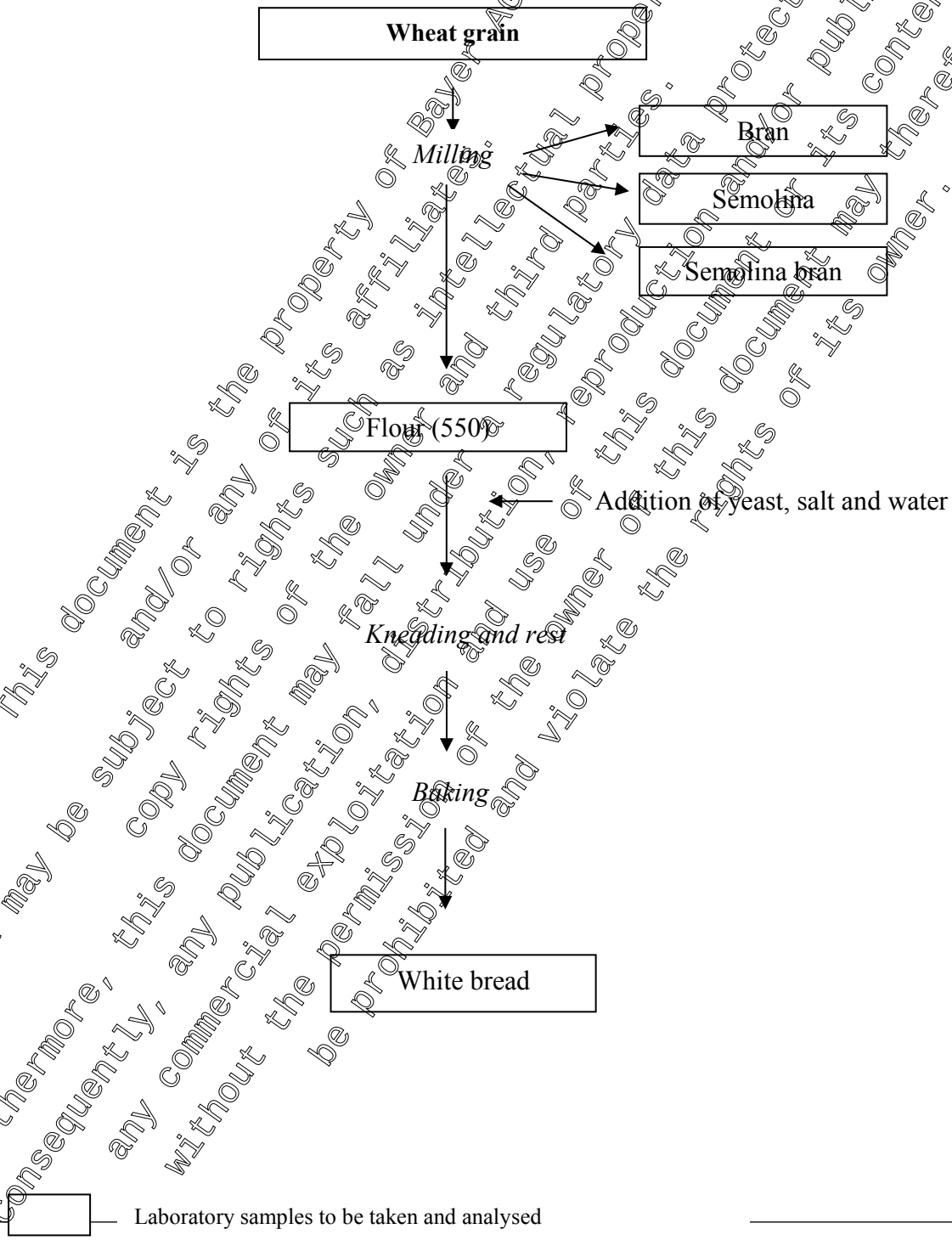

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Diagram 6.5.3- 5: Milling of whole-meal flour and baking of whole-meal bread

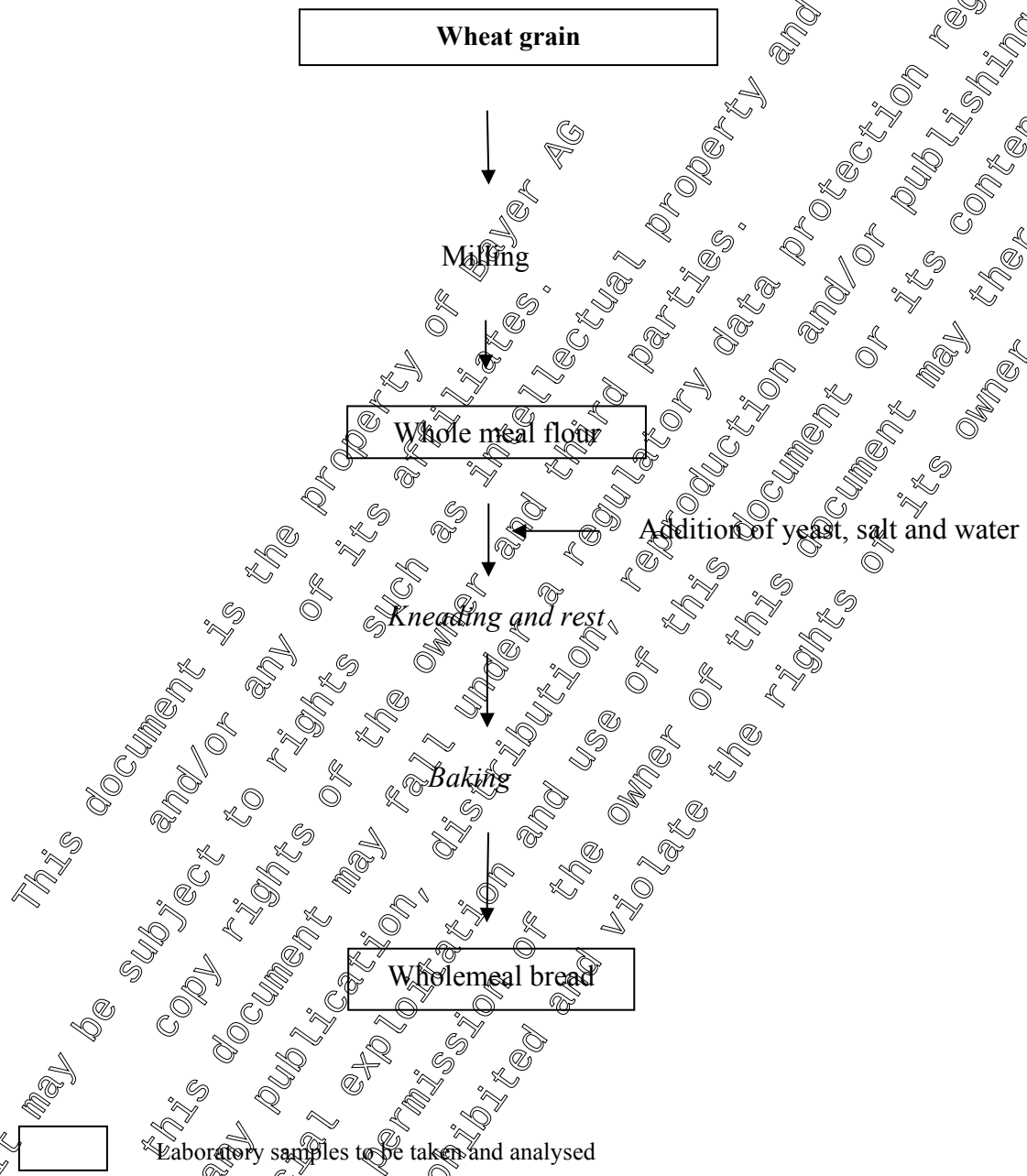

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Diagram 6.5.3- 6: Production of wheat germ

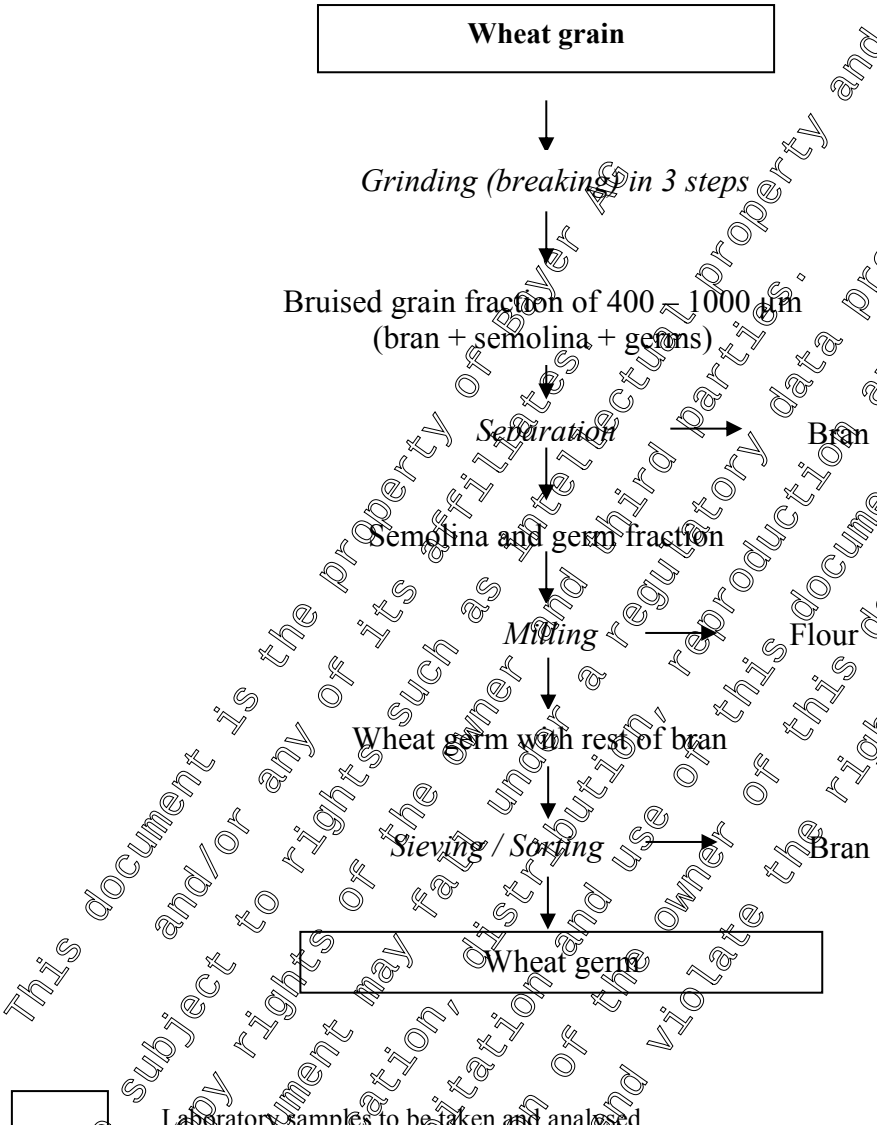

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Table 6.5.3- 11: Application data in residue processing trials conducted in/on wheat after spraying with 'Fluoxastrobin + Tebuconazole EC 150' in northern Europe

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	Application No.	Fluoa-strobin [kg/ha] (a.s.)	Fluoa-strobin [kg/hl] (a.s.)	GS (BBCH)	PHI (days)
RA-2060/00 *RA-3060/00* (R 2000 0269/6) [redacted] EU-N 2000	wheat Tarso (winter wheat)	150 EC	2 2	0.15 0.15	0.050 0.050	47 65-69	61
RA-2060/00 *RA-3060/00* (R 2000 0273/4) Germany [redacted] EU-N 2000	wheat Flair (winter wheat)	150 EC	2 2	0.15 0.15	0.050 0.050	47 81	36

FL = formulation GS = growth stage (BBCH-code) at treatment
EU-N = northern European residue region

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Table 6.5.3- 12: Results of residue processing trials conducted in/on wheat after spraying with 'Fluoxastrobin + Tebuconazole EC 150' in northern Europe

Study No. (Trial No.) Country GLP	Portion analysed	DALT (days)	Residues (mg/kg) Fluoxastrobin (HEC 5725 E-Isomer)	HEC 5725 Z-Isomer	total residue of HEC 5725
RA-3060/00 (R 2000 0269/6) [redacted] GLP: yes	grain (RAC)	61	<0.018	<0.002	<0.02
	milling of white flour and baking of white bread				
	bran		<0.018	0.003	0.02
	bran		<0.018	0.002	0.02
	semolina		<0.018	<0.002	<0.02
	semolina		<0.018	<0.002	<0.02
	semolina bran		<0.018	<0.002	<0.02
	semolina bran		<0.018	<0.002	<0.02
	flour		<0.018	<0.002	<0.02
	flour		<0.018	<0.002	<0.02
	white bread		<0.045	<0.005	<0.05
	white bread		<0.045	<0.005	<0.05
	milling of wholemeal and baking of wholemeal bread				
	whole meal flour		<0.018	<0.002	<0.02
	whole meal flour		<0.018	<0.002	<0.02
	whole-meal bread		<0.045	<0.005	<0.05
	whole-meal bread		<0.045	<0.005	<0.05
	germ production				
	wheat germ		<0.045	<0.005	<0.05
	wheat germ		<0.045	<0.005	<0.05
RA-3060/00 (R 2000 0273/4) Germany GLP: yes	grain (RAC)	36	<0.018	<0.002	<0.02
	milling of white flour and baking of white bread				
	bran		0.04	0.007	0.05
	bran		0.04	0.006	0.05
	semolina		<0.018	<0.002	<0.02
	semolina		<0.018	<0.002	<0.02
	semolina bran		0.03	0.003	0.04
	semolina bran		0.04	0.003	0.04
	flour		<0.018	<0.002	<0.02
	flour		<0.018	<0.002	<0.02
	white bread		<0.045	<0.005	<0.05
	white bread		<0.045	<0.005	<0.05
	milling of wholemeal and baking of wholemeal bread				
	whole meal flour		<0.018	<0.002	<0.02
	whole meal flour		<0.018	<0.002	<0.02
	whole-meal bread		<0.045	<0.005	<0.05
	whole-meal bread		<0.045	<0.005	<0.05
	germ production				
	wheat germ		<0.045	<0.005	<0.05
	wheat germ		<0.045	<0.005	<0.05

DALT = days after last treatment; RAC = raw agricultural commodity

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Table 6.5.3- 13: Recovery data for fluoxastrobin (HEC 5725 E -Isomer) and HEC 5725 Z-isomer in wheat grain and processed commodities

Study No.. GLP Year	Crop	Portion analysed	Analyte	n	Forti-fication level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2060/00 *RA-3060/00* GLP: yes 2000	wheat	grain	fluoxastrobin (HEC 5725 E-Isomer)	6	0.018	91; 92; 92; 95; 97; 103	91	103	95	4.8
			HEC 5725 Z-Isomer	6	0.002	90; 93; 86; 93; 96; 97	90	97	93	4.4
	wheat	germs	fluoxastrobin (HEC 5725 E-Isomer)	4	0.045	96; 99; 101; 101	96	101	99	2.4
			HEC 5725 Z-Isomer	4	0.008	100; 96; 108; 97	96	108	100	5.4
	wheat	bran, flour, whole-meal flour, semolina bran, semolina	fluoxastrobin (HEC 5725 E-Isomer)	3	0.018	92; 90; 94	90	94	92	2.2
				2	0.045	98; 91	91	98	95	-
				2	0.45	93; 88	88	93	91	-
				7	overall		88	98	92	3.5
			HEC 5725 Z-Isomer	3	0.002	100; 87; 103	87	103	97	8.8
				2	0.005	91; 87	87	91	89	-
				2	0.05	97; 84	84	97	91	-
				7	overall		84	103	93	7.9
	wheat	white bread, whole-meal bread	fluoxastrobin (HEC 5725 E-Isomer)	4	0.045	84; 87; 90; 86	84	90	87	2.9
			HEC 5725 Z-Isomer	4	0.005	83; 89; 89; 81	81	89	86	4.8

Fluoxastrobin use in/on onion

The use of fluoxastrobin in onion according to the representative use does not result in significant residues (i.e. > 0.1 mg/kg) of fluoxastrobin in onion bulb at harvest. Furthermore consumer intakes are low and consumption of the ADI or ARfD above 10% can be excluded. Therefore, supplementary processing studies are not considered necessary.

