

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: FF-075

Product name(s): EUSKATEL PRO

Chemical active substance:

Prothioconazole, 200 g/L

Azoxystrobin 150 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New Product Authorization)

Applicant: Rotam Agrochemical Europe Limited

Submission date: June 2021

MS Finalisation date: February 2022; 08/2022

Version history

When	What
1 June 2021	New product application in accordance with Article 33 of Regulation (EC) No.1107/2009
February 2022	zRMS evaluation
August 2022	Final version after commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS comment:

Noticed data gaps are: none. The analytical method proposed for determination of the active substances – **prothioconazole** and **azoxystrobin** in the formulation FF-075 (EUSKATEL PRO) is sufficiently sensitive and selective. Method has been validated in terms of specificity, linearity, precision (repeatability) and accuracy and fulfils the requirements of EEC guideline SANCO/3030/99 rev.5.

Presented analytical method for the determination of relevant impurities – **prothioconazole-deschloro** and **toluene** is specific, sensitive, precise, and accurate according to SANCO/3030/99 rev.5 guideline.

Validated analytical method for the determination of **prothioconazole-deschloro** was also submitted. According to the current consolidated version of the Reg. (EU) No 540/2011, prothioconazole-deschloro is not considered as a relevant impurity. According to the revision of SANCO/3923/07 from January 2021, prothioconazole-deschloro was indicated as the relevant impurity, with no maximum acceptable limit agreed. Still, it was not included in Appendix I, where information on prothioconazole and its impurities is summarised. zRMS conclusion: at the time of the evaluation the analytical method for prothioconazole-deschloro is not required and was not evaluated.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are:

- none

Prothioconazole:

- monitoring methods for body fluids and tissues (post-registration requirement – minor data gap: currently agreed EU endpoints for prothioconazole do not include a residue definition for body fluids and tissues)
- an independent laboratory validation (ILV) for drinking water (post-registration requirement – minor data gap)

Azoxystrobin

- monitoring methods for body fluids and tissues (post-registration requirement - minor data gap)

Commodity/crop	Supported/ Not supported
Cereals	Supported
Oilseed Rape	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods for analysis of prothioconazole and azoxystrobin in the plant

protection product FF-075 is provided below. The method / study has not been evaluated previously at EU level.

Comments of zRMS:	Accepted. The method for the determination of prothioconazole and azoxystrobin in the formulation FF-075 has been validated in accordance with SANCO/3030/99 rev.5.
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Reference:	KCP 5.2.1.1/01
Report	STUDY ON THE METHOD VALIDATION OF PROTHIOCONAZOLE 200 G/L + AZOXYSTROBIN 150 G/L SUSPENSION CONCENTRATE; Lu, J.; 2020; Study No.: 2878
Guideline(s):	Yes – SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (ca. 150 mg) is added to a volumetric flask (50 mL) and acetonitrile added (45 mL). Solutions are sonicated (2 minutes), equilibrated to room temperature and filled to the mark with acetonitrile. Solutions are filtered through a 0.22 µm filter.

Prothioconazole and azoxystrobin content are determined by **UHPLC- UVPDA** at 254 nm using a TC-C18 column (250 x 4.6 mm, 5 µm). Quantification is by calibration curve. Calibration solutions of prothioconazole and azoxystrobin reference standards are prepared in acetonitrile.

Injection volume:	5 µL
Flow rate:	1.0mL/min
Retention time:	Prothioconazole: about 16.9 min Azoxystrobin: about 12.1 min
Run time:	30 min

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of prothioconazole and azoxystrobin in plant protection product FF-075

	Prothioconazole	Azoxystrobin
Author(s), year	Lu, J.; 2020	
Principle of method	UHPLC- UVPDA	
Linearity	Linear range= 382.2 - 640.8 mg/L (purity corrected), equivalent to 12.74 - 21.36 %w/w in the sample (appropriate to the highest and lowest concentrations in test solutions) $r^2 = 0.9997$ $n = 5$ $y = 94849.891 - 120790.917$	Linear range= 299.0 - 501.3 mg/L (purity corrected), equivalent to 9.97 - 16.71 %w/w in the sample (appropriate to the highest and lowest concentrations in test solutions) $r^2 = 0.9997$ $n = 5$ $y = 88605.532 - 55527.946$
Precision (Repeatability)	$n = 6$ mean content = 16.64 %	$n = 6$ mean content = 12.79 %

	RSD = 1.05 % RSD _r = 1.76 % Horrat = 0.60	RSD = 0.32 % RSD _r = 1.863 % Horrat = 0.17
Accuracy (Recovery)	Concentration = 16.49 % w/w n = 5 mean recovery = 98.79 % (within guideline range of 97-103 % for nominal active content >10 % w/w) RSD = 0.97 % Horrat = 0.55	Concentration = 13.07 % w/w n = 5 mean recovery = 99.53 % (within guideline range of 97-103 % for nominal active content >10 % w/w) RSD = 0.34 % Horrat = 0.19
Interference/ Specificity	Representative chromatograms of solvent (acetonitrile) blank, formulation blank, reference standards, calibration standards at the lowest and highest levels, and test solutions are included in the report. No interferences are noted at the retention times of prothioconazole (16.9 min) or azoxystrobin (12.1 min) in the chromatograms of the solvent or blank formulation.	
	Confirmation of peak identity was achieved by retention time match with the analytical standards.	

Conclusion

The method is considered acceptable in terms of linearity, accuracy, precision and specificity for the determination of prothioconazole and azoxystrobin in the plant protection product FF-075, in accordance with SANCO/3030/99 rev.5.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

An overview on the acceptable methods for analysis of relevant impurities prothioconazole-desthio (M04) and toluene (Reg. (EU) No 540/2011 and SANCO/3923/07 – final, 10 December 2007) in the plant protection product FF-075 is provided below. The method/study has not been evaluated previously at EU level.

Comments of zRMS:	Accepted. The method for the determination of prothioconazole-desthio and toluene in the formulation FF-075 has been validated in accordance with SANCO/3030/99 rev.5.
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Reference:	KCP 5.2.1.2/01
Report	METHOD VALIDATION OF RELEVANT IMPURITIES IN PROTHIOCONAZOLE 200 g/L + AZOXYSTROBIN 150 g/L SUSPENSION CONCENTRATE Lu, J.; 2020; Study No.: 2959
Guideline(s):	Yes – SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item (ca. 1000 mg) is added to a volumetric flask (25 mL) and methanol added to just below the

mark. Solutions are sonicated (1 minute), equilibrated to room temperature and filled to the mark with methanol. Prothioconazole-desthio (M04) and toluene contents are determined by **UHPLC- UVPDA** at 220 nm using an EC-C18 column (150 x 4.6 mm, 2.7 µm).

Mobile phase:	Time (minutes)	Buffer (%)	Acetonitrile (%)
	0.00	66	34
	23.00	66	34
	23.01	20	80
	30.00	20	80
	30.10	66	34
	35.00	66	34
Column oven temperature:	40°C		
Flow rate:	1.0 mL/min		
Injection volume:	5 µL		
Detector wavelength:	220 nm		
Retention time:	Prothioconazole-desthio: about 20.0 min Toluene: about 15.5 min		
Run time:	35 min		

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities prothioconazole-desthio (M04) and toluene in plant protection product FF-075

	Prothioconazole-desthio (M04) Max. content: <0.1275 g/kg (<0.0109 % w/w) ^[1]	Toluene Max. content: <1.275 g/kg (<0.109 % w/w) ^[1]
Linearity	<p>Linear range (low level): 0.41 - 1.22 mg/L (purity corrected), equivalent to 0.0010 - 0.0030 % w/w in the sample $r^2 = 0.9996$ $n = 5$ $y = 1230899.488x - 4860.076$</p> <p>Linear range (high level): 1.47 - 4.47 mg/L (purity corrected), equivalent to 0.0037 - 0.0112 % w/w in the sample $r^2 = 0.9993$ $n = 5$ $y = 1159389.994x - 5280.406$</p> <p>Calibration ranges are appropriate to the highest and lowest concentrations of the analyte in relevant analytical solutions $\pm 20\%$</p>	<p>Linear range (low level): 1.22 - 3.41 mg/L (purity corrected), equivalent to 0.0030 - 0.0085 % w/w in the sample $r^2 = 0.9992$ $n = 5$ $y = 447134.182x - 5436.241$</p> <p>Linear range (middle level): 3.17 - 21.93 mg/L (purity corrected), equivalent to 0.0079 - 0.0548 % w/w in the sample $r^2 = 0.9991$ $n = 5$ $y = 340635.910x + 360.820$</p> <p>Linear range (high level): 20.95 - 65.78 mg/L (purity corrected), equivalent to 0.0524 - 0.1644 % w/w in the sample $r^2 = 0.9992$ $n = 5$ $y = 353832.804x + 6283.421$</p> <p>Calibration ranges are appropriate to the highest and lowest concentrations of the analyte in relevant analytical solutions $\pm 20\%$</p>
Precision (Repeatability)	$n = 6$ mean content = 0.0020 % RSD = 1.58 % RSD _r = 6.83 % Horrat = 0.23	Precision of the method was confirmed in the recovery analyses

Accuracy (Recovery) ^[2]	<p><i>At LOQ concentration (0.0019 %w/w):</i> n = 5 mean recovery = 104.88 % (within guideline range of 70 - 130 % for nominal impurity contents <0.01 % w/w) RSD = 0.85 % RSD_r = 6.87 % Horrat = 0.12</p> <p><i>At high concentration (0.075 %w/w):</i> n = 5 mean recovery = 102.39 % (within guideline range of 75 - 125 % for nominal impurity contents ≥0.01 - <0.1 % w/w) RSD = 1.58 % RSD_r = 5.59 % Horrat = 0.28</p>	<p><i>At LOQ concentration (0.0056 %w/w):</i> n = 5 mean recovery = 101.61 % (within guideline range of 70 - 130 % for nominal impurity contents <0.01 % w/w) RSD = 1.65 % RSD_r = 5.85 % Horrat = 0.28</p> <p><i>At high concentration (0.11 %w/w):</i> n = 5 mean recovery = 99.99 % (within guideline range of 80 - 120 % for nominal impurity contents ≥0.1 - <1.0 % w/w) RSD = 0.77 % RSD_r = 3.74 % Horrat = 0.21</p>
Interference/ Specificity	Representative chromatograms of solvent (methanol) blank, formulation blank, reference standards, calibration standards at the lowest and highest levels, test solutions and fortified samples are included in the report. No interferences were noted at the retention times of prothioconazole-desthio (M04) or toluene in the chromatograms of the solvent, prothioconazole reference or blank formulation. Confirmation of peak identities were achieved by retention time matching with analytical standards. Retention times for azoxystrobin, prothioconazole, prothioconazole-desthio and toluene were significantly different under the conditions of the method (23.0, 25.0, 20.0 and 15.5 minutes, respectively). The method is considered specific for prothioconazole-desthio (M04) / toluene in the presence of the other relevant impurity and active substances.	
LOQ	0.0019 % w/w	0.0056 % w/w

^[1] Based on the composition of FF-075 (containing 17.45 %w/w technical prothioconazole with a minimum purity of 980 g/kg and based on a density of 1.169 g/mL at 20 °C). Technical prothioconazole has a specification with limits for relevant impurities outlined in Reg. (EU) No 540/2011, i.e. prothioconazole-desthio (M04): <0.5 g/kg (LOD) and toluene: <5 g/kg.

^[2] Blank formulation was spiked with prothioconazole-desthio and toluene reference standards

Conclusion

The method is considered acceptable in terms of linearity, accuracy, precision and specificity for determination of prothioconazole-desthio (M04) and toluene in the plant protection product FF-075 in accordance with SANCO/3030/99 rev.5. The LOQs of the method (0.0019 and 0.0056 % w/w) are appropriate for the maximum allowed concentrations of the relevant impurities in plant protection products.

Comments of zRMS:	Regarding the analytical method for prothioconazole-deschloro, see point 5.1 above
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Reference: KCP 5.2.1.2/02

Report METHOD VALIDATION FOR IMPURITY-3 (PROTHIOCONAZOLE DESCHLORO) IN PROTHIOCONAZOLE 200 g/L + AZOXYSTROBIN 150 g/L SUSPENSION CONCENTRATE
Lu, J.; 2021; Study No.: 3030

Guideline(s): Yes – SANCO/3030/99 rev.5

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test item (ca. 1.5 g) is added to a volumetric flask (25 mL) filled to the mark with acetonitrile. Prothioconazole-desethio (M04) and toluene contents are determined by HPLC-UV at 220 nm using an EC-C18 column (150 x 4.6 mm, 2.7 µm).

Validation - Results and discussions

Table 5.2-3: Methods suitable for the determination of the relevant impurity prothioconazole-deschloro in plant protection product FF-075

	Prothioconazole-deschloro Max. content: <0.1275 g/kg (<0.0109 %w/w) ^[1]
Linearity	<p>Linear range (low level): 0.25 - 0.75 mg/L (purity corrected), equivalent to 0.006 - 0.018 %w/w in the sample $r^2 = 0.9995$ $n = 5$ $y = 326014.0490x + 637.0756$</p> <p>Linear range (middle level): 0.79 - 2.78 mg/L (purity corrected), equivalent to 0.020 - 0.070 %w/w in the sample $r^2: 0.9991$ $n = 5$ $y = 433462.2008x - 2811.1306$</p> <p>Linear range (high level): 2.94 - 5.00 mg/L (purity corrected), equivalent to 0.073 - 0.125 %w/w in the sample $r^2: 0.9992$ $n = 5$ $y = 354512.4853x - 3919.2597$</p> <p>Calibration ranges are appropriate to the highest and lowest concentrations of the analyte in relevant analytical solutions $\pm 20\%$</p>
Precision (Repeatability)	$n = 6$ mean content = 0.055 % RSD = 1.58 % $RSD_r = 4.15 \%$ Horrat = 0.38
Accuracy (Recovery) ^[2]	<p><i>At LOQ concentration (0.012 %w/w):</i> $n = 5$ mean recovery = 95.55 % (within guideline range of 75 - 125 % for nominal impurity contents ≥ 0.01 - <0.1 %w/w) RSD = 5.10 % $RSD_r = 5.18 \%$ Horrat = 0.98</p> <p><i>At high concentration (0.097 %w/w):</i> $n = 5$ mean recovery = 98.06 % (within guideline range of 75 - 125 % for nominal impurity contents ≥ 0.01 - <0.1 %w/w) RSD = 0.78 % $RSD_r = 3.81 \%$ Horrat = 0.21</p>
Interference/ Specificity	Representative chromatograms of solvent blank, formulation blank, reference standard, calibration standards at the lowest and highest levels, test solutions and fortified samples are included in the report. No interferences were noted at the retention times of prothioconazole-deschloro in the chromatograms of the solvent, reference standard or blank formulation. Confirmation of peak

	identities were achieved by retention time matching with analytical standards. Retention times for azoxystrobin, prothioconazole and prothioconazole-deschloro were significantly different under the conditions of the method (25.6, 27.9, and 23.7 minutes, respectively). The method is considered specific for the determination of prothioconazole-deschloro in FF-075.
LOQ	0.0012 % w/w

^[1] Based on the composition of FF-075 (containing 17.45 %w/w technical prothioconazole with a minimum purity of 980 g/kg and based on a density of 1.169 g/mL at 20 °C). Technical prothioconazole has a specification with limits for prothioconazole-deschloro of <0.5 g/kg outlined in the Review Report (SANCO/3923 /07 - final, 26 January 2021)

^[2] Blank formulation was spiked with prothioconazole-deschloro reference standard

Conclusion

The method is considered acceptable in terms of linearity, accuracy, precision and specificity for determination of prothioconazole-deschloro in the plant protection product FF-075 in accordance with SANCO/3030/99 rev.5. The LOQ of the method (0.0012 % w/w) is appropriate for the maximum allowed concentrations of prothioconazole-deschloro in plant protection products.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Not applicable – The formulation does not contain any relevant co-formulants.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A reverse phase HPLC method (CIPAC/5159) for the determination of prothioconazole in TC, EC, FS and SC formulations was accepted as a full CIPAC method at the 63rd CIPAC Meeting in Braunschweig, Germany on 19th June 2019.

