DRAFT REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: A9873C

Product name: Wakil XL

Chemical active substances:

Cymoxanil, 100 g/kg

Fludioxonil, 50 g/kg

Metalaxyl-M, 169.6 g/kg

~~United Kingdom~~

Great Britain (GB)

NATIONAL ASSESSMENT

~~(Renewal of authorisation)~~

Submitted to support Article 7 amendment of approval of Metalaxyl-M in GB

Applicant: Syngenta

Submission date: 21/10/2021

Finalisation date: 31/01/2024

Version history

When	What
October 2021	Applicant submission to support amendment of approval under Article 7 of retained Regulation (EC) No 1107/2009
December 2023	HSE (GB) assessment added in green boxes

This is an application from Syngenta for the renewal of WAKIL XL (A9873C) under Article 43 of Regulation (EC) No. 1107/2009 following the renewal of EU approval of the active substance metalaxyl-M.

No equivalence assessment is required.

This application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011, also called 'old' data requirements. Metalaxyl-M is an 'AIR-2' substance which approval has been renewed in accordance with Regulation (EU) No 1141/2010, therefore Regulations (EU) No 283/2013 and (EU) No 284/2013 are not applicable to the renewal of authorizations for metalaxyl-M-containing plant protection products (derogation by Commission Regulation (EU) No 2015/1475; further details in the guidance document SANTE/11509/2013 rev. 5.2).

Following the renewal of EU approval of the active substance metalaxyl-M, the submission for the product renewal of WAKIL XL (A9873C) was made by 01 September 2020, in accordance with Article 43 of Regulation (EC) No 1107/2009.

All data relied on are provided with this application. The reference lists at Appendix 1 of dRR Part B Sections 1-10 define the data owner and data access. Data protection is a national concern and is addressed in Part A, Appendix 4.

The guidance on Renewal of Authorization according to Art 43 (SANCO/2010/13170 rev 14) requests that within the dRR 'changes to the risk assessment are highlighted'. This is the first submission of WAKIL XL (A9873C) in the dRR format of April 2015, consequently all of the summary text is previously unreviewed and should be considered as 'changed'. To facilitate the review, Syngenta has highlighted the summaries of reports not previously reviewed by the zRMS in yellow.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	The applicant, Syngenta Crop Protection AG, submitted this application to amend the conditions of approval of metalaxyl-M in accordance to Article 7 of Regulation 1107/2009 in Great Britain (GB). On the 5 May 2020 the Commission Implementing Regulation (EU) 2020/617 renewing the approval of the active substance metalaxyl-M, and restricting the use of seed treat-

	ed with a plant protection product containing it to be sown only in greenhouses, was published[1]. The renewal of metalaxyl-M applies since 1 June 2020. Since this was before UK withdrawal from the EU, the Commission Implementing Regulation for the renewal of metalaxyl-M applies direct in GB. Two representative formulations were considered in the renewal of approval for metalaxyl-M, 'Apron XL' (A9642C) and 'Ridomil Gold Mz'/68 WG Fubol Gold' (A9651D). For this Article 7 amendment application in GB, two different formulations have been considered. The formulation 'Vibrance SB' (A20607B) containing 14.4 g/L metalaxyl-M, 22.5 g/L fludioxonil and 15.0 g/L sedaxane to support the field seed treatment use on sugar and fodder beet, and the formulation 'Wakil XL' (A9873C) containing 169.6 g/Kg metalaxyl-M, 100 g/Kg cymoxanil and 50 g/Kg fludioxonil) to support the field seed treatment use on peas (vining) are the basis of this Article 7 application for metalaxyl-M to GB. The applicant has re-submitted the draft registration reports prepared for the product renewals of 'Vibrance SB' and 'Wakil XL' under Article 43 of Regulation No 1107/2009 following the renewal of approval of the active substance metalaxyl-M. The information and data submitted within these draft registration reports have been considered previously by HSE for the applications for authorisation of a new product under Article 33 of Regulation No 1107/2009. Where relevant, re-evaluation of data or information has not occurred where studies have been performed in accordance with the current requirements and the results have been deemed acceptable. This draft registration report has been provided by the applicant, where required, comments have been inserted in green boxes by HSE or the text amended by the HSE in green (applicant's text has been struck through in green where necessary). HSE notes that the product authorisations for 'Vibrance SB' and 'Wakil XL' were withdrawn in GB by the applicant. This was based on the approval restriction provided for in Commission Implementing Regulation (EU) 2020/617 that only the treatment of seeds intended to be sown in greenhouses may be authorised. Since all authorised GB uses of 'Vibrance SB' and 'Wakil XL' products are on seeds which are direct drilled in the field, these products do not comply with the restriction and therefore could not be renewed under Article 43 of Regulation No 1107/2009. HSE notes that no authorisation for 'Vibrance SB' or 'Wakil XL' is sought within this Article 7 amendment application. Therefore, HSE has only considered the information presented in the draft registration reports that relate to metalaxyl-M. For a future GB authorisation of these products a separate application would be required with a full evaluation of the data and information for all active substances present in the formulation. Note that as of 1st January 2024, The Retained EU Law (Revocation and Reform) Act 2023 has taken effect and retained EU law are now known as assimilated law. As this assessment has been prepared prior to the Retained EU Law Act taking effect, assessment may still refer to "retained" regulation as opposed to "assimilated".

[1] Commission Implementing Regulation (EU) 2020/617 of 5 May 2020 renewing the approval of the active substance metalaxyl-M, and restricting the use of seeds treated with plant protection products containing it, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011

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5 Analytical methods

5.1 Conclusion and summary of assessment

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the UK approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further. 'Wakil XL' is a WG formulation containing 169.6 g/kg metalaxyl-M, 100 g/kg cymoxanil and 50 g/kg fludioxonil. The proposed uses considered under this application are for fresh peas. The applicant company has access to the data considered in the DAR/RAR for metalaxyl-M as they are the data owner. This evaluation has been carried out in accordance with the Uniform Principles (as defined in Article 29 of Regulation (EC) No. 1107/2009) for active substance and product evaluation concerning the placing of plant protection products on the market. The renewal of 'metalaxyl-M' was assessed in accordance with the data requirements outlined in Regulation (No) 544/2011. Therefore, as methods of analysis data is considered active substance data, in accordance with the guidance document SANTE/11509 /2013– rev. 5.2 this methods assessment has been conducted in accordance with the same data requirements applied to the active. The information presented below has been written by the applicant, where required, comments have been inserted in green boxes by the HSE or the text amended by the HSE in green (applicant's text has been struck through in green where necessary). Sufficiently validated analytical methods are available for: • the active substance, metalaxyl-M in the plant protection product • the relevant impurities: CGA72649, CGA363736 in the plant protection product; methods are not available for CGA226048, however the current application seeks to remove CGA226048 as a relevant impurity, therefore this has not been considered further, depending on the outcome of the Art 7 application, methods may be required for CGA226048 for a future authorisation. New data generation methods in support of support of efficacy, environmental fate, residues in plants, residues in animal products, toxicology and ecotoxicology studies were not submitted and are not required. Sufficiently validated analytical methods are available to allow monitoring of residues of metalaxyl-M in • plants in all crop groups (further data was submitted but not evaluated or required)

	• animal matrices (further data was submitted but not evaluated or required) • soil, water, and air • body fluids and tissues **Conclusion**: Sufficiently sensitive and selective analytical methods are available to support the plant protection product for the proposed uses. Standard authorisation for 'A9873C' can be recommended.

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are:

- data gap 1
- data gap 2
- data gap 3

Sufficiently sensitive and selective analytical methods are not available for all analytes included in the residue definitions.

Noticed data gaps are:

- data gap 1
- data gap 2
- data gap 3

Commodity/crop	Supported/ Not supported
Commodity/crop 1	
Commodity/crop 2	
Commodity/crop 3	

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)

The plant protection product A9873C has not been reviewed at EU level as a consequence of the review of Cymoxanil, Fludioxonil or Metalaxyl-M.

An overview on the acceptable methods and possible data gaps for analysis of Cymoxanil, Fludioxonil and Metalaxyl-M in plant protection product is provided as follows:

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD),**
Reviewer's comments	The applicant's summary as provided is an accurate representation of the study and validation data. The data below was previously relied upon for the original authorisation of the product. This was based on a following zonal application, and the data was not specifically assessed by HSE; nevertheless, as the product will not be authorised based on this application, no further consideration has been made.

Reference:	KCP 5.1.1
Report	[redacted] (1998), Analytical method AF-1318/2. Determination of CGA329351, CGA173506 and Cymoxanil in Formulation (WG) by Liquid Chromatography. Novartis Crop Protection Münchwilen AG, CH. Unpublished Report No. 3967690. Issued date 05.20.1998 Syngenta File No. VV-124572
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (1999), A9873C - Validation of analytical method AF-1318/2. Novartis Crop Protection Münchwilen AG, CH Unpublished Report No. 17594530. Issued date 24.06.1999 Syngenta File No. VV-292097

Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] I. & [redacted] (2002), Analytical method AFA-1318/2. Content of CGA329351 and CGA351920 in A9873C in A9873C and A9873D by Chiral LC. Syngenta Crop Protection Münchwilen AG, CH. Unpublished Report No. 78766384. Issued date 17.12.2002 Syngenta File No. VV-123832
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2003), A9873C - Validation of analytical method AFA-1318/2. Syngenta Crop Protection Münchwilen AG, CH Unpublished Report No. 80637340. Issued date 9.1.2003 Syngenta File No. VV-293344
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

Method AF-1318/2 – achiral method

Cymoxanil, Fludioxonil and Metalaxyl-M (including its S-enantiomer) are determined in A9873C by HPLC using a reversed phase column (Nucleosil C18, 250 mm length, 4.o mm i.d.). Detection was done with an UV detector operating at 230 nm. Elution was done using a 0.1% aqueous phosphoric acid/acetonitrile/methanol gradient. Quantification was achieved by comparing peak areas of test samples with the areas from calibrated analytical standard solutions.

Method AFA-1318/2 – chiral method

Metalaxyl-M (CGA329351) and its S-enantiomer (CGA351920) are determined by HPLC using an achiral (Nucleosil C18, 100 mm length, 4.0 mm i.d.) and a chiral (Chira-Grom2, 250 mm length, 2.0 mm

i.d.) column in series. Detection is done with an UV detector operating at 220 nm. Elution was done using an acetonitrile/water/methanol gradient with a flow rate of 0.3 ml/min. The peak area of metalaxyl-M (CGA329351) and the reference standard are measured by a data handling system and used to calculate the methalaxyl-M (CGA329351) content of the sample.

Validation - Results and discussions

The following validation of the analytical method (AF-1318/2, achiral method) for the determination of Cymoxanil, Fludioxonil and Metalaxyl-M (including its S-enantiomer) in the plant protection product A9873C has not previously been reviewed and is provided in support of this assessment.

The validation of AF-1318/2 has been conducted for A9873C.

Table 5.2-1: Methods suitable for the determination of active substances Cymoxanil, Fludioxonil and Metalaxyl-M (including its S-enantiomer) in plant protection product Wakil XL/A9873C

	Cymoxanil	**Fludioxonil**	**Metalaxyl-M (inc. S-enantiomer)**
Author(s), year	[redacted] (1999)	[redacted] (1999)	[redacted] (1999)
Principle of method	HPLC and UV detection	HPLC and UV detection	HPLC and UV detection
Linearity n = 5 Tested between 50% - 150% of declared content	r =0.99997 y = 0.995*X+0.625	r =0.99997 y = 0.997*X+0.084	r = 0.99999 y = 0.992*X+2.886
Precision – Repeatability Mean n = 5 (duplicate injections)	S_{rel} (%RSD) = 0.18 mean concentration = 10.6% w/w	S_{rel} (%RSD) = 0.16 mean concentration = 17.5% w/w	S_{rel} (%RSD) = 0.28 mean concentration = 5.09% w/w
Accuracy 6 samples Tested between 75% - 125% of declared content	mean recovery = 99.7%	mean recovery = 99.7%	mean recovery = 100.0%
Interference/ Specificity	no significant interference	no significant interference	no significant interference
Comment	The method is acceptably validated	The method is acceptably validated	The method is acceptably validated

Conclusion

The method is suitable for the specific, accurate and precise determination of Cymoxanil, Fludioxonil and Metalaxyl-M (including its S-enantiomer) in plant protection product Wakil XL (A9873C).

The following validation of the analytical method (AFA-1318/2, chiral method) for the determination of Metalaxyl-M (CGA329351) and its S-enantiomer (CGA351920) in the plant protection product A9873C has not previously been reviewed and is provided in support of this assessment.

The validation of AFA-1318/2 has been conducted for A9873C.

Table 5.2-2: Methods suitable for the determination of Metalaxyl-M (CGA329351) and its S-enantiomer (CGA351920) in plant protection product Wakil XL (A9873C)

	Metalaxyl-M CGA329351 (R-enantiomer)	**Metalaxyl-M CGA351920 (S-enantiomer)**
Author(s), year	Ph. [redacted] (2003)	Ph. [redacted] (2003)

	Metalaxyl-M CGA329351 (R-enantiomer)	**Metalaxyl-M CGA351920 (S-enantiomer)**
Principle of method	HPLC and UV detection	HPLC and UV detection
Linearity n = 5 Tested between 75% - 125% of declared content (duplicate injections)	r = 0.9997 y = 0.984X + 0.881	r = 0.99853 y = 0.996*X + 0.009
Precision – Repeatability Mean n = 5 (duplicate injections)	S_{rel} (%RSD) = 0.28 Mean = 17.5% w/w	S_{rel} (%RSD) = 3.38 Mean = 0.68% w/w
Accuracy 3 samples Tested between 75% - 125% of declared content (duplicate injections)	mean recovery =100.3%	mean recovery =101.4%
Interference/ Specificity	no significant interference	no significant interference
Comment	The method is acceptably validated	The method is acceptably validated

Conclusion

The method is suitable for the specific, accurate and precise determination of Metalaxyl-M (CGA329351) and its S-enantiomer (CGA351920) in plant protection product Wakil XL (A9873C).