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CA 6.6 Residues in rotational crops

CA 6.6.1 Metabolism in rotational crops

Confined rotational crops studies were conducted with [chlorophenyl-UL-^{14}C]fluoxastrobin ([redacted],; 2002; M-091191-02-1), [pyrimidine-2-^{14}C]fluoxastrobin ([redacted]; 2001; M-091162-01-1) and [methoxyiminotolyl-ring-UL-^{14}C]fluoxastrobin ([redacted]; 2001; M-090320-01-1). These studies were peer reviewed at EU level (see also introduction to chapter CA 6.2 and Table 6.2-2). A short summary is given below (see also overall conclusions on plant metabolism on page 85).

Following application at 683 - 846 g/ha on bare soil, the total radioactive residues in the plant matrices decreased from the first rotation planted 30 days to the third rotation planted 80 - 328 days after application. Maximum total radioactive residues were found in wheat straw at the first rotation at approx. 1.4 - 2.4 mg/kg falling to 0.21 - 0.75 mg/kg in the third rotation. Total radioactive residues in wheat grain and the matrices of Swiss chard and turnips were below 0.3 mg/kg.

After soil application and planting of the rotational crops, parent compound (sum of fluoxastrobin and its Z-isomer) was the predominant component of the residues in the plant matrices with up to 73.6% of the TRR in wheat, up to 32.6% of the TRR in Swiss chard and up to 50.8% of the TRR in turnips. It was the major component in the plant matrices of the first rotation and in most matrices of the second and third rotation. In turnip leaves of the second and third rotation and in Swiss chard of the second rotation it occurred at amounts comparable to the major identified metabolite. In the individual plant matrices of the three rotations the major identified metabolite represented up to 26.4% of the TRR. The residue levels of the metabolites were low and did not exceed 0.05 mg a.s. equiv./kg in the edible matrices wheat grain, leaves of Swiss chard and turnip roots.

CA 6.6.2 Magnitude of residues in rotational crops

Evaluation in the EU peer review (EFSA conclusion, 2007) and EFSA Reasoned Opinion on existing MRLs (2012)

Occurrence of fluoxastrobin residues in rotational crops was evaluated in the EU peer review. It was concluded that except for wheat straw fluoxastrobin related residues are unlikely to occur in succeeding crops. However, as fluoxastrobin is for use on cereals as a primary crop, it is unlikely that residues of fluoxastrobin in the soil would contribute significantly to the residue in following cereal crops treated with fluoxastrobin since:

- the metabolism studies were conducted at an overdose factor of 2N (relative to the evaluated GAP).
- in the rotational crop metabolism studies, fluoxastrobin was applied to bare soil, thus any reduction due to interception is not reflected in the residue levels found.
- the GAP evaluated in the EU peer review involved 3 applications and not one as in the rotational crop metabolism study. The critical field GAP involved seed treatment (12 months before the following crop planted) followed by two spray applications (5 months and 3 months before the following crop planted) which would allow for degradation during the intervals.

In the list endpoints it was concluded that no data on field trials are required.

In the EFSA Reasoned Opinion (2012), it was concluded *'that residues of fluoxastrobin resulting from soil uptake will not exceed the LOQ of 0.01 mg/kg, except in wheat straw.'*

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In wheat straw, higher amounts of fluoxastrobin (0.06 – 1.2 mg/kg) and metabolites (up to 0.51 mg/kg) were quantified in the above study [rotational crop metabolism study]. *Nevertheless, considering the overdosing factor and potential foliar interception, levels of parent compound are expected to be significantly lower than those resulting from the primary use.*
Furthermore, non-rat metabolites are neither considered for enforcement purpose nor relevant for risk assessment but further information about the toxicity of metabolite M82 found in straw in primary crops is anyhow required …. It is concluded that specific plant-back restrictions related to the use of fluoxastrobin are currently not required, provided that fluoxastrobin is applied in compliance with the GAPs reported in Appendix A [up to 2 x 200 g as/ha for cereals and 4 x 125 g as/ha for onions] '.

Thus, the reasoning applies all the more as the GAPs of the representative uses supported in the present dossier involve significantly lower application rates and/or less applications compared to those evaluated in the EFSA documents.

Additional information on the straw metabolites non common to the rat was submitted by the notifier as confirmatory data and evaluated by the RMS (UK CRD) in Addendum 8 to the DAR (January 2011). This was circulated to Member States and the EFSA for comments which were incorporated into a reporting table by the RMS in January 2012. As a result of these comments the evaluation has been updated to reflect both the northern and southern EU GAP involving higher application rates at that time. Changes from the January 2011 version are included in the revised and updated version of the DAR from April 2012. Information on the toxicological profile of metabolite M82 (2-chlorophenol) may be found in CA 5.8.1. The other straw metabolites are considered as sufficiently addressed by the arguments and data provided earlier and resulting in Addendum 8 to the DAR, 2012. This conclusion is supported by the EFSA reasoned opinion on existing MRLs (EFSA Journal 2012:10(12):3012) where reference is made exclusively to M82 and M84 (glucoside of M82).

Estimate of fluoxastrobin residues taken up from soil after repeated use of fluoxastrobin containing products
In the Inclusion Directive 2008/44/EC Part B and in the Review Report SANCO/3921/07 (2012) it is noted that Member States should pay particular attention to the risk of accumulation in the soil surface, if the substance is used in succeeding crops in rotation.

In the following paragraphs calculations are provided addressing the question whether the potential for accumulation may result in fluoxastrobin residues taken up from soil by replanted crops if fluoxastrobin containing products are used year on year. The calculations presented below provide an estimate of the contribution of the active substance present in soil due to previous applications. The overall soil loading arising from the maximum seasonal rate and supplemented by residues in soil from previous treatments are compared to the application rates used in the rotational crop metabolism studies.

In order to estimate the concentration in soil and the contribution of residues that might be present in soil from previous treatments predicted environmental concentrations (PEC) in soil – as presented in CP 9.1.3 ([redacted]; 2015; M-537905-01-1) are used for the calculation. Input parameters for the PEC$_{soil}$ calculation were derived from field soil dissipation studies. The behaviour of fluoxastrobin was investigated in a terrestrial field soil dissipation study designed to determine the dissipation under representative European field conditions on cropped and uncropped plots ([redacted]; 2001; M-136670-01-1), cf. baseline dossier CA 7.1.2.2.1.

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PEC_{soil} calculations were performed for (but not limited to) the following scenarios reflecting the GAPs of the representative uses in/on cereals and onions per climatic residue zone. The highest application rates per FOCUS crop are highlighted in bold.

Table 6.6.2- 1: Use pattern in small grain cereals and onions for which predicted environmental concentrations in soil were calculated

Crop Region	FOCUS crop	Rate [g a.s./ha]	Interval [days]	Plant interception [%]	BBCH stage	Amount reaching soil [g a.s./ha]
Wheat; EU-N	**cereals**	**2×150**	**14**	**2×80**	**30 - 69**	2×30.0
Barley; EU-N	cereals	2×125	14	2×80	30 - 61	2×25.0
Wheat; EU-S	cereals	2×87.5	14	2×80	30 - 69	2×17.5
Barley; EU-S	cereals	2×75	14	2×80	30 - 61	2×15.0
Onions; EU-S	**onions**	**2×125**	**10**	**2×10**	**15 - 47**	2×112.5

EU-N = northern climatic zone; EU-S = southern climatic zone

PEC_{soil} calculations were conducted for the maximum soil concentration after application of the maximum seasonal rate and for the long-term plateau concentration after repeated application.

The degradation of the active substance fluoxastrobin was predicted assuming a bi-phasic DFOP (double first-order in parallel) model from the data of field degradation trials. As input parameter for the PEC_{soil} calculation the longest DT_{50}/DT_{90} values (non-normalised) reflecting a worst case scenario were derived from 8 field dissipation trials. More detailed information on input parameters may be found in the original report [redacted] 2015; M-537905-01-1) . A bulk density of 1.5 kg/L and a soil mixing depth of 5 cm was used as recommended (European Commission, 1995, 2000; FOCUS, 1996) for calculation of the soil concentration after application of the maximum seasonal rate. Crop interception was taken into account according to the BBCH growth stage (EFSA guidance document to obtain DegT50 values, EFSA Journal 2014;12(5):3662).

The accumulation potential of fluoxastrobin after long term use was also assessed considering a soil mixing depth of 10 and 20 cm. Generally, for long-term assessments the substance distribution in soil for annual crops with tillage should be calculated for a 20 cm soil layer. However, when the soil loading is translated into an application rate in kg per hectare the soil layer taken into account for the calculation is of no relevance since the absolute amount of the compound in soil remains unchanged (cf.Table 6.6.2- 2). Soil concentrations after long-term use of the compound with applications in succeeding years is calculated taking into account some carry-over of residues from one year to the next. This results in the typical saw-tooth soil concentration curve, with annual peaks at the time of application, and with dissipation between the annual peaks. The lower limit of the saw-tooth curve converges to a constant value (plateau) when the equilibrium between application and dissipation is approached after long-term use of the compound.

The following characteristic parameters are calculated in [redacted]; 2015; M-537905-01-1) to address a scenario with applications at the maximum annual rate in succeeding years:
- long-term background ($PEC_{plateau}$): maximum of the lower saw tooth curve, which can be considered as background concentration after multiple year use. For fluoxastrobin, the plateau is reached after approximately 3 – 4 years.

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- PEC $_{soil, total}$: the maximum residue of one year (distributed in 5 cm) is added to the long-term background concentration ($PEC_{plateau}$) in a 20 cm soil layer to take into account a conservative shallow distribution just after an annual application.
The calculation for a 20 cm soil layer is appropriate to describe the situation for annual crops with tillage.

In the table below calculations are made for the highest application rates for cereals (and covering the remaining cereal uses) and for the use on onions involving two applications.

Table 6.6.2- 2: Predicted environmental soil concentrations [a] of fluoxastrobin following multi year use and conversion to the corresponding application rate
(application rate per hectare in italic and parentheses)

GAP	Residues distributed over [cm]	Maximum seasonal PEC soil [mg/kg] *(corresponding to g/ha)*	Long-term background/ PEC plateau [mg/kg] *(corresponding to g/ha)*	PEC soil total (from background + max seasonal rate [mg/kg] *(corresponding to g/ha)*
Representative GAP (N-EU): Winter cereals, spray, 2*150 g/ha, 14 d interval, 2*80% interception	5	0.075 [c] *(=56 g as/ha)* [b]	0.022 *(= 15 g as/ha)* [b]	0.097 *(=73 g as/ha)* [b]
	20		0.005 *(= 15 g as/ha)* [b]	0.080 *(= 71 g as/ha)* [b]
Representative GAP (S-EU): onion, spray, 2*125 g/ha, 10 d interval; 2*10% interception	5	0.286 [c] *(= 215 g as/ha)* [b]	0.081 *(= 61 g as/ha)* [b]	0.367 *(= 275 g as/ha)* [b]
	20		0.020 *(= 60 g as/ha)* [b]	0.306 *(= 275 g as/ha)* [b]

- Maximum seasonal PEC $_{soil}$: maximum soil residue calculated for one season
- long-term background or $PEC_{plateau}$: maximum of the lower saw-tooth curve which reflects background concentration after multiple year use
- PEC $_{soil, total}$: Combined concentration in a 5 or 20 cm soil layer arising from the long-term background concentration and the maximum annual rate from an additional individual application.

Note: The PEC soil (total) comprising the background resulting from previous applications plus an additional application is calculated based on the rounded values reported in the PEC soil report ([redacted] 2015; M-537905-01-1). Therefore slight deviations may occur for the amount per hectare calculated from the different soil layers (5 and 20 cm). Calculation example for conversion of max seasonal PEC soil into application rate (g a.s/ha):
0.075 mg/kg × 500,000 dm^3 × 1.5 kg/L/ 1000 = 56 g as/ha.
Calculation example for PEC soil total over 20 cm:
(0.075 mg/kg × 500,000 dm^3 × 1.5 kg/L/ 1000) + (0.005 mg/kg × 2,000,000 dm^3 × 1.5 kg/L/ 1000) =56 + 15 g as/ha

[a] calculation based on input parameters as reflected in ([redacted]; 2015; M-537905-01-1)
[b] Assuming soil density of 1.5 kg/L;
[c] Predicted soil concentration of fluoxastrobin on day 0. Soil concentration on day 28 which approximates the 30 day short-term plant back interval would correspond to 77% of the day 0 concentration.
as = active substance

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From the calculations above it can be concluded that the contribution due to the plateau concentration reached after repeated use to the overall predicted soil concentration is low.
The calculated PEC $_{soil\ total}$ (from background + extra max. seasonal rate) corresponds to a maximum of 275 g as/ha arising from the use on onions, while for cereals the combined soil concentration corresponds to a rate of 71 g as/ha.
Confined rotational crops studies were conducted at 846, 841 and 683 g a.s./ha. The exaggeration factor of the dose applied in the rotational crop metabolism studies amounts to 2.5-3.1N for onions and 9.4-11.6N for the cereals relative to the GAP involving the highest supported application rates (2 × 150 g as/ha) for the representative uses.