A reverse phase HPLC method (CIPAC 5251/m) for the determination of prothioconazole-desthio (M04) was adopted at the 64th CIPAC Virtual Meeting in June 2020.

A GC method (CIPAC 571/SC/M/3) for the determination of azoxystrobin in SC formulations is available in CIPAC Handbook M (2009).

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Prothioconazole

An overview on the acceptable methods for analysis of residues of prothioconazole for the generation of pre-authorization data is given in the following tables.

In the 2007 EFSA Conclusion, the risk assessment residue definitions for prothioconazole are:

- **Foodstuffs of plant and animal origin:** Sum of prothioconazole-desthio (M04) and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety expressed as prothioconazole-desthio (M04). This definition is *provisional*, depending on the outcome of the EU discussion regarding triazole derivative metabolites.
- **Soil:** Prothioconazole, prothioconazole-S-methyl (M01) and prothioconazole-desthio (M04)
- **Ground water:** Prothioconazole, prothioconazole-S-methyl (M01) and prothioconazole-desthio (M04)
- **Surface water:** Prothioconazole, prothioconazole-desthio (M04) and 1,2,4-triazole
- **Sediment:** Prothioconazole and prothioconazole-desthio (M04)

- **Air:** Prothioconazole and prothioconazole-desthio (M04)
- **Body fluids and tissues:** None*

*In the 2007 EFSA Conclusion, the residue definition was ‘Open’, based on whether the active would be classified as toxic. Currently, prothioconazole has no classification for human health hazards (Reg. (EC) No 1272/2008 – the CLP Regulation). On this basis alone, a residue definition in this matrix is not required for authorisation of FF-075. Rotam are aware that a CLH dossier for prothioconazole was submitted by the UK authorities in March 2018 and a RAC Opinion was adopted on 15th March 2019 (CLH-O-0000001412-86-269/F) supporting the following classification:

- Aquatic Acute 1 (H400)
- Aquatic Chronic 1 (H410)

The opinion is yet to be agreed by the COM (and feature in an amendment to the CLP Regulation), however, if accepted, a residue definition in body fluids and tissues is still not necessary.

Study summaries of any new studies are provided in Appendix 2.

Table 5.2-4: Validated methods for the generation of pre-authorization data

Component of residue definition: prothioconazole				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuffs of plant origin: High starch/ high protein content (dry) (Storage stability – Residues)	Primary	0.01 mg/kg (wheat grain) 0.05 mg/kg (wheat – forage and straw)	LC-MS/MS Method: 00598 and 00598/M001 Analyte: Prothioconazole i) Extraction with ACN/H ₂ O (with added cysteine HCl) ii) Partition with hexane and CH ₂ Cl ₂ m/z: Not stated	Heinemann, 2000a, Report no. 00598, KCP 5.2.2/01 and Heinemann, 2000b, Report no. 00598/M001, KCP 5.2.2/02 EU agreed (DAR, 2004, RMS: UK) Identical to post-registration method (see: KCP 5.3.2.2/03 and KCP 5.3.2.2/04)
	Confirmatory	Not required – The primary method is considered highly specific.		
Foodstuff of animal origin (Residues)	No specific methods for the support of residues (foodstuffs of animal origin) have been developed for the authorisation of FF-075.			
Soil (Storage stability and field studies – Environmental fate)	Primary	0.006 mg/kg	LC-MS/MS Analyte: Prothioconazole Method: Not stated, but similar to 00610 i) Extraction with ACN/H ₂ O/cysteine hydrochloride monohydrate ii) Filtered m/z: Not stated	Reference to validation study not given in DAR. Risk assessment study: Schramel, 2001, Report no. MR-644/99, KCP 5.2.2/03 00610: Schramel, 2000, Report no. 00610, KCP 5.2.2/04 EU agreed (DAR, 2004, RMS: UK) Not confirmed as identical, but method is similar to post-registration method (see: KCP 5.3.2.4/01)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	Analytical methods for the determination of prothioconazole in various matrices were relied on to support the toxicology package for Annex I approval. These same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and			

Component of residue definition: prothioconazole				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
	supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are anticipated to be acceptable for the purposes of this submission. Further, the dRAR (2018) stated: <i>The methods of analysis for the determination of prothioconazole in liquid application medium (0.5% (v:v) aqueous tylose solutions/suspension) were submitted and evaluated by the applicant. These methods supported studies which were originally accepted for the Annex I inclusion of prothioconazole. As such, the evaluation of these analytical methods is not required and no further consideration is required.</i>			
Garments used for operator protection (Exposure)	Primary	Not stated	LC-MS/MS Analyte: Prothioconazole Method: Adaption of 00598 and 00598/M001. Specific adaption not stated. m/z: Not stated	00598 and 00598/M001: Heinemann, 2000a, Report no. 00598, KCP 5.2.2/01 and Heinemann, 2000b, Report no. 00598/M001, KCP 5.2.2/02 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
Ecotoxicology	Analytical methods for the determination of prothioconazole in various matrices (e.g. soil, test water, diet) were relied on to support the ecotoxicology package for Annex I approval. A number of these same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are anticipated to be acceptable for the purposes of this submission.			
Ecotoxicology (Chronic oral toxicity test – Honey Bee)	Primary	0.017 mg/kg (in 50% w/v aq. sucrose solution)	LC-MS/MS Method: S20-00395-L3 Analyte: Prothioconazole Dilution: ACN/H ₂ O m/z: 344→266 (quantifier) 344→180 (qualifier)	Bogner, F.; 2020, Report no. S20-00395-L3, KCP 5.2.2/08 [Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00395] EU not agreed – new study
	Confirmatory	Not required – primary method is considered highly specific.		
Ecotoxicology (Larval toxicity test – Honey Bee)	Primary	0.017 mg/kg (larval diet)	LC-MS/MS Method: S20-00396-L3, QuEChERS Analyte: Prothioconazole m/z: 344→266 (quantifier) 344→180 (qualifier)	Bogner, F.; 2020, Report no. S20-00396-L3, KCP 5.2.2/09 [Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00396] EU not agreed – new study
	Confirmatory	Not required – primary method is considered highly specific.		
Ecotoxicology (Acute oral and contact toxicity test – Bumblebee)	Primary	21.3 mg/L (0.1% Triton X solution in deionised water, 50% w/v aq. sucrose solution)	LC-MS/MS Method: S19-03594-L3 Analyte: Prothioconazole Dilution: ACN/H ₂ O m/z: 344→189(quantifier)	Wendling, K.; 2020, Report no. S19-03594 (Appendix D and E), KCP 5.2.2/10 EU not agreed – new study

Component of residue definition: prothioconazole				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
			344→154 (qualifier)	
	Confirmatory	Not required – primary method is considered highly specific.		
Ecotoxicology (Algae Growth Inhibition Test, Acute immobilisation test – <i>Daphnia magna</i>)	Primary	0.0356 mg/L (OECD TG 201 medium, ISO standard dilution water, Swedish standard growth medium)	HPLC-UV Method: Not stated Analyte: Prothioconazole i) Dilution in matrix ii) SPE cartridge or filtered 254 nm	Yu, J.; 2021, Report no. 2856, KCP 5.2.2/11 EU not agreed – new study
	Confirmatory	No separate method presented. Confirmation of peak identity achieved through retention time matching with analytical standards.		
Plant protection product (Properties)	Refer to Section 5.2.1.1.			

Table 5.2-5: Validated methods for the generation of pre-authorization data

Component of residue definition: prothioconazole-desthio (M04)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuffs of plant origin: High starch/ high protein content (dry) (Storage stability – Residues)	Primary	0.01 mg/kg (wheat grain) 0.05 mg/kg (wheat – forage and straw)	LC-MS/MS Method: 00598 and 00598/M001 Analyte: Prothioconazole-desthio (M04) i) Extraction with ACN/H ₂ O (with added cysteine HCl) ii) Partition with hexane and CH ₂ Cl ₂ m/z: Not stated	Heinemann, 2000a, Report no. 00598, KCP 5.2.2/01 and Heinemann, 2000b, Report no. 00598/M001, KCP 5.2.2/02 EU agreed (DAR, 2004, RMS: UK) Identical to post-registration method (see: KCP 5.3.2.2/03 and KCP 5.3.2.2/04)
	Confirmatory	Not required – primary method is considered highly specific.		
Foodstuff of plant origin: High water High oil High starch/ high protein content (dry) (Supervised residue trials – Residues)	Primary	0.01 mg/kg (wheat and barley – grain, rape seed) 0.05 mg/kg (wheat and barley – forage and straw, rape – straw, pods and green material)	LC-MS/MS Analyte: Prothioconazole-desthio (M04) Method: 00647 i) Extraction with ACN/H ₂ O m/z: 312→70	Heinemann, 2001a, Report no. 00647, KCP 5.2.2/05 EU agreed (DAR, 2004, RMS: UK) Identical to post-registration method (see: KCP 5.3.2.2/05)
	Confirmatory	Not required – primary method is considered highly specific.		
Foodstuff of	Primary	0.01 mg/kg	LC-MS/MS	Heinemann, 2001b, Report no. 00655,

Component of residue definition: prothioconazole-desthio (M04)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
animal origin: Milk Meat Liver Kidney Fat (Feeding studies – Residues)		(meat, liver, kidney, fat) 0.004 mg/kg (milk)	Analyte: Prothioconazole-desthio (M04) Method: 00655 and 00655/M001 i) Extraction with ACN/H ₂ O ii) Refluxed with HCl iii) Purified on ChemElut cartridge m/z: Not stated	KCP 5.2.2/06 and Heinemann, 2001c, Report no. 00655/M001, KCP 5.2.2/07 EU agreed (DAR, 2004, RMS: UK) Identical to post-registration method (see: KCP 5.3.2.3/01 and KCP 5.3.2.3/02)
	Confirmatory	Not required – The primary method is considered highly specific.		
Soil (Storage stability and field studies – Environmental fate)	Primary	0.006 mg/kg	LC-MS/MS Analyte: Prothioconazole-desthio (M04) Method: Not stated, but similar to 00610 i) Extraction with ACN/H ₂ O/cysteine hydrochloride monohydrate ii) Filtered m/z: Not stated	Reference to validation study not given in DAR. Risk assessment study: Schramel, 2001, Report no. MR-644/99, KCP 5.2.2/03 00610: Schramel, 2000, Report no. 00610, KCP 5.2.2/04 EU agreed (DAR, 2004, RMS: UK) Not confirmed as identical, but method is similar to post-registration method (see: KCP 5.3.2.4/01)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Garments used for operator protection (Exposure)	Primary	Not stated	LC-MS/MS Analyte: Prothioconazole-desthio (M04) Method: Adaption of 00598 and 00598/M001. Specific adaption not stated. m/z: Not stated	00598 and 00598/M001: Heinemann, 2000a, Report no. 00598, KCP 5.2.2/01 and Heinemann, 2000b, Report no. 00598/M001, KCP 5.2.2/02 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
Ecotoxicology	Analytical methods for the determination of prothioconazole-desthio (M04) in various matrices (e.g. soil, test water, diet) were relied on to support the ecotoxicology package for Annex I approval. These same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are assumed to still be acceptable for the purposes of this submission.			
Plant protection product (Properties)	Refer to Section 5.2.1.2.			

Table 5.2-6: Validated methods for the generation of pre-authorization data

Component of residue definition: prothioconazole-S-methyl (M01)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuff of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin (Residues)	No specific methods for the support of residues (foodstuffs of animal origin) have been developed for the authorisation of FF-075.			
Soil (Storage stability and field studies – Environmental fate)	Primary	0.006 mg/kg	LC-MS/MS Analyte: Prothioconazole-S-methyl (M01) Method: Not stated, but similar to 00610 i) Extraction with ACN/H ₂ O/cysteine hydrochloride monohydrate ii) Filtered m/z: Not stated	Reference to validation study not given in DAR. Risk assessment study: Schramel, 2001, Report no. MR-644/99, KCP 5.2.2/03 00610: Schramel, 2000, Report no. 00610, KCP 5.2.2/04 EU agreed (DAR, 2004, RMS: UK) Not confirmed as identical, but method is similar to post-registration method (see: KCP 5.3.2.4/01)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Exposure	No specific methods for the support of exposure have been developed for the authorisation of FF-075.			
Ecotoxicology	Analytical methods for the determination of prothioconazole-S-methyl (M01) in various matrices (e.g. soil, test water, diet) were relied on to support the ecotoxicology package for Annex I approval. These same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are assumed to still be acceptable for the purposes of this submission.			
Properties	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.			

Table 5.2-7: Validated methods for the generation of pre-authorization data

Component of residue definition: 1,2,4-triazole				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuff of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin (Residues)	No specific methods for the support of residues (foodstuffs of animal origin) have been developed for the authorisation of FF-075.			
Environmental	No specific methods for the support of environmental fate have been developed for the			

fate	authorisation of FF-075.
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.
Exposure	No specific methods for the support of exposure have been developed for the authorisation of FF-075.
Test water (Ecotoxicology)	Analytical methods for the determination of 1,2,4-triazole in test water were relied on to support the ecotoxicology package for Annex I approval. These same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are assumed to still be acceptable for the purposes of this submission.
Properties	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.

Table 5.2-8: Validated methods for the generation of pre-authorization data

Analyte: JAU6476-3-hydroxy-desthio (M14) [Note: This analyte does not form part of any risk assessment residue definition, however has been quantified using a validated analytical method to support the data package, so is included in Section 5.2.2 for completeness]				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuff of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin: Milk Meat Liver Kidney Fat (Feeding studies – Residues)	Primary	0.01 mg/kg (meat, liver, kidney, fat) 0.004 mg/kg (milk)	LC-MS/MS Analyte: JAU6476-3-hydroxy-desthio (M14) Method: 00655 and 00655/M001 i) Extraction with ACN/H ₂ O ii) Refluxed with HCl iii) Purified on ChemElut cartridge m/z: Not stated	Heinemann, 2001b, Report no. 00655, KCP 5.2.2/06 and Heinemann, 2001c, Report no. 00655/M001, KCP 5.2.2/07 EU agreed (DAR, 2004, RMS: UK) Identical to post-registration method (see: KCP 5.3.2.3/01 and KCP 5.3.2.3/02)
	Confirmatory	Not required – The primary method is considered highly specific.		
Environmental fate	No specific methods for the support of environmental fate have been developed for the authorisation of FF-075.			
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Exposure	No specific methods for the support of exposure have been developed for the authorisation of FF-075.			
Ecotoxicology	No specific methods for the support of ecotoxicology have been developed for the authorisation of FF-075.			
Properties	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.			

Table 5.2-9: Validated methods for the generation of pre-authorization data

Analyte: JAU6476-4-hydroxy-desthio (M15) [Note: This analyte does not form part of any risk assessment residue definition, however has been quantified using a validated analytical method to support the data package, so is included in Section 5.2.2 for completeness]				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuff of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin: Milk Meat Liver Kidney Fat (Feeding studies – Residues)	Primary	0.01 mg/kg (meat, liver, kidney, fat) 0.004 mg/kg (milk)	LC-MS/MS Analyte: JAU6476-4-hydroxy-desthio (M15) Method: 00655 and 00655/M001 i) Extraction with ACN/H ₂ O ii) Refluxed with HCl iii) Purified on ChemElut cartridge m/z: Not stated	Heinemann, 2001b, Report no. 00655, KCP 5.2.2/06 and Heinemann, 2001c, Report no. 00655/M001, KCP 5.2.2/07 EU agreed (DAR, 2004, RMS: UK) Identical to post-registration method (see: KCP 5.3.2.3/01 and KCP 5.3.2.3/02)
	Confirmatory	Not required – The primary method is considered highly specific.		
Environmental fate	No specific methods for the support of environmental fate have been developed for the authorisation of FF-075.			
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Exposure	No specific methods for the support of exposure have been developed for the authorisation of FF-075.			
Ecotoxicology	No specific methods for the support of ecotoxicology have been developed for the authorisation of FF-075.			
Properties	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.			