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The levels of the relevant impurities was not determined in the storage stability studies, neither pre- or post-storage. Nevertheless, the following methods have been described below. The Commission Implementing Regulation (EU) 2020/617 and GB approvals register state a maximum content of 0.5 g/kg (500 ppm) CGA72649 (2,6-dimethylphenylamine), 1.0 g/kg (1000 ppm) CGA363736 (4-methoxy-5-methyl-5H-[1,2]oxathiole 2,2-dioxide) and 0.18 g/kg CGA226048 (2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester in the technical material for metalaxyl-M. Considering a technical content of 169.6 g metalaxyl-M/kg in 'A9873C', the theoretical maximum level of CGA72649, CGA363736 and CGA226048 are 0.085 g/kg, 0.170 g/kg and 0.031 g/kg respectively. A method (AG-1837/2) for the determination of relevant impurity CGA72649 was presented in the RAR. However linearity and repeatability were determined using two other formulations (A9407A and A9408B). Therefore the RMS concluded that the method is not fully validated for linearity and repeatability. In addition, a method for the relevant impurity CGA363736 was not provided.

Therefore at renewal there was a data gap for a method(s) to determine relevant impurities CGA72649 and CGA363736 in 'Apron XL'. The method SD-1751/1 for the determination of CGA72649 and CGA363736 in 'Apron XL' has been submitted under this application to address this data gap.

A new method for the determination of CGA72649 and CGA363736 in 'A9651D', a water dispersible granule formulation (WG) fungicide containing metalaxyl-M and mancozeb, has been submitted in the framework of the current application, but was also submitted for the previous evaluation of 'Apron XL'.

Three study reports have been submitted: Study report numbers 'VV-411110' and 'VV-128413' have been evaluated for a previous product evaluation). Study number 'VV-28929' is a statement which includes data, which describes the methods applicability to the current formulation ('A9873C').

Studies 'VV-411110' and 'VV-128413' were evaluated previously for 'Apron XL', using formulation 'A9651D', a WG formulation containing 3.88 w/w % metalaxyl-M and 64 w/w % mancozeb. The different nominal concentration of metalaxyl-M in the formulation is not considered to be an issue, the sample weighting is adjusted accordingly, to give a consistent concentration of metalaxyl-M, and the corresponding levels of impurities.

Method SD-1751/1 uses the standard addition procedure, calibration solutions are prepared by adding known amounts of CGA72649 and CGA363736 directly to formulation samples and diluting all samples to the same final volume. The resulting spiked solutions contain different known levels of CGA72649, ranging from between approx. 100 ppm to 700 ppm relative to metalaxyl-M, and of CGA363736, ranging from between approx. 200 ppm to 1400 ppm relative to metalaxyl-M, and by plotting the amounts of CGA72649 and CGA363736 added against their respective instrument responses (area of CGA72649 and CGA363736), the calibration curve is generated. One of the samples is prepared without the addition of CGA72649 and CGA363736, as it is from this sample that the actual content of CGA72649 and CGA363736 can be calculated using the calibration curve generated. Due to the fact that the analytes of interest, in this case CGA72649 and CGA363736, are directly added to the sample, all sample matrix effects with a potential influence on specificity, linearity, recovery, repeatability or the limit of quantification, can be accounted for.

The nominal for which the concentrations used in method validation are compared against for determination of the acceptability of the method are the maximum theoretical content of the impurities. For CGA72649 this is 500 ppm and for CGA363736 this is 1000 ppm (based on implementing reg).

The pre-spiking levels of the impurities were not given, for a future product authorisation, this information should be provided.

GC Conditions:

Chromatograph:	Thermo Trace GC ultra
Detector (mass spectrometer):	Thermo TSQ Quantum XLS For CGA72649: the selective reaction monitoring transition is m/z 121 → 106 using 15 V collision energy, segment start time 7 min, segment end time 9 min For CGA363736: the selective reaction monitoring transition is m/z

	164 → 121 using 12 V collision energy, segment start time 9 min, segment end time 13 min
Column:	type: fused silica length: 15 m inside diameter: 0.32 mm stationary phase: DB-1701 film thickness: 1 µm
Column temperature:	75 °C,1 minute isothermal 10 °C/minute to 140 °C 25 °C/minute to 305 °C, 10 minute isothermal
Transfer line temperature:	295 °C
Injector temperature:	250 °C, split injector equipped with a split liner (5 mm straight without wool)
Carrier gas:	helium, flow rate 2.5 ml/minute, constant flow
Size of sample:	1 µl of test solution / spiked test solution
Split ratio:	20:1
Duration of chromatography:	approx. 24 minutes
Retention times:	CGA72649: approx. 7.8 min CGA363736: approx. 11.1 min

Summary of method validation data

Matrix	Analyte	LOQ (ppm)	Recovery fortification level (ppm)	% Recovery	Linearity	Specificity
A9873C	CGA72649	100 (equiva-lent to 0.1 g/kg)	98 (equivalent to 0.098 g/kg)	98.1	98 - 660 ppm (0.098 – 0.660 g/kg CGA72649 in TGAI in formulation, equivalent to 19.6 - 132% theoretical maximum CGA72649 in test solutions) y = 1488.58 x + 812.27 r = 0.9999	Using MS/MS and standard addition mode, the specificity is established and no significant interference was observed.
			191 (equivalent to 0.191 g/kg)	101.3		
			292 (equivalent to 0.292 g/kg)	101.0		
			488 (equivalent to 0.488 g/kg)	99.0		

			660 (equivalent to 0.660 g/kg)	100.3		
A9873C	CGA363736	200 (equiva-lent to 0.2 g/kg)	188 (equivalent to 0.188 g/kg)	106.8	188 -1264 ppm (0.188 – 1.264 g/kg CGA363736 in TGAI in formulation, equivalent to 19 – 126 % theoretical maximum CGA363736 in test solutions) y = 78.84 x - 1638.36 r = 0.9982	Using MS/MS and standard addition mode, the specificity is established and no significant interference was observed.
			367 (equivalent to 0.367 g/kg)	95.8		
			560 (equivalent to 0.560 g/kg)	96.3		
			935 (equivalent to 0.935 g/kg)	96.2		
			1264 (equivalent to 1.264 g/kg)	103.0		

Specificity:
Using a specific detection technique (MS/MS) and standard addition mode, the specificity was established and no significant interference was observed. Chromatograms of 'A9873C', batch KWL0K111 blank and spiked with approx. 300 ppm CGA72649 and 600 ppm CGA363736, relative to the amount of metalaxyl-M present in formulation, were presented. There was no interference of peaks. Therefore the method has been shown to be specific for the determination of CGA72649 and CGA363736 in formulation A9873C.

Linearity:
Linearity was demonstrated by the analysis of five standards of increasing concentration in duplicate. The range of standard concentrations used was 98 - 660 ppm for CGA72649, equivalent to 0.098 – 0.660 g/kg in the product and 188 - 1264 ppm for CGA363736, equivalent to 0.188 – 1.264 g/kg in the product. The response was linear with a correlation coefficient (r) > 0.999.

Accuracy:
Recovery samples were prepared by spiking blank formulation with active substance standard at

	concentrations of 98 - 660 ppm for CGA72649 and 188 - 1264 ppm for CGA363736 and analysing them by the method described. Two samples were prepared at each fortification level. The spike concentrations were equivalent to 19.6 - 132% of the nominal concentration of 500 ppm for CGA72649 and 19 - 126% of the nominal concentration of 1000 ppm for CGA363736. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 98.1 to 101.3% for CGA72649 and 95.8 to 106.8% for CGA363736. **Precision:** Repeatability was only tested for formulation 'A9651D'; as the proposed formulation is also a WG formulation, and as all other parameters tested for 'A9873C' are acceptable, the repeatability is considered sufficiently addressed for 'A9873C' based on the data generated using 'A9651D'. **Conclusion:** The method for the determination of CGA72649 and CGA363736 in 'A9873C' is satisfactory validated in accordance with SANCO/3030/99 rev.5. The LOQ is 100 ppm (0.1 g/kg) CGA72649 and 200 ppm (0.2 g/kg) for CGA363736. This is sufficient to cover the respective limits of 0.5 g/kg (500 ppm) CGA72649 (2,6-dimethylphenylamine) and 1.0 g/kg (1000 ppm) in the technical material as given in the approval conditions for metalaxyl-M (Commission Implementing Regulation (EU) 2020/617 and GB approvals register). **The pre-spiking levels of the impurities were not given, for a future product authorisation, this information should be provided.**

CGA72649 and CGA363736 in Metalaxyl-M

Analytical method SD-1751/1 has been developed for the determination of the relevant impurities CGA72649 and CGA363736 in A9873C. CGA72649 and CGA363736 are impurities which may be found in A9873C at trace levels as a result of the Metalaxyl-M active ingredient manufacturing process. CGA72649 and CGA363736 is not formed during manufacture or storage of Metalaxyl-M. The analytical method for the determination of CGA72649 and CGA363736 in A9873C has not previously been reviewed and is provided in support of this assessment.

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Reference:	KCP 5.1.1
Report	[redacted] A., [redacted], [redacted]. (2014) Analytical Method SD-1751/1 Determination of CGA72649 and CGA363736 in formulation by GC/MS/MS. Syngenta Crop Protection Münchwilen AG Switzerland. Unpublished Report No. 300021240. Issued date 11.12.2014 Syngenta File No. VV-128413 (A9651D_10487)
Guideline(s):	None
Deviations:	None

GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2014). A9651D - Validation of analytical method SD-1751/1. Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report No. CHMU140410. Issued date 25.11.2014 Syngenta File No. VV-411110 (A9651D_10488)
Guideline(s):	no
Deviations:	no
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2014). Statement on validation of the analytical method SD-1751/1 for the determination of CGA72649 and CGA363736 in A9873C metalaxyl-M/cymoxanil/fludioxonil WG, Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished report no. 300031476. Issued date 15.12.2014 Syngenta File No. VV-28929
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Materials and methods

The relevant impurities CGA72649 and CGA363736 are determined in formulation by gas chromatography on a 15 m fused silica DB-1701 column using helium as a carrier gas. Column temperature: 75°C up to 305°C. Detection was by MS using a Thermo TSQ Quantum XLS, monitoring for CGA72649 the transition m/z 121-> 106 and for CGA363736 the transition m/z 164-121. Quantification is by standard addition method (internal standard).

Validation - Results and discussions

Full validation of the method SD-1751/1 has been conducted. The method has been shown to be specific for the determination of CGA72649 and CGA363736 in product A9873C and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of CGA72649 and CGA363736 in product A9873C.

The following validation of the analytical method (SD-1751/1) for the determination of CGA72649 and CGA363736 in the plant protection product A9873C has not previously been reviewed and is provided in support of this assessment.

Table 5.2-3: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) A9873C

Relevant impurities in Metalaxyl-M	CGA72649 max. content in PPP ≤ 0.5 g/kg	CGA363736 max. content in PPP ≤ 1.0 g/kg
Author(s), year	[redacted] (2014)	[redacted] (2014)
Principle of method	GC/MS/MS	GC/MS/MS
Linearity n = 5	r = 0.9999 y = 1488.9*X+812 Tested between 98 and 660 ppm of CGA72649 relative to the amount of metalaxyl-M	r = 0.9982 y = 78.8*X-1638.4 Tested between 188 and 1264 ppm of CGA363736 relative to the amount of metalaxyl-M
Precision as repeatability n = 6 (duplicate injections)	mean conc.= 291 ppm S_{rel} (%RSD) = 4.22	mean conc.= 554 ppm S_{rel} (%RSD) = 2.94
Accuracy n = 5	mean recovery = 99.9 % Tested between 98 and 660 ppm of CGA72649 relative to the amount of metalaxyl-M	mean recovery = 99.6 % Tested between 188 and 1264 ppm of CGA363736 relative to the amount of metalaxyl-M
Interference/Specificity	no significant interference	no significant interference
LOQ	100 ppm	200 ppm
Comment	The method is acceptably validated	The method is acceptably validated

Conclusion

The method is suitable for the specific, accurate and precise determination of CGA72649 and CGA363736 in plant protection product Wakil XL (A9873C).

CGA226048 in Metalaxyl-M

Please note: Regarding impurity CGA226048 (2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester) as stated in Annex 1 of the Metalaxyl-M Implementing Regulation (EU) 2020/617 of 5 May 2020, an on-going EU evaluation is currently being finalised by the active substance RMS Belgium under Article 7 (submission of documentation 25th July 2019). Impurity CGA226048 is shown to be non-genotoxic and non-relevant. Hence, there is no need to provide an analytical method for the determination of CGA226048 within this product.

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	There are no relevant co-formulants in 'A9873C', therefore methods are not required

There are no relevant formulants in formulation A9873C

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	'A9873C' contains more than one active substance. CIPAC methods for products that contain more than one active substance are not available

There are no CIPAC methods for the determination of Cymoxanil, Fludioxonil or Metalaxyl-M.

There are no CIPAC methods for the determination of Cymoxanil, Fludioxonil and Metalaxyl-M in WG formulations.

5.2.2 Methods for the determination of residues of cymoxanil (KCP 5.1.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further.

An overview on the acceptable methods and possible data gaps for analysis of residues of cymoxanil for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

No analytical methods were used to support the efficacy data generated on this product.

No specific analytical methods were used to support the toxicological data generated on this product.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-4: Validated methods for the generation of pre-authorization data for cymoxanil in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition: Cymoxanil				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**

Component of residue definition: Cymoxanil				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
DFG 513	High water content *Lettuce, tomato, potato*	0.05 mg/kg	GC-NPD	Method [redacted], 2001a & 2001b Reports: DFG 513 Validation: [redacted], 2001a & 2001b Reports: A0087, SIP 1264, SIP 1277, SIP 1279, SIP 1297, R 6124, R 7180, SIP 1353, SIP 1298, SIP1265 EU agreed (Austria, 2007)
	High acid content *Grapes*	0.05 mg/kg		
	Dry matrices *Pea, dry* *Pea empty pods*	0.02 mg/kg		Validation in reports: [redacted], 1999: 2010/98 [redacted], 1999: 2011/98 [redacted], 2002: 0140501 [redacted] 2003: gpe14201 [redacted] 2003: gpe541002 [redacted], 1999: 2012/98 [redacted], 1999: 2013/98 [redacted], 1999: 2014/98 [redacted], 1999: 2015/98 **New data**
-	Animal products, food of animal origin (Residues)	Pre-authorisation methods are not required for animal products for the supported uses of A9873C		

Methods and relationship to studies presented in the Part B Section 7 document.