From the table 6.6.2-2 above it is evident that the individual application at the maximum seasonal rate (simulating a crop failure) contributes most to the overall soil concentration (PEC $_{soil\ total}$). Taking into account that in a rotational crop scenario crops are replanted after 30 days in the short-term plant back interval and fluoxastrobin degrades fast directly after application only 77% of the day 0 amount would be present after 28 days.
Moreover, cultivation of the same crop on the same field for more than 3 years and using the same product at the maximum label rate is unlikely and not according to agronomic practice and would contradict any resistance management strategy.

In order to estimate whether the properties of the soil used in the confined rotational crops study and the characteristics of the soil showing the slowest degradation in the terrestrial field dissipation study which was from an uncropped trial can be considered comparable both soils are compared in the table below.

Table 6.6.2- 3: Comparison of soil characteristics from the rotational crop metabolism study and the trial site providing the slowest degradation in the field dissipation study

	Confined rotational crop study (same soil for the three radiolabels; e.g. [redacted],; 2001; M-090320-01-1) (texture analysis according to USDA soil diagram: = sandy loam	Field dissipation study ([redacted],; 2001; M-136670-01-1) - Trial site [redacted] (Great Britain) providing longest DT_{50} (119 d); texture analysis according to USDA soil diagram (0-30 cm): = sandy clay loam
sand[%]	58.2%	52.1%
silt[%]	31.0%	18.1%
clay[%]	10.8%	29.8%
Organic carbon [%]	1.98	1.34
pH($CaCl_2$)	6.2	7.6

Both soils were characterized by a high content of sand and a medium content of organic carbon. The trials from the soil dissipation study also show that the DT_{50} values for soil were higher for trials conducted on uncropped fields. Therefore, residues found in the rotational crop metabolism study where the compound was also applied on bare soil are not considered to reflect an underestimate due to differences in the DT_{50} values from different soil types or cropping.

It can be concluded that the application rates used in the rotational crop metabolism studies cover a scenario where succeeding crops might be exposed to residues present in soil from previous applications and where an additional maximum seasonal rate is added simulating a crop failure situation. Residues in succeeding crops are not anticipated when considering

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- the exaggerations in the dose applied in the rotational crop metabolism studies (2.5-3.1N for onions and 9.4-11.6N for the cereals at the highest rates)
- agricultural practice where the total spray amount is split into 2 individual applications
- a crop failure situation and short-term replanting would be unlikely after a second application made at late growth stages
- DT_{50} values are shorter when the fields are cropped

Based on these considerations no additional field studies are considered necessary in view of the representative GAPs. The evaluation EFSA made in the Conclusion (2007) and in the Reasoned Opinion on existing MRLs (2012) is still appropriate and does not need to be changed in the view of representative uses.

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CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

The results of the metabolism studies in tomato and peanuts (see summaries in chapter CA 6.2.1) confirmed the metabolic pathways and distribution of fluoxastrobin and metabolites already observed in the wheat metabolism and confined rotational crops studies. Parent compound, *i.e.* the sum of fluoxastrobin and its Z-isomer, was observed as the predominant portion of the residues and it was stable under conditions simulating industrial and domestic processing (see chapter CA 6.5.1). Therefore, it is proposed that the residue definition for risk assessment and for enforcement purposes (monitoring) in plant commodities is the sum of fluoxastrobin and its Z-isomer.

The results of the livestock metabolism studies (see chapters CA 6.2.2 and CA 6.2.3) showed that parent compound (sum of fluoxastrobin and its Z-isomer) and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55, HEC 7154) were observed as the predominant components of the residues. Therefore, it is proposed that the residue definition for risk assessment and for enforcement purposes (monitoring) in animal commodities is the sum of fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55), expressed as fluoxastrobin.

These plant and animal residue definitions have also been proposed by EFSA (see Table 6.7.1-1).

Table 6.7.1-1 Proposed residue definitions by EFSA

Matrices		Residue definition	Reference
Food of plant origin	Risk assessment	Sum of fluoxastrobin and its Z-isomer[#]	EFSA Scientific Report (2007) 102, 1-84, and EFSA Reasoned Opinion according to Art 12 of Reg. (EC) No 396/2005 (EFSA Journal 2012;10(12):3012)
	Monitoring*	Sum of fluoxastrobin and its Z-isomer[#]	
Food of animal origin	Risk assessment	Sum of fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxy-pyrimidine (M55), expressed as fluoxastrobin	
	Monitoring*	Sum of fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxy-pyrimidine (M55), expressed as fluoxastrobin	

* In the EFSA Scientific Report the residue definition for monitoring food of plant origin is given as the sum of E-isomer (fluoxastrobin) and Z-isomer. The residue definition for monitoring food of animal origin is given as sum of E-isomer (fluoxastrobin), Z-isomer and the metabolite HEC 5725-phenoxy-hydroxypyrimidine (M55) expressed as fluoxastrobin. In Regulation EC 396/2005 amended by Regulation (EC) 839/2008 the residue definition refers only to fluoxastrobin (HEC 5725 E-isomer).

[#] In the EFSA Scientific Report (2007) 102, 1-84 the proposed plant residue definitions were limited to cereals only.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The EU MRLs for fluoxastrobin were set in Annex III A of Commission Regulation No 839/2008 amending Regulation (EC) 396/2005. Following the publication of the EFSA Reasoned Opinion on existing MRLs according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal

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2012;10(12):3012 a draft Regulation (SANCO/11739/2013) is under preparation but not yet published when the present dossier was prepared. The most recent draft Regulation SANTE/11195/2015 does not consider the modifications made following the MRL review according to Art 12.

In the EFSA Reasoned Opinion (2012) it was noted that for barley and oat grain the critical GAP identified for the southern climatic region was not adequately supported by the residue data since the application rates used in the trials were overdosed. Since the established MRLs for barley and oats do not present a consumer health concern these MRLs were flagged as tentative MRLs.

For wheat and rye grain the Reasoned Opinion proposed to lower the MRLs from 0.05 mg/kg and 0.5 mg/kg, respectively, to 0.02 mg/kg for both crops. This proposal was likely based on the fact that residues were found to be below or at the LOQ of 0.02 mg/kg in wheat grain.
The more recent supplementary data reported in the present dossier were generated using a method providing an LOQ of 0.01 mg/kg for the sum of HEC 5725 E-and Z-isomers, but nevertheless resulting in residue levels of 0.02 mg/kg in some residue trials. Therefore it is proposed to apply the OECD calculator to the new data set in order to derive appropriate MRLs for wheat and rye grain.

Important note:
Since there is a delay with the finalization of the MRL review according to Art 12 and the voting of the draft Regulation SANCO/11739/2013 it is open which MRL will be in place when this dossier will be evaluated. As a precautionary measure Bayer CropScience submits an MRL application (M-543078-01-1) along with and as part of this supplementary dossier in order to avoid an unsuitable lowering of the wheat and rye MRLs.
In addition this supplementary dossier provides appropriate residue data for the new critical GAP for barley in the southern climatic zone to address the defiency identified in the EFSA Reasoned Opinion. Bayer CropScience proposes to use this data set to define appropriate MRLs for barley and oat in order to convert the tentative MRLs into final MRLs. The new proposed barley and oat MRLs are also part of the MRL application submitted along with the present dossier.

The residue levels obtained from the trials reported in the supplementary dossier which were conducted according to the critical GAPs were used for calculation of the proposed new MRLs. All the trials are included in chapter 6.3.1 (barley) and 6.3.2 (wheat) of the supplementary dossier.

Relative to onions, the use in the northern zone has been evaluated in the EFSA reasoned opinion on existing MRLs according to Art 12. The residue data were found to comply with the critical GAP and an MRL of 0.04 mg/kg was proposed. The use in the southern zone forming the representative use was found to not result in residues exceeding this MRL and thus a modification is not required.

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Table 6.7.2- 1: Established EU MRLs (Regulation EC 839/2008) and proposed MRLs (EFSA Reasoned Opinion [EFSA Journal 2012;10(12):3012]) of fluoxastrobin

Code number	Commodity	MRL EC 839/2008 [mg/kg]	Proposed in EFSA RO, 2012 *(draft Regulation SANCO/11739/2013)* [mg/kg]
Commodities of plant origin *Enforcement residue definition (existing): fluoxastrobin* *Enforcement residue definition (proposed): sum of fluoxastrobin and its Z-isomer*			
0220020	Onion	0.05*	0.04
0500010	Barley grain	0.5	0.5 (a)
0500050	Oat grain	0.5	0.5 (a)
0500070	Rye grain	0.5	0.02
0500090	Wheat grain	0.05*	0.02
Commodities of animal origin *Enforcement residue definition (existing): fluoxastrobin* *Enforcement residue definition (proposed): Sum of fluoxastrobin, its Z-isomer and the metabolite HEC 5725-phenoxy-hydroxy-pyrimidine metabolite (M55), expressed as fluoxastrobin*			
1011010	Meat (swine)	0.05	0.02* (a)
1011020	Fat (swine)	0.05	0.02*(a)
1011030	Liver (swine)	0.1	0.04*(a)
1011040	Kidney (swine)	0.1	0.04*(a)
1012010, 1013010, 1014010, 1015010	Muscle (bovine, sheep, goat, equine)	0.05	0.02*(a)
1012020, 1013020, 1014020, 1015020	Fat (bovine, sheep, goat, equine)	0.05	0.05(a)
1012030, 1013030, 1014030, 1015030	Liver (bovine, sheep, goat, equine)	0.05	0.04*(a)
1012040, 1013040, 1014040, 1015040	Kidney (bovine, sheep, goat, equine)	0.1	0.1(a)
1016010	Muscle (poultry)	0.05	No new proposal
1016020	Fat (poultry)	0.05	No new proposal
1016030	Liver (poultry)	0.05	No new proposal
1016040	Kidney (poultry)	0.1	No new proposal
1020010,1020020. 1020030	Milk (cattle, sheep, goat)	0.2	0.02*
1030000	Bird's eggs	0.01*	No new proposal

*: indicates that the MRL is set at the LOQ

(a) MRLs proposed by EFSA for barley and oat as well as for the animal commodities are tentative

New MRL proposals for wheat, rye, barley and oats

For easy reference the table below provides an overview on the GAPs per crop and region for which new MRL calculations are provided (Table 6.7.2- 2). The GAPs from the northern zone reflect the cGAP which will be applicable post AIR. The GAPs from the southern zone reflect the cGAPs already in place today.

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Table 6.7.2- 2: GAPs for which MRL calculations are provided

GAP no (cf. Table 6.3.1- 2 *and* Table 6.3.2- 2*)*	Crop	Maximum Number of Applications	Minimum Application Interval (days)	Growth stage (BBCH)	Maximum Rate per Application (g a.s./ha)	Minimum PHI (days)
Northern Europe						
EU-N 2	Wheat, Rye	2	14-21	30-69	150	*
EU-N 2	Barley, Oat	2	14-21	30-61	125	*
Southern Europe						
EU-S 2	Wheat Rye	2	14-21	30-69	100	*
EU-S 2	Barley, Oat	2	14-21	30-61/ 69 (oat)**	87.5/ 100 (oat)**	*
--	Onion	2	10	15-47	125	21

* as per growth stage; the PHI is defined by the growth stage at the last application

** The residue trials on barley conducted at the individual application rate of 75 – 87.5 g a.s./ha and supporting the critical GAP for barley (individual rate of 87.5 g a.s./ha) are also suitable to support oats although the critical GAP for oats involves a slightly higher individual application rate (i.e. 100 g a.s./ha), because the trials rates fulfill the requirement to stay within the ±25% tolerance range. Therefore the extrapolation from barley to oats is considered acceptable.

The following Table 6.7.2- 3 provides key information on the residue data summarised above in CA 6.3.1, CA 6.3.2 and CA 6.3.3 (median and highest residues) and the corresponding results of the MRL calculations. All data are based on the proposed residue definition involving the sum of fluoxastrobin (E-isomer) and its Z-isomer.

The table includes a hyperlink to the respective MRL calculation (Table 6.7.2- 5 to Table 6.7.2- 11).

In addition to the individual MRL calculations per crop, commodity (grain, straw) and region (EU-N, EU-S) a calculation is presented for barley grain from the southern region merging the data sets from the new cGAP and the previous cGAP evaluated during the EU peer review. The reason for this exercise is to define appropriate MRLs for barley and oat grain based on a broader data set and to assess the relevance of the individual elevated residue finding in barley grain. For this purpose all residue results are scaled to the application rate of the cGAP (0.0875 kg as/ha) applying the proportionality approach (cf. EFSA document on 'Residue 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; September 2015). For this exercise, all data were scaled to the cGAP rate, also those which fall in the ± 25% range. From the 18 (grain) and 19 (straw) individual data points 10 results reflect the cGAP, 4 (5) results fall in the ±25% range and 4 data points are outside this range (results from applications later than BBCH 69 or from fields with laying down at application are not considered).

This approach is also in line with the most recent 'OECD Draft Guidance Document on Crop Field Trials' (August 2015):

Paragraph 27: '*The OECD decided to use the principles and guidance as adopted by Codex Alimentarius Commission.*

...

c) 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.'