Azoxystrobin

An overview on the acceptable methods for analysis of residues of azoxystrobin for the generation of pre-authorisation data is given in the following tables.

In the 2010 EFSA Conclusion, the risk assessment residue definitions for azoxystrobin are:

- **Foodstuffs of plant origin:** Azoxystrobin
- **Foodstuffs of animal origin:** Azoxystrobin (provisional)*
- **Soil:** Azoxystrobin, R234886, R401553 and R402173
- **Ground water:** Azoxystrobin, R234886, R401553 and R402173
- **Surface water:** Azoxystrobin, R234886, R401553 and R402173
- **Sediment:** Azoxystrobin, R234886, R401553 and R402173
- **Air:** Azoxystrobin
- **Body fluids and tissues:** n/a

* It is noted that the residue definition for risk assessment remains provisional following the evaluation of

confirmatory data following the Article 12 MRL review and modification of the existing maximum residue levels for azoxystrobin (EFSA Journal 2020;18(8):6231).

Study summaries of any new studies are provided in Appendix 2.

Table 5.2-10: Validated methods for the generation of pre-authorization data

Component of residue definition: azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuffs of plant origin: Dry, high-acid, high-water and high-oil crops (Storage stability – Residues)	Primary	0.01 mg/kg (all crops)	LC-MS/MS Method: RAM305/01 Analyte: Azoxystrobin Extraction with ACN/H ₂ O m/z: Not stated	Lister, N.; 1999, Report no. RJ2770B, KCP 5.2.2/12 EU agreed (DAR, 2009, RMS: UK) Identical to post-registration method (see section 5.3 below)
	Confirmatory	Not required – The primary method is considered highly specific.		
Foodstuff of animal origin (Residues)	Primary	0.01 mg/kg (egg, liver, muscle, fat) 0.001 mg/kg (milk)	GC-NPD Method: RAM 255/03 Analyte: Azoxystrobin Extraction with ACN	Sapiets, A.; 1996, Report no. RJ1089B, KCP 5.2.2/13 EU agreed (DAR, 2009, RMS: UK) Identical to post-registration method (see section 5.3 below)
	Confirmatory	Not required – The primary method is considered highly specific.		
Soil (Storage stability and field studies – Environmental fate)	Primary	0.02 mg/kg	LC-MS/MS Method: RAM 269/03 Analyte: Azoxystrobin, Extraction with MeOH/HCl, DCM/NaCl m/z: Not stated	Johnson, R. 2000, Syngenta File No. ICI5504/0751, KCP 5.2.2/14 EU agreed (DAR, 2009, RMS: UK) Identical to post-registration method (see section 5.3 below)
	Confirmatory	Not required – The primary method is considered highly specific.		
Water: Surface, ground and drinking (Environmental fate)	Primary	0.1 µg/L	GC-MSD Method: RAM 358/01 Analyte: Azoxystrobin Extraction with SPE, EA/DCM, ACN m/z: 344 (388 and 372 qualifier ions)	Robinson, N., 2000, Syngenta File No. ICI5504/0758, KCP 5.2.2/15 EU agreed (DAR, 2009, RMS: UK) Identical to post-registration method (see section 5.3 below)
	Confirmatory	Not required – The primary method is considered highly specific.		
Water: Surface, ground and drinking (Environmental fate)	Primary	0.01 µg/L	LC-MS/MS Method: RAM 292/02 Analyte: Azoxystrobin Extraction with SPE, m/z: Not stated	Hurt, A., 1999, Syngenta File No. ICI5504/0767, KCP 5.2.2/16 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		

Component of residue definition: azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Air (Operator Exposure)	Primary	0.003 mg/m ³	GC-MSD Method: RAM 376/01 Analyte: Azoxystrobin Extraction with ACN m/z: 344 (388 and 403 qualifier ions)	Crawford, N., 2001, Report No. TMJ4658B, KCP 5.2.2/17 EU agreed (DAR, 2009, RMS: UK) Identical to post-registration method (see section 5.3 below)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	Analytical methods for the determination of azoxystrobin in various matrices were relied on to support the toxicology package for Annex I approval. These same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are anticipated to be acceptable for the purposes of this submission.			
Ecotoxicology	Analytical methods for the determination of azoxystrobin in various matrices (e.g. soil, test water, diet) were relied on to support the ecotoxicology package for Annex I approval. A number of these same studies are being relied on here for the authorisation of FF-075. Detailed information on these analytical methods (and supporting validation data) are not available in the EU literature and Rotam do not have access to the original studies. Though, the methods are anticipated to be acceptable for the purposes of this submission.			
Ecotoxicology (Chronic oral toxicity test – Honey Bee)	Primary	0.0127 mg/kg (in 50% w/v aq. sucrose solution)	LC-MS/MS Method: S20-00395-L3 Analyte: Azoxystrobin Dilution: ACN/H ₂ O m/z: 404→372 (quantifier) 404→329 (qualifier)	Bogner, F.; 2020, Report no. S20-00395-L3, KCP 5.2.2/08 <i>[Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00395]</i> EU not agreed – new study
	Confirmatory	Not required – primary method is considered highly specific.		
Ecotoxicology (Larval toxicity test – Honey Bee)	Primary	0.0127 mg/kg (larval diet)	LC-MS/MS Method: S20-00396-L3, QuEChERS Analyte: Azoxystrobin m/z: 404→372 (quantifier) 404→329 (qualifier)	Bogner, F.; 2020, Report no. S20-00396-L3, KCP 5.2.2/09 <i>[Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00396]</i> EU not agreed – new study
	Confirmatory	Not required – primary method is considered highly specific.		
Ecotoxicology (Acute oral and contact toxicity test – Bumblebee)	Primary	15.9 mg/L (0.1% Triton X solution in deionised water, 50% w/v aq. sucrose solution)	LC-MS/MS Method: S19-03594-L3 Analyte: Azoxystrobin Dilution: ACN/H ₂ O m/z: 404→372 (quantifier) 404→344 (qualifier)	Wendling, K.; 2020, Report no. S19-03594 (Appendix D and E), KCP 5.2.2/10 EU not agreed – new study

Component of residue definition: azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
	Confirmatory	Not required – primary method is considered highly specific.		
Ecotoxicology (Algae Growth Inhibition Test, Acute immobilisation test – <i>Daphnia magna</i>)	Primary	0.0297 mg/L (OECD TG 201 medium, ISO standard dilution water, Swedish standard growth medium)	HPLC-UV Method: Not stated Analyte: Azoxystrobin iii) Dilution in matrix iv) SPE cartridge or filtered 254 nm	Yu, J.; 2021, Report no. 2856, KCP 5.2.2/11 EU not agreed – new study
	Confirmatory	No separate method presented. Confirmation of peak identity achieved through retention time matching with analytical standards.		
Plant protection product (Properties)	Refer to Section 5.2.1.1.			

Table 5.2-11: Validated methods for the generation of pre-authorization data

Component of residue definition: R234886				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuffs of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin (Residues)	No specific methods for the support of residues (foodstuffs of animal origin) have been developed for the authorisation of FF-075.			
Soil (Storage stability and field studies – Environmental fate)	Primary	0.02 mg/kg	LC-MS/MS Method: RAM 269/03 Analyte: R234886 Extraction with MeOH/HCl, DCM/NaCl m/z: Not stated	Johnson, R. 2000, Syngenta File No. ICI5504/0751, KCP 5.2.2/14 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		
Water: Surface, ground and drinking (Environmental fate)	Primary	0.01 µg/L	LC-MS/MS Method: RAM 292/02 Analyte: R234886 Extraction with SPE, m/z: Not stated	Hurt, A., 1999, Syngenta File No. ICI5504/0767, KCP 5.2.2/16 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Operator Exposure	No specific methods for the support of operator exposure have been developed for the authorisation of FF-075.			

Component of residue definition: R234886				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Ecotoxicology	No specific methods for the support of ecotoxicology have been developed for the authorisation of FF-075.			
Plant protection product (Properties)	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.			

Table 5.2-12: Validated methods for the generation of pre-authorization data

Component of residue definition: R401553				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuffs of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin (Residues)	No specific methods for the support of residues (foodstuffs of animal origin) have been developed for the authorisation of FF-075.			
Soil (Storage stability and field studies – Environmental fate)	Primary	0.02 mg/kg	LC-MS/MS Method: RAM 269/03 Analyte: R234886 Extraction with MeOH/HCl, DCM/NaCl m/z: Not stated	Johnson, R. 2000, Syngenta File No. ICI5504/0751, KCP 5.2.2/14 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		
Water: Surface, ground and drinking (Environmental fate)	Primary	0.01 µg/L	LC-MS/MS Method: RAM 292/02 Analyte: R234886 Extraction with SPE, m/z: Not stated	Hurt, A., 1999, Syngenta File No. ICI5504/0767, KCP 5.2.2/16 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Operator Exposure	No specific methods for the support of operator exposure have been developed for the authorisation of FF-075.			
Ecotoxicology	No specific methods for the support of ecotoxicology have been developed for the authorisation of FF-075.			
Plant protection product (Properties)	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.			

Table 5.2-13: Validated methods for the generation of pre-authorization data

Component of residue definition: R402173				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Foodstuffs of plant origin (Residues)	No specific methods for the support of residues (foodstuffs of plant origin) have been developed for the authorisation of FF-075.			
Foodstuff of animal origin (Residues)	No specific methods for the support of residues (foodstuffs of animal origin) have been developed for the authorisation of FF-075.			
Soil (Storage stability and field studies – Environmental fate)	Primary	0.02 mg/kg	LC-MS/MS Method: RAM 269/03 Analyte: R234886 Extraction with MeOH/HCl, DCM/NaCl m/z: Not stated	Johnson, R. 2000, Syngenta File No. ICI5504/0751, KCP 5.2.2/14 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		
Water: Surface, ground and drinking (Environmental fate)	Primary	0.01 µg/L	LC-MS/MS Method: RAM 292/02 Analyte: R234886 Extraction with SPE, m/z: Not stated	Hurt, A., 1999, Syngenta File No. ICI5504/0767, KCP 5.2.2/16 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – The primary method is considered highly specific.		
Efficacy	No specific methods for the support of efficacy have been developed for the authorisation of FF-075.			
Toxicology	No specific methods for the support of toxicology have been developed for the authorisation of FF-075.			
Operator Exposure	No specific methods for the support of operator exposure have been developed for the authorisation of FF-075.			
Ecotoxicology	No specific methods for the support of ecotoxicology have been developed for the authorisation of FF-075.			
Plant protection product (Properties)	No specific methods for the support of physicochemical properties have been developed for the authorisation of FF-075.			

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Refer to Section 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of prothioconazole (KCP 5.2)

Rotam were not a notifier at Annex I inclusion of prothioconazole, nor are they a notifier for the renewal of approval process. Rotam only have access to publicly available data, e.g. the 2004 DAR and 2007

DAR Addendum (RMS: UK), 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98) and 2007 Review Report (SANCO/3923/07 – final, 10 December 2007).

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Prothioconazole is currently under review at EU level. However, this application relies on existing, EU agreed endpoints. The residue definitions proposed in the Draft Assessment Report (incl. its addenda) and the current legal residue definitions are **not identical** for some matrices.

In the DAR (October 2004, RMS:UK), the residue definition for foodstuff of plant origin was 'Prothioconazole and prothioconazole-desthio' and in the 2007 EFSA Conclusion, the definition was 'Prothioconazole-desthio' only. However, the current legal definition (Reg. (EC) No 149/2008, latest amendment Reg (EU) 2019/552) is 'Prothioconazole-desthio (sum of isomers)'.

For foodstuff of animal origin, the residue definition in the DAR was 'Prothioconazole-desthio' only. In the EFSA Conclusion, this was changed to the 'Sum of prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio'. The need for including the glucuronide conjugate resulted from the fact that the free metabolite was not found in milk and therefore could not act as a valid marker compound. However, the current legal definition (Reg. (EC) No 149/2008, latest amendment Reg (EU) 2019/552) is 'Prothioconazole-desthio (sum of isomers)'.

Residues definitions in environmental matrices (soil, water, air) are unchanged from the DAR (i.e. 'Prothioconazole and prothioconazole-desthio').

A residue definition in body fluids and tissues has not been set.

zRMS comment (analytical methods for body fluids and tissues):

In the case of the Euskatel Pro application provided for in Art. 33 the evaluation was carried out on existing endpoints in the EU, in line with the data requirements and guidance in force at the time of inclusion or last renewal of the active substance. Currently agreed EU endpoints for prothioconazole do not include a residue definition for monitoring in body fluids and tissues.

The development of monitoring methods for body fluids and tissues will be required once the active substance is renewed and the residue definitions in these matrices are finalized at EU level.

On the other hand, we agree that such a method is required under Reg (EU) No 283/2013. This data gap should be fulfilled as a post-registration requirement.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Prothioconazole-desthio (sum of isomers) ^[3]	0.01* mg/kg (lowest MRL)	Reg. (EC) No 149/2008, latest amendment Reg (EU) 2019/552
Plant, high acid content		0.01* mg/kg (lowest MRL)	
Plant, high protein/high starch content (dry commodities)		0.01* mg/kg (lowest MRL) 0.05 mg/kg (rye, oat) 0.1 mg/kg (wheat, triticale) 0.2 mg/kg (barley)	
Plant, high oil content		0.01* mg/kg (lowest MRL) 0.15 mg/kg (OSR)	
Muscle	Prothioconazole-desthio	0.01* mg/kg (lowest MRL)	Reg. (EC) No 149/2008,

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Milk	(sum of isomers) ^[3]	0.01* mg/kg (lowest MRL)	latest amendment Reg (EU) 2019/552
Eggs		0.01* mg/kg (lowest MRL)	
Fat		0.01* mg/kg (lowest MRL)	
Liver, kidney		0.1 mg/kg (lowest MRL)	
Soil (Ecotoxicology)	Prothioconazole and prothioconazole-desthio (M04) ^[1,2]	0.05 mg/kg	Common limit (SANCO/825/00 rev.8.1; the toxic concentrations (LC ₅₀) for the most sensitive non-target organism is >0.05 mg/kg ^[2])
Drinking water (Human toxicology)	Prothioconazole and prothioconazole-desthio (M04) ^[1,2]	0.1 µg/L	General limit (SANCO/825/00 rev.8.1)
Surface water (Ecotoxicology)	Prothioconazole and prothioconazole-desthio (M04) ^[1,2]	308 µg/L (prothioconazole) 73 µg/L (prothioconazole-desthio (M04))	0.308 mg a.s./L (lowest NOEC – <i>Oncorhynchus mykiss</i> (ELS), a.s., prothioconazole) ^[2] 0.073 mg p.m./L (lowest E _b C ₅₀ – <i>Scenedesmus subspicatus</i> , prothioconazole-desthio (M04)) ^[2]
Air	Prothioconazole and prothioconazole-desthio (M04) ^[1,2]	60 µg/m ³ (prothioconazole) 3 µg/m ³ (prothioconazole-desthio (M04))	AOEL: 0.2 mg/kg bw/d (prothioconazole) ^[2, 4] AOEL: 0.01 mg/kg bw/d (prothioconazole-desthio (M04)) ^[2, 5]
Tissue (meat or liver)	Not defined ^[1, 2]	Not required	Not classified as T / T+
Body fluids			

^[1] DAR, Volume 1, October 2004, RMS: UK

^[2] 2007 EFSA Conc., EFSA Scientific Report (2007) 106, 1-98

^[3] Reg. (EC) No 149/2008, latest amendment Reg (EU) 2019/552

^[4] Based on the combined developmental NOAEL of 20 mg/kg bw/day applying a safety factor of 100

^[5] Based on a rat developmental study, with a SF 100.

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods for analysis of prothioconazole-desthio (sum of isomers) in plant matrices is given in the following tables. Difficult to analyse crops are not considered, as the intended GAP does not include these matrices.

Data have been taken from peer-reviewed EU documents, published during or since Annex I listing of prothioconazole. These are:

- 2004 DAR (RMS: UK)
- 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
- 2014 Art. 12 MRL Review (EFSA Journal 2014;12(5):3689)
- 2018 dRAR (B.5, Volume 3, RMS: UK) – considered for additional information purposes on out-of-protection (Annex I) data only.