The below table indicates which method has been used for each of the studies included within the Part B Section 7 document as well as the respective data point and report reference.

Method	**Study (Part B Section 7)**	
Identifier	**Data Point**	**Report Reference**
GRM023.11A	*KCA2 6.1*	*T012299-05-REG*
GRM027.08A		*KP-2009-02*
GRM023.010A	*KCA2 6.4.1-6.4.3*	*30634*

No specific analytical methods were used to support the ecotoxicology data generated on this product.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.3 Methods for the determination of residues of fludioxonil (KCP 5.1.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further.

An overview on the acceptable methods and possible data gaps for analysis of residues of fludioxonil for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.2-5: Validated methods for the generation of pre-authorization data for fludioxonil in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Component of residue definition: Fludioxonil				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 133.04	Soil	0.02 mg/kg	HPLC-UV	Method: [redacted], 1993 Report No. REM 133.04 EU agreed (Denmark, 2005) Validation: [redacted], 2001 Report No. 210/01 EU agreed (Denmark, 2005)

No analytical methods were used to support the efficacy data generated on this product.

No specific analytical methods were used to support the toxicological data generated on this product.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-6: Validated methods for the generation of pre-authorization data for fludioxonil in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition for plant and animal products: Fludioxonil				
Method type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 133.01	High acid content *grape*	0.02 mg/kg	HPLC-UV	***Method:*** [redacted] E, 1989 Report No.:: REM 133.01 ***Validation:*** [redacted] E, 1989 Report No.: REM 133.01 EU agreed
	No group *wine*	0.005 mg/kg		

Component of residue definition for plant and animal products: Fludioxonil				
Method type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				(Denmark 2005)
REM 133.04	High water content *tomato* *aubergine* *apple*	0.02 mg/kg	HPLC-UV	***Method:*** [redacted], 1993 Report No.: REM 133.04 ***Validation:*** [redacted], 1993 Report No.: REM 133.04 [redacted], 2001 Report No.: 210/01 EU agreed (Denmark 2005)
	High acid content *grape* *strawberry*	0.02 mg/kg		
	High starch content *Wheat grain*	0.02 mg/kg		
	No group *wine*	0.005 mg/kg		
AG-597	High water content: *corn forage* *sorghum fodder* *rice stalks*	0.01 mg/kg	HPLC-UV	***Method:*** [redacted], 1993 Report No.: AG-597 ***Validation:*** [redacted], 1993 Report No.: AG-597 EU agreed (Denmark 2005)
	High starch content *corn grain* *sorghum grain* *rice grain* *potato tuber*	0.01 mg/kg (0.05 mg/kg sorghum grain)		
	No group *sorghum hay*	0.01 mg/kg		
AG-631A	High water content: *cherry* *apple* *pear* *peach* *plum* *forage/fodder*	0.02 mg/kg	HPLC-UV GP-NPD	***Method:*** [redacted] & van [redacted]., 1996 Report No.: AG-631A ***Validation:*** [redacted] & van [redacted]., 1996 Report No.: AG-631A EU agreed (Denmark 2005)
	High starch content *Cereal grain*	0.02 mg/kg		
	High acid content *grape*	0.02 mg/kg		
	No group *prune* *straw* *wine*	0.02 mg/kg 0.05 mg/kg 0.01 mg/kg		
-	Animal products, food of animal origin, (Residues)	Pre-authorisation methods are not required for animal products for the supported uses of A14918E as a seed treatment.		

Details of new studies not previously reviewed at EU level are given in Appendix 2.

Methods and relationship to studies presented in the Part B Section 7 document.

The below table indicates which method has been used for each of the studies included within the Part B Section 7 document as well as the respective data point and report reference.

Method Identifier	Study (Part B Section 7)	
	Data Point	Report Reference
REM 133.01	KCA1 6.1	621/7-1012
REM 133.04		131/93, 221/98, 222/98, 210/00
AG-597		115-93
REM 133.04	KCA2 6.3.1	2010/98
	KCA2 6.3.1	2011/98
	KCA2 6.3.1	0140501
	KCA2 6.3.1	gpe14201
	KCA2 6.3.1	gpe514002
	KCA2 6.3.1	2012/98
	KCA2 6.3.1	2013/98
	KCA2 6.3.1	2014/98
	KCA2 6.3.1	2015/98
	KCA2 6.3.2	2296/97
	KCA2 6.3.2	2297/97
	KCA2 6.3.2	2298/97
	KCA2 6.3.2	2299/97
	KCA2 6.3.2	2008/98
	KCA2 6.3.2	2009/98
	KCA2 6.3.2	0140501
	KCA2 6.3.2	gpe14201
	KCA2 6.3.2	gpe514002
AG-631A	KCA1 6.6.2	174-97

No specific analytical methods were used to support the ecotoxicology data generated on this product.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.4 Methods for the determination of residues of metalaxyl-M (KCP 5.1.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	No new methods have been evaluated in the context of this evaluation, the sections below describe the available methods for various matrices.

An overview on the acceptable methods and possible data gaps for analysis of residues of metalaxyl-M for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

No specific analytical methods for the generation of pre-authorization data for metalaxyl-M in soil, water,

air (in support of environmental fate studies) were used. Please see post-authorization methods for relevant soil, water and air methods.

No analytical methods were used to support the efficacy data generated on this product.

No specific analytical methods were used to support the toxicology data generated on this product.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-7: Validated methods for the generation of pre-authorization data for metalaxyl-M in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition for plant and animal products: metalaxyl-M				
Method type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 181.01	High water content *Tomato*	0.02 mg/kg	GC-NPD (original method) GC-MSD (confirmation) LC-MS/MS (updated method)	**Method and Validation[(a)]:** [redacted], 1995 Report No.: REM 181.01 EU agreed (Belgium, 2014)
	High starch content *Potato*	0.02 mg/kg		
	High acid content *Grape*	0.02 mg/kg		
	High acid content *Citrus*	0.02 mg/kg		Validation: [redacted], 1999 Report No.: 517/99 EU agreed (Belgium, 2014)
	High acid content *Citrus peel, citrus pulp*	0.04 mg/kg		
	High oil content *Cotton*	0.02 mg/kg		Validation: [redacted], 1999 Report No.: 518/99 EU agreed (Belgium, 2014)
	No group *Cotton hulls*	0.04mg/kg		
	High oil content *Sunflower*	0.02 mg/kg		Validation: [redacted], 1999 Report No.: 519/99 EU agreed (Belgium, 2014)
	High water content *Witloof chicory leaves*	0.01 mg/kg		Validation: [redacted], 2005 Report No.: T004798-04 EU agreed (Belgium, 2014)
	High water content *Pome fruit, stone fruit pulp, carrot, onion, tomato, pepper, cucumber, melon, melon peel, melon pulp, flowering brassica, cabbage, lettuce, spinach, witloof chicory sprouts, bean pods,*	0.02 mg/kg		

Component of residue definition for plant and animal products: metalaxyl-M				
Method type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	bean seeds, globe artichoke, leek, potato			
	High water content *Tobacco green leaves*	0.1 mg/kg		
	High protein content *Dry bean*	0.02 mg/kg		
	High starch content *witloof chicory roots*	0.02 mg/kg		
	High acid content *Citrus, citrus peel, citrus pulp, berries, strawberry, kiwi peel, kiwi pulp*	0.02 mg/kg		
	High acid content *Kiwi peel*	0.04 mg/kg		
	No group *Wine, cocoa*	0.02 mg/kg		
	No group *Tobacco dried leaves*	0.2 mg/kg		
-	Animal products, food of animal origin, (Residues)	Pre-authorisation methods are not required for animal products for the supported uses of A14918E as a seed treatment.		

(a) Metalaxyl-M was indicated as analyte because recovery was determined with samples spiked with metalaxyl-M but it has to be noted that the method is not enantioselective. Therefore, metalaxyl (R+S) is detected.

Component of residue definition: metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 181.13/A	High water content *Peach, tomato*	0.01 mg/kg	LC-MS/MS	Method[(a)]: [redacted] 2005 Reports: REM 181.13 Validation: [redacted] 2005 Report: RJ3585B 04-S624 [redacted] 2005 Reports: REM 181.13A[(b)] EU agreed (Belgium, 2014)
	High oil content *Oilseed rape*	0.01 mg/kg		
	High starch content *Carrot*	0.01 mg/kg		
	High acid content *Orange*	0.01 mg/kg		
	No group *Hops*	0.01 mg/kg		

a) Metalaxyl-M was indicated as analyte because recovery was determined with samples spiked with metalaxyl-M but it has to be noted that the method is not enantioselective. Therefore, metalaxyl (R+S) is detected.

(b) This method is a minor modification of REM 181.13, due to the addition of text to the method. No further validation was performed.

Methods and relationship to studies presented in the Part B Section 7 document.

The below table indicates which method has been used for each of the studies included within the Part B Section 7 document as well as the respective data point and report reference.

Method	Study (Part B Section 7)	
Identifier	**Data Point**	**Report Reference**
REM181.06	KCA3 6.1	201/01 (plant)
REM181.01	KCA3 6.3.1	2016/00, 2017/00, 2017/00, 2114/00, 2115/00, 2093/01, 2094/01, 2008/01, 2009/01
REM181.01	KCA3 6.3.2	2010/98, 2011/98, 0140501, gpe14201, gpe14002, 2012/98, 2013/98, 2014/98, 2015/98, gr 31197, gr 32297, 34497, gr 36497, GR 35297, 139/97, 2004/97
REM181.01	KCA2 6.3.3	2296/97, 2297/97, 2298/97, 2299/97, 2008/98, 0140501, gpe 14201, gpe 14002, 140/97, gr31197, gr32297, 2153/98, gr35297, 141/97, 2003/97, 2004/97, 2012/98, 2013/98, 2014/98, 2015/98
REM181.01	KCA2 6.6.2	208/98
REM181.01		209/98
REM181.01		210/98
REM 181.13A		S11-00510
REM 181.13A		S11-00511

No specific analytical methods were used to support the ecotoxicology data generated on this product.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

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)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**

Reviewer's comments	'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further.

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

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	Cymoxanil	0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Plant, high acid content		0.01 mg/kg	LOQ MRL according to Reg. (EU) 978/2011
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Plant, high oil content		0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Plant, difficult matrices (hops, spices, tea)		0.1 mg/kg	LOQ MRL for difficult matrices according to Reg. (EU) 2016/1785
Muscle	Cymoxanil	0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Milk		0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Eggs		0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Fat		0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Liver, kidney		0.01 mg/kg	LOQ MRL according to Reg. (EU) 2016/1785
Soil (Ecotoxicology)	Cymoxanil	0.01 mg/kg	AOEL for cymoxanil (EFSA 2008)
Drinking water (Human toxicology)	Cymoxanil and IN-KQ960	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Cymoxanil and IN-KQ960	0.1 µg/L	general limit for drinking water
Air	Cymoxanil	3 µg/m^3	AOEL sys: 0.01 mg/kg bw/d (EFSA 2008)
Tissue (meat or liver)	Not applicable	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.2.2 Description of analytical methods for the determination of residues of cymoxanil in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of cymoxanil in plant matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

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

Component of residue definition: cymoxanil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	DFG S19	0.04 mg/kg	GC-NPD (multi-residue)	DFG S19 (NPD) Method: [redacted] & [redacted] 1999 Report P-14.141.04 Validation: [redacted] & [redacted] 1999 Report: DuPont Report No. 2158 ILV: [redacted], 1999 Report: DuPont Report No. 2946 EU agreed (Austria, 2008) --- DFG S19 (LC-MS/MS) Method & validation: [redacted] & [redacted], 2013 Report: DuPont Report No. 35769 ILV: [redacted] 2013 Report: DuPont Report No. 35770 **New data**
	ILV (DFG S19)	0.04 mg/kg		
	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
High acid content	DFG S19	0.04 mg/kg	GC-NPD (multi-residue)	
	ILV (DFG S19)	0.04 mg/kg		
	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
High oil content	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
High protein/high starch content (dry)	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Data according to SANTE 2017/10632 Rev. 3 is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the product renewal/AIR submission of cymoxanil.

5.3.2.3 Description of analytical methods for the determination of residues of cymoxanil in animal matrices (KCP 5.2.2)

No residue definition for commodities of animal origin is proposed; therefore no analytical method is required (Austria 2008).

5.3.2.4 Description of methods residues of cymoxanil for the analysis of body fluids and tissues (KCP 5.2.3)

Cymoxanil is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required.

5.3.2.5 Description of methods for residues of cymoxanil in the analysis of soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of cymoxanil in soil is given in the following table.

Table 5.3-4: Validated methods for soil

Component of residue definition: Cymoxanil			
Method name	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	HPLC-UV HPLC-DAD	Method & validation: [redacted] 2000a Report: Notox No. 281802 EU agreed (Austria, 2008)

5.3.2.6 Description of methods residues of cymoxanil for the analysis of water (KCP 5.2.5)

An overview on the acceptable methods and possible data gaps for analysis of cymoxanil in surface and drinking water is given in the following table. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-5: Validated methods for water

Component of residue definition: cymoxanil and metabolite IN-KQ960				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water (tap & well)	Primary	0.1 µg/L	LC-MS/MS	Method & validation: [redacted], 2010 Report: DuPont-27500 rev. 1 **New data**
	ILV	0.1 µg/L	LC-MS/MS	ILV: [redacted] J, 2013 Report: DuPont -35792 **New data**
Surface water (well, pond & stream)	Primary	0.1 µg/L	LC-MS/MS	Method & validation: [redacted], 2010 Report: DuPont-27500 rev. 1 **New data**

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.2.7 Description of methods residues of cymoxanil for the analysis of air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of cymoxanil in air is given in the following table.