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Table 6.7.2- 3: Summary of MRL calculations, median and highest residues on <u>small grain cereals (grain and straw)</u> and <u>onion bulb</u> in northern and southern Europe

				Results of MRL calculation (OECD calculator)			
Crop	**Region**	**GAP Dose rate (kg a.s./ha)**	**Commodities**	**MRL proposal (mg/kg)**	**Median residue (mg/kg)**	**Highest residue (mg/kg)**	**MRL input data and result tables**
Barley Oat	NEU	2 x 0.125 BBCH 61 (new trials)	Grain	0.05 (n=11)	0.02	0.03	Table 6.7.2- 5
			Straw	**4** (n=11)	0.44	2.7	
	SEU	2 x 0.0875 BBCH 61 (barley) (new trials)	Grain	**0.4** (n=15)	(<0.02)	0.34	Table 6.7.2- 6
			Straw	3 (n=15)	0.31	1.7	
	SEU	Merged data sets 0.075 – 0.2 kg/ha, BBCH 61-69 scaled to cGAP	Grain	0.4 (n=18)	0.02	0.34	Table 6.7.2- 7 and Table 6.7.2- 8
			Straw	3 (n=19)	0.31	2.06	
Wheat Rye	NEU	2 x 0.150 BBCH 69 (new trials)	Grain	**0.04** (n=8)	0.01	<0.02	Table 6.7.2- 9
			Straw	4 (n=8)	0.71	2.3	
	SEU	2 x 0.100 BBCH 69 (new trials)	Grain	**0.04** (n=11)	0.01	0.02	Table 6.7.2- 10
			Straw	**6** (n=11)	1.0	3.7	
Onion	S-EU	2 x 0.125 BBCH 15-47 PHI 21 d interval 10 d (new trials)	Bulb	No new MRL proposal [0.03] (n=12)	0.01	0.021	Table 6.7.2- 11

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Conclusion

Barley: The highest residue level of the total residue of HEC 5725 (sum of fluoxastrobin and its Z-isomer) found in barley grain was 0.34 mg/kg from the southern European data set, while the highest residue in straw appeared in the northern region (2.7 mg/kg). Merging the data sets of the new critical GAP and suitable trials (application during flowering) from the previous cGAP evaluated during the EU peer review by application of the proportionality approach still results in an MRL proposal of 0.4 mg/kg which is driven by the highest residue of 0.34 mg/kg from trial 10-2157-04. When disregarding this result an MRL of 0.15 mg/kg would be adequate.

The applicant suggests that the competent authority takes the new data into account for setting new MRLs for barley grain (0.4 mg/kg) and straw (4 mg/kg) based on the submitted data in order to convert the tentative MRLs into final MRLs. By means of extrapolation these MRLs shall apply also to oat.

Wheat: The highest residue levels of the total residue of HEC 5725 (sum of fluoxastrobin and its Z-isomer) found in wheat grain was 0.02 mg/kg from the southern European data set. Highest residues in straw appeared also in the southern region (3.7 mg/kg).

All residue findings obtained from the supplementary residue trials are covered by the existing MRLs for wheat of 0.05 mg/kg or rye of 0.5 mg/kg. However, in the EFSA Reasoned Opinion (2012) new MRLs for wheat / rye of 0.02 mg/kg are proposed. Taking into account that residues at 0.02 mg/kg (LOQ of the method 0.01 mg/kg) may appear it is proposed to set the MRL at 0.04 mg/kg in wheat / rye grain and not to lower to 0.02 mg/kg. For wheat and rye straw an MRL of 6 mg/kg derived from the data of the southern zone is considered adequate.

Onion: Residues in onion bulb originating from the southern European GAP are covered by the existing (0.05 mg/kg*) or the proposed (EFSA RO, 2012) MRL of 0.04 mg/kg which is derived from the northern European GAP. Residue levels of the total residue of HEC 5725 (sum of fluoxastrobin and its Z-isomer) up to 0.021 mg/kg were found in onion bulbs in the southern zone. An MRL calculation for the southern European data results in an MRL proposal of 0.03 mg/kg when using the OECD calculator methodology. Thus a modification of the existing or proposed MRL is not necessary.

MRLs in commodities of animal origin

Table 6.7.2- 4 below compiles the information on the dietary burden arising from the representative uses on small grain cereals and the dose levels used in the feeding studies on dairy cows and laying hen and compares to the proposed MRLs (EFSA Reasoned Opinion (2012) and draft Reg. SANCO/11739/2013) for animal tissues, milk and eggs.

The proposed or the established MRLs (Reg. (EC) 839/2008) MRLs are found to sufficiently cover the residue situation arising from the representative uses supported in the supplementary dossier.

Note:
In Reg (EC) 839/2008 the MRL for eggs was set at the default level of 0.01 mg/kg. No new proposal was made for eggs in the draft Regulation SANCO/11739/2013. Since the proposed residue definition for enforcement includes fluoxastrobin and its Z-isomer and the metabolite HEC 5725 phenoxy-hydroxypyrimidine (M55), expressed as fluoxastrobin it is proposed to set the MRL for eggs to the

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*LOQ of the enforcement method, i.e. 0.02*mg/kg for the total residue.*

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Table 6.7.2- 4: Overview of the dietary burden, the residue levels derived from livestock feeding studies and proposed MRLs (EFSA Reasoned Opinion, 2012)

Commodity	EU Dietary Burden [a]	Results of livestock feeding study				Highest residue [e]	MRL EFSA Journal 2012;10(12):3012 and SANCO/11739/2013 [f]	Remark
		Dose level	No of animals	Results for enforcement = results for risk assessment *Proposed residue definition: [d] Fluoxastrobin + Z-isomer + HEC 7154 (=M55)*				
	(mg/kg bw/d)	(mg/kg bw/d)		Mean (mg/kg)	Max (mg/kg)	(mg/kg)	(mg/kg)	
Pig meat	0.001	0.22 [b), c)]	3	0.02	0.02	0.02	0.02*	Dietary burden from swine finishing and breeding
Pig fat			3	0.02	0.02	0.02	0.02*	
Pig liver			3	0.02	0.02	0.02	0.04* [f)]	
Pig kidney			3	0.04*	0.05	0.02	0.04* [f)]	
Cattle meat	0.036	0.22 [c)]	3	0.02	0.02	0.02	0.02*	Dietary burden from dairy cattle
Cattle fat			3	0.02	0.02	0.02	0.05	
Cattle liver			3	0.02	0.02	0.02	0.04* [f)]	
Cattle kidney			3	0.04	0.05	0.02	0.1	
Cattle milk			30	0.02	0.02	0.02	0.02*	
Sheep meat	0.078	0.22 [c)]	3	0.02	0.02	0.02	0.02*	Dietary burden from lamb
Sheep fat			3	0.02	0.02	0.02	0.05	
Sheep liver			3	0.02	0.02	0.02	0.04* [f)]	
Sheep kidney			3	0.04	0.05	0.02	0.1	
Sheep milk	0.061	0.22 [c)]	30	0.02	0.02	0.02	0.02*	Dietary burden from ewe
Poultry Meat	0.030	0.032 [c)]	12	0.02	0.02	0.02	0.05 [g)]	Dietary burden from poultry layer
Poultry fat			12	0.02	0.02	0.02	0.05 [g)]	
Poultry Liver			12	0.02	0.02	0.02	0.05 [g)]	
Eggs			12	0.02	0.02	0.02	0.01* [g)]	

a) The dietary burden was calculated for the European livestock diet using the OECD feeding tables issued with the OECD guidance Document No 73 without taking into account immature cereals (forage, hay, silage) as feeding items (cf. Table 6.4- 2). The dietary burden was calculated for the representative uses.

b) The dose levels used for the cow feeding study can be used to extrapolate to pig.

c) Lowest dose level of ruminant feeding study was 0.22 mg/kg bw/d and for the poultry feeding study the lowest dose level was 0.032 mg/kg bw/d. Results from other dose levels are not listed here since the anticipated dietary burden is less than the lowest dose level of the feeding studies.

d) Data based on anticipated residue definition for enforcement and risk assessment involving fluoxastrobin (E-isomer) and its Z-isomer and HEC 5725-phenoxy-hydroxypyrimidine (M55). Residues of individual components of the residue definition below the LOQ were calculated as being *at* the LOQ and summed up

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e) Highest residue value (tissues, eggs) or mean residue value (milk) according to the enforcement residue definition, derived by transfer factor (OECD guidance document No 73) or interpolation/extrapolation of the maximum dietary burden between the relevant feeding groups of the study (FAO, 2009).

f) With the previous enforcement method the LOQ for liver and kidney was 0.02 mg/kg for fluoxastrobin and its Z-isomer and 0.02 mg/kg for HEC 5725-phenoxy-hydroxypyrimidine (HEC 7154, M55) resulting in an LOQ of 0.04 mg/kg for the total residue.

g) MRLs for poultry tissues and eggs were set with Reg. (EC) 839/2008. No new proposals were made in the EFSA Reasoned Opinion on existing MRLs (2012). It is suggested to elevate the MRL for eggs to 0.02* mg/kg based on the findings of the poultry feeding study and the proposed residue definition.

Table 6.7.2- 5: Input data for MRL calculation on barley in northern Europe from supplementary trials supporting the critical GAP (OECD calculator)

N°	Crop	FL Type	GAP Dose rate FXA (kg a.s./ha)	Commodities	Residue level of total residue HEC 5725 and DALT (mg/kg)	Report No. Trial No.
1	Barley	EC 200	2 x 0.125	Grain Straw	0.02 0.41 (47)	RA-2013/00 0152-00
2	Barley	EC 200	2 x 0.125	Grain Straw	0.02 0.22 (54)	RA-2013/00 0153-00
3	Barley	EC 200	2 x 0.125	Grain Straw	0.02 0.44 (56)	RA-2013/00 0154-00
4	Barley	EC 200	2 x 0.125	Grain Straw	<0.02 0.14 (71)	RA-2013/00 0156-00
5	Barley	EC 150	2 x 0.150	Grain Straw	0.02 0.72 (47)	RA-2062/00 0278-00
6	Barley	EC 150	2 x 0.150	Grain Straw	0.03 0.58 (43)	RA-2062/00 0279-00
7	Barley	EC 150	2 x 0.150	Grain Straw	0.02 0.47 (56)	RA-2062/00 0280-00
	Barley	EC 150	2 x 0.150	Grain Straw	Not considered since last application at BBCH 83	RA-2062/00 0281-00
8	Barley	EC 200	2 x 0.125	Grain Straw	<0.01 0.18 (69)	13-2137 13-2137-01
9	Barley	EC 200	2 x 0.125	Grain Straw	0.026 0.17 (68)	13-2137 13-2137-02
10	Barley	EC 200	2 x 0.125	Grain Straw	0.020 (43) 0.44 (43)	13-2158 13-2158-01
11	Barley	EC 200	2 x 0.135	Grain Straw	0.011 2.7 (35)	13-2158 13-2158-01

( ) days after last treatment
FL = formulation
DALT = days after last application

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Results for barley grain (northern Europe)

Total number of data (n)	11	Standard deviation (SD)	0.006
Lowest residue	0.01	Percentage of censored data	27
Highest residue	0.03	Number of non-censored data	8
Median residue	0.020	Correction factor for censoring (CF)	0.818
Mean	0.020		

Proposed MRL estimate for barley/oat grain (northern Europe)

Highest residue	0.03
Mean + 4 SD	0.042
CF x 3 mean	0.048
Unrounded MRL	0.048
Rounded MRL	**0.05**

Results for barley straw (northern Europe)

Total number of data (n)	11	Standard deviation (SD)	0.724
Lowest residue	0.14	Percentage of censored data	0
Highest residue	2.7	Number of non-censored data	11
Median residue	0.440	Correction factor for censoring (CF)	1.000
Mean	0.588		

Proposed MRL estimate for barley / oat straw (northern Europe)

Highest residue	2.7
Mean + 4 SD	3.485
CF x 3 mean	1.765
Unrounded MRL	3.485
Rounded MRL	**4**

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Table 6.7.2- 6: Input data for MRL calculation on barley in southern Europe from supplementary trials supporting the critical GAP (OECD calculator)

N°	Crop	FL Type	GAP Dose rate FXA (kg a.s./ha)	Commodities	Residue level of total residue HEC 5725 and DALT (mg/kg)	Report No. Trial No. [2]
1	Barley	EC 300	2 x 0.075	Grain Straw	<0.02 0.74 (35)	RA-2017/03 0132-03
2	Barley	EC 300	2 x 0.075	Grain Straw	0.04 0.15 (45)	RA-2017/03 0253-03
3	Barley	EC 300	2 x 0.075-0.080	Grain Straw	0.02 0.61 (69)	RA-2017/03 0254-03
4	Barley	EC 300	2 x 0.0875	Grain Straw	0.02 0.23 (40)	RA-2017/03 0256-03
5	Barley	EC 190	2 x 0.075	Grain Straw	0.02 (34) 0.16 (47)	10-2206 10-2206-01
6	Barley	EC 190	2 x 0.075	Grain Straw	0.01 0.03 (52)	10-2206 10-2206-02
7	Barley	EC 150	2 x 0.0875	Grain Straw	0.02 0.97 (50)	10-2157 10-2157-01
	Barley	EC 150	2 x 0.0875	Grain Straw	Not considered due to an interval of 3 days	10-2157 10-2157-02
8	Barley	EC 150	2 x 0.0875	Grain Straw	0.01 0.31 (41)	10-2157 10-2157-03
9	Barley	EC 150	2 x 0.0875	Grain Straw	0.34 1.7 (35)	10-2157 10-2157-04
10	Barley	EC 150	2 x 0.0875	Grain Straw	0.01 (28) 0.23 (28)	10-2157 10-2157-05
11	Barley	EC 150	2 x 0.0875	Grain Straw	0.03 0.73 (51)	11-2111 11-2111-01
12	Barley	EC 150	2 x 0.0875	Grain Straw	0.013 0.28 (55)	11-2111 11-2111-02
13	Barley	EC 150	2 x 0.0875	Grain Straw	0.015 0.29 (63)	11-2111 11-2111-03
14	Barley	EC 150	2 x 0.0875	Grain Straw	0.019 1.4 (43)	11-2111 11-2111-04
15	Barley	EC 150	2 x 0.0875	Grain Straw	0.010 0.52 (49)	11-2111 11-2111-05

( ) days after last treatment
FL = formulation
DALT = days after last application

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Results for barley grain (southern Europe)

Total number of data (n)	15	Standard deviation (SD)	0.083
Lowest residue	0.01	Percentage of censored data	7
Highest residue	0.34	Number of non-censored data	14
Median residue	0.000	Correction factor for censoring (CF)	0.956
Mean	0.040		