Two new (publicly available) studies have been submitted. For the detailed evaluation of these new studies, refer to Appendix 2.

zRMS comment (The LOQs of the EU agreed methods cited in the table do not meet the current legal MRLs in Reg (EU) No 2019/552):

The proposed GAP uses of Euskatel Pro are on cereal and oil seed crops only, for which the analytical methods relied upon have sufficient LOQs to meet the current legal MRLs as listed in Reg (EU) No 2019/552.

Under Article 33 of Regulation (EC) No 1107/2009, the current EU-agreed endpoints are relevant. Analytical methods data accepted in the 2004 DAR and 2007 DAR Addendum (RMS: UK), and the 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98) that were sufficient to fulfil the data requirements and guidance relevant at the time of the active substance inclusion, remain sufficient to support the product application.

The assessment should be revised when the active substance is renewed and the new methods should be provided by the applicant for re-evaluation.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: Prothioconazole-desthio (sum of isomers)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
High water High acid High oil High starch/ high protein content (dry)	Primary	0.02 mg/kg (wheat and barley – grain, rape seed, tomato, orange) 0.05 mg/kg (wheat and barley – forage and straw) 0.01 mg/kg (wheat and barley – grain, canola – seed) ^[1]	GC-MS Analyte: Prothioconazole- desthio (M04) ^[3] Method: 00086/M033 (DFG S19) i) Extraction with acetone/H ₂ O [cereals, tomato, orange] or extraction with acetone/H ₂ O and ACN [rape seed] ii) Partition with EtOAc and cyclohexane [tomato, orange, wheat only] iii) GPC m/z: Not stated	Weeren and Pelz, 2000, Report no. 00086/M033, KCP 5.3.2.2/01 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	None presented – however in the Art. 12 MRL review, it is stated that: “ <i>This method can be confirmed by an independent analytical method using HPLC-MS/MS fully validated for the determination of prothioconazole-desthio in high water content commodities and in straw with an LOQ of 0.05 mg/kg and in high oil content and in dry commodities with an LOQ of 0.01 mg/kg (United Kingdom, 2004).</i> ” The method described is presented below (KCP 5.3.2.2/03, KCP 5.3.2.2/04 and /or KCP 5.3.2.2/05).		
	ILV	0.02 mg/kg (tomato, orange)	Same as primary method.	Class, 2001, Report no. P/B 484 G, KCP 5.3.2.2/02 EU agreed (DAR, 2004, RMS: UK)
High water High oil High starch/	Primary	0.01 mg/kg (wheat and barley – grain, rape	LC-MS/MS Method: 00598 and 00598/M001	Heinemann, 2000a, Report no. 00598, KCP 5.3.2.2/03 and Heinemann, 2000b, Report no.

Component of residue definition: Prothioconazole-desthio (sum of isomers)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
high protein content (dry)		seed) 0.05 mg/kg (wheat and barley – forage and straw, rape – straw, pods, green material)	Analyte: Prothioconazole-desthio (M04) ^[2, 3] i) Extraction with ACN/H ₂ O ii) Partition with hexane and CH ₂ Cl ₂ m/z: Not stated	00598/M001, KCP 5.3.2.2/04 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV	None presented.		
High water High oil High starch/ high protein content (dry)	Primary	0.01 mg/kg (wheat and barley – grain, rape seed) 0.05 mg/kg (wheat and barley – forage and straw, rape – straw, pods and green material)	LC-MS/MS Analyte: Prothioconazole-desthio (M04) ^[3, 6] Method: 00647 i) Extraction with ACN/H ₂ O m/z: 312→70	Heinemann, 2001a, Report no. 00647, KCP 5.3.2.2/05 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV	None presented.		
High starch/ high protein content (dry)	Primary	0.01 mg/kg (maize)	LC-MS/MS Method: QuEChERS Analyte: Prothioconazole-desthio (M04) ^[3] m/z: 312→70, 314→127	Herrmann, 2014, Validation Report 17 (EURL for Cereals and Feeding stuff) ^[4] , KCP 5.3.2.2/06 EU not agreed – new study (publicly available)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV	None presented.		
High starch/ high protein content (dry)	Primary	0.01 mg/kg (wheat, rye, rice, barley) 0.02 mg/kg (oat)	LC-MS/MS Method: QuEChERS Analyte: Prothioconazole-desthio (M04) ^[3] m/z: 312→70, 314→127	Poulsen, 2012, Validation Report 9 (EURL for Cereals and Feeding stuff) ^[5] , KCP 5.3.2.2/07 EU not agreed – new study (publicly available)
	Confirmatory	Not required – the primary method is considered highly selective.		
	ILV	None presented.		

^[1] Additional information given in the dRAR (Volume 3, February 2018, RMS: UK).

^[2] The method was also validated for determination of prothioconazole. Though, such data are not summarised in the table, given prothioconazole does not form part of the residue definition in this matrix.

^[3] Although prothioconazole-desthio (M04) is stated as the analyte, the method is not enantioselective, hence the sum of isomers will be analysed.

^[4] [https://www.eurl-pesticides.eu/userfiles/file/\(17\)%20Appendix%203%20Validating%202014%20Feed%20Quechers%20report%2017_150204.pdf](https://www.eurl-pesticides.eu/userfiles/file/(17)%20Appendix%203%20Validating%202014%20Feed%20Quechers%20report%2017_150204.pdf)

^[5] [https://www.eurl-pesticides.eu/userfiles/file/\(9\)%20Validating%202011%20Cerealier%20LC-MSMS%20report%209.pdf](https://www.eurl-pesticides.eu/userfiles/file/(9)%20Validating%202011%20Cerealier%20LC-MSMS%20report%209.pdf)

^[6] The method was also validated for determination of JAU6476-sulfonic acid (M02). Though, such data are not summarised in the table, given this analyte does not form part of the residue definition in this matrix.

Conclusion

A DFG S19 GC-MS method (00086/M033), that was fully accepted in the DAR and EFSA Conclusion, is acceptable to monitor all components of the monitoring residue definition in the crop commodities applied for, i.e. high protein/starch (dry) and high oil commodities, to at least the lowest MRLs in these groups (i.e. 0.01 mg/kg). The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional data are necessary.

In addition, the multi-residue QuEChERS method in combination with LC-MS/MS is available to analyse prothioconazole-desthio (M04) in high protein/high starch commodities to a LOQ of 0.01 mg/kg. Validation data is publicly available for this method.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	<p>SANTE 2017/10632 Rev. 3 of 22 November 2017 (i.e. the Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods), with application from December 2019, states that:</p> <p><i>“For renewal of product authorisations or for new product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In case this document did not yet apply, when the data for the latest renewal or approval were submitted, at this stage no new studies or data related to extraction efficiency are required. This means in practice that for renewal of product authorisations or for new product authorisations for which no new MRL is required, no additional proof of extraction efficiency will be required.”</i></p> <p>The guidance document did not apply when the data for the latest renewal of approval of prothioconazole were submitted. As such, no additional data are required to address extraction efficiency to support authorisation of FF-075. Nonetheless, an evaluation of the existing data is provided below.</p> <p>Several monitoring methods are available for the quantification of residues from crop matrices, that involve extractions with the following solvent systems:</p> <ul style="list-style-type: none"> • acetone/H₂O or acetone/H₂O and ACN [method 00086/M033] • ACN/H₂O [methods 00598, 00598/M001, 00647 and QuEChERS] <p>The crop metabolism study – Haas and Bornatsch, 2000, Report no. MR-198/99, KCP 5.3.2.2/08 – on wheat is available that uses the ACN/H₂O extraction solvent system. Large fractions of the TRR for wheat fodder [high water commodity] and grain [high protein/high starch commodity] samples were extractable (≥82.5%) with prothioconazole-desthio (M04) having the largest contribution to each total. Extraction efficiency is therefore considered sufficient for methods 00598, 00598/M001, 00647 and QuEChERS in at least high protein/starch crops (covering the intended GAP) and high water commodities.</p> <p>On comparing between ACN and acetone as extraction solvents, there are little significant differences – both are simple organic polar aprotic systems that are miscible in water and capable of weak intramolecular hydrogen bonding and Van der Waals interactions with the target analyte (important for solvation and therefore extraction). On this basis, sufficient extraction efficiency for method 00086/M033 can also be assumed.</p> <p>It is further noted that all monitoring methods relied on for the authorisation of FF-075 have been accepted at EU level (in the DAR) with the exception of the QuEChERS method – which is a widely known and reliable method for extraction of a multitude of pesticide residues from crop matrices – including prothioconazole (and residues thereof), as demonstrated in the reports by the EURL validation studies.</p>
Not required, because:	-

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods for analysis of prothioconazole-desthio (sum of isomers) in

animal matrices is given in the following tables.

Data have been taken from peer-reviewed EU documents, published during or since Annex I listing of prothioconazole. These are:

- 2004 DAR (RMS: UK)
- 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
- 2014 Art. 12 MRL Review (EFSA Journal 2014;12(5):3689)

No new studies are presented.

zRMS comment:

1. Analytical methods (primary, confirmatory, ILV) for the determination of residues in eggs are missing

2. The EU agreed methods cited in the table do not meet the requirements for confirmatory purposes, since only one ion transition was validated

Under Article 33 of Regulation (EC) No 1107/2009, the current EU-agreed endpoints are relevant. Analytical methods data accepted in the 2004 DAR and 2007 DAR Addendum (RMS: UK), and the 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98) were considered sufficient to fulfil the data requirements and guidance relevant at the time of the active substance inclusion, and are considered sufficient to support the product application.

The assessment should be revised when the active substance is renewed and the new methods should be provided by the applicant for re-evaluation.

Table 5.3-4: Validated methods for food and feed of animal origin

Component of residue definition: Prothioconazole (sum of isomers)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Milk Meat Liver Kidney Fat	Primary	0.01 mg/kg (meat, liver, kidney, fat) 0.004 mg/kg (milk)	LC-MS/MS Analyte: Prothioconazole-desthio (M04) ^[1, 2] Method: 00655 and 00655/M001 iv) Extraction with ACN/H ₂ O v) Refluxed with HCl vi) Purified on ChemElut cartridge m/z: Not stated	Heinemann, 2001b, Report no. 00655, KCP 5.3.2.3/01 and Heinemann, 2001c, Report no. 00655/M001, KCP 5.3.2.3/02 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV	0.01 mg/kg (meat, liver, kidney, fat) 0.004 mg/kg (milk)	Same as primary method.	Dubey, 2001, Report no. A-14-01-01, KCP 5.3.2.3/03 EU agreed (DAR, 2004, RMS: UK)

^[1] Method also validated for determination of JAU6476-3-hydroxy desthio (M14) and JAU6476-4-hydroxy desthio (M15). These data are not summarised in the table, as these analytes do not form part of the residue definition for this matrix.

^[2] Although prothioconazole-desthio (M04) is stated as the analyte, the method is not enantioselective, hence the sum of isomers will be analysed.

Conclusion

A LC-MS/MS method (00655 and 00655/M001), that was fully accepted in the DAR and EFSA Conclusion, is acceptable to monitor all components of the monitoring residue definition in animal commodities to a LOQ of 0.01 mg/kg (meat, liver, kidney, fat) and 0.004 mg/kg (milk). The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional data are necessary.

It is noted that in the EFSA Conclusion, it was stated that “*a method is not available to monitor the glucuronide conjugate in products of animal origin*”. The need for including the glucuronide conjugate in the residue definition stemmed from the fact that the free metabolite was not found in milk. However, in the Art. 12 MRL Review for the active, actual residue levels of the glucuronide in milk were expected at a trace level at the calculated dietary burden (<0.01 mg/kg) and so analysing the conjugates of prothioconazole-desthio (M04) would have negligible impact on the residue levels enforced in milk. No further data are necessary on this topic.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	<p>The extraction efficiency of the residue method 00655 (and 00655/M001) in animal matrices was demonstrated in study: Weber, Weber and Spiegel, 2002, Report no. MR-091-01, KCP 5.3.2.3/04 using aged radioactive residues from the goat metabolism study. Comparison of the 00655 (and 00655/M001) residue analytical method with the method used in the metabolism study demonstrated the suitability of the extraction step (i.e. extracting with an acetonitrile/water solvent system) for the determination of the relevant residue in animal matrices.</p> <p>According to SANTE 2017/10632 Rev. 3 of 22 November 2017 (i.e. the Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods), with application from December 2019:</p> <p><i>“For renewal of product authorisations or for new product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In case this document did not yet apply, when the data for the latest renewal or approval were submitted, at this stage no new studies or data related to extraction efficiency are required. This means in practice that for renewal of product authorisations or for new product authorisations for which no new MRL is required, no additional proof of extraction efficiency will be required.”</i></p> <p>The guidance document did not apply when the data for the latest renewal of approval of prothioconazole were submitted. As such, no additional data are required to address extraction efficiency to support authorisation of FF-075.</p>

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods for the analyses of prothioconazole and prothioconazole-desthio (M04) in soil is given in the following tables.

Data have been taken from peer-reviewed EU documents, published during Annex I listing of prothioconazole. These are:

- 2004 DAR (RMS: UK)
- 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)

No new studies are presented.

Table 5.3-6: Validated methods for soil

Component of residue definition: Prothioconazole			
Method type	Method LOQ	Principle of method	Author(s), year
Primary	0.006 mg/kg	LC-MS/MS Method: 00610 ^[1] iii) Extraction with ACN/H ₂ O/cysteine hydrochloride monohydrate iv) Filtered m/z: Not stated	Schramel, 2000, Report no. 00610, KCP 5.3.2.4/01 EU agreed (DAR, 2004, RMS: UK)
Confirmatory	Not required – the primary method is considered highly specific.		

^[1] The method was also validated for determination of prothioconazole-desthio (M04) (refer to Table 5.3-7) and JAU6476-3-hydroxy desthio. A summary for the JAU6476-3-hydroxy desthio analysis is not included in either table, given that the analyte does not form part of the residue definition in this matrix.

Table 5.3-7: Validated methods for soil

Component of residue definition: Prothioconazole-desthio (M04)			
Method type	Method LOQ	Principle of method	Author(s), year
Primary	0.006 mg/kg	LC-MS/MS Method: 00610 ^[1] i) Extraction with ACN/H ₂ O/cysteine hydrochloride monohydrate m/z: Not stated	Schramel, 2000, Report no. 00610, KCP 5.3.2.4/01 EU agreed (DAR, 2004, RMS: UK)
Confirmatory	Not required – the primary method is considered highly specific.		
Primary	0.01 mg/kg	GC-MS Method: 00086/M038, DFG S19 i) Extraction with acetone/H ₂ O ii) Partition with ethyl acetate and cyclohexane iii) GPC m/z: Not stated	Steinhauer, 2001, Report no. 00086/M038, KCP 5.3.2.4/02 EU agreed (DAR, 2004, RMS: UK)
Confirmatory	None presented.		

^[1] The method was also validated for determination of prothioconazole (refer to Table 5.3.6) and JAU6476-3-hydroxy desthio. A summary for the JAU6476-3-hydroxy desthio analysis is not included in either table, given that the analyte does not form part of the residue definition in this matrix.

Conclusion

A LC-MS/MS method (00610) is available to monitor all components of the residue definition (i.e. prothioconazole and prothioconazole-desthio (M04)) in soil to a LOQ of 0.006 mg/kg. The primary method is highly specific therefore negating the need for a confirmatory method. The analytical method was fully accepted in the DAR and EFSA Conclusion for monitoring purposes. The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional information is necessary.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods for the analyses of prothioconazole and prothioconazole-desthio (M04) in surface and drinking water is given in the following tables.

Data have been taken from peer-reviewed EU documents, published during Annex I listing of prothioconazole. These are:

- 2004 DAR (RMS: UK)
- 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)

No new studies are presented.

zRMS comment:

1. An independent laboratory validation (ILV) for drinking water is missing.

Under Article 33 of Regulation (EC) No 1107/2009, the current EU-agreed endpoints are relevant. Analytical methods data accepted in the 2004 DAR and 2007 DAR Addendum (RMS: UK), and the 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98) were considered sufficient to fulfil the data requirements and guidance relevant at the time of the active substance inclusion, and are considered sufficient to support the product application.