Table 5.3-6: Validated methods for air

Component of residue definition: Cymoxanil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.46 µg/m^3	HPLC-UV HPLC-DAD	Method: [redacted] 2000b Report Notox No. 257805 EU agreed (Austria, 2008)

Summary cymoxanil:

All analytical methods are active substance date and are evaluated during the EU review of cymoxanil. For further information please refer to data submitted by Corteva for which a Letter of Access is available with this document.

5.3.2.8 Other studies/ information

No other new or additional studies have been submitted.

5.3.3 Description of analytical methods for the determination of residues of fludioxonil (KCP 5.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	**'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further.**

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-7: Relevant residue definitions for monitoring/enforcement and levels of fludioxonil for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Fludioxonil	0.01* mg/kg	LOQ MRL according to Reg. (EU) No 2019/1791
Plant, high acid content		0.01* mg/kg	
Plant, high protein/high starch content (dry commodities)		0.01* mg/kg	
Plant, high oil content		0.01* mg/kg	
Plant, difficult matrices (hops, spices, tea)		0.05* mg/kg	
Muscle	Sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole- 4 carboxylic acid (CGA 192155), expressed as fludioxonil	0.01* mg/kg	LOQ MRL according to Reg. (EU) No 2019/1791
Milk		0.01* mg/kg	
Eggs		0.05* mg/kg	
Fat		0.05* mg/kg	
Liver, kidney		0.05* mg/kg	
Soil (Ecotoxicology)	Fludioxonil	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Fludioxonil	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Fludioxonil	5 µg/L	NOEC Daphnia magna EFSA Scientific Report (2007) 110, 1-85. ASB2012- 3640
Air	Fludioxonil	177 µg/m^3	AOEL sys: 0.59 mg/kg bw/d EFSA Scientific Report (2007) 110, 1-85. ASB2012- 3640
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.3.2 Description of analytical methods of fludioxonil for the determination of residues in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of fludioxonil in plant matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-8: Validated methods for food and feed of plant origin

Component of residue definition: fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	DFG S19	0.02 mg/kg	LC-MS/MS (multi-residue)	**DFG S19** **DFG S19** Method: [redacted], 2001 Validation (tomato, orange, oilseed rape, wheat grain): [redacted], 2001 Report: SYN-0103V Validation (citrus, kiwi, wheat grain): [redacted], 2005 Report: SYN-0503V ILV (tomato): [redacted], 2001 Report: SYN-0104V ILV (kiwi, oilseed rape, avocado [redacted], 2006 Report: IF-05/00362984 EU agreed (Denmark, 2005) ---------- **QuEChERS** Validation (lettuce, orange, dried bean, oilseed rape seed, wheat straw): [redacted], [redacted] (2014) Report: P-3446 G ILV (lettuce, dried bean, oilseed rape seed, wheat straw): [redacted], [redacted] (2014) Report: 20140189 **New data**
	ILV (DFG S19)	0.02 mg/kg		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		
High acid content	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	--		
High oil content	DFG S19	0.02 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		
High protein/high starch content (dry)	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	--		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	**DFG S19:** The polarity of the acetone/water extraction solution used in DFG S19 is similar to that of the extraction procedures used in metabolism studies previously reviewed (see section 7.3 of the dRR). Therefore, DFG S19 method for crops extraction efficiency has

	Method for products of plant origin
	been adequately demonstrated.
Not required, because:	**QuEChERS** (EN 15662:2009-02) is a standard multi-residue method Data according to SANTE 2017/10632 Rev. 3 is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the product renewal of fludioxonil.

5.3.3.3 Description of analytical methods of fludioxonil for the determination of residues in animal matrices (KCP 5.2.2)

The use of A9873C is expected to result in residues of fludioxonil below the LOQ in relevant animal feed items. Therefore, the use of A9873C will not result in residues of fludioxonil in animal feed items, and so the possible transfer of residues in animal commodities from the proposed uses does not need to be considered. Methods of analysis for residues in animal matrices are not required; however an overview on the acceptable methods for monitoring and possible data gaps for analysis of residues of fludioxonil in animal matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-10: Validated methods for food and feed of animal origin

Component of residue definition: Sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluorobenzo[1,3]dioxole- 4 carboxylic acid (CGA 192155), expressed as fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	AG-616B	0.01 mg/kg	HPLC-UV	**AG-616B** *Method:* Vienneau K, 1996 *Validation (milk, eggs, muscle, fat liver, kidney):* Vienneau K, 1996 Report: AG-616B *ILV (milk, eggs, liver):* Tang J and Baldi B, 1996 Report: 96-0010 EU agreed (Denmark, 2005) ---------- **GRM025.03A** *Method:* Sole C, 2008 (*not submitted*) Report: GRM025.03A Sole C, 2009 Report: GRM025.03A version 2 *Validation (milk, eggs, muscle, fat, liver, kidney, whole blood):* Sole C, 2009
	ILV (AG-616B)	0.01 mg/kg		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	--		
Eggs	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	0.05 mg/kg		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Muscle	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	--		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Fat	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	--		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Kidney, liver	AG-616B	0.05 mg/kg	HPLC-UV	

Component of residue definition: Sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole- 4 carboxylic acid (CGA 192155), expressed as fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	ILV (AG-616B)	0.05 mg/kg		Report: T001341-08-REG *ILV (Eggs, muscle, fat, liver):* [redacted], 2009 Report: 1983/108-D2149 (T001339-08) **New data**
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Blood (whole)	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	--		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-11: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	**AG-616B:** Radio validation of analytical method AG-616 was reported for the EU review (Denmark, 2005). Radiovalidation of analytical method AG-616 has been carried out and reported (ref. "Validation of "Draft" Analytical Method AG-616 for the Determination of Total Resiudes of CGA-173506 and Metabolites as CGA-192155 in Animal Tissues, Milk and Eggs", [redacted] 1993, ABR-95063). Fludioxonil is shown to be effectively extracted from animal matrices **GRM025.03A** The extraction procedures used in analytical methods AG-616B and GRM025.03 are very similar, so extractability efficiency of analytical method GRM02.03 has been adequately demonstrated. Data according to **SANTE 2017/10632 Rev. 3** is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the product renewal of fludioxonil.

5.3.3.4 Description of methods of fludioxonil for the analysis of body fluids and tissues (KCP 5.2.3)

Fludioxonil is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required. Should a method be required for monitoring of fludioxonil in body fluids method GRM025.03A has been successfully validated in whole blood.

5.3.3.5 Description of methods of fludioxonil for the analysis of soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of fludioxonil in soil is given in the following tables.

Table 5.3-12: Validated methods for soil

Component of residue definition: Fludioxonil			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS with two mass transitions	RAM 423/01 Method: [redacted] & [redacted], 2004 Report: RAM 423/01 Validation: [redacted], 2004 Report: RJ3493B EU agreed (Denmark, 2007)
Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

5.3.3.6 Description of methods of fludioxonil for the analysis of water (KCP 5.2.5)

An overview on the acceptable methods and possible data gaps for analysis of fludioxonil in surface and drinking water is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-13: Validated methods for water

Component of residue definition: Fludioxonil and metabolites CGA339833 and CGA192155 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS with two mass transitions	GRM025.01A Method: [redacted], 2007 Report : GRM025.01A Validation: [redacted], 2007 Report: T003490-06-REG EU agreed (Denmark, 2007)
	ILV	0.05 µg/L		ILV [redacted], 2016 Report: CGA173506DW **New data**
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS with two mass transitions	GRM025.01A Method: [redacted], 2007 Report : GRM025.01A Validation: [redacted], 2007 Report: T003490-06-REG

Component of residue definition: Fludioxonil and metabolites CGA339833 and CGA192155 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				EU agreed (Denmark, 2007)
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

* Metabolites CGA192155 and CGA339833 are not part of the residue definition for monitoring, but included in the method and fully validated.

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.3.7 Description of methods of fludioxonil for the analysis of air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of fludioxonil in air is given in the following tables.

Table 5.3-14: Validated methods for air

Component of residue definition: Fludioxonil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	2 µg/m^3	Normal phase HPLC-UV	REM 133.03 Method: [redacted], 1992 Report: REM 133.03 Validation (normal phase): [redacted], 1996 Report: 103/96 EU agreed (Denmark, 2007)
Confirmatory	2 µg/m^3	Reverse phase HPLC-UV	REM 133.03 Method: [redacted], 1992 Report: REM 133.03 Validation (reverse phase): [redacted], 2001 Report: 133.03 29/11/2001 EU agreed (Denmark, 2007)

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.3.8 Other studies/ information

No new or additional studies have been submitted.

5.3.4 Description of analytical methods for the determination of residues of metalaxyl-M (KCP 5.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The table below gives a mostly accurate representation of the residue definitions for monitoring methods for metalaxyl-M; where necessary the text has been amended by HSE in green (applicant's text has been struck through in green where necessary).

5.3.4.1 Overview of residue definitions and levels of metalaxyl-M for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical. In the most recent EU assessment a residue definition for animal products was not proposed, however as part of the MRL review conducted in 2015[2] EFSA proposed a residue definition for monitoring of the sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6- dimethylaniline moiety, expressed as metalaxyl. The current EU MRL legislation (Regulation (EU) No 2017/1164 amending Annexes II and III to Regulation (EC) No 396/2005) states the residue definition for products of animal origin as: metalaxyl and metalaxyl-M (metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)). Although the uses of A9873C do not give rise to residues in animal products (See dRR Part B Section 7) methods of analysis to determine the "EFSA MRL" residue definition of sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6- dimethylaniline moiety, expressed as metalaxyl are available.

Table 5.3-15: Relevant residue definitions for monitoring/enforcement and levels of metalaxyl-M for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Metalaxyl and metalaxyl-M (metalaxyl including other mixtures of constituents isomers including metalaxyl-M (sum of isomers))	0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, high acid content		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, high protein/high starch content (dry commodities)		0.05mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, high oil content		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, difficult matrices (hops, spices, tea)		0.1 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Muscle	~~sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6- dimethylaniline moiety,~~	0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Milk		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164

[2] Combined review of the existing maximum residue levels (MRLs) for the active substances metalaxyl and metalaxyl-M, EFSA Journal 2015; 13(4):4076

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Eggs	~~expressed as metalaxyl~~ Not required for the representative uses EFSA, 2015a	0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Fat		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Liver, kidney		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Soil (Ecotoxicology)	Metalaxyl including other mixtures of constituents isomers including metalaxyl-M (sum of isomers)	0.05 mg/kg	General limit
Drinking water (Human toxicology)	Metalaxyl including other mixtures of constituents isomers including metalaxyl-M (sum of isomers)	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Metalaxyl including other mixtures of constituents isomers including metalaxyl-M (sum of isomers)	1.2 mg/L	NOEC (Daphnia) (EFSA, 2015a)
Air	Metalaxyl including other mixtures of constituents isomers including metalaxyl-M (sum of isomers)	24 µg/m^3	AOEL sys: 0.08 mg/kg bw/d (EFSA, 2015a)
Body fluids	~~sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6-dimethylaniline moiety, expressed as metalaxyl~~ The active substance is not classified as a Health Hazard under CLP and therefore a method of analysis is not required for body fluids and tissues. EFSA, 2015a	0.01 mg/L	Default LOQ

5.3.4.2 Description of analytical methods of metalaxyl-M for the determination of residues in plant matrices (KCP 5.2.1)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of metalaxyl-M in plant matrices were evaluated for the active approval (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. The available methods are validated for the crops in the high water, high acid, high oil, high protein, high starch crop groups. These are considered sufficient to cover the proposed uses of

	'A9873C' on fresh peas. The EFSA conclusion states '*The compounds in the residue definition for plants can be determined with a multi-residue method (QuEChERs) however a data gap was identified for extraction efficiency.*' Data to address the extraction efficiency will be addressed the next renewal of the active substance. The applicant has provided a justification for not requiring extraction efficiency data. The proposed uses are expected to give to residues <LOQ therefore extraction efficiency of the method are not critical in the framework of this assessment. Hence, this case is accepted. The applicant has provided new data however this is not required in the context of this assessment so has not been evaluated. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of metalaxyl-M in plant matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-16: Validated methods for food and feed of plant origin

Component of residue definition: metalaxyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Validation (tomato, potato, orange oilseed rape seed, dried bean): [redacted] & [redacted], 2011 Report: S11-01731 (two mass transitions validated) EU agreed (Belgium, 2014) ILV (tomatoes and oilseed rape) : [redacted], 2012 Report: S11-03712 **New data** New data is not required in the context of this assessment so has not been evaluated.
High acid content	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
High oil content	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
High protein/high starch content (dry)	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
Difficult (if required, depends on intended use)	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Validation (hop, cocoa bean): [redacted], 2016 Report: RES-00055 (two mass transitions validated) **New data** Not required in the context of this assessment; however this method was evaluated for a GB import tolerance
	ILV (QuEChERS)	0.01 mg/kg		

Component of residue definition: metalaxyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				application. ILV (hop, cocoa bean): [redacted], 2016 Report: YB27DB **New data** Not required in the context of this assessment; however this method was evaluated for a GB import tolerance application.