Proposed MRL estimate for barley / oat grain (southern Europe)

Highest residue	0.34
Mean + 4 SD	0.374
CF x 3 mean	0.114
Unrounded MRL	0.374
Rounded MRL	**0.4**

Results for barley straw (southern Europe)

Total number of data (n)	15	Standard deviation (SD)	0.484
Lowest residue	0.03	Percentage of censored data	0
Highest residue	1.7	Number of non-censored data	15
Median residue	0.310	Correction factor for censoring (CF)	1.000
Mean	0.557		

Proposed MRL estimate for barley /oat straw (southern Europe)

Highest residue	1.7
Mean + 4 SD	2.493
CF x 3 mean	1.670
Unrounded MRL	2.493
Rounded MRL	**3**

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Table 6.7.2- 7: Input data for MRL calculation on barley in southern Europe for merged data sets from supplementary trials (new critical GAP) and scaled trials conducted according to the previous critical GAP

N°	FL Type	Report No.	Trial No.	GAP Dose rate FXA (kg a.s./ha)	Residue level of total residue HEC 5725 GRAIN (mg/kg)		Residue level of total residue HEC 5725 and DALT STRAW (mg/kg)	
					measured	Scaled to cGAP rate (0.0875 kg/ha)	measured	Scaled to cGAP rate (0.0875 kg/ha)
19 (straw only)	EC 300	RA-2017/03	0132-03	2 x 0.075	0.02	Not considered since residues < LOQ	0.74 (35)	0.863
1	EC 300	RA-2017/03	0253-03	2 x 0.075	0.04	0.047	0.15 (45)	0.175
2	EC 300	RA-2017/03	0254-03	2 x 0.075-0.080	0.02	0.023	0.61 (69)	0.712
3	EC 300	RA-2017/03	0256-03	2 x 0.0875	0.02	--	0.23 (40)	--
4	EC 190	10-2206	10-2206-01	2 x 0.075	0.02 (34)	0.023	0.16 (47)	0.187
5	EC 190	10-2206	10-2206-02	2 x 0.075	0.01	0.012	0.03 (52)	0.035
6	EC 150	10-2157	10-2157-01	2 x 0.0875	0.02	--	0.97 (50)	--
	EC 150	10-2157	10-2157-02	2 x 0.0875	Not considered due to an interval of 3 days			
7	EC 150	10-2157	10-2157-03	2 x 0.0875	0.010	--	0.31 (41)	--
8	EC 150	10-2157	10-2157-04	2 x 0.0875	0.34	--	1.7 (35)	--
9	EC 150	10-2157	10-2157-05	2 x 0.0875	0.01 (28)	--	0.23 (28)	--
10	EC 150	11-2111	11-2111-01	2 x 0.0875	0.03	--	0.73 (51)	--
11	EC 150	11-2111	11-2111-02	2 x 0.0875	0.013	--	0.28 (55)	--
12	EC 150	11-2111	11-2111-03	2 x 0.0875	0.015	--	0.29 (63)	--
13	EC 150	11-2111	11-2111-04	2 x 0.0875	0.019	--	1.4 (43)	--
14	EC 150	11-2111	11-2111-05	2 x 0.0875	0.010	--	0.52 (49)	--
	EC 100	*RA-2026/99*	*0126-99*	*2 x 200*	*Not considered, last application at early milk stage (BBCH 73)*			
15	*EC 100*	*RA-2026/99*	*0126-99*	*2 x 200*	*0.02*	*0.01*	*2.6 (35)*	*1.14*
	EC 100	*RA-2026/99*	*0129-99*	*2 x 200*	*Not considered last application at watery ripe stage (BBCH 71)*			
	EC 100	*RA-2026/99*	*0130-99*	*2 x 200*	*Not considered, laying down of barley since BBCH 52*			
	EC 100	*RA-2027/99*	*0123-99*	*2 x 200*	*Not considered , last application at soft dough stage (BBCH 83)*			
	EC 100	*RA-2027/99*	*0124-99*	*2 x 200*	*Not considered, last application at early milk stage (BBCH 73)*			
16	*EC100*	*RA-2027/99*	*0127-99*	*2 x 200*	*0.08 (35)*	*0.035*	*0.25 (42)*	*0.109*
17	*EC 100*	*RA-2027/99*	*0128-99*	*2 x 200*	*0.02*	*0.01*	*0.38 (57)*	*0.166*
18	*EC 100*	*RA-2027/99*	*0710-99*	*2 x 200*	*0.24*	*0.105*	*4.7 (35)*	*2.056*

Results in italic originate from the data included in the Annex II dossier; for details please refer to Tier 1 summary forms. In case two sets of residue data were available from the same trial the highest residue value was selected.

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Table 6.7.2- 8: MRL calculation on barley grain and straw in southern Europe for merged data sets from supplementary trials (new critical GAP) and scaled trials conducted according to the previous critical GAP

Crop	Barley grain	Barley straw	
Region	EU-S	EU-S	
PHI			
other	last appl. BBCH 61-69	last appl. BBCH 61-69	
Number of values	18	19	
mean	0.04	0.64	
Dixon Test	0.89	0.35	
Q10%	0.42	0.41	
Result	**Outlier**	**No outlier**	
Rber	0.06	1.94	
Rmax	0.23	2.05	
Rounded **EU-MRL**	**0.30**	**2.00**	
Highest residue	0.34	2.06	
mean+ 4 SD	0.35	2.07	
CF x 3 mean	0.13	1.91	
Rounded MRL$_{OECD}$	**0.40**	**3.00**	
HR	0.34	2.06	
STMR	0.02	0.31	
1	0.01	1	0.04
2	0.01	2	0.11
3	0.01	3	0.17
4	0.01	4	0.18
5	0.01	5	0.19
6	0.01	6	0.23
7	0.01	7	0.23
8	0.02	8	0.28
9	0.02	9	0.29
10	0.02	10	0.31
11	0.02	11	0.52
12	0.02	12	0.71
13	0.02	13	0.73
14	0.03	14	0.86
15	0.04	15	0.97
16	0.05	16	1.14
17	0.11	17	1.40
18	0.34	18	1.70
19		19	2.06

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Table 6.7.2- 9: Input data for MRL calculation for <u>wheat in northern Europe</u> from <u>supplementary trials</u> supporting the new critical GAP (OECD calculator)

N°	Crop	FL Type	GAP Dose rate FXA (kg a.s./ha)	Commodities	Residue level of total residue HEC 5725 and DALT (mg/kg)	Report No. Trial No. [1]
	Wheat	EC 200	2 x 0.150	Grain Straw	Not considered because side-by side with trial 0269/00 from study RA-2060/00	RA-2011/00 0144-00
1	Wheat	EC 200	2 x 0.150	Grain Straw	<0.02 0.59 (48)	RA-2011/00 0145-00
2	Wheat	EC 200	2 x 0.150	Grain Straw	<0.02 0.86 (58)	RA-2011/00 0146-00
	Wheat	EC 200	2 x 0.150	Grain Straw	Not considered since last application at BBCH 81-83	RA-2011/00 0147-00
3	Wheat	EC 150	2 x 0.150	Grain Straw	<0.02 0.64 (61)	RA-2060/00 0269-00
	Wheat	EC 150	2 x 0.150	Grain Straw	Not considered because side-by side with trial 0145-00 from study RA-2011/00	RA-2060/00 0271-00
	Wheat	EC 150	2 x 0.150	Grain Straw	Not considered because side-by side with trial 0146-00 from study RA-2011/00	RA-2060/00 0272-00
	Wheat	EC 150	2 x 0.150	Grain Straw	Not considered since last application at BBCH 81-83	RA-2060/00 0273-00
4	Wheat	EC 200	2 x 0.150	Grain Straw	0.01 0.10 (57)	13-2138 13-2138-01
5	Wheat	EC 200	2 x 0.150	Grain Straw	0.01 0.77 (54)	13-2138 13-2138-02
6	Wheat	EC 200	2 x 0.150	Grain Straw	0.04 2.3 (35)	13-2138 13-2138-03
7	Wheat	EC 200	2 x 0.150	Grain Straw	0.01 0.58 (49)	13-2159 13-2159-01
8	Wheat	EC 200	2 x 0.150	Grain Straw	<0.01 1.5 (35)	13-2159 13-2159-02

[1] Trials from study RA-2011/00 and RA-2060/00 were conducted side-by-side at the same location using different products. From a pair of trials residues in grain were always < 0.02 mg/kg. From the pairs, the trial showing the highest residues in straw was selected for MRL calculation as recommended in the EFSA document on 'Residue trials and MRL calculations, proposals for a harmonised approach for the selection of the trials and data used in the estimation of MRL, STMR and HR' (September 2015).

( ) days after last treatment

FL = formulation

DALT = days after last application

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Results for wheat grain (northern Europe)

Total number of data (n)	8	Standard deviation (SD)	0.005
Lowest residue	0.01	Percentage of censored data	75
Highest residue	0.02	Number of non-censored data	2
Median residue	0.010	Correction factor for censoring (CF)	0.500
Mean	0.014		

Proposed MRL estimate for wheat / rye grain (northern Europe)

Highest residue	0.02
Mean + 4 SD	0.034
CF x 3 mean	0.021
Unrounded MRL	0.034
Rounded MRL	**0.04**

Results for wheat straw (northern Europe)

Total number of data (n)	8	Standard deviation (SD)	0.681
Lowest residue	0.1	Percentage of censored data	0
Highest residue	2.3	Number of non-censored data	8
Median residue	0.705	Correction factor for censoring (CF)	1.000
Mean	0.918		

Proposed MRL estimate for wheat / rye straw (northern Europe)

Highest residue	2.3
Mean + 4 SD	3.640
CF x 3 mean	2.753
Unrounded MRL	3.640
Rounded MRL	4

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Table 6.7.2- 10: Input data for MRL calculation on <u>wheat in southern Europe from supplementary trials</u> supporting the new critical GAP (OECD calculator)

N°	Crop	FL Type	GAP Dose rate FXA (kg a.s./ha)	Commodities	Residue level of total residue HEC 5725 and DALT (mg/kg)	Report No. Trial No.
1	Wheat	EC 300	2 x 0.075	Grain Straw	0.02 1.0 (41)	RA-2019/03 0134-03
2	Wheat	EC 300	2 x 0.071	Grain Straw	0.02 1.5 (46)	RA-2019/03 0257-03*
3	Wheat	EC 190	2 x 0.0875	Grain Straw	<0.01 0.70 (38)	10-2207 10-2207-01
4	Wheat	EC 190	2 x 0.0875	Grain Straw	<0.01 0.41 (63)	10-2207 10-2207-02
5	Wheat	EC 150	2 x 0.100	Grain Straw	<0.01 0.79 (41)	10-2156 10-2156-01
6	Wheat	EC 150	2 x 0.100	Grain Straw	<0.01 1.4 (43)	10-2156 10-2156-02
7	Wheat	EC 150	2 x 0.100	Grain Straw	<0.01 (35) 1.3 (42)	10-2156 10-2156-03
8	Wheat	EC 150	2 x 0.100	Grain Straw	<0.01 1.0 (35)	10-2156 10-2156-04
9	Wheat	EC 150	2 x 0.100	Grain Straw	0.01 0.47 (53)	10-2156 10-2156-05
10	Wheat	EC 150	2 x 0.100	Grain Straw	0.02 2.7 (48)	10-2156 10-2156-06
11	Wheat	EC 150	2 x 0.100	Grain Straw	0.02 3.7 (35)	10-2156 10-2156-07

*trial was underdosed relative to cGAP, however residues in grain and straw were in the upper range of the residue distribution and are therefore considered for MRL calculation.
( ) days after last treatment
FL = formulation
DALT = days after last application

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Results for wheat grain (southern Europe)

Total number of data (n)	11	Standard deviation (SD)	0.005
Lowest residue	0.01	Percentage of censored data	64
Highest residue	0.02	Number of non-censored data	4
Median residue	0.000	Correction factor for censoring (CF)	0.576
Mean	0.014		

Proposed MRL estimate for wheat / rye grain (southern Europe)

Highest residue	0.02
Mean + 4 SD	0.034
CF x 3 mean	0.024
Unrounded MRL	0.034
Rounded MRL	**0.04**

Results for wheat straw (southern Europe)

Total number of data (n)	11	Standard deviation (SD)	1.000
Lowest residue	0.41	Percentage of censored data	0
Highest residue	3.7	Number of non-censored data	11
Median residue	1.000	Correction factor for censoring (CF)	1.000
Mean	1.362		

Proposed MRL estimate for wheat / rye straw (southern Europe)

Highest residue	3.7
Mean + 4 SD	5.361
CF x 3 mean	4.085
Unrounded MRL	5.361
Rounded MRL	**6**

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Table 6.7.2- 11: Input data for MRL calculation on <u>onion in</u> <u>southern Europe for supplementary trials</u> supporting the GAP (OECD calculator)

N°	Crop	FL Type	GAP Dose rate FXA (kg a.s./ha)	Commo-dities	Days after application	Residue level of total residue HEC 5725 (mg/kg)	Trial No. Report No.	Country
1	Onion	EC 200	2 x 0.125	bulb	19	<0.01	14-2175-02-T / 14-2175	Italy
2	Onion	EC 200	2 x 0.115-0.116	bulb	20	<0.01	12 F CL BY P04 / 12 F CL BY P/A	Spain
3	Onion	EC 200	2 x 0.125	bulb	21	0.010	13-2139-01-T / 13-2139	Spain
4	Onion	EC 200	2 x 0.125	bulb	21	0.017	14-2176-03-T / 14-2175	France
5	Onion	EC 200	2 x 0.114-0.124	bulb	20	0.01	12 F CL BY P09 / 12 F CL BY P/A	Spain
6	Onion	EC 200	2 x 0.114-0.116	bulb	20	<0.01	12 F CL BY P01 / 12 F CL BY P/A	Spain
7	Onion	EC 200	2 x 0.125	bulb	21	<0.01	14-2175-04-T / 14-2175	Greece
8	Onion	EC 200	2 x 0.125	bulb	21	0.010	14-2175-01-T / 14-2175	Spain
9	Onion	EC 200	2 x 0.125	bulb	21	0.021	13-2139-04-T / 13-2139	Portugal
10	Onion	EC 200	2 x 0.125	bulb	21	0.011	13-2139-03-T / 13-2139	France
11	Onion	EC 200	2 x 0.116	bulb	20	<0.01	12 F CL BY P02 / 12 F CL BY P/A	Spain
12	Onion	EC 200	2 x 0.125	bulb	21	<0.01	13-2139-02-T / 13-2139	Italy

FL = formulation

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Results for <u>onion</u> (southern Europe)

Total number of data (n)	12	Standard deviation (SD)	0.004
Lowest residue	0.01	Percentage of censored data	58
Highest residue	0.021	Number of non-censored data	5
Median residue	0.010	Correction factor for censoring (CF)	0.611
Mean	0.012		

Proposed MRL estimate for <u>onion</u> (southern Europe)

Highest residue	0.021
Mean + 4 SD	0.026
CF x 3 mean	0.021
Unrounded MRL	0.026
Rounded MRL	**0.03**

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

There are no relevant import tolerances established at EU level and no CXLs are set.