On the other hand, we agree that such a method is required under Reg (EU) No 283/2013. This data gap should be fulfilled as a post-registration requirement.

2. The EU agreed methods cited in the table do not meet the requirements for confirmatory purposes, since only one ion transition was validated.

The assessment should be revised when the active substance is renewed and the new methods should be provided by the applicant for re-evaluation.

Table 5.3-8: Validated methods for water

Component of residue definition: Prothioconazole				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Surface water	Primary	6 µg/L	HPLC-UV Method: 00586 ^[1] i) Direct injection	Sommer, 1999, Report no. 00586, KCP 5.3.2.5/01 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	None stated		
Surface water Drinking water	Primary	0.1 µg/L	LC-MS/MS Method: 00684 ^[1] i) Direct injection m/z: not stated	Sommer, 2001, Report no. 00684, KCP 5.3.2.5/02 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV (drinking water)	None stated		

^[1] The method was also validated for determination of prothioconazole-desthio (M04) (refer to Table 5.3-9).

Table 5.3-9: Validated methods for water

Component of residue definition: Prothioconazole-desthio (M04)				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Surface water	Primary	6 µg/L	HPLC-UV Method: 00586 ^[1] i) Direct injection	Sommer, 1999, Report no. 00586, KCP 5.3.2.5/01 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	None stated		
Surface water Drinking water	Primary	0.05 µg/L	LC-MS/MS Method: 00684 ^[1] i) Direct injection m/z: not stated	Sommer, 2001, Report no. 00684, KCP 5.3.2.5/02 EU agreed (DAR, 2004, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV (drinking water)	None stated		

^[1] The method was also validated for determination of prothioconazole (refer to Table 5.3.8).

Conclusion

A LC-MS/MS method (00684) is available to monitor all components of the residue definition (i.e. prothioconazole and prothioconazole-desthio (M04)) in surface and drinking water to a LOQ of 0.1 µg/L (prothioconazole) and 0.05 µg/L (prothioconazole-desthio (M04)). The primary method is highly specific therefore negating the need for a confirmatory method. The analytical method was fully accepted in the DAR and EFSA Conclusion for monitoring purposes.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods for the analyses of prothioconazole and prothioconazole-desthio (M04) in air is given in the following tables.

Data have been taken from peer-reviewed EU documents, published during Annex I listing of prothioconazole. These are:

- 2004 DAR (RMS: UK)
- 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)

No new studies are presented.

Table 5.3-10: Validated methods for air (if appropriate)

Component of residue definition: Prothioconazole			
Method type	Method LOQ	Principle of method	Author(s), year
Primary	0.015 mg/m ³	LC-MS/MS Method: 00724 ^[1] i) Concentrated through TENAX absorption tube ii) Extracted with ACN m/z: Not stated	Maasfeld, 2002a, Report no. 00724, KCP 5.3.2.6/01 EU agreed (DAR, 2004, RMS: UK)
Confirmatory	Not required – the primary method is considered highly specific.		

^[1] The method was also validated for determination of prothioconazole-desthio (M04) (refer to Table 5.3-11).

Table 5.3-11: Validated methods for air

Component of residue definition: Prothioconazole-desthio (M04)			
Method type	Method LOQ	Principle of method	Author(s), year
Primary	0.0006 mg/m ³	LC-MS/MS Method: 00731 ^[1] i) Concentrated through TENAX absorption tube ii) Extracted with ACN m/z: Not stated	Maasfeld, 2002b, Report no. 00731, KCP 5.3.2.6/02 EU agreed (DAR, 2004, RMS: UK)
Confirmatory	Not required – the primary method is considered highly specific.		

^[1] The method was also validated for determination of prothioconazole (refer to Table 5.3.10)

Conclusion

A LC-MS/MS method (00724) is available to monitor prothioconazole and prothioconazole-desthio (M04) in air to a LOQ of 0.015 mg/m³ (prothioconazole) and 0.0006 mg/m³ (prothioconazole-desthio (M04)). The primary method is highly specific therefore negating the need for a confirmatory method. The analytical method was fully accepted in the DAR and EFSA Conclusion for monitoring purposes. The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional information is necessary.

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Monitoring methods in body fluids and tissues are not required as the active substance (and the relevant impurities) are not classified as toxic nor highly toxic or classified according to CLP Regulation as acute toxic (cat. 1 - 3), CMR (cat. 1) or STOT (cat. 1) as specified in section 8 of the European Commission Guidance document on pesticide residue analytical methods (reference SANCO/825/00 rev. 8.1). Further, no residue definition has been set in this matrix.

zRMS comment (analytical methods for body fluids and tissues):

In the case of the Euskatel Pro application provided for in Art. 33 the evaluation was carried out on existing endpoints in the EU, in line with the data requirements and guidance in force at the time of inclusion or last renewal of the active substance. Currently agreed EU endpoints for prothioconazole do not include a residue definition for monitoring in body fluids and tissues.

The development of monitoring methods for body fluids and tissues will be required once the active substance is renewed and the residue definitions in these matrices are finalized at EU level.

On the other hand, we agree that such a method is required under Reg (EU) No 283/2013. This data gap should be fulfilled as a post-registration requirement.

5.3.2.8 Other studies/ information

None.

5.3.3 Description of analytical methods for the determination of residues of

azoxystrobin (KCP 5.2)

Rotam were not a notifier at Annex I inclusion of azoxystrobin, nor are they a notifier for the renewal of approval process. Rotam only have access to publicly available data, e.g. the 2009 DAR (RMS: UK), 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542) and 2011 Review Report (SANCO/11027/2011 Rev 2 - 17 June 2011).

5.3.3.1 Overview of residue definitions and levels for which compliance is required

Azoxystrobin is currently under review at EU level. However, this application relies on existing, EU agreed endpoints. In the 2010 EFSA Conclusion, the residue definitions for monitoring purposes in all matrices is 'azoxystrobin'.

Table 5.3-12: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Azoxystrobin	0.01* mg/kg	LOQ of method
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)			
Plant, high oil content			
Milk	Azoxystrobin	0.001* mg/kg	LOQ of method
Eggs		0.01* mg/kg	
Muscle			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Azoxystrobin	0.02 mg/kg	LOQ of method
Drinking water (Human toxicology)	Azoxystrobin	0.1 µg/L	General limit and LOQ of method (SANCO/825/00 rev.8.1)
Surface water (Ecotoxicology)			
Air	Azoxystrobin	3 µg/m³	AOEL: 0.2 mg/kg bw/d
Tissue (meat or liver)	Azoxystrobin	0.01* mg/kg (tissues)	LOQ of method for residues in foodstuff of animal origin
Body fluids			

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods for analysis of azoxystrobin in plant matrices is given in the following table. Difficult to analyse crops are not considered, as the intended GAP does not include these

matrices. Data have been taken from peer-reviewed EU documents, published during or since Annex I listing of azoxystrobin. These are:

- 2009 DAR (RMS: UK)
- 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542)
- 2013 Art. 12 MRL Review (EFSA Journal 2013;11(12):3497)

No new studies are presented.

Table 5.3-13: Validated methods for food and feed of plant origin

Component of residue definition: Azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
High water High acid High oil High starch/ high protein content (dry)	Primary	0.01 mg/kg (all crops)	LC-MS/MS Method: RAM/305 Analyte: Azoxystrobin Extraction with ACN/H ₂ O m/z: Not stated	Lister, N.; 1999, Report no. RJ2770B, KCP 5.2.2/12 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV	0.01 mg/kg (all crops)	Same as primary method.	Kang, J, 2003, Report no. CEMR- 1708 v3, KCP 5.3.3/01 Croucher, A., 2002, Syngenta File No. ICI5504/1336, KCP 5.3.3/02 EU agreed (DAR, 2009, RMS: UK)
High water High acid High oil High starch/ high protein content (dry)	Primary	0.01 mg/kg (all crops)	LC-MS/MS Method: DFG-S19 Analyte: Azoxystrobin	EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV	None presented.		

Conclusion

HLPC-MS/MS (RAM/305) and DFG S19 GC-MS methods were accepted in the DAR and EFSA Conclusion, are acceptable to monitor all components of the monitoring residue definition in the crop commodities applied for, i.e. high protein/starch (dry) and high oil commodities, to at least an LOQ of 0.01 mg/kg. The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional data are necessary.

Table 5.3-14: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	SANTE 2017/10632 Rev. 3 of 22 November 2017 (i.e. the Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods), with application from December 2019, states that: <i>“For renewal of product authorisations or for new product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In case this document did not yet apply, when the data for the latest renewal or approval were submitted, at this stage no new studies or data related to extraction efficiency are required. This means in practice that for renewal of product</i>

	Method for products of plant origin
	<p><i>authorisations or for new product authorisations for which no new MRL is required, no additional proof of extraction efficiency will be required.”</i></p> <p>The guidance document did not apply when the data for the latest renewal of approval of azoxystrobin were submitted, and no change of MRL is required as a result of the proposed uses of FF-075. As such, no additional data are required to address extraction efficiency to support authorisation of FF-075.</p>

5.3.3.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods for analysis of azoxystrobin in animal matrices is given in the following table. Data have been taken from peer-reviewed EU documents, published during or since Annex I listing of azoxystrobin. These are:

- 2009 DAR (RMS: UK)
- 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542)
- 2013 Art. 12 MRL Review (EFSA Journal 2013;11(12):3497)

No new studies are presented.

Table 5.3-15: Validated methods for food and feed of animal origin

Component of residue definition: Azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Milk Meat Liver Kidney Fat	Primary	0.01 mg/kg (egg, liver, muscle, fat) 0.001 mg/kg (milk)	GC-NPD Method: RAM 255/03 Analyte: Azoxystrobin Extraction with ACN	Sapiets, A.; 1996, Report no. RJ1089B, KCP 5.2.2/13 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	The EFSA Conclusion stated: “Residues of azoxystrobin in animal matrices can be monitored by GC-NPD”, with no data gap for a confirmatory method set.		
	ILV	The Article 12 review (EFSA Journal 2013;11(12):3497) stated: “During the peer review under Directive 91/414/EEC, an analytical method using GC-NPD <u>and its ILV</u> were evaluated and validated for determination of parent azoxystrobin with an LOQ of 0.001 mg/kg in milk and 0.01 mg/kg in eggs, liver, fat, muscle.” Though it is noted that details of this ILV method do not appear in the 2009 DAR or in the 2010 EFSA Conclusion.		

Conclusion

A GC-NPD method (RAM 255/03), that was fully accepted in the DAR and EFSA Conclusion, is acceptable to monitor all components of the monitoring residue definition in animal commodities to a LOQ of 0.01 mg/kg (meat, liver, kidney, fat) and 0.001 mg/kg (milk). The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional data are considered necessary.

It is noted that in the Article 12 review (EFSA Journal 2013;11(12):3497), it was stated that a second fully validated analytical method (RAM 399), based on LC-MS/MS, and its ILV, were evaluated in the 2008 JMPR evaluation with a LOQ of 0.01 mg/kg in muscle, milk, kidney, liver and egg.

Table 5.3-16: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	<p>SANTE 2017/10632 Rev. 3 of 22 November 2017 (i.e. the Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods), with application from December 2019, states that:</p> <p><i>“For renewal of product authorisations or for new product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In case this document did not yet apply, when the data for the latest renewal or approval were submitted, at this stage no new studies or data related to extraction efficiency are required. This means in practice that for renewal of product authorisations or for new product authorisations for which no new MRL is required, no additional proof of extraction efficiency will be required.”</i></p> <p>The guidance document did not apply when the data for the latest renewal of approval of azoxystrobin were submitted, and no change of MRL is required as a result of the proposed uses of FF-075. As such, no additional data are required to address extraction efficiency to support authorisation of FF-075.</p>

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods for the analyses of azoxystrobin in soil is given in the following table. Data have been taken from peer-reviewed EU documents, published during Annex I listing of azoxystrobin. These are:

- 2009 DAR (RMS: UK)
- 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542)

No new studies are presented.

zRMS comment (Johnson analytical method, 2000, does not meet the requirements for a confirmatory method due to the use of chloroform and dichloromethane):

Under Article 33 of Regulation (EC) No 1107/2009, the current EU-agreed endpoints are relevant. Analytical methods data accepted in the DAR were considered sufficient to fulfil the data requirements and guidance relevant at the time of the active substance inclusion, and are considered sufficient to support the product application.

The assessment should be revised when the active substance is renewed and the new methods should be provided by the applicant for re-evaluation.

Table 5.3-17: Validated methods for soil

Component of residue definition: Azoxystrobin			
Method type	Method LOQ	Principle of method	Author(s), year
Primary	0.02 mg/kg	LC-MS/MS Method: RAM 269/03 Analyte: Azoxystrobin Extraction with MeOH/HCl, DCM/NaCl m/z: Not stated	Johnson, R. 2000, Syngenta File No. ICI5504/0751, KCP 5.2.2/14 EU agreed (DAR, 2009, RMS: UK)
Confirmatory	Not required – The primary method is considered highly specific.		

Conclusion

A LC-MS/MS method (RAM 269/03) is available to monitor all components of the residue definition in soil to a LOQ of 0.02 mg/kg. The primary method is highly specific therefore negating the need for a confirmatory method. The analytical method was fully accepted in the DAR and EFSA Conclusion for monitoring purposes. The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional information is necessary.

5.3.3.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods for the analyses of azoxystrobin surface and drinking water is given in the following tables. Data have been taken from peer-reviewed EU documents, published during Annex I listing of azoxystrobin. These are:

- 2009 DAR (RMS: UK)
- 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542)

No new studies are presented.

zRMS comment (Robinson analytical method, 2000, does not meet the requirements for a confirmatory method. Chromatograms for higher concentration levels are missing):

Under Article 33 of Regulation (EC) No 1107/2009, the current EU-agreed endpoints are relevant. Analytical methods data accepted in the 2004 DAR and 2007 DAR Addendum (RMS: UK), and the 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98) were considered sufficient to fulfil the data requirements and guidance relevant at the time of the active substance inclusion, and are considered sufficient to support the product application.

The assessment should be revised when the active substance is renewed and the new methods should be provided by the applicant for re-evaluation.

Table 5.3-18: Validated methods for water

Component of residue definition: Azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Surface water Drinking water	Primary	0.1 µg/L	GC-MSD Method: RAM 358/01 Analyte: Azoxystrobin Extraction with SPE, EA/DCM, ACN m/z: 344 (388 and 372 qualifier ions)	Robinson, N., 2000, Syngenta File No. ICI5504/0758, KCP 5.2.2/15 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	Not required – the primary method is considered highly specific.		
	ILV (drinking water)	None presented.		

Conclusion

A GC-MSD method (RAM 358/01) is available to monitor all components of the residue definition in surface and drinking water to a LOQ of 0.1 µg/L. The primary method is highly specific therefore negating the need for a confirmatory method. The analytical method was fully accepted in the DAR and EFSA Conclusion for monitoring purposes.

5.3.3.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods for the analyses of azoxystrobin in air is given in the following table. Data have been taken from peer-reviewed EU documents, published during Annex I listing of azoxystrobin. These are:

- 2009 DAR (RMS: UK)
- 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542)

No new studies are presented.

Table 5.3-19: Validated methods for air (if appropriate)

Component of residue definition: Azoxystrobin			
Method type	Method LOQ	Principle of method	Author(s), year
Primary	0.003 mg/m ³	GC-MSD Method: RAM 376/01 Analyte: Azoxystrobin Extraction with ACN m/z: 344 (388 and 403 qualifier ions)	Crawford, N., 2001, Report No. TMJ4658B, KCP 5.2.2/17 EU agreed (DAR, 2009, RMS: UK)
Confirmatory	Not required – the primary method is considered highly specific.		

Conclusion

A GC-MSD method (RAM 376/01) is available to monitor azoxystrobin in air to a LOQ of 0.003 mg/m³. The primary method is highly specific therefore negating the need for a confirmatory method. The analytical method was fully accepted in the DAR and EFSA Conclusion for monitoring purposes. The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075. No additional information is necessary.