Table 5.3-17: Validated methods for food and feed of plant origin

Component of residue definition: Metalaxyl-M (enantiomer specific)				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	<u>REM 181.06</u> Method: [redacted], 2001 Report: REM 181.06 Validation: [redacted], 2001 Report: 212/00 ILV: [redacted], 2001 Report: NOV/MET00111 EU agreed (Belgium, 2014) --- <u>DFG S19</u> Validation: [redacted] & [redacted], 2012 Report: S11-03698 EU agreed (Belgium, 2014)
	ILV (REM 181.06)	0.02 mg/kg		
	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
High acid content	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg		
High oil content	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg		
High protein/high starch content (dry)	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg	GC-MSD (single residue)	

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-18: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	**Extraction Efficiency (SANTE 2017/10632 Rev. 3)**

	Method for products of plant origin
	Based on SANTE 2017/10632, for renewal of 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 the case of Metalaxyl-M as an AIR2 compound this application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011. Therefore, when considering these data requirements, no additional proof of extraction efficiency is required in the context of this product submission as in SANTE 2017/10632 Rev. 3 guidance. (page 19) In addition, the uses under consideration as part of this product submission results in <LOQ residues based on seed treatment metabolism data scaled to the proposed cGAP (33.92 g ai/100 kg seed) for components of the plant definition of the residues for risk assessment and enforcement. This finding is also carried over into the magnitude of residues data for the crops asscoiated with this submission which are also <0.02 mg/kg in all cases. On the basis of <0.02 mg/kg exposure, extraction efficiency is not needed as per the decision tree for post registration methods (figure 1) and the decision tree for pre-registration methods (figure 2) outlined in the guidance.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	See green box above.

5.3.4.3 Description of analytical methods of metalaxyl-M for the determination of residues in animal matrices (KCP 5.2.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of metalaxyl-M in products of animal origin were presented in the RAR (EU RAR, 2014) and summarised below. It is noted that the EFSA conclusion states: '*Analytical methods for food of animal origin are not required in this regulatory context as there is no significant intake by livestock, when solely considering the supported representative uses.'* Based on the proposed uses of 'A9873C' residues in animal products will not be significant; therefore methods are not required. The applicant has provided new data for milk, eggs, muscle, fat, liver, kidney and blood. However

	this is not required in the context of this assessment so has not been evaluated. Data will be considered at the next renew of the active. No further consideration is required.

The use of A9873C is expected to result in residues of metalaxyl-M below the LOQ in animal feed items. Therefore, the use of A9873C will not result in residues of metalaxyl-M in animal feed items, and so the possible transfer of residues in animal commodities from the proposed uses does not need to be considered. Methods of analysis for residues in animal matrices are not required; however an overview on the acceptable methods and possible data gaps for analysis of residues of metalaxyl-M in animal matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-19: Validated methods for food and feed of animal origin

Component of residue definition: Metalaxyl-M				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	QuEChERS Validation (milk, eggs, muscle, fat, liver, kidney and blood): [redacted], 2011 Report: S11-01732 **New data** New data is not required in the context of this assessment so has not been evaluated. ILV (milk, eggs, muscle, liver, and fat) : [redacted], 2018 Report: MM87YQ **New data** New data is not required in the context of this assessment so has not been evaluated.
	ILV (QuEChERS)	0.01 mg/kg		
Eggs	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		
Muscle/meat	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	--		
Fat	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		
Liver	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		
Kidney	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		

Table 5.3-20: Validated methods for food and feed of animal origin

Component of residue definition: 2,6-dimethylaniline				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	GRM031.06A Method: [redacted] & [redacted], 2012
	ILV	0.01 mg/kg		

Component of residue definition: 2,6-dimethylaniline				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(GRM031.06A)			Report: GRM031.06A Validation: [redacted] & [redacted], 2012 Report: S11-03382 ILV (milk, eggs, liver, kidney) : [redacted], 2012 Report: S12-03412 EU agreed (Belgium, 2014) ILV (fat): [redacted], 2016 Report: TK0261461 **New data** New data is not required in the context of this assessment so has not been evaluated.
Eggs	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	0.01 mg/kg		
Muscle/meat	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	--		
Fat	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	0.01 mg/kg		
Liver, Kidney	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	0.01 mg/kg		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-21: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	**Extraction Efficiency (SANTE 2017/10632 Rev. 3)** Based on SANTE 2017/10632, for renewal of 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 the case of Metalaxyl-M as an AIR2 compound this application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011. Therefore, when considering these data requirements, no additional proof of extraction efficiency is required in the context of this product submission as in SANTE 2017/10632 Rev. 3 guidance. (page 19) Also, according to SANTE 2017/10632, it is “not expected that new animal metabolism studies or new animal feeding studies should be set up only in order to evaluate aspects of analytical methods and extraction efficiency”, as these would have to be carried out with vertebrate animals.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The extraction efficiency of the monitoring method for the determination of residues of metalaxyl-M in animal matrices was not considered during the active substance renewal (EU RAR, 2014). Data to address the extraction efficiency will be addressed at the next renewal of the active substance. In addition, significant residues in products of animal origin are not expected based on the proposed uses therefore the extraction efficiency is not a critical concern. No further consideration is required.

5.3.4.4 Description of methods of metalaxyl-M for the analysis of body fluids and tissues (KCP 5.2.3)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of Metalaxyl-M in body fluids and tissues were not evaluated for the active approval. Metalaxyl-M was assessed using the data requirements under Reg (EU) 544/2011, and is not classified as toxic or very toxic, therefore it was not a requirement to provide methods of analysis. The EFSA Conclusion states *'The active substance is not classified as a Health Hazard under CLP and therefore a method of analysis is not required for body fluids and tissues.*' The applicant has provided a study; however, it is not necessary to consider these data until renewal of the active substance and the study has not been evaluated in this RR. No further consideration is required.

Metalaxyl-M is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required. However the following method can be used to determine residues of metalaxyl-M in body fluids and tissues.

Table 5.3-22: Methods for body fluids and tissues

Component of residue definition: Metalaxyl-M				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**

Component of residue definition: Metalaxyl-M				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Blood	QuEChERS	0.01 mg/kg	LC-MS/MS (single residue) LC-MS/MS (single residue)	QuEChERS Validation (milk, eggs, muscle, fat, liver, kidney and **blood**): [redacted], 2011 Report: S11-01732 **New data** New data is not required in the context of this assessment so has not been evaluated.

Table 5.3-23: Methods for body fluids and tissues

Components of residue method: Metalaxyl-M and 2,6-dimethylaniline			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg 0.01 mg/L	LC-MS/MS two mass transitions validated	Method: [redacted] & [redacted], 2012 Report: GRM031.06A Validation: [redacted] & [redacted], 2012 Report: S11-03382 ILV: [redacted], 2012 Report: S12-03412 EU agreed (Belgium, 2014)

5.3.4.5 Description of methods of metalaxyl-M for the analysis of soil (KCP 5.2.4)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The table below gives an accurate representation of the available monitoring methods for residues of metalaxyl-M in soil. Monitoring methods for the determination of residues of metalaxyl-M in soil were evaluated for the active renewal (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of metalaxyl-M in soil is given in the following tables.

Table 5.3-24: Validated methods for soil

Component of residue definition: Metalaxyl-M (sum of isomers) and NOA409045*			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.03A Method and validation: [redacted], 2008a Report: GRM031.03A EU agreed (Belgium, 2014)
Confirmatory	-	-	Not required: two mass transitions validated in primary method

* Metabolite NOA409045 is not part of the residue definition for monitoring, but included in the method and fully validated. Metalaxyl-M and NOA409045 each contain 1 chiral centre. NOA409045 is the R-enantiomer of this metalaxyl metabolite, the RS-enantiomer of which is CGA62826. GRM031.03A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of each analyte as single chromatographic peaks.

5.3.4.6 Description of methods of metalaxyl-M for the analysis of water (KCP 5.2.5)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of metalaxyl-M in water were evaluated for the active renewal (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. The applicant has provided new studies for drinking water, surface water and ground water. However, it is not necessary to consider these data until renewal of the active substance and therefore studies have not been evaluated in this RR. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of metalaxyl-M in surface and drinking water is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-25: Validated methods for water

Component of residue definition: Metalaxyl-M (sum of isomers) and metabolites NOA409045 and CGA108906*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.02A [redacted], 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	ILV	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	[redacted], 2016 Report: IF-15/03469803-TK **New data**

Component of residue definition: Metalaxyl-M (sum of isomers) and metabolites NOA409045 and CGA108906*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.02A [redacted], 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.02A [redacted], 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

* Metabolites NOA409045 and CGA108906 are not part of the residue definition for monitoring, but included in the method and fully validated. Metalaxyl-M, NOA409045 and CGA108906 each contain 1 chiral centre. NOA409045 is the R-enantiomer of this metalaxyl metabolite, the RS-enantiomer of which is CGA62826. CGA108906 is the racemic form (RS-enantiomer) of metalaxyl diacid metabolite, the R-enantiomer of which is SYN546520. GRM031.02A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of each analyte as single chromatographic peaks.

Table 5.3-26: Validated methods for water

Component: Metalaxyl-M (sum of isomers) and metabolites NOA409045, CGA108906 and CGA67868*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.08A [redacted] & [redacted], 2015 Report: GRM031.08A **New data** New data is not required in the context of this assessment so has not been evaluated. Validation: [redacted], 2015 Report: TK0222545 **New data** New data is not required in the context of this assessment so has not been evaluated.
	ILV	0.05 µg/L	LC-MS/MS two mass transitions validated	[redacted], 2016 Report: IF-15/03469803-TK

Component: Metalaxyl-M (sum of isomers) and metabolites NOA409045, CGA108906 and CGA67868*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
			(*non-enantiospecific*)	**New data** New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.08A [redacted] & [redacted], 2015 Report: GRM031.08A **New data** New data is not required in the context of this assessment so has not been evaluated. Validation: [redacted], 2015 Report: TK0222545 **New data** New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.08A [redacted] & [redacted], 2015 Report: GRM031.08A **New data** New data is not required in the context of this assessment so has not been evaluated. Validation: [redacted], 2015 Report: TK0222545 **New data** New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

* Metabolites NOA409045, CGA108906 and CGA67868 are not part of the residue definition for monitoring, but included in the method and fully validated. Metalaxyl-M, NOA409045 and CGA108906 each contain 1 chiral centre. NOA409045 is the R-enantiomer of this metalaxyl metabolite, the RS-enantiomer of which is CGA62826. CGA108906 is the racemic form (RS-enantiomer) of metalaxyl diacid metabolite, the R-enantiomer of which is SYN546520. GRM031.02A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of each analyte as single chromatographic

peaks. CGA67868 is not a chiral molecule.

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.4.7 Description of methods of metalaxyl-M for the analysis of air (KCP 5.2.6)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The table below gives an accurate representation of the available monitoring methods for residues of metalaxyl-M in air. Monitoring methods for the determination of residues of metalaxyl-M in air were evaluated for the active renewal (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of metalaxyl-M in air is given in the following tables.

Table 5.3-27: Validated methods for air

Component of residue definition: Metalaxyl-M (sum of isomers)			
Method	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	10 µg/m^3	LC-MS/MS with 2 mass transitions. *(non-enantiospecific)*	GRM011.01A [redacted], 2006 Report: T003619-05-REG EU agreed (Belgium, 2014)
Confirmatory	-	-	Not required: 2 ion transitions validated in primary method

* Metalaxyl-M contains 1 chiral centre. GRM011.01A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of metalaxyl as single chromatographic peaks.

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.4.8 Other studies/ information

No new or additional studies have been submitted.

5.4 References

Cymoxanil

Austria, 2007. Draft assessment report on the active substance cymoxanil prepared by the rapporteur Member State Austria in the framework of Council Directive 91/414/EEC, October 2007.

Austria, 2008. Final addendum to the draft assessment report on the active substance cymoxanil prepared by the rapporteur Member State Austria in the framework of Council Directive 91/414/EEC, September 2008.

EFSA (European Food Safety Authority), 2008. Conclusion on the peer review of the pesticide risk assessment of the active substance cymoxanil. EFSA Scientific Report (2008) 167, 1-116.

Fludioxonil

Denmark, 2005. Draft assessment report on the active substance fludioxonil prepared by the rapporteur Member State Denmark in the framework of Council Directive 91/414/EEC, June 2005.

Denmark, 2007. Final addendum to the draft assessment report on the active substance fludioxonil prepared by the rapporteur Member State Denmark in the framework of Council Directive 91/414/EEC, compiled by EFSA, June 2007.

EFSA (European Food Safety Authority), 2007. Conclusion regarding the peer review of the pesticide risk assessment of the active substance fludioxonil. EFSA Scientific Report (2007) 110, 1-85.

Metalaxyl-M

Belgium, 2014. Renewal assessment report on the active substance metalaxyl-M prepared by the rapporteur Member State Belgium under Regulation (EC) No 1107/2009, December 2013.

EFSA (European Food Safety Authority), 2015a. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015; 13(3):3999, [105 pp.] doi:10.2903/j.efsa.2015.3999.

EFSA (European Food Safety Authority), 2015b. Combined review of the existing maximum residue levels (MRLs) for the active substances metalaxyl and metalaxyl-M, EFSA Journal 2015; 13(4):4076, [56 pp.] doi:10.2903/j.efsa.2015.4076.