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CA 6.8 Proposed safety intervals

Proposed pre-harvest intervals for envisaged uses, or withholding periods and justification

The intervals and waiting periods proposed all pertain to the herein supported representative uses, namely foliar applications in small grain cereals (wheat, rye, barley and oat) and onions. The representative uses on small grain cereals in the northern climatic zone and on onion in the southern zone are represented by the GAPs for the product 'Fluoxastrobin + Prothioconazole EC 200'. The representative uses on small grain cereals in the southern zone are represented by the product 'Bixafen + Fluoxastrobin + Prothioconazole EC 190'.

Pre-harvest interval for each relevant crop

The use patterns for the representative uses of fluoxastrobin containing products in small grain cereals and onions are specified in Table 6.3.1-1, Table 6.3.2-1 and Table 6.3.3-1. No PHI is proposed for wheat, rye, triticale, barley and oat. The proposed use patterns specify that applications be made at particular growth stages and thus the pre-harvest interval is defined by the period between the growth stage at the last application and harvest.
For the representative use on onions, the use pattern involves a PHI of 21 days.
The results of the calculations on dietary exposure demonstrate that the supported use patterns are acceptable with regard to consumer protection.

Re-entry period for livestock to areas to be grazed

It is not relevant to define a re-entry period for livestock after use of the products on small grain cereals or onions since these crops are not intended to be grazed by livestock.

Re-entry period for man to crops, buildings or spaces treated

The products are applied in the field to cereals or onions via tractor mounted field crop sprayers and there is no reason to enter the crop shortly after treatment. No manual activities are necessary for maintaining the crops. Harvesting is performed by appropriate machines. It is therefore not necessary to define particular re-entry times for workers. As a general rule, however, treated fields should not be re-entered until the spray deposit is completely dry.

Withholding period for animal feedingstuffs

According to the agreements in the EFSA document on 'Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin' (September 2015) the representative uses on small grain cereals are understood as uses for grain production and therefore, only residues in grain and straw from cereals are considered as relevant feeding items of the EU livestock diet.
The highest levels of fluoxastrobin related residues likely to be present in these commodities were taken into account as appropriate, to estimate the dietary burden of livestock. The calculation of chronic and acute intakes by consumers shows that the residue levels in animal tissues, milk and eggs are low and do not cause a consumer health concern. It is not necessary to define a withholding period for animal feeding stuff.
Onions are not a relevant feeding item according to the OECD guidance (N°73) document.

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Waiting period between last application and sowing or planting the crops to be protected

The product is always applied as a foliar application after sowing cereals or onions to be protected. Therefore, there is no need to define a waiting period between last application and sowing or planting the crops to be protected.

Waiting period between last application and handling treated products

Handling of treated cereals is generally not required before harvest, which is always done mechanically. The use on onions is restricted to mechanical harvest after a minimum waiting period of 21 days. Furthermore, the residue levels of fluoxastrobin in grain or onion bulbs are low. Therefore, there is no need to define a waiting period between application and handling treated products.

Waiting period between last application and sowing or planting succeeding crops

Rotational crop metabolism studies were conducted on wheat, Swiss chard and turnips at exaggerated rates. Residues of the parent compound (HEC 5725 E-and Z-isomer) and metabolites are not anticipated in cereal grain, in leafy or root crops taking into account the overdose of the confined rotationalcrop studies, interception of the crop at application and agricultural practice of applying split applications. Possible residues in straw are low and not expected to significantly contribute to the residues found in straw from the primary crops.

In the EFSA reasoned opinion on existing MRLs (2012) it is concluded that specific plant-back restrictions related to the use of fluoxastrobin are not required, provided that fluoxastrobin is applied in compliance with the GAPs evaluated in the document and which involve higher application rates or more treatments compared to the representative uses.

As outlined in CA 6.6.2 the contribution of fluoxastrobin residues present in soil from previous treatments to the overall soil concentration is low. The exaggerations used in the CRC studies and applied to bare soil by far exceed predicted soil concentrations resulting from the maximum seasonal rate and potential left-over after year-on-year use.

Therefore, it is not necessary to set a waiting period before sowing or planting succeeding crops for the purpose of limiting the residue levels in these crops.

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CA 6.9 Estimation of the potential and actual exposure through diet and other sources

The toxicological reference values (ADI, ARfD) as published in the EFSA Scientific Report (2007) and the Review Report (SANCO/3921/07 final – 28 September 2012) are summarized in the table below.

Table 6.9- 1: Toxicological endpoints for fluoxastrobin

Endpoint	Value (mg/kg bw/day)	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.015	dog- 1year study	100	EFSA Scientific Report (2007) 102, 1-84 and Review Report (SANCO/3921/07 final)
Acute Reference Dose (ARfD)	0.3	dog- first week of 90days and 1year study	100	

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Since the MRL application jointed to the present dossier concerns only the representative uses (barley, oats, wheat, rye) and the same sets of residue data are used to support the MRL application as well as the representative uses the TMDI or NEDI calculation according to EFSA PRIMo do not differ (cf. LoEP) for

- a consumer risk assessment when including the representative uses and uses related to an MRL application and
- a consumer risk assessment limited to the representative uses.

TMDI calculation

The Theoretical Maximum Daily Intake (TMDI) was calculated using the EFSA PRIMo rev. 2 and compared with the toxicological reference value. Input values for three scenarios are used.

- Established MRLs as published in the EU Pesticides database on the EU Commission website which are in place when the dossier was prepared (Reg. (EC) 839/2008).
- MRLs as proposed in the EFSA Reasoned Opinion (review according to Art 12 of Reg. 396/2005) and drafted in Regulation SANCO/11739/2013 rev 0.
- As above for scenario 2 but with MRLs for barley, oat, wheat and rye as proposed in the present dossier.

All input data were entered at the lowest level of aggregation. Table 6.9- 2 compiles the input data for the calculations.

Table 6.9- 3, Table 6.9- 4 and Table 6.9- 5 summarise the results of the TMDI calculations. When running the calculation with the existing MRLs, the total calculated intake values accounted up to 43.1% of the ADI for the Dutch child with milk and milk products as the highest contributing commodities. The MRL for milk of 0.2 mg/kg is unrealistically high and was subject to change already in the EFSA Reasoned Opinion (2012).

The 2nd calculation using the MRLs as drafted in the Regulation SANCO/11739/2013 rev 0 result in a maximum usage of 5.9% of the ADI for the IE sdult with barley grain contributing most.

Replacing the MRLs as proposed in the EFSA Reasoned Opinion (2012) by the new MRL proposals for small cereal grain (cf. Table 6.7.2- 3) and eggs in the present dossier results in a slightly higher

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usage of the ADI (6.4%). Milk and milk products are then again the highest contributors to the diet of the Dutch child.

Table 6.9- 2: TMDI calculation: Input values according to the representative uses for the chronic consumer risk assessment

Commodity	EU MRL (EC) 839/2008 (mg/kg)	EFSA Reasoned Opinion (2012) [SANCO/11739/2013 (draft)]	EFSA Reasoned Opinion (2012) [SANCO/11739/2013 (draft)] and new MRL proposals for small grain cereals and eggs in present dossier (mg/kg)
Commodities of plant origin			
Onions	0.05*	0.04	**0.04**
Barley	0.5	0.5	**0.4**
Oat	0.5	0.5	**0.4**
Rye	0.5	0.02	**0.04**
Wheat (spelt, triticale)	0.05*	0.02	**0.04**
Commodities of animal origin			
Swine meat	0.05	0.02*	0.02*
Swine fat	0.05	0.02*	0.02*
Swine liver	0.1	0.04*	0.04*
Swine kidney	0.1	0.04*	0.04*
Bovine, sheep, goat, equine, other farm animals: meat	0.05	0.02*	0.02*
Bovine, sheep, goat, horse other farm animals : fat	0.05	0.05	0.05
Bovine, sheep, goat, horse, other farm animals: liver	0.05	0.04*	0.04*
Bovine, sheep, goat, horse, other farm animals: kidney	0.1	0.1	0.1
Poultry meat	0.05	0.05	0.05
Poultry fat	0.05	0.05	0.05
Poultry liver	0.05	0.05	0.05
Poultry kidney	0.1	0.1	0.1
Milk	0.2	0.02*	0.02*
Eggs	0.01*	0.01*	**0.02***
Other terrestrial animals and their products	0.01*	0.01*	0.01*

* indicates that the MRL is set at the LOQ of the analytical method

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Table 6.9- 3: TMDI calculation for the representative uses of Fluoxastrobin and based on MRLs as published with Regulation (EC) 839/2008 (in place when the dossier is prepared); EFSA PRIMo rev. 2

				fluoxastrobin					
				Status of the active substance:		Code no.			
				LOQ (mg/kg bw):		proposed LOQ:			
				Toxicological end points					
				ADI (mg/kg bw/day):	**0,015**	ARfD (mg/kg bw):	**0,3**		
				Source of ADI:	**EFSA, 2007**	Source of ARfD:	**EFSA, 2007**		
				Year of evaluation:		Year of evaluation:			
Explain choice of toxicological reference values.									
The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).									
The pTMRLs have been submitted to EFSA in September 2006.									
Chronic risk assessment									
				TMDI (range) in % of ADI minimum - maximum					
				0	43				
				No of diets exceeding ADI:					
	Highest calculated TMDI values in % of ADI	MS Diet	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 contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
	43,1	NL child	39,1	Milk and milk products: Cattle	1,6	Wheat	0,6	Rye	
	35,1	FR infant	34,3	Milk and milk products: Cattle	0,3	Wheat	0,2	Bovine: Meat	
	24,4	DE child	19,0	Milk and milk products: Cattle	2,6	Rye	1,4	Wheat	
	19,8	ES child	16,7	Milk and milk products: Cattle	1,5	Wheat	0,5	Bovine: Meat	
	18,8	SE general population 90th percentile	16,5	Milk and milk products: Cattle	1,1	Wheat	1,0	Rye	
	18,0	DK child	14,7	Rye	1,8	Wheat	1,3	Oats	
	12,6	WHO Cluster diet F	7,2	Milk and milk products: Cattle	2,5	Rye	2,0	Barley	
	12,0	WHO cluster diet D	9,3	Milk and milk products: Cattle	2,2	Wheat	1,4	Rye	
	11,8	NL general	8,7	Milk and milk products: Cattle	1,2	Barley	0,7	Wheat	
	10,9	WHO cluster diet E	4,0	Milk and milk products: Cattle	3,7	Barley	1,4	Rye	
	10,5	IE adult	4,1	Barley	3,7	Milk and milk products: Cattle	0,8	Wheat	
	10,3	LT adult	6,3	Milk and milk products: Cattle	3,6	Rye	0,4	Wheat	
	10,2	WHO regional European diet	6,4	Milk and milk products: Cattle	1,1	Barley	1,0	Wheat	
	9,9	ES adult	6,6	Milk and milk products: Cattle	1,6	Barley	0,8	Wheat	
	9,7	WHO Cluster diet B	4,0	Milk and milk products: Cattle	2,8	Wheat	0,9	Barley	
	5,2	FR all population	3,6	Milk and milk products: Cattle	1,1	Wheat	0,2	Poultry: Meat	
	3,6	DK adult	2,3	Rye	0,7	Wheat	0,4	Oats	
	3,0	FI adult	2,3	Rye	0,3	Wheat	0,3	Oats	
	2,3	IT kids/toddler	2,2	Wheat	0,0	Onions	0,0	Barley	
	2,1	PT General population	1,3	Wheat	0,5	Rye	0,1	Onions	
	1,9	FR toddler	0,9	Wheat	0,4	Bovine: Meat	0,3	Poultry: Meat	
	1,8	UK Infant	0,9	Wheat	0,8	Oats	0,1	Onions	
	1,6	UK Toddler	0,9	Wheat	0,2	Oats	0,1	Onions	
	1,4	IT adult	1,4	Wheat	0,0	Onions	0,0	Barley	
	1,1	UK vegetarian	0,7	Wheat	0,2	Oats	0,1	Onions	
	0,8	UK Adult	0,6	Wheat	0,1	Barley	0,1	Oats	
	0,1	NL general population	0,1	Onions		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	

Prepare workbook for refined calculations

Undo refined calculations

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Table 6.9- 4: TMDI calculation for the representative uses of Fluoxastrobin based on proposed MRLs in the EFSA Reasoned Opinion (EFSA Journal 2012;10(12):3012 EFSA PRIMo rev. 2

fluoxastrobin			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0,015**	ARfD (mg/kg bw):	**0,3**
Source of ADI:	**EFSA, 2007**	Source of ARfD:	**EFSA, 2007**
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2009. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range in % of ADI
minimum maximum
6