5.3.3.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods for the analyses of azoxystrobin in body fluids and tissues is given in the following table. Data have been taken from peer-reviewed EU documents, published during Annex I listing of azoxystrobin. These are:

- 2009 DAR (RMS: UK)
- 2010 EFSA Conclusion (EFSA Journal 2010; 8(4):1542)

No new studies are presented.

zRMS comment (The method of xxxxxx, 1999, is not accepted after peer review and also reported as data gap in EFSA conclusion – data gap):

This data gap was included as a post-registration requirement.

Table 5.3-20: Validated methods for body fluids and tissues (if appropriate)

Component of residue definition: Azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Meat	Primary	0.01 mg/kg	GC-NPD Method:	Sapiets, A.; 1996, Report no.

Component of residue definition: Azoxystrobin				
Matrix type	Method type	Method LOQ	Principle of method	Author(s), year
Liver Kidney Fat		(egg, liver, muscle, fat)	RAM 255/03 Analyte: Azoxystrobin Extraction with ACN	RJ1089B, KCP 5.2.2/13 EU agreed (DAR, 2009, RMS: UK)
	Confirmatory	The EFSA Conclusion stated: “ <i>Residues of azoxystrobin in animal matrices can be monitored by GC-NPD</i> ”, with no data gap for a confirmatory method set.		
Plasma	Primary	0.05 µg/mL	LC-MS/MS Method CTL/R/1401 Analyte: Azoxystrobin Extraction with SPE	xxxxxx, Report no. CTL/R/1401, KCP 5.3.3/03
	Confirmatory	Not required – the primary method is considered highly specific.		

Conclusion

A GC-NPD method (RAM 255/03), that was fully accepted in the DAR and EFSA Conclusion, is acceptable to monitor all components of the monitoring residue definition in animal tissues to a LOQ of 0.01 mg/kg (meat, liver, kidney, fat). It is noted that in the Article 12 review (EFSA Journal 2013;11(12):3497), it was stated that a second fully validated analytical method (RAM 399), based on LC-MS/MS, and its ILV, were evaluated in the 2008 JMPR evaluation with a LOQ of 0.01 mg/kg in muscle, milk, kidney, liver and egg. A LC-MS/MS method (CTL/R/1401), that was fully accepted in the DAR and EFSA Conclusion, is acceptable to monitor all components of the monitoring residue definition in body fluids to a LOQ of 0.05 µg/mL (plasma). The data package already agreed at EU level for Annex I listing is therefore acceptable to support the authorisation of FF-075.

5.3.3.8 Other studies/ information

None.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.1.1/01	Lu, J.	2020	Study on the method validation of prothioconazole 200 g/l + azoxystrobin 150 g/l suspension concentrate Study No.: 2878 GLP Unpublished	N	Rotam
KCP 5.2.1.2/01	Lu, J.	2020	Method validation of relevant impurities in prothioconazole 200 g/l + azoxystrobin 150 g/l suspension concentrate Study No.: 2959 GLP Unpublished	N	Rotam
KCP 5.2.1.2/02	Lu, J.	2021	Method validation for impurity-3 (prothioconazole deschloro) in prothioconazole 200 g/l + azoxystrobin 150 g/l suspension concentrate Study No.:3030 GLP Unpublished	N	Rotam
KCP 5.2.2/08	Lozano, J	2020	Analytical phase report. Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Report no. S20-00395-L3 GLP Unpublished <i>Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00395 - see dRR B9]</i>	N	Rotam

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.2/09	Lozano, J	2020	Analytical phase report. Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Report no. S20-00396-L3 GLP Unpublished <i>Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00396 - see dRR B9]</i>	N	Rotam
KCP 5.2.2/10	Wendling, K.	2020	Final Report. Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC: Acute Oral and Contact Toxicity to the Bumble Bee, <i>Bombus terrestris</i> L. under Laboratory Conditions Report no. S19-03594 GLP Unpublished	N	Rotam
KCP 5.2.2/11	Yu, J.	2021	Method validation, solubility and stability of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) in aquatic test mediums Report no. 2856 GLP Unpublished	N	Rotam
KCP 5.3.2.2/06	Herrmann, S.S.	2014	Determination of pesticide residues in maize for livestock feed by GC-MS/MS and LC-MS/MS (QuEChERS method) Validation Report 17 EURL for Cereals and Feeding stuff, National Food Institute, Technical University of Denmark GLP status not specified Published	N	EURL for Cereals and Feeding Stuff
KCP 5.3.2.2/07	Poulsen, M.E.	2012	Determination of pesticide residues in wheat, oat, rye, rice and barley by LC-MS/MS (QuEChERS method) Validation Report 9 EURL for Cereals and Feeding stuff, National Food Institute, Technical University of Denmark GLP status not specified Published	N	EURL for Cereals and Feeding Stuff

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.2/01	Heinemann, O.	2000a	Analytical determination of residues of JAU 6476 and desthio-JAU 6476 in/on cereals by HPLC/MS/MS 00598 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.2.2/02	Heinemann, O.	2000b	Analytical determination of residues of JAU6476 and JAU6476-desthio in/on cereals and canola by HPLC-MS/MS (method modification 00598/M001) 00598/M001 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.2.2/03	Schramel, O.	2001	Determination of the storage stability of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil MR-644/99 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.2.2/04	Schramel, O.	2000	Residue analytical method 00610 (MR-643/99) for the determination of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLC-MS/MS 00610 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.2.2/05	Heinemann, O.	2001a	Analytical determination of residues of JAU6476-sulfonic acid and JAU6476-desthio in/on cereals and canola by HPLC-MS/MS 00647 Bayer AG GLP Unpublished	N	Bayer AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.2/06	Heinemann, O.	2001b	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS 00655 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.2.2/07	Heinemann, O.	2001c	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in milk by HPLC-MS/MS (00655/M001) 00655/M001 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.2.2/12	Lister, N.	1999	Azoxystrobin: Validation of RAM 305/01 for the determination of azoxystrobin and R230310 in crops Report no. RJ2770B GLP Unpublished	N	Syngenta
KCP 5.2.2/13	Sapiets, A.	1996	ICIA5504 and R230310: Validation of a method for the determination of residues in animal tissue, eggs and milk RAM 255/03 Report no. RJ1089B GLP Unpublished	N	Syngenta
KCP 5.2.2/14	Johnson, R.	2000	Residue analytical method for the analysis of azoxystrobin, R230310, R2334886, R401553 and R402173 in soil RAM 269/03 Syngenta File No. ICI5504/0751 Non-GLP Unpublished	N	Syngenta
KCP 5.2.2/15	Robinson, N.	2000	Analytical method for the determination of residues of azoxystrobin in water RAM 358/01	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Syngenta File No. ICI5504/0758 Non-GLP Unpublished		
KCP 5.2.2/16	Hurt, A.	1999	Residue analytical method for the analysis of azoxystrobin, R230310, R2334886, R401553 and R402173 in water RAM 292/02 Syngenta File No. ICI5504/0767 GLP Unpublished	N	Syngenta
KCP 5.2.2/17	Crawford, N.	2001	Azoxystrobin: Validation of an analytical method for the determination of residues in air Report No. TMF4658B	N	Syngenta
KCP 5.3.2.2/01	Weeren, R.D. and Pelz, S.	2000	Modification M033 of method 00086: Validation of DFG method S 19 (extended revision) for the determination of residues of JAU 6476-desthio in materials of plant and animal origin 00086/M033 Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany GLP Unpublished	N	Bayer AG
KCP 5.3.2.2/02	Class, Th.	2001	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residues of JAU 6476-desthio (BAYER method 00086/M033) in plant materials P/B 484 G PTRL Europe, Ulm, Germany GLP Unpublished	N	Bayer AG
KCP 5.3.2.2/03	Heinemann, O.	2000a	Analytical determination of residues of JAU 6476 and desthio-JAU 6476 in/on cereals by HPLC/MS/MS 00598 Bayer AG GLP Unpublished	N	Bayer AG
KCP	Heinemann, O.	2000b	Analytical determination of residues of JAU6476 and JAU6476-desthio in/on cereals and canola by	N	Bayer AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
5.3.2.2/04			HPLC-MS/MS (method modification 00598/M001) 00598/M001 Bayer AG GLP Unpublished		
KCP 5.3.2.2/05	Heinemann, O.	2001a	Analytical determination of residues of JAU6476-sulfonic acid and JAU6476-desthio in/on cereals and canola by HPLC-MS/MS 00647 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.3.2.3/01	Heinemann, O.	2001b	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS 00655 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.3.2.3/02	Heinemann, O.	2001c	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in milk by HPLC-MS/MS (00655/M001) 00655/M001 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.3.2.3/03	Dubey, L.	2001	Independent laboratory validation of Bayer methods 00655 and 00655/M001 for the determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio and JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS A-14-01-01 Battelle, Geneva Research Centres, Carouge/Geneva, Switzerland GLP Unpublished	N	Bayer AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.3.2.3/04	Weber, H., Weber, E. and Spiegel, K.	2002	Validation of the residue analytical method for the determination of JAU6476-desthio , JAU6476-3-hydroxy-desthio and JAU6476-4-hydroxy-desthio residues in animal matrices using aged radioactive residues MR-091-01 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.3.2.4/01	Schramel, O.	2000	Residue analytical method 00610 (MR-643/99) for the determination of JAU6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLC-MS/MS Report no. 00610 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.3.2.4/02	Steinhauer, S.	2001	Enforcement method 00086/M038 for the determination of the residues of JAU6476-desthio in soil – validation of DFG method S19 (extended revision) 00086/M038 Dr Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany GLP Unpublished	N	Bayer AG
KCP 5.3.2.5/01	Sommer, H.	1999	Method for the determination of JAU6476 and SXX0665 in test water from aquatic toxicity test by HPLC [Tox/Ecotox method] 00586 Bayer AG Non-GLP Unpublished	N	Bayer AG
KCP 5.3.2.5/02	Sommer, H.	2001	Enforcement method 00684 for determination of JAU6476 and JAU6476-desthio in drinking and surface water by HPLC-MS/MS 00684 Bayer AG GLP	N	Bayer AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.3.2.6/01	Maasfeld, W.	2002a	Method for the determination of JAU6476 in air by HPLC-MS/MS 00724 Bayer AG GLP Unpublished	N	Bayer AG
KCP 5.3.2.6/02	Maasfeld, W.	2002b	<i>[The study was relied on in the DAR however reference details were not provided in the DAR. Reference details below are instead taken from the dRAR]</i> Method for the determination of JAU6476-desthio (SXX0665) in air by HPLC-MS/MS 00731 Bayer AG, Leverkusen, Germany GLP Unpublished	N	Bayer AG
KCP 5.3.3/01	Kang, J.	2003	Independent laboratory validation of SPO RAM 305/02 analytical method for the determination of residues of azoxystrobin and R2303010 in leafy crops, brassicae and root/tuber crops Report no. CEMR-1708 v3 GLP Unpublished	N	Syngenta
KCP 5.3.3/02	Croucher, A.	2002	Independent laboratory validation of SPO RAM 305/02 analytical method for the determination of residues in crops (brassicae, maize and root crops) Syngenta File No. ICI5504/1336 GLP Unpublished	N	Syngenta
KCP 5.3.3/03	xxxxxx	1999	Method validation, solubility and stability of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) in aquatic test mediums Report no. CTL/R/1401 Non-GLP Unpublished	Y	Syngenta
IIA 6.3	Burke, S.R.	1995	ICIA5504 and R23031: Validation of a method for the determination of residues in cereals and vines.	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Final report. Zeneca RJ 1729B RIP96-00474		
IIA 6.3	Clarke, D.M.	1994	ICIA5504 and R230310: Validation of a method [RAM 243/02] for the determination of residues in cereals and vines Zeneca RJ 1557B RIP96-00475	N	Syngenta

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for prothioconazole

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

New studies have been submitted for the determinations of the active substance (prothioconazole) and relevant impurities (prothioconazole-desthio and toluene) in the plant protection product FF-075 – that have not previously been evaluated at EU level. Refer to Section 5.2.1 for complete summaries of the methods and supporting validation data.

New analytical methods supporting other areas of the dossier have been submitted for the authorisation of FF-075. The validation data of these methods are described below. All other studies relied on in other areas of the dossier have either been previously evaluated at EU level for Annex I inclusion and accepted without provision of further data, and/or do not involve the detection of (non-radiolabelled) analytical residues.

A 2.1.1.1 Analytical method 1

A 2.1.1.1.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2.2/08
Report	Analytical phase report. Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Bogner, F.; Report No. S20-00395-L3 <i>Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00395</i>
Guideline(s):	SANCO/3029/99, rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of 50 % (w/v) aqueous sucrose solution (2 mL) fortified with test item (FF-075) at 0.1 and 7700 mg/kg (nominal content of 0.0170 and 1309 mg/kg prothioconazole, respectively) were vortexed. Samples were diluted with acetonitrile/water (1:1, v/v) to a 50 mL volume and again with acetonitrile/water (1:1, v/v) by a factor of 5. High level recovery samples were further diluted to be within calibration range.

Test samples (from the chronic oral toxicity test) were quantitatively transferred to a plastic tube with use of water (2 x 2 mL) and acetonitrile (2 x 2 mL). Samples were vortexed then diluted with acetonitrile/water (1:1, v/v) to a 25 mL volume. All samples were diluted with acetonitrile/water (1:1, v/v) by a factor of 5, and then further diluted as appropriate with acetonitrile/water (1:1, v/v) to be within

calibration range.

Samples were analysed by LC-MS/MS using a Phenomenex Kintex 2.6 µm Biphenyl column (100 x 2.1 mm, 2.6 µm) monitoring two ion transitions: 344→266 and 344→180 m/z.

Solvent standards were prepared in acetonitrile and water.

Results and discussions

Table A 1: Recovery results from method validation of prothioconazole using the analytical method for the ion transition 344→266 m/z

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
50 % (w/v) aqueous sucrose solutions	Prothioconazole	0.0170 (n = 5)	107	9	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		1309 (n = 5)	110*	2	

* One recovery of 125 % was identified according to the Grubbs test and was not included in the calculation of the mean and relative standard deviation

Table A 2: Characteristics for the analytical method used for validation of prothioconazole residues in 50 % (w/v) aqueous sucrose solutions

	Prothioconazole
Specificity	A mass spectrum is provided Representative chromatograms (solvent standards, lowest fortification levels, blank samples) for each matrix/ion transitions are provided. Blank values < 30 % LOQ Highly specific primary method monitoring two ion transitions
Calibration (type, number of data points)	Representative calibration plot presented n ≥ 5 y = -9.056E+01 +7.433E+01x (344→266 m/z) r ² ≥ 0.99
Calibration range	0.05 - 5 ng/mL, from <30% LOQ to 20% above the highest analyte concentration in any diluted sample extract (based on LOQ level, higher fortification levels were diluted to be within calibration range)
Assessment of matrix effects is presented	Yes – matrix effects were > ±20 %, therefore matrix-matched standards were used for quantification.
Limit of quantification	0.017 mg/kg - representing the lowest validated level supported by recovery and precision data
Limit of detection	0.0051 mg/kg (set at 30 % of the LOQ)
Extract stability	Prothioconazole was stable under refrigerated conditions (1 - 10 °C) in 50 % (w/v) aqueous sucrose solution diluted by a factor of 25 with acetonitrile/water (1:1, v/v) for at least 1 day. Samples were analysed on the extraction day. If samples were not analysed on the extraction day extract stability was confirmed.

Conclusion

The LC-MS/MS method for the determination of prothioconazole residues in 50 % (w/v) aqueous sucrose solutions was sufficiently validated to a LOQ of 0.017 mg/kg in accordance with SANCO/3029/99 rev.4.

A 2.1.1.1.2 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.1.1.1.2 Analytical method 2

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2.2/09
Report	Analytical phase report. Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Bogner, F.; Report No. S20-00396-L3 <i>Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00396</i>
Guideline(s):	SANCO/3029/99, rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Sample preparation follows the QuEChERS method.