Appendix 1 Lists of data considered in support of the evaluation

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**
List of data submitted by the applicant and relied on – A9873C					
KCP 5.1.1	[redacted], B.	05/10/1998	Analytical method CGA 329351, CGA 173506 and cymoxanil in formulation (WG) by liquid chroma-tography Report No. AF-1318/2 Document No. VV-124572 , A9873C_10312 | CGA173506/1254 Test Facility Novartis Crop Protection Münchwilen AG GLP Unpublished	N	SYN
KCP 5.1.1	[redacted], B.	24/06/1999	Report on validation of analytical method AF-1318/2 Report No. 59202 Document No. VV-292097 , CGA173506/4974 | A9873C_10313 Test Facility Novartis Crop Protection Münchwilen AG GLP Unpublished	N	SYN
KCP 5.1.1	[redacted], I. [redacted], P.	17/12/2002	Analytical method AFA-1318/2 - Content of CGA329351 and CGA351920 in A9873C and A9873D by chiral LC Report No. AFA-1318/2 Document No. VV-123832 , CGA173506/5568 | A9873C_10314 Test Facility Syngenta Crop Protection Münchwilen AG Not GLP Unpublished	N	SYN
KCP 5.1.1	[redacted], P.	09/01/2003	Report on validation of analytical method - AFA-1318/2 Report No. 109644 Document No. VV-293344 , CGA173506/5571 | A9873C_10315 Test Facility Syngenta Crop Protection AG	N	SYN

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**
			Not GLP Unpublished		
KCP 5.1.1	[redacted]	11/12/2014	A9651D - Analytical Method SD-1751/1 Report No. 300021240 Document No. VV-128413 , A9651D_10487 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	SYN
KCP 5.1.1	[redacted]	25/11/2014	A9651D - Validation Analytical Method SD-1751/1 Report No. CHMU140410 Document No. VV-411110 , A9651D_10488 Test Facility Syngenta Crop Protection GLP Unpublished	N	SYN
KCP 5.1.1	[redacted]	15/12/2014	Statement on Validation of the Analytical Method SD-1751/1 for the determination of CGA72649 and CGA363736 in A9873C metalaxyl-M/cymoxanil/fludioxonil WG Report No. 300031476 Document No. VV-28929 , A9873C_10344 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	SYN
~~**List of data submitted by the applicant and relied on – Cymoxanil**~~					
~~KCP 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (North)~~ ~~Report No. 2010/98~~ ~~Document No. VV-312513 , CGA173506/4962~~ ~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~

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 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (North)~~ ~~Report No. 2011/98~~ ~~Document No. VV-312406 , CGA173506/4963~~ ~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South)~~ ~~Report No. 2012/98~~ ~~Document No. VV-312407 , CGA173506/4964~~ ~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~07/08/2002~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (North)~~ ~~Report No. 0140501~~ ~~Document No. VV-330998 , CGA173506/5506~~ ~~Test Facility ADME - Bioanalyses~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~30/06/2003~~	~~Determination of Residues of Metalaxyl-M, Fludioxonil and Cymoxanil after seed treatment with WAKIL XL in Peas in Germany (2001)~~ ~~Report No. gpe14201~~ ~~Document No. VV-328561 , CGA173506/5666~~ ~~Test Facility Syngenta Agro GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~01/08/2003~~	~~Residues of Metalaxyl-M, Fludioxonil and Cymoxanil after seed treatment with WAKIL XL (A9873C) in Peas, Germany 2002~~	~~N~~	~~SYN~~

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
			~~Report No. gpe514002~~ ~~Document No. VV-340015 , CGA173506/5765~~ ~~Test Facility Syngenta Agro GmbH~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South)~~ ~~Report No. 2012/98~~ ~~Document No. VV-312407 , CGA173506/4964~~ ~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South)~~ ~~Report No. 2013/98~~ ~~Document No. VV-312408 , CGA173506/4965~~ ~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South)~~ ~~Report No. 2014/98~~ ~~Document No. VV-312409 , CGA173506/4966~~ ~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2.5~~	[redacted]	~~20/05/1999~~	~~Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South)~~ ~~Report No. 2015/98~~ ~~Document No. VV-312410 , CGA173506/4967~~	~~N~~	~~SYN~~

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
			~~Test Facility Novartis Crop Protection AG~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.1~~	[redacted]	~~2013~~	~~Validation of Multi-Residue Method DFG S19 (LC-MS/MS module) for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain. DuPont 35769. DuPont Report No. 35769~~ ~~Eurofins Agroscience Services Chem GmbH (EAS Chem)~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Corteva (SYN access)~~
~~KCP 5.2.1~~	[redacted]	~~2013~~	~~Independent Laboratory Validation of Multi-Residue Method DFG S19 for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain using LC-MS/MS - DuPont-35770.~~ ~~Výzkumný ústav organických syntéz a.s. (Research Institute for Organic Syntheses, Inc.)~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Corteva (SYN access)~~
~~KCP 5.2.5~~	[redacted]	~~2010~~	~~Analytical method for the determination of cymoxanil and IN-KQ960 in water (pond, stream, well, and tap) using LC/MS/MS~~ ~~Report DuPont-27500, Revision No. 1~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Corteva (SYN access)~~
~~KCP 5.2.5~~	[redacted]	~~2013~~	~~Independent Laboratory Validation for the Determination of Residues of Cymoxanil and IN-KQ960 in Water (Drinking and Stream) Using LC-MS/MS.~~ ~~Report DuPont-35792~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Corteva (SYN access)~~
~~**List of data submitted by the applicant and relied on – Fludioxonil**~~					
~~KCP 5.2.1~~	[redacted]	~~15/10/2014~~	~~Fludioxonil – Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS~~ ~~Report No. P 3446 G~~	~~N~~	~~SYN~~

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
			~~Document No. VV-410631 , CGA173506_11710~~ ~~Test Facility PTRL Europe~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.1~~	[redacted]	~~05/12/2014~~	~~Fludioxonil - Independent Laboratory Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS~~ ~~Report No. 20140189~~ ~~Document No. VV-410968 , CGA173506_11723~~ ~~Test Facility Innovative Environmental Services~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~26/02/2009~~	~~Fludioxonil - Analytical Method for the Determination of Residues of Total Fludioxonil (CGA173506) and Metabolites as CGA192155 in Animal Matrices (milk, eggs, muscle, fat, liver, kidney and whole blood). Final Determination by LC-MS/MS~~ ~~Report No. GRM025.03A~~ ~~Document No. VV-127758 , CGA173506_11402~~ ~~Test Facility ADME - Bioanalyses~~ ~~GCP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~24/02/2009~~	~~Validation of residue method GRM025.03A for total fludioxonil (CGA173506) and metabolites as CGA192155 in animal matrices (milk, eggs, muscle, fat, liver, kidney and whole blood)~~ ~~Report No. T001341-08-REG~~ ~~Document No. VV-382790 , CGA173506_11403~~ ~~Test Facility ADME - Bioanalyses~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~02/04/2009~~	~~Fludioxonil - Magnitude of residues in animal tissues following repeated oral administration to the laying hen~~ ~~Report No. T001339-08|1983/108-D2149~~ ~~Document No. VV-383645 , CGA173506_11440~~	~~Y~~	~~SYN~~

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
			~~Test Facility Covance Laboratories Ltd.~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.5~~	[redacted]	~~04/04/2016~~	~~Fludioxonil – Independent Laboratory Validation (ILV) of Analytical Method GRM025.01A for the Determination of Residues of Fludioxonil (CGA173506) and its Metabolites CGA192155 and CGA339833 in Water~~ ~~Report No. CGA173506DW~~ ~~Document No. VV-462757 , CGA173506_11942~~ ~~Test Facility CIP Chemisches Institut Pforzheim GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
List of data submitted by the applicant and relied on – Metalaxyl-M					
~~KCP 5.2.1~~	[redacted]	~~07/01/2014~~	~~Metalaxyl-M – Independent Laboratory Validation (ILV) of an Analytical Method for Determination of Residues of Metalaxyl-M in Crops~~ ~~Report No. S11-03712~~ ~~Document No. VV-407367 , CGA329351_11643~~ ~~Test Facility Eurofins Agroscience Services EcoChem GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.1~~	[redacted]	~~15/06/2016~~	~~Metalaxyl-M – Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans~~ ~~Report No. RES-00055~~ ~~Document No. VV-465427 , CGA329351_11743~~ ~~Test Facility ResChem Analytical Limited~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.1~~	[redacted]	~~16/08/2016~~	~~Metalaxyl-M: Independent Laboratory Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans~~ ~~Report No. YB27DB~~ ~~Document No. VV-465743 , CGA329351_11745~~ ~~Test Facility Envigo CRS Limited~~	~~N~~	~~SYN~~

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
			~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.2~~	[redacted]	~~10/10/2011~~	~~Metalaxyl-M – Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices~~ ~~Report No. S11-01732~~ ~~Document No. VV-400487 , CGA329351_11472~~ ~~Test Facility Eurofins Agroscience Services Chem GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~19/11/2018~~	~~Metalaxyl-M – Independent Laboratory Validation of Analytical Method QuEChERS for the Determination of Residues of Metalaxyl-M in Animal Matrices by LC-MS/MS~~ ~~Report No. MM87YQ~~ ~~Document No. VV-470901 , CGA329351_11851~~ ~~Test Facility Envigo CRS Limited~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~30/03/2016~~	~~Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.06A for the Determination of Metalaxyl-M and Structurally Related Metabolites as the Common Moiety 2,6-Dimethylaniline (CGA72649) in Animal Fat~~ ~~Report No. S16-00573~~ ~~Document No. VV-463097 , CGA329351_11737~~ ~~Test Facility Eurofins Agroscience Services EcoChem GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.5~~	[redacted]	~~01/10/2015~~	~~Metalaxyl-M – Residue Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and Metabolites NOA409045, CGA108906 and CGA67868 in water. Non-enantiospecific method. Final determination by LC-MS/MS~~ ~~Report No. GRM031.08A~~ ~~Document No. VV-132583 , CGA329351_11693~~ ~~Test Facility Syngenta – Jealott's Hill~~	~~N~~	~~SYN~~

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
			~~Not GLP~~ ~~Unpublished~~		
~~KCP 5.2.5~~	[redacted]	~~01/07/2015~~	~~Metalaxyl-M – Validation of Analytical Method for the Determination of Metalaxyl-M Metabolite CGA67868 in Water~~ ~~Report No. S14-05740~~ ~~Document No. VV-412805 , CGA092370_10006~~ ~~Test Facility Eurofins Agroscience Services Chem SAS~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.5~~	[redacted]	~~12/02/2016~~	~~Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and its Metabolites NOA409045, CGA108906 and CGA67868 in Drinking Water~~ ~~Report No. IF-15/03469803-TK~~ ~~Document No. VV-415481 , CGA329351_11732~~ ~~Test Facility SGS Germany GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~

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 XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for cymoxanil

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	**'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further.**

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

A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted

A 2.1.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

A 2.1.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted

A 2.1.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted

A 2.1.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

A 2.1.1.5.1 DFG 513

A 2.1.1.5.1.1 Method validation

Reference: KCP 5.1.2

Reports

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (North)

[redacted] (1999)

Syngenta File No. CGA173506/4962, Syngenta Report No. 2010/98.

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (North).

[redacted] (1999a)

Syngenta File No. CGA173506/4963, Syngenta Report No. 2011/98.

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (North)

[redacted] (2002)

Syngenta File No. CGA173506/5506, Syngenta Report No. 0140501.

Determination of Residues of Metalaxyl-M, Fludioxonil and Cymoxanil after seed treatment with WAKIL XL in Peas in Germany (2001)

[redacted] (2003)

Syngenta File No. CGA173506/5666, Syngenta Report No. gpe14201.

Determination of Residues of Metalaxyl-M, Fludioxonil and Cymoxanil after seed treatment with WAKIL XL in Peas in Germany (2002)

[redacted] (2003a)

Syngenta File No. CGA173506/5765, Syngenta Report No. gpe541002.

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South).

[redacted] (1999b)

Syngenta File No. CGA173506/4964, Syngenta Report No. 2012/98

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South).

[redacted] (1999c)

Syngenta File No. CGA173506/4965, Syngenta Report No. 2013/98

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South).

[redacted] (1999d)

Syngenta File No. CGA173506/4966, Syngenta Report No. 2014/98

Residue Study with Fludioxonil (CGA 173506), Metalaxyl-M (CGA 329351) and Cymoxanil (ASF 331) in or on Peas in France (South).

[redacted] (1999e)

Syngenta File No. CGA173506/4967, Syngenta Report No. 2015/98

Guideline(s): No. Methods used comparable to EU guideline.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

In all residue trials, the analytical method for the determination of cymoxanil in crops was the method 133.04 based on the published multi-residue method DFG 513. This method has already been validated for high water content and high acid content matrices (Austria 2008).

Cymoxanil is extracted from crops by blending with ethyl acetate. The extract is washed with hexane, which is discarded and then partitioned into dichloromethane. The extract is further purified using a silica gel column. Determination is by capillary gas chromatography with a DB-5 column (30m x 0.53 mm i.d., 1.5 µm film thickness; J&W) and a nitrogen phosphorus detector.

Results and discussions

The results from the procedural recovery data generated during analysis of the residue trials are summarised in the tables below.

Table A 1: Recovery results from method validation of cymoxanil using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Recoveries (%)	Mean recovery (%)	RSD (%)
Peas (whole plant)	Cymoxanil	0.05	85, 109, 100, 88	96	12
		0.5	79, 124, 99, 92	99	19
Peas (green seeds)	Cymoxanil	0.02	81	-	-
		0.2	76	-	-
Peas (dry seed, empty pods)	Cymoxanil	0.02	92, 104, 78, 81, 110, 73, 96, 74, 90, 92	89	14
		0.05	95 , 99, 120, 111, 77, 81, 101, 99	98	14
		0.2	97, 80, 74, 76, 113, 73, 73, 101, 97	87	17
		0.5	72, 89, 104, 111, 102, 94, 88, 89	94	13

Table A 2: Characteristics for the analytical method used for validation of cymoxanil residues in plants

	Cymoxanil
Specificity	Not required for pre-registration methods.

	Cymoxanil
Specificity	Not required for pre-registration methods.
Calibration (type, number of data points)	No data concerning the linearity were presented. Nevertheless, as the validation of the linearity has been performed in solvent (and there were no adverse matrix effects), the linearity validated for high water matrices content can be considered acceptable for the dry matrices.
Calibration range	
Assessment of matrix effects is presented	Yes - No interference > 30 % LOQ.
Limit of determination/quantification	0.02 mg/kg.

Conclusion

The validation data provided for pre-registration are considered sufficient and acceptable.

A 2.1.1.5.1.2 Confirmatory method

No confirmatory method is required for data generation methods.

A 2.1.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted

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 5.2.1)

A 2.1.2.1.1 DFG S19 (LC-MS/MS)

A 2.1.2.1.1.1 Method validation

Reference:	KCP 5.2.1
Report	Validation of multi-residue method DFG S19 (LC-MS/MS module) for the determination of residues of cymoxanil in tomato, grapes, oilseed rape and wheat grain

[redacted] & [redacted] (2013)

Report No: DuPont-35769

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Extraction modules E1, E2, and E3 – Tomato, Wheat Grain, and Grapes

A 25 g specimen (10 g for wheat grain) is extracted with acetone using a homogeniser. Water is added beforehand in an amount that takes into account the natural water content of the specimen so that during extraction the acetone/water ratio remains constant at 2/1 (v/v). For E2 (wheat grain) the water is heated to 40°C and samples are allowed to soak for approx. 30 min.