No of diets exceeding ADI: ---

Highest calculated TMDI values in % of ADI	MS Diet	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 contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
5,9	IE adult	4,1	Barley	0,6	Oats	0,4	Milk and milk products: Cattle	
5,8	NL child	3,9	Milk and milk products: Cattle	0,6	Wheat	0,4	Oats	
4,8	WHO cluster diet E	2,7	Barley	0,5	Wheat	0,4	Milk and milk products: Cattle	
4,2	WHO Cluster diet F	2,0	Barley	0,5	Milk and milk products: Cattle	0,5	Oats	
3,9	FR infant	3	Milk and milk products: Cattle	0,2	Poultry: Meat	0,1	Wheat	
3,7	DE child	1,9	Milk and milk products: Cattle	0,7	Oats	0,5	Wheat	
3,5	WHO Cluster diet B	1,1	Wheat	0,9	Barley	0,4	Milk and milk products: Cattle	
3,2	ES child	1,7	Milk and milk products: Cattle	0,6	Wheat	0,4	Poultry: Meat	
3,2	WHO regional European diet	1,1	Barley	0,6	Milk and milk products: Cattle	0,4	Wheat	
3,2	ES adult	1,6	Barley	0,7	Milk and milk products: Cattle	0,3	Wheat	
3,1	WHO cluster diet D	0,9	Wheat	0,7	Barley	0,6	Milk and milk products: Cattle	
3,0	NL general	1,2	Barley	0,9	Milk and milk products: Cattle	0,3	Wheat	
2,7	DK child	1,3	Oats	0,7	Wheat	0,6	Rye	
2,3	SE general population 90th percentile	1,7	Milk and milk products: Cattle	0,4	Wheat	0,1	Onions	
1,6	LT adult	0,5	Milk and milk products: Cattle	0,3	Oats	0,2	Barley	
1,3	UK Infant	0,8	Oats	0,3	Wheat	0,0	Onions	
1,2	FR all population	0,4	Wheat	0,4	Milk and milk products: Cattle	0,2	Poultry: Meat	
1,0	FR toddler	0,3	Wheat	0,3	Poultry: Meat	0,2	Bovine: Meat	
1,0	IT kids/toddler	0,9	Wheat	0,0	Barley	0,0	Onions	
0,9	DK adult	0,4	Oats	0,3	Wheat	0,1	Rye	
0,8	PT General population	0,5	Wheat	0,1	Barley	0,1	Barley	
0,8	UK Toddler	0,5	Wheat	0,2	Oats	0,1	Onions	
0,6	FI adult	0,3	Oats	0,1	Wheat	0,1	Rye	
0,6	IT adult	0,6	Wheat	0,0	Barley	0,0	Onions	
0,6	UK vegetarian	0,3	Wheat	0,2	Oats	0,1	Barley	
0,4	UK Adult	0,2	Wheat	0,1	Barley	0,1	Oats	
0,1	PL general population	0,1	Onions		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	

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Table 6.9- 5 TMDI calculation for the representative uses of Fluoxastrobin based on proposed MRLs in the EFSA Resoned Opinion (EFSA Journal 2012;10(12):3012 and new MRL proposals for small grain cereals and eggs; EFSA PRIMo rev. 2

fluoxastrobin

Prepare workbook for refined calculations

Undo refined calculations

Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,015	ARfD (mg/kg bw):	0,1
Source of ADI:	EFSA, 2007	Source of ARfD:	EFSA, 2007
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum	maximum
No of diets exceeding ADI:	0	0

Highest calculated TMDI values in % of ADI	MS Diet	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 contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
6,4	NL child	3,9	Milk and milk products: Cattle	1,1	Wheat	0,3	Oats	
5,3	IE adult	3,3	Barley	0,6	Wheat	0,5	Oats	
4,8	WHO cluster diet E	2,2	Barley	1,1	Wheat	0,4	Milk and milk products: Cattle	
4,5	WHO Cluster diet B	2,3	Wheat	0,8	Barley	0,4	Milk and milk products: Cattle	
4,3	WHO Cluster diet F	1,6	Barley	1,0	Wheat	0,5	Milk and milk products: Cattle	
4,2	DE child	1,6	Milk and milk products: Cattle	1,1	Wheat	0,5	Oats	
4,0	FR infant	3,4	Milk and milk products: Cattle	0,2	Wheat	0,2	Poultry: Meat	
3,8	ES child	1,7	Milk and milk products: Cattle	1,2	Wheat	0,4	Poultry: Meat	
3,8	DK child	1,5	Wheat	1,1	Rye	1,1	Oats	
3,8	WHO cluster diet D	1,7	Wheat	0,6	Milk and milk products: Cattle	0,6	Barley	
3,3	WHO regional European diet	0,9	Barley	0,8	Wheat	0,6	Milk and milk products: Cattle	
3,1	ES adult	1,3	Barley	0,7	Milk and milk products: Cattle	0,6	Wheat	
3,0	NL general	1,0	Barley	0,9	Milk and milk products: Cattle	0,6	Wheat	
2,8	SE general population 90th percentile	1,7	Milk and milk products: Cattle	0,9	Wheat	0,1	Onions	
1,8	IT kids/toddler	0,9	Wheat	0,0	Onions	0,0	Barley	
1,8	LT adult	0,5	Milk and milk products: Cattle	0,3	Rye	0,3	Wheat	
1,6	FR all population	0,9	Wheat	0,2	Milk and milk products: Cattle	0,2	Poultry: Meat	
1,5	UK Infant	0,7	Wheat	0,0	Oats	0,0	Onions	
1,3	FR toddler	0,7	Wheat	0,3	Poultry: Meat	0,2	Bovine: Meat	
1,3	PT General population	1,0	Wheat	0,1	Onions	0,1	Barley	
1,3	UK Toddler	1,0	Wheat	0,1	Oats	0,1	Onions	
1,2	DK adult	0,5	Wheat	0,3	Oats	0,2	Rye	
1,2	IT adult	0,9	Wheat	0,0	Onions	0,0	Barley	
0,8	UK vegetarian	0,5	Wheat	0,1	Oats	0,1	Onions	
0,8	FI adult	0,3	Wheat	0,2	Oats	0,2	Rye	
0,6	UK adult	0,4	Wheat	0,1	Barley	0,0	Oats	
0,1	PL general population	0,1	Onions		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	

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<u>NEDI calculation</u>

For wheat, rye, barley, oat and onions the median residues as described in the present dossier are used as input data. For wheat, rye, barley and oat the median values are derived from the residue data reflecting the critical GAPs for these crops. Residue levels in food of animal origin are estimated to remain at the LOQ of the analytical method.

Table 6.9- 6 compiles the input values for the NEDI calculation using EFSA PRIMo rev 2. In addition the chronic exposure is calculated using the UK 10 consumer group model based on the same input values. Calculation details are compiled in Table 6.9- 7 and Table 6.9- 8 below.

Table 6.9- 6: Input values for the chronic consumer risk assessment (NEDI) for fluoxastrobin

Commodity	Input value (mg/kg)	Comment	Source
Proposed risk assessment residue definition: *sum of fluoxastrobin (E-isomer) and Z-isomer*			
Onions	0.01	STMR	Present dossier
Barley and oats grain	0.02	STMR	Present dossier
Rye and wheat grain	0.01	STMR	Present dossier
Proposed risk assessment residue definition: *sum of fluoxastrobin (E-isomer) and Z- isomers and the metabolite M55 (HEC 5725 phenoxy-hydroxypyrimidine)*			
Swine meat	0.02*	Median a)	Values reported in EFSA Journal 2012; 10(12):3012 adjusted to the residue levels as anticipated from the dietary burden using the OECD feeding tables and the residue levels reported in the present dossier
Swine fat (free of lean meat)	0.02*	Median a)	
Swine liver	0.02*b)	Median a) b)	
Swine kidney	0.02*b)	Median a) b)	
Meat (bovine, sheep, goat)	0.02*	Median a)	
Fat (bovine, sheep, goat)	0.02*	Median a)	
Liver (bovine, sheep, goat)	0.02*	Median a) b)	
Kidney (bovine, sheep, goat)	0.02*	Median a) b)	
Poultry meat	0.02*	Median a)	Present dossier
Poultry fat	0.02*	Median a)	
Poultry liver	0.02*	Median a) b)	
Poultry kidney	0.02*	Median a) b)	
Milk	0.02*	Median a)	EFSA Journal 2012; 10(12):3012
Eggs	0.02*	Median a)	Present dossier

* indicates that the input value is set at the LOQ of the analytical method

a) In the EFSA Reasoned Opinion (2012) the median residue for food of animal origin was used for the consumer risk assessment derived by interpolation/extrapolation from the feeding study for the median dietary burden.

b) In the EFSA Reasoned Opinion (2012) the median was estimated at 0.04 mg/kg for ruminant and pig liver and kidney derived from the calculated EU dietary burden and the EU feeding tables (guidance document 7031/VI/95 rev 4) and taking into account the LOQ of the previous enforcement method. The new method based on QuEChERS reported in CA 4.2 achieves an LOQ of 0.01 mg/kg for the sum of fluoxastrobin and its Z-isomer and 0.01 mg/kg for M55. Therefore, it deems justified to use an input value of 0.02 mg/kg for the total residue derived from the feeding study.

The highest NEDI was calculated to exhaust 4.8 % of the ADI. The highest contributor (3.9%) was milk and milk products from cattle for the Dutch children diet. According to the UK model the highest intake was determined for the consumer group of infants where the NEDI was found to represent about 14% of the ADI.

It is concluded that long-term exposure to fluoxastrobin residues in food does not cause any unacceptable risk to consumers.

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Table 6.9- 7: NEDI calculation for the representative uses of Fluoxastrobin; EFSA PRIMo rev. 2

				fluoxastrobin							
				Status of the active substance:		Code no.					
				LOQ (mg/kg bw):		proposed LOQ:					
				Toxicological end points							
				ADI (mg/kg bw/day):	**0,015**	ARfD (mg/kg bw):	**0,3**				
				Source of ADI:	**EFSA, 2007**	Source of ARfD:	**EFSA, 2007**				
				Year of evaluation:		Year of evaluation:					
Explain choice of toxicological reference values.											
The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).											
The pTMRLs have been submitted to EFSA in September 2006.											
Chronic risk assessment											
				TMDI (range) in % of ADI minimum - maximum							
					5						
				No of diets exceeding ADI:							
	Highest calculated TMDI values in % of ADI	MS Diet	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 contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)		
	4,8	NL child	3,9	Milk and milk products: Cattle	0,3	Wheat	0,2	Swine: Meat			
	3,7	FR infant	3,4	Milk and milk products: Cattle	0,1	Bovine: Meat	0,1	Poultry: Meat			
	2,6	ES child	1,7	Milk and milk products: Cattle	0,3	Wheat	0,2	Bovine: Meat			
	2,6	DE child	1,9	Milk and milk products: Cattle	0,3	Wheat	0,1	Birds' eggs			
	2,0	SE general population 90th percentile	1,7	Milk and milk products: Cattle	0,2	Wheat	0,1	Birds' eggs			
	1,6	WHO Cluster diet B	0,6	Wheat	0,4	Milk and milk products: Cattle	0,1	Poultry: Meat			
	1,5	WHO regional European diet	0,6	Milk and milk products: Cattle	0,2	Wheat	0,2	Swine: Meat			
	1,5	WHO cluster diet D	0,6	Milk and milk products: Cattle	0,4	Wheat	0,1	Bovine: Meat			
	1,4	NL general	0,9	Milk and milk products: Cattle	0,1	Wheat	0,1	Swine: Meat			
	1,4	WHO Cluster diet F	0,5	Milk and milk products: Cattle	0,3	Wheat	0,2	Swine: Meat			
	1,3	WHO cluster diet E	0,4	Milk and milk products: Cattle	0,3	Wheat	0,1	Poultry: Meat			
	1,3	ES adult	0,7	Milk and milk products: Cattle	0,2	Wheat	0,1	Bovine: Meat			
	1,0	IE adult	0,4	Milk and milk products: Cattle	0,2	Barley	0,2	Wheat			
	1,0	LT adult	0,5	Milk and milk products: Cattle	0,1	Swine: Meat	0,1	Rye			
	0,9	DK child	0,4	Wheat	0,3	Rye	0,1	Birds' eggs			
	0,8	FR all population	0,4	Milk and milk products: Cattle	0,2	Wheat	0,1	Poultry: Meat			
	0,7	FR toddler	0,2	Bovine: Meat	0,2	Wheat	0,1	Birds' eggs			
	0,5	IT kids/toddler	0,4	Wheat	0,0	Onions	0,0	Barley			
	0,4	UK Infant	0,2	Birds' eggs	0,2	Wheat	0,0	Oats			
	0,4	UK Toddler	0,3	Wheat	0,1	Birds' eggs	0,0	Onions			
	0,3	DK adult	0,1	Wheat	0,1	Bovine: Meat	0,0	Birds' eggs			
	0,3	PT General population	0,3	Wheat	0,0	Onions	0,0	Rye			
	0,3	IT adult	0,3	Wheat	0,0	Onions	0,0	Barley			
	0,2	UK vegetarian	0,1	Wheat	0,0	Birds' eggs	0,0	Onions			
	0,2	UK Adult	0,1	Wheat	0,0	Birds' eggs	0,0	Onions			
	0,2	FI adult	0,1	Wheat	0,0	Rye	0,0	Birds' eggs			
	0,0	PT general population	0,0	Onions		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)			

Prepare workbook for refined calculations

Undo refined calculations

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Table 6.9- 8: Fluoxastrobin - Details of the NEDI-calculation according to the UK 10 consumer group model (2006)

ADI: 0.015 mg/kg body weight/day Body weight: see individual populations

Commodities	Residue level (STMR) [mg/kg]	Total Maximum Daily Intake [mg/kg bw/d] Adult (76.0 kg bw)	Infant (8.7 kg bw)	Toddler (14.5 kg bw)	Child 4-6 yrs. (20.5 kg bw)	Child 7-10 yrs. (30.9 kg bw)
commodities of plant origin						
Onions	0.01	0.00001	0.00001	0.00001	0.00001	0.00001
Oats	0.02	0.00001	0.00004	0.00002	0.00002	0.00001
Barley	0.02	0.00000	L/C	0.00001	0.00001	0.00002
Wheat	0.01	0.00004	0.00003	0.00008	0.00009	0.00007
Rye	0.01	0.00001	0.00001	0.00000	0.00000	0.00000
commodities of animal origin						
Poultry	0.02	0.00003	0.00003	0.00006	0.00006	0.00004
Meat fat	0.02	0.00000	0.00001	0.00001	0.00001	0.00001
meat excl. poultry	0.02	0.00004	0.00008	0.00008	0.00007	0.00006
All types of kidney	0.02	0.00001	0.00001	0.00003	0.00001	0.00000
All types of liver	0.02	0.00001	0.00004	0.00005	0.00001	0.00001
All types of offal	0.02	0.00001	0.00003	0.00004	0.00002	0.00002
Eggs	0.02	0.00002	0.00009	0.00007	0.00005	0.00003
Milk	0.02	0.00016	0.00195	0.00112	0.00059	0.00036
Total dietary intake: [mg/kg bw/d]		**0.00024**	**0.00212**	**0.00126**	**0.00074**	**0.00048**
% ADI exhaustion:		**2%**	**14%**	**8%**	**5%**	**3%**