Larval diet (Diet C) (500 mg) was fortified with test item (FF-075) at 0.1 and 4700 mg/kg (nominal content of 0.0170 and 799 mg/kg prothioconazole, respectively). Fortified samples were mixed with acetonitrile/water (1:1, v/v) + 0.5% formic acid solution (8 mL). A Citrat-Kit-01 was added. Samples were shaken and centrifuged. Supernatant (1 mL) was transferred into a PSA-Kit-01. Samples were shaken and centrifuged. A 500 µL aliquot was diluted with water + 1% formic acid solution (500 µL). High-level recovery samples were further diluted as appropriate with acetonitrile/water (1:1, v/v) + 0.5% formic acid solution to be within the calibration range.

Test samples (from the larval toxicity test) were quantitatively transferred to a plastic tube with use of water + 1% formic acid (3 x 2 mL) and acetonitrile (3 x 2 mL). Samples were mixed with acetonitrile/water (1:1, v/v) + 0.5 % formic acid solution (23 mL), vortexed, and four Citrat-Kit-01 added. Samples were shaken and centrifuged. Supernatant (1 mL) was transferred into a PSA-Kit-01. Samples were shaken and centrifuged. A 500 µL aliquot was diluted with water + 1% formic acid solution (500 µL). The treated larval diet samples were further diluted as appropriate with acetonitrile/water (1:1, v/v) + 0.5% formic acid solution to be within the calibration range.

Samples were analysed by LC-MS/MS using a Phenomenex Kintex 2.6 µm Biphenyl column (100 x 2.1 mm, 2.6 µm) monitoring two ion transitions: 344→266 and 344→180 m/z.

Solvent standards were prepared in acetonitrile and water.

Results and discussions

Table A 3: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Larval diet (Diet C)	Prothioconazole	0.017 (n = 5)	84*	11	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		799 (n = 5)	87	6	

* One recovery of 263 % was identified according to the Grubbs test and was not included in the calculation of the mean and relative standard deviation

Table A 4: Characteristics for the analytical method used for validation of prothioconazole residues in Larval diet (Diet C)

	Prothioconazole
Specificity	Representative chromatograms (solvent standards, lowest fortification levels, blank samples) for each matrix/ion transitions are provided. Blank values < 30 % LOQ Highly specific primary method monitoring two ion transitions
Calibration (type, number of data points)	Representative calibration plots presented $n \geq 5$ $y = 9.740E-01 + 1.167E+02x$ (344 → 266 m/z) $r^2 \geq 0.99$
Calibration range	0.025 - 5 ng/mL, from <30% LOQ to 20% above the highest analyte concentration in any diluted sample extract (based on LOQ level, higher fortification levels were diluted to be within calibration range)
Assessment of matrix effects is presented	Yes – matrix effects were > ±20 %, therefore matrix-matched standards were used for quantification.
Limit of quantification	0.017 mg/kg - representing the lowest validated level supported by recovery and precision data
Limit of detection	0.0051 mg/kg (set at 30 % of the LOQ)
Extract stability	Prothioconazole was stable under refrigerated conditions (1 - 10 °C) in larval diet extracted with acetonitrile/water (1:1, v/v) + 0.5 % formic acid and QuEChERS Citrat-Kit-01 for at least 1 day. Samples were analysed on the extraction day. If samples were not analysed on the extraction day extract stability was confirmed.

Conclusion

The LC-MS/MS method for the determination of prothioconazole residues in larval diet was sufficiently validated to a LOQ of 0.017 mg/kg in accordance with SANCO/3029/99 rev.4.

A 2.1.1.1.2.2 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.1.1.1.3 Analytical method 3

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2.2/10
Report	Final Report. Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC: Acute Oral and Contact Toxicity to the Bumble Bee, <i>Bombus terrestris</i> L. under Laboratory Conditions Wendling, K.; Report No. S19-03594 (Appendix D)
Guideline(s):	SANCO/3029/99, rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of test item in 50 % w/v aqueous sucrose solutions or 0.1 % Triton X solutions in deionised water were diluted with acetonitrile:water (1:1, v/v) to be within calibration range.

Samples were analysed by LC-MS/MS using a Supelco Ascentis Express C18 column (50 x 2.0 mm, 2.7 µm) with 2.1 mm C18 guard column, monitoring two ion transitions: 344→189 and 344→154 m/z.

Calibration standards were prepared in acetonitrile and water.

Results and discussions

Table A 5: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Quantifier ion transition: 344→189 m/z					
50 % w/v aqueous sucrose solutions	Prothioconazole	21.3 (n = 5)	105	5	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		4420 (n = 5)	107	5	
0.1 % Triton X solutions in deionised water		425 (n = 5)	96	2	
		88000 (n = 5)	98	12	

Table A 6: Characteristics for the analytical method used for validation of prothioconazole residues in 50% w/v aq. sucrose solution and 0.1 % Triton X solution in deionised water

	Prothioconazole
Specificity	Representative chromatograms (solvent standards, lowest fortification levels, blank samples) for each matrix/ion transitions are provided. Blank values < 30 % LOQ Highly specific primary method monitoring two ion transitions
Calibration (type, number of data points)	Representative calibration plot presented $n \geq 5$ $y = 2.19e+005 x + 9.86e+003$ (50 % w/v aqueous sucrose solutions) $y = 3.42e+005 x + 4.34e+004$ (0.1 % Triton X solutions in deionised water) $r^2 \geq 0.99$
Calibration range	1.0 - 11 ng/mL, covers from 30% the LOQ to 20% above the highest analyte concentration in any (diluted) sample extract
Assessment of matrix effects is presented	No - matrix-matched calibration solutions used
Limit of quantification	21.3 mg/L (50 % w/v aqueous sucrose solutions) 425 mg/L (0.1 % Triton X solutions in deionised water) Representing the lowest validated level supported by recovery and precision data
Limit of detection	6.39 mg/L (set at 30 % of the LOQ in 50 % w/v aqueous sucrose solutions) 128 mg/L (set at 30 % of the LOQ in 0.1 % Triton X solutions in deionised water)
Extract stability	The maximum storage period from sampling to analysis was 8 days for samples of aqueous sucrose solution and 42 days for water samples containing 0.1 % Triton X-100. Storage stability data are included within the study report and demonstrate that prothioconazole was stable under deep-frozen conditions ($\leq -18^\circ\text{C}$) in demineralized water containing 0.1 % Triton X-100 for at least 42 days.

Conclusion

The LC-MS/MS method for the determination of prothioconazole residues in 50% w/v aq. sucrose solution and 0.1 % Triton X solution in deionised water was sufficiently validated to a LOQ of 21.3 mg/L and 425 mg/L, respectively, in accordance with SANCO/3029/99 rev.4.

A 2.1.1.1.3.2 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.1.1.1.4 Analytical method 4

A 2.1.1.1.4.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference: KCP 5.2.2/11

Report Method validation, solubility and stability of Prothioconazole 200 g/L +

Azoxystrobin 150 g/L SC (FF-075) in aquatic test mediums
Yu, J.; Report No. 2856

Guideline(s): SANCO/3029/99 rev.4
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Blank test media (OECD TG 201 alga medium, ISO standard dilution water or Swedish standard growth medium) was fortified with test item and either cleaned by SPE cartridge (LOQ level samples) and eluting with acetonitrile (2 mL) or filtered (higher level samples).

Samples were analysed by HPLC-UV at 254 nm using a EC-C18 column (150 x 4.6 mm, 2.7 µm).

Calibration standards were prepared in acetonitrile.

Results and discussions

Table A 7: Recovery results from method validation of prothioconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
OECD TG 201 alga medium	Prothioconazole	0.0356 (n = 5)	95.30	2.01	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		2.029 (n = 5)	90.77	0.59	
ISO standard dilution water		0.0356 (n = 5)	94.39	2.27	
		2.043 (n = 5)	94.13	1.01	
Swedish standard growth medium		0.0356 (n = 5)	97.94	3.05	
		8.157 (n = 5)	91.77	1.44	

Table A 8: Characteristics for the analytical method used for validation of prothioconazole residues in OECD TG 201 medium, ISO standard dilution water and Swedish standard growth medium

	Prothioconazole		
	OECD TG 201 medium	ISO standard dilution water	Swedish standard growth medium
Specificity	Representative chromatograms (acetonitrile solvent, blank test media, blank formulation, active substance reference, test substance, lowest and highest calibration standards, fortified samples at LOQ and higher levels) provided for each matrix. Blank values < 30 % LOQ Confirmation of peak identity was achieved by retention time matching with analytical standards.		

Calibration (type, number of data points)	Calibration plot presented $n = 5$ $y = 504058.4563x - 33.2179$ $r^2 = 0.9997$		
Calibration range	Low level: 0.032 – 0.988 mg/L Linearity covers from ca. 20% below the LOQ to ca. 5% above the highest analyte concentration in any (diluted) sample extract		
Assessment of matrix effects is presented	No – though recoveries were acceptable, so matrix effects not deemed significant.		
Limit of quantification	0.0356 mg/L – representing the lowest validated level (per matrix) supported by recovery and precision data		
Limit of detection	0.01 mg/L (set at 30 % of the LOQ)		
Extract stability	Prothioconazole (0.0338 and 1.6900 mg/L solutions) was stable in the test media under test conditions (21 - 24 °C, 6920 - 7980 Lux) for at least 96 hours.	Prothioconazole (0.0676 and 1.6900 mg/L solutions) was stable in the test media under test conditions (19 - 20 °C, 16 hours light, 8 hours dark) for at least 48 hours.	Prothioconazole (0.0068 and 6.7600 mg/L solutions) was stable in the test media under test conditions (24 ± 2 °C, 7080 - 7920 Lux) for at least 168 hours.

Conclusion

The HPLC-UV method for the determination of prothioconazole residues in OECD TG 201 medium, ISO standard dilution water and Swedish standard growth medium was sufficiently validated to a LOQ of 0.0356 mg/L (all matrices) in accordance with SANCO/3029/99 rev.4.

A 2.1.1.1.4.2 Confirmatory method

A separate method was not provided. Confirmation of peak identity was achieved by retention time matching with analytical standards.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

Two new studies are presented in this application to support the authorisation of FF-075.

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.3.2.2/06
Report	Determination of pesticide residues in maize for livestock feed by GC-MS/MS and LC-MS/MS (QuEChERS method) Herrmann, S.S., Andersen, G, Poulsen, M.E., 2014, Validation Report 17.
Guideline(s):	Not stated – complies with principles of SANCO/825/00 rev.8.1
Deviations:	n/a
GLP:	Not stated
Acceptability:	Yes

Materials and methods

The procedure follows the QuEChERS method. Maize samples (ca. 5 g) were added to centrifuge tubes and internal standard (¹³C₆-carbaryl) added. Cold water (10 g) was added and samples homogenised. Acetonitrile (10 mL) was added and samples shaken. Salt mixture containing MgSO₄ (4 g), NaCl (1 g), Na₃ citrate dihydrate (1 g) and Na₂H citrate sesquihydrate (0.5 g) was added and samples shaken and centrifuged. Supernatants (8 mL) were transferred to separate centrifuge tubes and stored in the freezer (-80 °C for 1 hr or overnight). When extracts were almost thawed, samples were centrifuged. Cold extracts (6 mL) were transferred to separate centrifuge tubes containing PSA (150 mg) and MgSO₄ (900 mg). Samples were shaken and centrifuged. Extracts (4 mL) were transferred to separate centrifuge tubes, 5% formic acid in acetonitrile solution (40 µL; 10 µL/mL) added. Extracts were diluted 1:1 with acetonitrile.

Samples were analysed by LC-MS/MS (ESI) on a reversed-phase column monitoring two ion transitions: 312→70 m/z and 314→127 m/z.

Results and discussions

Table A 9: Recovery results from method validation of prothioconazole-desthio using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Maize	Prothioconazole-desthio	0.01 (n = 5-6)	97	8	Mean recoveries and %RSD values are within acceptable limits, i.e. 70-110% and ≤20% respectively.
		0.02 (n = 5-6)	89	5	
		0.04 (n = 5-6)	90	10	
		0.2 (n = 5-6)	86	4	

Table A 10: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in maize

	Prothioconazole-desthio
Specificity	Representative chromatograms (lowest fortification level) for each ion transitions are provided. Blank (maize) samples were analysed, but results were not reported. Highly specific primary method monitoring two ion transitions.
Calibration (type, number of data points)	Example calibration line presented $n \geq 4$ $y = 217707.x - 7.85827$ $r^2 = 0.995284$
Calibration range	0.003-0.1 µg/mL
Assessment of matrix effects is presented	Not required – calibration standards were matrix-matched.
Limit of quantification	0.01 mg a.i./kg – representing the lowest validated level with sufficient recovery and precision

Conclusion

The LC-MS/MS method for the determination of prothioconazole-desthio in maize (i.e. dry, high protein/high starch commodities) was sufficiently validated to a LOQ of 0.01 mg as/kg in accordance with the principles of SANCO/825/00 rev. 8.1.

A 2.1.2.1.1.2 Independent laboratory validation

None available.

A 2.1.2.1.1.3 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.1.2.1.1.4 Extraction efficiency

Refer to Section 5.3.2.2.

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.3.2.2/07
Report	Determination of pesticide residues in wheat, oat, rye, rice and barley by LC-MS/MS (QuEChERS method) Poulsen, M.E., 2012, Validation Report 9.
Guideline(s):	Not stated – complies with principles of SANCO/825/00 rev.8.1
Deviations:	n/a
GLP:	Not stated
Acceptability:	Yes

Materials and methods

The procedure follows the QuEChERS method. Cereal samples (ca. 5 g) were added to centrifuge tubes and internal standard ($^{13}\text{C}_6$ -carbaryl) added. Cold water (10 g) was added and samples homogenised. Acetonitrile (10 mL) was added and samples shaken. Salt mixture containing MgSO_4 (4 g), NaCl (1 g), Na_3 citrate dihydrate (1 g) and Na_2H citrate sesquihydrate (0.5 g) was added and samples shaken and centrifuged. Supernatants (8 mL) were transferred to separate centrifuge tubes and stored in the freezer (-80°C for 1 hr or overnight). When extracts were almost thawed, samples were centrifuged. Cold extracts (6 mL) were transferred to separate centrifuge tubes containing PSA (150 mg) and MgSO_4 (900 mg). Samples were shaken and centrifuged. Extracts (4 mL) were transferred to separate centrifuge tubes, 5% formic acid in acetonitrile solution (40 μL ; 10 $\mu\text{L}/\text{mL}$) added. Extracts were diluted 1:1 with acetonitrile.

Samples were analysed by LC-MS/MS (ESI) on a reversed-phase column monitoring two ion transitions: $312 \rightarrow 70$ m/z and $314 \rightarrow 127$ m/z.

Results and discussions

Table A 11: Recovery results from method validation of prothioconazole-desthio using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Wheat	Prothioconazole-desthio	0.01 (n = 5-6)	85	16	Mean recoveries and %RSD values are within acceptable limits, i.e. 70-110% and ≤20% respectively.
		0.02 (n = 5-6)	103	14	
		0.1 (n = 5-6)	110	17	
Oat		0.01 (n = 5-6)	93	28	Mean recovery is within the guideline limit (70-110%) however %RSD is above the acceptable limit of 20%.
		0.02 (n = 5-6)	87	14	Mean recoveries and %RSD values are within acceptable limits, i.e. 70-110% and
		0.1 (n = 5-6)	89	7	

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
					≤20% respectively.
Rye		0.01 (n = 5-6)	113	20	Mean recoveries and %RSD values are within acceptable limits, i.e. 70-110% and ≤20% respectively.
		0.02 (n = 5-6)	79	14	
		0.1 (n = 5-6)	90	18	
Rice		0.01 (n = 5-6)	98	20	Mean recoveries and %RSD values are within acceptable limits, i.e. 70-110% and ≤20% respectively.
		0.02 (n = 5-6)	78	10	
		0.1 (n = 5-6)	100	6	
Barley		0.01 (n = 5-6)	91	15	Mean recoveries and %RSD values are within acceptable limits, i.e. 70-110% and ≤20% respectively.
		0.02 (n = 5-6)	88	10	
		0.1 (n = 5-6)	87	9	

Table A 12: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in cereal

	Prothioconazole-desthio
Specificity	Blank (cereal) samples were analysed, but results were not reported. Highly specific primary method monitoring two ion transitions.
Calibration (type, number of data points)	No calibration line presented specifically for analysis of prothioconazole-desthio, though the report states: <i>The calibration curves were best fitted to a linear curve. The quantification was performed from the mean of two bracketing calibration curves. The majority of the correlation coefficients (R) were higher or equal to 0.99.</i> n≥4
Calibration range	0.003-0.1 µg/mL
Assessment of matrix effects is presented	Not required – calibration standards were matrix-matched.
Limit of quantification	0.01 mg a.i./kg (wheat, barley, rye, rice), 0.02 mg a.i./kg (oat) – representing the lowest validated levels with sufficient recovery and precision

Conclusion

The LC-MS/MS method for the determination of prothioconazole-desthio in wheat, oat, rye, rice and barley (i.e. dry, high protein/high starch commodities) was validated to a LOQ of 0.01 mg as/kg (wheat, barley, rye, rice) or 0.02 mg as/kg (oat) in accordance with the principles of SANCO/825/00 rev. 8.1.