For E3 (grapes) only: A small amount of sodium bicarbonate (~2.0 g) is added to adjust the pH value to pH 7.

After addition of sodium chloride and ethyl acetate/cyclohexane (1/1, v/v) and repeated homogenisation, the organic layer containing cymoxanil is allowed to separate from the aqueous layer. The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 (polystyrene gel) using a mixture of ethyl acetate and cyclohexane (1/1, v/v) as eluent and an automated gel permeation chromatograph. The residue-containing GPC fraction is concentrated, re-dissolved in the HPLC solvent and analysed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS) in positive ion mode.

Extraction module E7 – Oilseed Rape

A 10 g specimen is extracted with 25 mL of acetone and 225 mL of acetonitrile in the presence of 20 g Calflo E and 10 g Celite. The suspension is blended intensively and filtered with suction through a paper filter in a Buechner porcelain funnel. Then the filtrate is filtered through a dry fluted filter covered with Calflo E into a graduated cylinder. The volume of the filtrate is measured, and transferred, rinsing with acetone, into a round-bottomed flask. Isooctane is added, and the solution is reduced using rotary-evaporation to approximately 1 mL. Last traces of solvent are removed with a gentle stream of air at room temperature. The weight of the residue is determined. The evaporated residue of the organic phase is dissolved in 10 mL of ethyl acetate/cyclohexane (1/1, v/v) and cleaned up by gel permeation chromatography on Bio Beads S-X3. The residue-containing GPC fraction is concentrated, re-dissolved in the HPLC solvent and analysed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS) in positive ion mode.

Results and discussions

Table A 3: Recovery results from method validation of cymoxanil using method DFG S19

Matrix	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range
Tomato	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	71	12	59-78
	0.1 (n = 5)	83	9.0	74-88
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	77	10	64-85
	0.1 (n = 5)	86	8.3	76-94
Grape	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	87	7.4	76-92
	0.1 (n = 5)	74	4.3	71-78
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	87	4.4	82-92
	0.1 (n = 5)	75	3.9	73-80
Oilseed rape	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	87	5.9	81-93
	0.1 (n = 5)	88	7.6	84-100
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	94	6.5	86-100
	0.1 (n = 5)	91	8.9	86-105
Wheat grain	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	91	6.4	85-100
	0.1 (n = 5)	102	7.3	89-107
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	91	2.5	85-100
	0.1 (n = 5)	100	4.4	92-103

Table A 4: Characteristics for the analytical method used for validation of cymoxanil residues in plant products

	Cymoxanil
Specificity	The concentration of cymoxanil in the final extracts was determined by LCMS/MS with two mass transitions. No significant interferences from the specimen matrix (tomato, grape, oilseed rape and wheat grain) were detected at the retention time corresponding to cymoxanil in any of the control samples.
Calibration (type, number of data points)	0.250 µg/L to 25 µg/L (n=6) for both transitions. Correlation coefficients of $r^2 > 0.99$ were achieved.
Calibration range	Calibration range: 0.250 - 25 µg/L for both transitions.

Assessment of matrix effects is presented	Yes.
Limit of determination/quantification	LOQ: 0.010 mg/kg. LOD: 0.003 mg/kg.

Conclusion

The method meets the EU criteria with respect to linearity, precision (repeatability), accuracy (recovery), and specificity. This method is suitable for enforcement purposes.

A 2.1.2.1.1.2 Independent laboratory validation

Reference: KCP 5.2.1

Report: Independent Laboratory Validation of Multi-Residue Method DFG S19 for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain using LC-MS/MS.

[redacted] (2013)

Report No: DuPont-35770

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

The method was independently validated in tomato, wheat grain, grapes and oilseed rape.

Results and discussions

Table A 5: Recovery results from independent laboratory validation of cymoxanil using method DFG S19

Matrix	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Recovery Range**
Tomato	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	77	5.3	71-82

Matrix	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Recovery Range**
	0.1 (n = 5)	81	2.0	79-83
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	77	7.0	71-83
	0.1 (n = 5)	84	2.2	82-86
Grape	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	104	4.1	100-111
	0.1 (n = 5)	99	3.3	95-102
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	103	7.9	93-111
	0.1 (n = 5)	96	6.7	89-104
Oilseed rape	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	75	1.7	74-77
	0.1 (n = 5)	74	3.5	70-77
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	72	2.3	71-74
	0.1 (n = 5)	75	1.8	74-77
Wheat grain	Mass transition 199→128 (quantification)			
	0.01 (n = 5)	91	11	83-107
	0.1 (n = 5)	105	3.8	99-109
	Mass transition 199→111(confirmation)			
	0.01 (n = 5)	91	14	81-110
	0.1 (n = 5)	108	1.5	106-110

Table A 6: Characteristics for the analytical method used for independent laboratory validation of cymoxanil residues in plant products

	Cymoxanil
Specificity	The concentration of cymoxanil in the final extracts was determined by LCMS/MS with two mass transitions. No significant interferences from the specimen matrix (tomato, grape, oilseed rape and wheat grain) were detected at the retention time corresponding to cymoxanil in any of the control samples.
Calibration (type, number of data points)	0.250 µg/L to 25 µg/L (n=6) for both transitions. Correlation coefficients of $r^2 > 0.99$ were achieved.
Calibration range	Calibration range: 0.250 - 25 µg/L for both transitions.
Assessment of matrix effects is presented	Yes.
Limit of determination/quantification	LOQ: 0.010 mg/kg. LOD: 0.003 mg/kg.

Conclusion

The method meets the EU criteria with respect to linearity, precision (repeatability), accuracy (recovery), and specificity. This method is suitable for enforcement purposes.

A 2.1.2.1.1.3 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.1.2.1.1.4 Extraction efficiency

Not required.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

A 2.1.2.5.1 Method DuPont

A 2.1.2.5.1.1 Method validation

Reference:	KCP 5.2.5
Report	Analytical method for the determination of cymoxanil and IN-KQ960 in water (pond, stream, well, and tap) using LC/MS/MS [redacted] (2010) Report No.: DuPont-27500, Revision No. 1
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Resi-

due Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

EPA Field Test Data Reporting Guideline, Environmental Chemistry Methods and Associated Independent Laboratory Validation, OCSPP 850.6100

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Water samples were prepared by placing 10 mL of test sample into a culture tube, removing an aliquot of water equal to the volume of the intended fortification, and fortifying with appropriate spiking solution. Analyses were accomplished by direct injection of the water samples without any cleanup or concentration onto the LC/MS/MS using a Phenomenex Luna C8 (150 × 2 mm, 5-µm particle) column and mobile phases of methanol and 0.1% formic acid in water. Detection of the analytes was by electrospray mass spectrometry (ESI-MS) in the positive ion mode.

Results and discussions

Fortification recovery data for samples analysed for cymoxanil and IN-KQ960 are summarised in the tables below. The mean cymoxanil and IN-KQ960 recoveries at each fortification level were in the range 79% to 104%. The overall RSD for both fortification levels was less than 5% for both cymoxanil and IN-KQ960 which indicate that the method demonstrates good precision for both analytes at the validation levels (LOQ and 10x LOQ). These results demonstrate that the method has satisfactory accuracy and repeatability.

Table A 7: Recovery results from the method validation of cymoxanil in water from different sources

Matrix	**Analyte**	**Fortification level (µg/L) (n = x)**	**Mean recovery (%)**	**RSD (%)**
Pond water	Cymoxanil *m/z* 199→128	0.1 (n=5)	81	5.3
		1.0 (n=5)	79	3.3
Stream water	Cymoxanil *m/z* 199→128	0.1 (n=5)	84	3.8
		1.0 (n=5)	85	1.0
Well water	Cymoxanil *m/z* 199→128	0.1 (n=5)	96	2.8
		1.0 (n=5)	93	3.2
Drinking water	Cymoxanil *m/z* 199→128	0.1 (n=5)	92	2.8

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
		1.0 (n=5)	94	0.8

Table A 8: Recovery results from the method validation of IN-KQ960 in water from different sources

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Pond water	IN-KQ960 *m/z* 217→146	0.1 (n=5)	93	4.7
		1.0 (n=5)	98	2.2
Stream water	IN-KQ960 *m/z* 217→146	0.1 (n=5)	98	3.8
		1.0 (n=5)	99	1.4
Well water	IN-KQ960 *m/z* 217→146	0.1 (n=5)	99	6.4
		1.0 (n=5)	104	2.1
Drinking water	IN-KQ960 *m/z* 217→146	0.1 (n=5)	98	6.1
		1.0 (n=5)	103	2.1

Table A 9: Characteristics for the analytical method used for validation of cymoxanil and IN-KQ960 residues in drinking and surface water

	Cymoxanil	IN-KQ960
Specificity	No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil and IN-KQ960 in any of the control specimens.	
Calibration (type, number of data points)	-	-
Calibration range	-	-
Assessment of matrix effects is presented	No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil and IN-KQ960 in any of the control specimens.	
Limit of determination/quantification	The LOQ determined in this method was 0.1 µg/L for all matrices tested. The limit of detection (LOD) was estimated to be one-third of the LOQ, or 0.03 µg/L.	

Conclusion

The method meets the EU criteria with respect to precision (repeatability), accuracy (recovery), and specificity. This method is suitable for enforcement purposes.

A 2.1.2.5.1.2 Independent laboratory validation

Reference:	KCP 5.2.5
Report	Independent Laboratory Validation for the Determination of Residues of Cymoxanil and IN-KQ960 in Water (Drinking and Stream) using LC-MS/MS [redacted] (2013) Report No.: DuPont-35792
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. EPA Field Test Data Reporting Guideline, Environmental Chemistry Methods and Associated Independent Laboratory Validation, OCSPP 850.6100.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Water samples were prepared by placing 9.9 mL of test sample into a culture tube, and fortifying with appropriate spiking solution (100 µL). Analyses were accomplished using direct injection with LC/MS/MS using a Nucleodur C8 Gravity EC 150/2 (150 × 2 mm, 5-µm particle) column and mobile phases of 0.1% ammonium hydroxide in methanol and 0.1% formic acid in water. Detection of the analytes was by electrospray mass spectrometry (ESI-MS) in the positive ion mode. Two mass transitions (quantifier and qualifier) per analyte were monitored during LC-MS/MS analysis.

Results and discussions

Fortification recovery data for samples analysed for cymoxanil and IN-KQ960 are summarised in the tables below. The mean cymoxanil and IN-KQ960 recoveries for both mass transitions at each fortification level were in the range 94% to 110%. The relative standard deviations (RSDs) of recoveries for all analytes for both mass transitions at each fortification level were in the range 1 to 6%. These results demonstrate that the method has satisfactory accuracy and repeatability.

Table A 10: Recovery results from the method validation of cymoxanil using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
Drinking water	Cymoxanil *m/z* 199→128	0.1 (n=5)	100	3	97 - 104

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
		1.0 (n=5)	101	6	96 - 110
	Cymoxanil *m/z* 199→111	0.1 (n=5)	95	4	91 - 101
		1.0 (n=5)	99	6	95 - 108
Stream water	Cymoxanil *m/z* 199→128	0.1 (n=5)	99	4	95 - 104
		1.0 (n=5)	96	2	94 - 98
	Cymoxanil *m/z* 199→111	0.1 (n=5)	97	3	94 - 100
		1.0 (n=5)	94	2	93 - 97

Table A 11: Recovery results from the method validation of IN-KQ960 using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
Drinking water	IN-KQ960 *m/z* 217→146	0.1 (n=5)	99	3	94 - 102
		1.0 (n=5)	96	6	91 - 106
	IN-KQ960 *m/z* 217→71	0.1 (n=5)	98	4	95 - 104
		1.0 (n=5)	95	6	91 - 105
Stream water	IN-KQ960 *m/z* 217→146	0.1 (n=5)	110	3	106 - 116
		1.0 (n=5)	108	1	106 - 110
	IN-KQ960 *m/z* 217→71	0.1 (n=5)	108	4	103 - 113
		1.0 (n=5)	108	2	105 - 110

Table A 12: Characteristics for the analytical method used for validation of cymoxanil and IN-KQ960 residues in drinking and surface water

	Cymoxanil	IN-KQ960
Specificity	No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil and IN-KQ960 in any of the control specimens.	
Calibration (type, number of data points)	-	-
Calibration range	-	-

	Cymoxanil	**IN-KQ960**
Assessment of matrix effects is presented	No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil and IN-KQ960 in any of the control specimens.	
Limit of determination/quantification	The LOQ determined in this method was 0.1 µg/L for all matrices tested. The limit of detection (LOD) was estimated to be one-third of the LOQ, or 0.03 µg/L.	

Conclusion

The method meets the EU criteria with respect to precision (repeatability), accuracy (recovery), and specificity. This method is suitable for enforcement purposes.

A 2.1.2.5.1.3 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.1.2.5.1.4 Extraction efficiency

Not required.

A 2.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for fludioxonil

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	**'Wakil XL' was not the representative product for the approval of metalaxyl-M. 'Wakil XL' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Wakil XL' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and cymoxanil have not been considered further.**

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

A 2.2.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted

A 2.2.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

A 2.2.1.3 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new or additional studies have been submitted.

A 2.2.1.4 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted

A 2.2.1.5 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted

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

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 2.2.2.1.1 QuEChERS (EN 15662:2009-02)

A 2.2.2.1.1.1 Method validation

Reference:	KCP 5.2.1
Report	Fludioxonil – Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS. [redacted] & [redacted] (2014). Report No. P-3446 G. Syngenta document No. CGA173506/11710.
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

The analytical method was derived from the QuEChERS (EN 15662:2009-02) multi-residue method. It is based on extraction and clean-up procedures, and subsequent LC-MS/MS determination.