Commodities	Residue level (STMR) [mg/kg]	Total Maximum Daily Intake [mg/kg bw/d] Child 11-14 yrs. (48.0 kg bw)	Child 15-18 yrs. (63.8 kg bw)	Vegetarian (66.7 kg bw)	Elderly (own home) (70.8 kg bw)	Elderly (residential) (61.6 kg bw)
commodities of plant origin						
Onions	0.01	0.00001	0.00001	0.00001	0.00001	0.00000
Oats	0.02	0.00001	0.00001	0.00001	0.00001	0.00001
Barley	0.02	0.00000	0.00000	0.00001	0.00001	0.00000
Wheat	0.01	0.00005	0.00004	0.00004	0.00003	0.00003
Rye	0.01	0.00000	0.00000	0.00001	0.00000	0.00000
commodities of animal origin						
Poultry	0.02	0.00003.	0.00003	0.00003	0.00003	0.00002
Meat fat	0.02	0.00001	0.00000	0.00000	0.00000	0.00000
meat excl. poultry	0.02	0.00004	0.00004	0.00001	0.00004	0.00003
All types of kidney	0.02	0.00000	0.00001	L/C	0.00001	0.00001
All types of liver	0.02	0.00001	0.00001	L/C	0.00001	0.00001
All types of offal	0.02	0.00002	0.00002	0.00001	0.00002	0.00001
Eggs	0.02	0.00003	0.00002	0.00002	0.00002	0.00003
Milk	0.02	0.00024	0.00019	0.00019	0.00017	0.00024
Total dietary intake: [mg/kg bw/d]		**0.00032**	**0.00027**	**0.00025**	**0.00024**	**0.00030**
% ADI exhaustion:		**2%**	**2%**	**2%**	**2%**	**2%**

L/C = Low consumption (<0.1 g/day) or low number of consumers (<4)

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Acute Reference Dose (ARfD) and Dietary Exposure Calculation

In order to evaluate the potential acute exposure to fluoxastrobin residues through the diet the National Estimated Short Term Intakes (NESTI)/International Estimated Short Term Intakes (IESTI) were estimated using the EFSA PRIMo model (revision 2.0).
In addition, also the short-term consumer risk for the active substance according to the UK 10 consumer group model was estimated.

The assessment of the acute intake of residues as a result of fluoxastrobin treated crops is based on the highest residue values (sum of fluoxastrobin and its Z-isomer) determined for the critical GAPs in/on wheat, rye, barley, oat and onions and derived from the data sets described under CA 6.3 in the present dossier. Since the same residue data support the critical GAPs relevant to the MRL application for wheat, rye, barley and oat submitted as part of this dossier and the representative uses the same input data (HR values) are applicable for the acute risk assessments.

For commodities of animal origin the highest residues are estimated based on the findings from the livestock feeding studies extrapolated to the calculated dietary burden presented in CA 6.4, Table 6.4-2. Actually no residues according to the proposed residue definition for animal commodities above the LOQ of the data generation method or the new enforcement method reported in CA 4.2 are anticipated.
Table 6.9- 9 below compiles the input data for the acute dietary risk assessments. Table 6.9- 10 shows the output of the EFSA PRIMo (rev. 2) calculation. Table 6.9- 11 summarises the calculation according to the UK 10 consumer group model (2006).

The highest calculated NESTI according to the EFSA model results in an ARfD usage of 0.8% for adults by intake of barley and 0.8% for children by intake of milk and milk products. The calculation according to the UK model yields the highest result (0.8% of the ARfD) for milk for intake by infants.
Therefore, the short term intake of fluoxastrobin residues is unlikely to present a public health concern.

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Table 6.9- 9: Input values for the acute consumer risk assessment (NESTI) for fluoxastrobin

Commodity	Input value (mg/kg)	Comment	Source
Proposed risk assessment residue definition: *sum of fluoxastrobin (E-isomer) and Z-isomer*			
Onions	0.02	HR	Present dossier
Barley and oats grain	0.34	HR	
Rye and wheat grain	0.02	HR	
Proposed risk assessment residue definition: *sum of fluoxastrobin (E-isomer) and Z- isomers and the metabolite M55 (HEC 5725 phenoxy-hydroxypyrimidine*			
Swine meat	0.02*,	HR [a)]	Values reported in EFSA Journal 2012; 10(12):3012 adjusted to the residue levels as anticipated from the dietary burden using the OECD feeding tables and the residue levels reported in the present dossier
Swine fat (free of lean meat)	0.02*,	HR [a)]	
Swine liver	0.02*,	HR [a) b)]	
Swine kidney	0.02*,	HR [a) b)]	
Meat (bovine, sheep, goat)	0.02*,	HR [a)]	
Fat (bovine, sheep, goat)	0.02*,	HR [a)]	
Liver (bovine, sheep, goat)	0.02*	HR [a) b)]	
Kidney (bovine, sheep, goat)	0.02*	HR [a) b)]	
Poultry meat	0.02*,	HR	Present dossier
Poultry fat	0.02*	HR	
Poultry liver	0.02*	HR	
Poultry kidney	0.02*	HR	
Milk	0.02*	HR [a)]	EFSA Journal 2012; 10(12):3012 and present dossier
Eggs	0.02*	HR	Present dossier

* indicates that the input value is set at the LOQ of the analytical method

a) In the EFSA Reasoned Opinion (2012) the highest residue for food of animal origin was used for the acute consumer risk assessment derived by interpolation/extrapolation from the relevant dose level of the feeding study and for the highest dietary burden.

b) In the EFSA Reasoned Opinion (2012) the highest residue was estimated at 0.03 mg/kg for ruminant fat, 0.04 mg/kg for ruminant liver and 0.06 mg/kg for ruminant kidney derived from the calculated EU dietary burden and the EU feeding tables (guidance document 7031/VI/95 rev 4). The HR of 0.04 mg/kg for pig liver and kidney is based on the LOQ of the previous enforcement method.

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Table 6.9- 10: NESTI calculation for Fluoxastrobin; EFSA PRIMo rev. 2

	Acute risk assessment /children						Acute risk assessment / adults / general population					
	The acute risk assessment is based on the ARfD.											
	For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.											
	In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
	Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
Unprocessed commodities	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**			**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
	IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	0,8	Milk and milk	0,02 / -	0,8	Milk and milk	0,02 / -	0,8	Barley	0,34 / -	0,8	Barley	0,34 / -
	0,5	Oats	0,34 / -	0,5	Oats	0,34 / -	0,2	Oats	0,34 / -	0,2	Oats	0,34 / -
	0,3	Onions	0,02 / -	0,2	Barley	0,34 / -	0,1	Milk and milk	0,02 / -	0,1	Milk and milk products: Cattle	0,02 / -
	0,2	Barley	0,34 / -	0,2	Onions	0,02 / -	0,1	Onions	0,02 / -	0,1	Poultry: Meat	0,02 / -
	0,2	Milk and milk	0,02 / -	0,2	Milk and milk	0,02 / -	0,1	Poultry: Meat	0,02 / -	0,1	Onions	0,02 / -

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Table 6.9- 11: NESTI calculations for fluoxastrobin according to the UK CRD model (2006)

Acute Intakes (97.5th percentiles)

			adult		infant		toddler		4-6 year old child		7-10 year old child	
Commo-dity	**HR**	**P**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**
Onions	0.02		0,00024	0,1	0,00045	0,2	0,00036	0,1	0,00038	0,1	0,00036	0,1
Oats	0.34		0.00031	0.1	0.00107	0.4	0.00105	0.4	0.00062	0.2	0.00070	0.2
Barley	0.34		0.00023	0.1	0.00000	0.0	0.00025	0.1	0.00060	0.2	0.00091	0.6
Wheat	0.02		0.00012	0.0	0.00026	0.1	0.00026	0.1	0.00029	0.1	0.00022	0.1
Rye	0.02		0.00003	0.0	0.00013	0.0	0.00002	0.0	0.00004	0.0	0.00003	0.0
Poultry	0.02		0.00011	0.0	0.00014	0.0	0.00017	0.1	0.00019	0.1	0.00014	0.0
Meat fat	0.02		0.00001	0.0	0.00004	0.0	0.00004	0.0	0.00004	0.0	0.00003	0.0
Meat excl. poultry & offal	0.02		0.00010	0.0	0.00024	0.1	0.00020	0.1	0.00018	0.1	0.00016	0.1
All types of kidney	0.02		0,00003	0,0	0,00005	0,0	0,00008	0,0	0,00005	0,0	0,00003	0,0
All types of liver	0.02		0,00005	0,0	0,00016	0,1	0,00013	0,0	0,00004	0,0	0,00005	0,0
Other types of offal	0.02		0,00006	0,0	0,00015	0,0	0,00014	0,0	0,00011	0,0	0,00011	0,0
Eggs	0.02		0.00006	0.0	0.00025	0.1	0.00016	0.1	0.00013	0.0	0.00010	0.0
Milk	0.02		0.00026	0.1	0.00248	0.8	0.00147	0.5	0.00093	0.3	0.00060	0.2

			11-14 year old child		15-18 year old child		Vegetarian		Elderly - own home		Elderly - residential	
Commo-dity	**HR**	**P**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**	**NESTI**	**% ARfD**
Onions	0.02		0,00031	0,1	0,00025	0,1	0,00030	0,1	0,00019	0,1	0,00012	0,0
Oats	0.34		0.00031	0.1	0.00049	0.2	0.00041	0.1	0.00025	0.1	0.00022	0.1
Barley	0.34		0.00015	0.1	0.00021	0.1	0.00025	0.1	0.00017	0.1	0.00011	0.0
Wheat	0.02		0.00018	0.1	0.00017	0.1	0.00016	0.1	0.00009	0.0	0.00009	0.0
Rye	0.02		0.00001	0.0	0.00002	0.0	0.00003	0.0	0.00002	0.0	0.00001	0.0
Poultry	0.02		0.00012	0.0	0.00011	0.0	0.00023	0.1	0.00009	0.0	0.00005	0.0
Meat fat	0.02		0.00002	0.0	0.00002	0.0	0.00001	0.0	0.00001	0.0	0.00001	0.0
Meat excl. poultry & offal	0.02		0.00011	0.0	0.00011	0.0	0.00005	0.0	0.00007	0.0	0.00006	0.0
All types of kidney	0.02		0,00003	0,0	0,00004	0,0	0,00000	0,0	0,00003	0,0	0,00003	0,0
All types of liver	0.02		0,00008	0,0	0,00004	0,0	0,00000	0,0	0,00005	0,0	0,00004	0,0
Other types of offal	0.02		0,00009	0,0	0,00005	0,0	0,00002	0,0	0,00005	0,0	0,00005	0,0
Eggs	0.02		0.00008	0.0	0.00006	0.0	0.00008	0.0	0.00004	0.0	0.00005	0.0
Milk	0.02		0.00041	0.1	0.00035	0.1	0.00030	0.1	0.00022	0.1	0.00029	0.1

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CA 6.10 Other studies

The studies reported in the baseline dossier and already evaluated for Annex I inclusion according to Directive 91/414 as well as the studies submitted in the present supplementary dossier are considered to provide the necessary information on the metabolism and residue behaviour of fluoxastrobin.
No other studies are considered necessary.

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of such studies is the determination of the residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom (Section 6.10.1 of Com. Reg (EU) No 283/2013).
The Annex of Commission Regulation (EC) No 283/2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 states that the type and conditions of studies to be performed shall be discussed with national competent authorities.
At the time when the present dossier was prepared, no corresponding guidance document was published which addresses reference 6.10.1 of the Annex to Regulation (EU) No 283/2013 and which lists the crops concerned or provides an agreed test methodology.

In the EFSA guidance document on the risk assessment of plant protection products on bees (EFSA Journal 2013;11(7):3295; Appendix D) a list of crops visited by bees for the collection of nectar and/or pollen as complete as possible based on the data available in the literature is compiled. In Appendix D to the guidance document small grain cereals (barley, oat, wheat, rye) are considered as non relevant for nectar collection and they are not attractive for pollen collection.
According to the French proposal for a guideline on setting MRLs in honey and bee products (Hazards of pesticides to bees – 10th international symposium of the ICP-Bee Protection Group) it is proposed in a decision tree that if a crop is not attractive to bees and has no melliferous capacity a specific MRL would not be required.
Onions intended for human consumption can also be considered being not a relevant bee feed item because they are harvested before flowering during the normal production.

The last application of fluoxastrobin containing products to cereals may be before (all small grain cereals) or during (only wheat and rye) flowering according to the GAPs of the representative uses. However, as evident from the residue decline studies fluoxastrobin residues decline well with time and residues are very low in cereal grain. There is no indication from the residue findings that the compound shows persistence in plant commodities or a high potential for translocation in the upper parts of the plant. The findings in the plant metabolism studies demonstrate that the parent compound (E-and Z-isomers) constitutes by far the most abundant residue in cereal grain (52-86% TRR) and measurable residue levels for metabolites are not anticipated.

Small grain cereals are typically wind pollinated and no feeding item for bees. Since according to Appendix D of the EFSA guidance document on the risk assessment of plant protection products on bees (EFSA Journal 2013;11(7):3295) small grain cereals are not relevant for nectar collection fluoxastrobin residues in mature honey are very unlikely (pollen content in honey 0.5%). If in individual cases honey bees may visit small grain cereals during flowering and forage pollen this would result in mixtures of pollen from various plant species and no genuine or homogeneous bee product solely from cereals would be produced which results in further dilution of potential residues..

The current MRL for fluoxastrobin residues in honey and bee products (Code 1040000) is 0.01* mg/kg according to Part A of Annex I to Reg. 396/2005). No exceedance of this MRL was

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noted in the past, although fluoxastrobin products are authorized and marketed in the EU since more than 10 years.

In the absence of a test guideline about how to investigate the residues in pollen and bee products this point was not addressed experimentally. Since the bee-relevant commodities are not expected to contain residues of fluoxastrobin a dietary risk for consumers due consumption of honey and bee products as well as an exceedance of the current MRL of 0.01* mg/kg can be excluded.

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