A 2.1.2.1.2.2 Independent laboratory validation

None available.

A 2.1.2.1.2.3 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.1.2.1.3 Extraction efficiency

Refer to Section 5.3.2.2.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for azoxystrobin

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

New studies have been submitted for the determinations of the active substance (azoxystrobin) in the plant protection product FF-075 – that have not previously been evaluated at EU level. Refer to Section 5.2.1 for complete summaries of the methods and supporting validation data.

New analytical methods supporting other areas of the dossier have been submitted for the authorisation of FF-075. The validation data of these methods are described below. All other studies relied on in other areas of the dossier have either been previously evaluated at EU level for Annex I inclusion and accepted without provision of further data, and/or do not involve the detection of (non-radiolabelled) analytical residues.

A 2.2.1.1.1 Analytical method 1

A 2.2.1.1.1.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2.2/08
Report	Analytical phase report. Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Bogner, F.; Report No. S20-00395-L3 <i>Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00395</i>
Guideline(s):	SANCO/3029/99, rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of 50 % (w/v) aqueous sucrose solution (2 mL) fortified with test item (FF-075) at 0.1 and 7700 mg/kg (nominal content of 0.0127 and 978 mg/kg azoxystrobin, respectively) were vortexed. Samples were diluted with acetonitrile/water (1:1, v/v) to a 50 mL volume and again with acetonitrile/water (1:1, v/v) by a factor of 5. High level recovery samples were further diluted to be within calibration range.

Test samples (from the chronic oral toxicity test) were quantitatively transferred to a plastic tube with use of water (2 x 2 mL) and acetonitrile (2 x 2 mL). Samples were vortexed then diluted with acetonitrile/water (1:1, v/v) to a 25 mL volume. All samples were diluted with acetonitrile/water (1:1, v/v) by a factor of 5, and then further diluted as appropriate with acetonitrile/water (1:1, v/v) to be within calibration range.

Samples were analysed by LC-MS/MS using a Phenomenex Kintex 2.6 µm Biphenyl column (100 x 2.1 mm, 2.6 µm) monitoring two ion transitions: 404→372 and 404→329 m/z.

Solvent standards were prepared in acetonitrile and water.

Results and discussions

Table A 13: Recovery results from method validation of azoxystrobin using the analytical method for the ion transition 344→266 m/z

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
50 % (w/v) aqueous sucrose solutions	Azoxystrobin	0.0127 (n = 5)	100	5	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		978 (n = 5)	108*	2	

* One recovery of 125 % was identified according to the Grubbs test and was not included in the calculation of the mean and relative standard deviation

Table A 14: Characteristics for the analytical method used for validation of azoxystrobin residues in 50 % (w/v) aqueous sucrose solutions

	Azoxystrobin
Specificity	A mass spectrum is provided Representative chromatograms (solvent standards, lowest fortification levels, blank samples) for each matrix/ion transitions are provided. Blank values < 30 % LOQ Highly specific primary method monitoring two ion transitions
Calibration (type, number of data points)	Representative calibration plot presented $n \geq 5$ $y = -1.404E+01 + 2.672E+04x$ (404→372 m/z) $r^2 \geq 0.99$
Calibration range	0.05 - 5 ng/mL, from <30% LOQ to 20% above the highest analyte concentration in any diluted sample extract (based on LOQ level, higher fortification levels were diluted to be within calibration range)
Assessment of matrix effects is presented	Yes – matrix effects were < ±20 % and deemed to be insignificant for azoxystrobin
Limit of quantification	0.0127 mg/kg - representing the lowest validated level supported by recovery and precision data
Limit of detection	0.00381 mg/kg (set at 30 % of the LOQ)
Extract stability	Azoxystrobin was stable under refrigerated conditions (1 - 10 °C) in 50 % (w/v) aqueous sucrose solution diluted by a factor of 25 with acetonitrile/water (1:1, v/v) for at least 1 day. Samples were analysed on the extraction day. If samples were not analysed on the extraction day extract stability was confirmed.

Conclusion

The LC-MS/MS method for the determination of azoxystrobin residues in 50 % (w/v) aqueous sucrose solutions was sufficiently validated to a LOQ of 0.0127 mg/kg in accordance with SANCO/3029/99rev.4.

A 2.2.1.1.2 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.2.1.1.2 Analytical method 2

A 2.2.1.1.2.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference: KCP 5.2.2/09

Report: Analytical phase report. Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions
Bogner, F.; Report No. S20-00396-L3
Contained in Annex 2 of Lozano, J.; 2020, Report no. S20-00396

Guideline(s): SANCO/3029/99, rev.4
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Sample preparation follows the QuEChERS method.

Larval diet (Diet C) (500 mg) was fortified with test item (FF-075) at 0.1 and 4700 mg/kg (nominal content of 0.0127 and 597 mg/kg azoxystrobin, respectively). Fortified samples were mixed with acetonitrile/water (1:1, v/v) + 0.5% formic acid solution (8 mL). A Citrat-Kit-01 was added. Samples were shaken and centrifuged. Supernatant (1 mL) was transferred into a PSA-Kit-01. Samples were shaken and centrifuged. A 500 µL aliquot was diluted with water + 1% formic acid solution (500 µL). High-level recovery samples were further diluted as appropriate with acetonitrile/water (1:1, v/v) + 0.5% formic acid solution to be within the calibration range.

Test samples (from the larval toxicity test) were quantitatively transferred to a plastic tube with use of water + 1% formic acid (3 x 2 mL) and acetonitrile (3 x 2 mL). Samples were mixed with acetonitrile/water (1:1, v/v) + 0.5 % formic acid solution (23 mL), vortexed, and four Citrat-Kit-01 added. Samples were shaken and centrifuged. Supernatant (1 mL) was transferred into a PSA-Kit-01. Samples were shaken and centrifuged. A 500 µL aliquot was diluted with water + 1% formic acid solution (500 µL). The treated larval diet samples were further diluted as appropriate with acetonitrile/water (1:1, v/v) + 0.5% formic acid solution to be within the calibration range.

Samples were analysed by LC-MS/MS using a Phenomenex Kintex 2.6 µm Biphenyl column (100 x 2.1 mm, 2.6 µm) monitoring two ion transitions: 304→372 and 404→329 m/z.

Solvent standards were prepared in acetonitrile and water.

Results and discussions

Table A 15: Recovery results from method validation of azoxystrobin using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)	Comments
Larval diet (Diet C)	Azoxystrobin	0.0127 (n = 5)	93*	4	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		597 (n = 5)	98	5	

* One recovery of 348 % was identified according to the Grubbs test and was not included in the calculation of the mean and relative standard deviation

Table A 16: Characteristics for the analytical method used for validation of azoxystrobin residues in Larval diet (Diet C)

	Azoxystrobin
Specificity	Representative chromatograms (solvent standards, lowest fortification levels, blank samples) for each matrix/ion transitions are provided. Blank values < 30 % LOQ Highly specific primary method monitoring two ion transitions

Calibration (type, number of data points)	Representative calibration plots presented $n \geq 5$ $y = -9.091E+00 + 1.999E+04x$ (404→372 m/z) $r^2 \geq 0.99$
Calibration range	0.025 - 5 ng/mL, from <30% LOQ to 20% above the highest analyte concentration in any diluted sample extract (based on LOQ level, higher fortification levels were diluted to be within calibration range)
Assessment of matrix effects is presented	Yes – matrix effects were < ±20 % and deemed to be insignificant for azoxystrobin
Limit of quantification	0.0127 mg/kg - representing the lowest validated level supported by recovery and precision data
Limit of detection	0.00381 mg/kg (set at 30 % of the LOQ)
Extract stability	Azoxystrobin was stable under refrigerated conditions (1 - 10 °C) in larval diet extracted with acetonitrile/water (1:1, v/v) + 0.5 % formic acid and QuEChERS Citrat-Kit-01 for at least 1 day. Samples were analysed on the extraction day. If samples were not analysed on the extraction day extract stability was confirmed.

Conclusion

The LC-MS/MS method for the determination of azoxystrobin residues in larval diet was sufficiently validated to a LOQ of 0.0127 mg/kg in accordance with SANCO/3029/99 rev.4.

A 2.2.1.1.2.2 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.2.1.1.3 Analytical method 3

A 2.2.1.1.3.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2.2/10
Report	Final Report. Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC: Acute Oral and Contact Toxicity to the Bumble Bee, <i>Bombus terrestris</i> L. under Laboratory Conditions Wendling, K.; Report No. S19-03594 (Appendix D)
Guideline(s):	SANCO/3029/99, rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of test item in 50 % w/v aqueous sucrose solutions or 0.1 % Triton X solutions in deionised water were diluted with acetonitrile:water (1:1, v/v) to be within calibration range.

Samples were analysed by LC-MS/MS using a Supelco Ascentis Express C18 column (50 x 2.0 mm, 2.7 µm) with 2.1 mm C18 guard column, monitoring two ion transitions: 344→189 and 344→154 m/z.

Calibration standards were prepared in acetonitrile and water.

Results and discussions

Table A 17: Recovery results from method validation of azoxystrobin using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Quantifier ion transition: 344→189 m/z					
50 % w/v aqueous sucrose solutions	Azoxystrobin	15.9 (n = 5)	95	7	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤20 % respectively)
		3300 (n = 5)	108	2	
0.1 % Triton X solutions in deionised water		318 (n = 5)	85	15	
		66000 (n = 5)	107	7	

Table A 18: Characteristics for the analytical method used for validation of azoxystrobin residues in 50% w/v aq. sucrose solution and 0.1 % Triton X solution in deionised water

	Azoxystrobin
Specificity	Representative chromatograms (solvent standards, lowest fortification levels, blank samples) for each matrix/ion transitions are provided. Blank values < 30 % LOQ Highly specific primary method monitoring two ion transitions
Calibration (type, number of data points)	Representative calibration plot presented $n \geq 5$ $y = 9.02e+005 x + 2.38e+005$ (50 % w/v aqueous sucrose solutions) $y = 1.28e+006 x + 6.66e+005$ (0.1 % Triton X solutions in deionised water) $r^2 \geq 0.99$
Calibration range	1.0 - 11 ng/mL, covers from 30% the LOQ to 20% above the highest analyte concentration in any (diluted) sample extract
Assessment of matrix effects is presented	No - matrix-matched calibration solutions used
Limit of quantification	15.9 mg/L (50 % w/v aqueous sucrose solutions) 318 mg/L (0.1 % Triton X solutions in deionised water) Representing the lowest validated level supported by recovery and precision data
Limit of detection	4.77 mg/L (set at 30 % of the LOQ in 50 % w/v aqueous sucrose solutions) 94.5 mg/L (set at 30 % of the LOQ in 0.1 % Triton X solutions in deionised water)
Extract stability	The maximum storage period from sampling to analysis was 8 days for samples of aqueous sucrose solution and 42 days for water samples containing 0.1 % Triton

	X-100. Storage stability data are included within the study report and demonstrate show that azoxystrobin was stable under deep-frozen conditions ($\leq -18^{\circ}\text{C}$) in demineralized water containing 0.1 % Triton X-100 for at least 42 days.
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Conclusion

The LC-MS/MS method for the determination of azoxystrobin residues in 50% w/v aq. sucrose solution and 0.1 % Triton X solution in deionised water was sufficiently validated to a LOQ of 15.9 mg/L and 318 mg/L, respectively, in accordance with SANCO/3029/99 rev.4.

A 2.2.1.1.3.2 Confirmatory method

No confirmatory method is required. The primary method – LC-MS/MS monitoring two ion transitions – is considered highly specific.

A 2.2.1.1.4 Analytical method 4

A 2.2.1.1.4.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2.2/11
Report	Method validation, solubility and stability of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) in aquatic test mediums Yu, J.; Report No. 2856
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Blank test media (OECD TG 201 alga medium, ISO standard dilution water or Swedish standard growth medium) was fortified with test item and either cleaned by SPE cartridge (LOQ level samples) and eluting with acetonitrile (2 mL) or filtered (higher level samples).

Samples were analysed by HPLC-UV at 254 nm using a EC-C18 column (150 x 4.6 mm, 2.7 μm).

Calibration standards were prepared in acetonitrile.

Results and discussions

Table A 19: Recovery results from method validation of azoxystrobin using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
OECD TG 201 alga medium	Azoxystrobin	0.0297 (n = 5)	97.06	2.88	Mean recoveries and %RSD values within acceptable limits (70 - 110 % and ≤ 20 % respectively)
		1.525 (n = 5)	94.80	0.27	
ISO standard dilution water		0.0297 (n = 5)	97.89	2.05	
		1.536 (n = 5)	98.93	0.21	
Swedish standard growth medium		0.0297 (n = 5)	97.63	2.32	
		6.130 (n = 5)	98.28	0.42	

Table A 20: Characteristics for the analytical method used for validation of azoxystrobin residues in OECD TG 201 medium, ISO standard dilution water and Swedish standard growth medium

	Azoxystrobin		
	OECD TG 201 medium	ISO standard dilution water	Swedish standard growth medium
Specificity	Representative chromatograms (acetonitrile solvent, blank test media, blank formulation, active substance reference, test substance, lowest and highest calibration standards, fortified samples at LOQ and higher levels) provided for each matrix. Blank values < 30 % LOQ Confirmation of peak identity was achieved by retention time matching with analytical standards.		
Calibration (type, number of data points)	Calibration plot presented n = 5 $y = 561693.6680x + 82.4680$ $r^2 = 0.9996$		
Calibration range	Low level: 0.026 – 1.012 mg/L Linearity covers from ca. 20% below the LOQ to ca. 5% above the highest analyte concentration in any (diluted) sample extract		
Assessment of matrix effects is presented	No – though recoveries were acceptable, so matrix effects not deemed significant.		
Limit of quantification	0.0297 mg/L – representing the lowest validated level (per matrix) supported by recovery and precision data		
Limit of detection	0.008 mg/L (set at 30 % of the LOQ)		
Extract stability	Azoxystrobin (0.0254 and 1.2700 mg/L solutions) was stable in the test media under test conditions (21 - 24 °C, 6920 - 7980 Lux) for at least 96 hours.	Azoxystrobin (0.0508 and 1.2700 mg/L solutions) was stable in the test media under test conditions (19 - 20 °C, 16 hours light, 8 hours dark) for at least 48 hours.	Azoxystrobin (0.0051 and 5.0800 mg/L solutions) was stable in the test media under test conditions (24 \pm 2 °C, 7080 - 7920 Lux) for at least 168 hours.

Conclusion

The HPLC-UV method for the determination of azoxystrobin residues in OECD TG 201 medium, ISO standard dilution water and Swedish standard growth medium was sufficiently validated to a LOQ of 0.0297 mg/L (all matrices) in accordance with SANCO/3029/99 rev.4.

A 2.2.1.1.4.2 Confirmatory method

A separate method was not provided. Confirmation of peak identity was achieved by retention time matching with analytical standards.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

No new or additional studies have been submitted.