Sample material (lettuce, oilseed rape seed, dried broad bean, wheat straw and whole orange) was extracted by shaking with acetonitrile, after the addition of a suitable volume of water if necessary (i.e. taking into account the natural water content of the samples). After the addition of a mixture of magnesium sulphate, sodium chloride, and buffering citrate salts (available pre-mixed commercially: dispersive SPE citrate extraction tube, Supelco 55227-U) the extracts were shaken and then centrifuged. In the case of oilseed rape seed and dried broad bean the fat was frozen out, and then an aliquot of each extract (for all matrices) was cleaned up using a pre-mixed, commercially available dispersive SPE clean up tube (Supelco 55228-U). For oilseed rape seed and dried broad bean extracts, a portion of C18 was added prior to shaking. After centrifugation, extracts were acidified with a small amount of 5% formic acid solution and diluted to within the calibration range with acetonitrile/water (20/80, v/v, containing 0.1% formic acid) and blank matrix (if necessary). Final determination was by high-performance liquid chromatography with triple quadrupole mass-spectrometric detection (LC MS/MS), monitoring for a primary (m/z 247→ 169) and confirmatory (m/z 247→ 126) transition.

The QuEChERS method was validated for a wide range of crop types:

Lettuce (high water content), oilseed rape seed (high oil content), dried broad bean (high protein content), wheat straw (dry commodity) and whole orange (high acid content).

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at a higher level: 0.10 mg/kg (oilseed rape seed), 0.50 mg/kg (dried broad bean and wheat straw), 10 mg/kg (whole orange) and 15 mg/kg (lettuce). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 825/00 rev.8.1 16/11/10) demonstrate the method has satisfactory accuracy.

The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore according to the EU guidance (SANCO 825/00 rev.8.1 16/11/10) demonstrate the method has satisfactory repeatability.

Table A 13: Recovery results from method validation of fludioxonil using the QuEChERS analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Lettuce	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	110	1	109-112
		15 (n = 5)	107	3	102-109
		Overall	*108*	*3*	*102-112*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	110	2	108-113
		15 (n = 5)	107	2	103-110
		Overall	*109*	*2*	*103-113*
Whole orange	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	110	2	106-113
		10 (n = 5)	99	5	93-103
		Overall	*105*	*7*	*93-113*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	110	4	104-116
		10 (n = 5)	97	4	91-101
		Overall	*104*	*8*	*91-116*
Wheat straw	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	91	3	87-95
		0.5 (n = 5)	96	5	88-98
		Overall	*93*	*5*	*87-98*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	91	4	86-95
		0.5 (n = 5)	96	4	88-98
		Overall	*93*	*5*	*86-98*
Dried broad bean	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	85	4	81-89
		0.5 (n = 5)	83	5	81-90
		Overall	*84*	*4*	*81-90*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	85	3	82-87
		0.5 (n = 5)	83	4	81-89
		Overall	*84*	*3*	*81-89*
Oil seed rape	Fludioxonil	0.01 (n = 5)	100	2	98-102

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
seed	*m/z* 247→ 169 (quantification)	0.1 (n = 5)	98	1	97-100
		Overall	*99*	*2*	*97-102*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	100	1	98-101
		0.1 (n = 5)	98	2	95-101
		Overall	*99*	*2*	*95-101*

Table A 14: Characteristics for the analytical method used for validation of fludioxonil residues in plants

	Fludioxonil
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention times of interest.
Calibration (type, number of data points)	The linearity was tested using standard solutions in solvent (for lettuce and dried broad bean) or matrix-matched standard solutions (for whole orange, oilseed rape seed, wheat straw), over a concentration range of 0.05 ng/mL to 5.0 ng/mL. Linearity was tested for both the primary and confirmatory MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9993 to 1.000 were obtained for fludioxonil.
Calibration range	0.05 ng/mL to 5.0 ng/mL.
Assessment of matrix effects is presented	Yes: Insignificant matrix effects (suppression or enhancement, ≤ ± 20%) were observed for lettuce and dried broad bean matrices when matrix-matched standards and standards in solvent (0.1% formic acid in acetonitrile/water (20/80, v/v)) were compared. Significant matrix effects on LC-MS/MS response were observed for whole orange, oilseed rape seed and wheat straw matrices; thus, whole orange, oilseed rape seed and wheat straw extracts were evaluated using matrix-matched standards
Limit of determination/quantification	The limit of quantification was established at 0.01 mg/kg. The limit of detection (LOD) was demonstrated to be ≤ 0.002 mg/kg for both the primary and confirmatory transitions, for all matrices tested.

Conclusion

The QuEChERS method has been demonstrated to be a reliable and accurate procedure for the determination of fludioxonil in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.1.1.2 Independent laboratory validation

Reference: KCP 5.2.1

Report	Fludioxonil – Independent Laboratory Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS. [redacted] & [redacted] (2014). Report No. 20140189. Syngenta document No. CGA173506/11723.
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

The QuEChERS method was independently validated in lettuce (high water content), oilseed rape seed (high oil content), dried broad bean (high protein content) and wheat straw (high starch content).

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and 10 x LOQ (0.10 mg/kg) for oilseed rape seed matrix; at the LOQ (0.01 mg/kg) and 50 x LOQ (0.50 mg/kg) for dried broad bean and wheat straw matrices; and at the LOQ (0.01 mg/kg) and 1500 x LOQ (15 mg/kg) for lettuce matrix. Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) demonstrate the method has satisfactory accuracy.

The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each crop tested during method validation were ≤20% and therefore according to the EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) demonstrate the method has satisfactory repeatability.

Table A 15: Recovery results from independent laboratory validation of fludioxonil using the QuEChERS analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Lettuce	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	109	4.1	101-112
		15 (n = 5)	100	2.5	97-103
		Overall	*105*	*5.3*	*97-112*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	104	6.8	92-109
		15 (n = 5)	102	1.4	100-104
		Overall	*103*	*4.7*	*92-109*

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Wheat straw	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	94	10.5	82-107
		0.5 (n = 5)	94	15.5	76-112
		Overall	*94*	*12.5*	*76-112*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	84	8.8	78-97
		0.5 (n = 5)	93	15.0	78-112
		Overall	*89*	*12.9*	*78-112*
Oilseed rape seed	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	77	18.1	59-90
		0.1 (n = 5)	107	2.7	103-109
		Overall	*92*	*19.9*	*59-109*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	83	3.8	78-87
		0.1 (n = 5)	110	2.1	107-112
		Overall	*96*	*15.2*	*78-112*
Dried broad beans	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	74	15.5	60-86
		0.5 (n = 5)	91	7.4	82-98
		Overall	*82*	*15.4*	*60-98*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	81	14.2	61-89
		0.5 (n = 5)	90	6.5	81-95
		Overall	*85*	*11.5*	*61-95*

Table A 16: Characteristics for the analytical method used for independent laboratory validation of fludioxonil residues in plants

	Fludioxonil
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention times of interest.
Calibration (type, number of data points)	The linearity was tested using standard solutions in solvent or matrix-matched standard solutions, over a concentration range of 0.05 ng/mL to 10 ng/mL. Linearity was tested for both the primary and confirmatory MS/MS transitions At least six different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9987 to 1.000 were obtained for fludioxonil.
Calibration range	0.05 ng/mL to 10 ng/mL.
Assessment of matrix effects is presented	Yes: Insignificant matrix effects (suppression or enhancement, < ± 20%) were observed for oilseed rape seed matrix when matrix-matched standards and standards in solvent (acetonitrile: water (20:80, v/v, containing 0.1% formic acid)) were compared. Significant matrix effects (suppression) on LC-MS/MS response were observed for wheat straw, lettuce and dried broad bean matrices. Matrix matched linearity standards were used for quantification for all crop matrices.

	Fludioxonil
Limit of determination/quantification	The limit of quantification was established at 0.01 mg/kg. The limit of detection (LOD) was demonstrated to be ≤ 0.002 mg/kg for both the primary and confirmatory transitions, for all matrices tested.

Conclusion

The repeatability and specificity of the method have been independently demonstrated, and QuEChERS method EN 15662:2009-2 is therefore considered valid for the determination of residues of fludioxonil in crop matrices to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.1.1.3 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

A 2.2.2.2.1 GRM025.03A

A 2.2.2.2.1.1 Method validation

Reference:	KCP 5.2.2
Report	Fludioxonil – Analytical Method for the Determination of Residues of Total Fludioxonil (CGA173506) and Metabolites as CGA192155 in Animal Matrices (Milk, Eggs, Muscle, Fat, Liver, Kidney and Whole Blood). Final Determination by LC-MS/MS [redacted] (2009) Report No. GRM025.03A Version 2. Syngenta document No. CGA173506_11402, VV-127758
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.7). OPPTS 860.1340.
Deviations:	No (analytical method description).
GLP:	No (analytical method description).
Acceptability:	Yes.

Reference:	KCP 5.2.2
Report	Fludioxonil: Validation of Residue Method GRM025.03A for Total Fludioxonil (CGA173506) and Metabolites as CGA192155 in Animal Matrices (Milk, Eggs, Muscle, Fat, Liver, Kidney and Whole Blood). [redacted] (2009) Report No. T-001341-08-REG. Syngenta document No. CGA173506/11403, VV-382790
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.7). OPPTS 860.1340.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Method GRM025.03A determines fludioxonil and its metabolites oxidisable to CGA192155 as CGA192155 (expressed as fludioxonil equivalents).

Samples are homogenised and then extracted by refluxing with ammonium hydroxide/acetonitrile (80/20, v/v). After filtration the aqueous phase is acidified and partitioned with toluene following addition of sodium chloride. Conversion of CGA173506 and its metabolites to CGA192155 is carried out by heating in the presence of potassium permanganate and aqueous sodium hydroxide; the oxidation is then quenched with sodium metabisulfite, the extracts are filtered, acidified, and partitioned into dichloromethane/ ethyl acetate (80/20, v/v). After evaporation, the residues are dissolved in acetonitrile/water (50/50, v/v) and determined as total fludioxonil by LC-MS/MS.

The analytical procedure converts fludioxonil and structurally-related metabolites to the common moiety CGA192155. A molecular weight correction factor of 1.23 is applied when calculating procedural recovery values and quantifying residues of CGA192155.

Results and discussions

A reagent blank sample was analysed, control samples were analysed in duplicate and samples fortified with fludioxonil were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) for each matrix tested.

Acceptable mean recoveries of between 70% and 110% with relative standard deviations (RSD) of <20% were found for both transitions for all analytes in all matrices.

Table A 17: Recovery results from method validation of total fludioxonil residues using the analytical method GRM025.03A

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
Eggs	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	109	2	107-113
		0.1 (n=5)	97	2	95-99
		Overall	*103*	*6*	*95-113*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	103	2	99-105
		0.1 (n=5)	97	2	95-100
		Overall	*100*	*3*	*95-105*
Milk	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	87	11	74-98
		0.1 (n=5)	78	2	76-80
		Overall	*83*	*9*	*74-98*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	84	6	80-92
		0.1 (n=5)	80	2	77-81
		Overall	*82*	*5*	*77-92*
Muscle	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	77	6	70-82
		0.1 (n=5)	78	3	75-80
		Overall	*78*	*4*	*70-82*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	79	4	74-82
		0.1 (n=5)	79	2	76-80
		Overall	*79*	*3*	*74-82*
Liver	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	86	2	85-89
		0.1 (n=5)	87	1	87-88
		Overall	*87*	*1*	*85-89*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	88	1	86-89
		0.1 (n=5)	87	1	86-87
		Overall	*87*	*1*	*86-89*
Kidney	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	79	4	74-82
		0.1 (n=5)	82	3	80-84
		Overall	*80*	*4*	*74-84*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	79	6	73-83
		0.1 (n=5)	81	2	80-83
		Overall	*80*	*4*	*73-83*
Fat	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	78	2	76-81
		0.1 (n=5)	79	2	77-81
		Overall	*78*	*2*	*76-81*
	CGA192155 *m/z* 201→ 91	0.01 (n=5)	72	5	65-75
		0.1 (n=5)	79	2	77-81

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
	(confirmation)	*Overall*	*75*	*6*	*65-81*
Blood	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	80	2	77-82
		0.1 (n=5)	83	1	82-84
		Overall	*81*	*2*	*77-84*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	79	3	77-82
		0.1 (n=5)	83	1	82-84
		Overall	*81*	*3*	*77-84*

Table A 18: Characteristics for the analytical method used for validation of fludioxonil residues in animal matrices

	Total fludioxonil
Specificity	Residues of fludioxonil as CGA192155 measured in control samples were <30% of the limit of quantification (LOQ) in any of the control or reagent blank samples.
Calibration (type, number of data points)	Standard solutions containing CGA192155 at concentrations ranging from 0.0005 to 0.05 µg/mL were analysed. The response of the LC-MS/MS system was shown to be linear for CGA192155 primary transition and the confirmatory transition over the concentration range tested. Correlation coefficients ranged from 0.9994 to 0.9999.
Calibration range	0.0005 to 0.05 µg/mL.
Assessment of matrix effects is presented	The effect of matrix on the LC-MS/MS response was assessed by preparing standards with and without matrix and comparing the peak areas of CGA192155 at equivalent concentrations. Matrix effects (enhancement or suppression) were greater than 10% for several matrices, and the use of matrix-matched standards is recommended.
Limit of determination/quantification	The validated limit of quantification for fludioxonil and metabolites as CGA192155 in animal tissues was 0.01 mg/kg for fludioxonil (= 0.0081 mg/kg for CGA192155) when measured as CGA192155 for all animal matrices tested.

Conclusion

Method GRM025.03A has been demonstrated to be a reliable and accurate procedure for the determination of fludioxonil as CGA192155 in animal matrices using commercially available laboratory equipment and reagents. Bovine milk, muscle, liver, fat, kidney, hen's eggs and bovine blood have been used as representative matrices. The limit of quantification is 0.01 mg/kg for all matrices tested using either the primary or confirmatory transition. The method complies with the requirements of SANCO 3029/99 rev 4 11/07/00 and SANCO 825/00 rev.8.1 16/11/10.

A 2.2.2.2.1.2 Independent laboratory validation

Reference: KCP 5.2.2

Report	Fludioxonil: Magnitude of Residues in Animal Tissues Following Repeated Oral Administration to the Laying Hen. [redacted] (2009). Report No. 1983/108-D2149. Syngenta document No CGA 173506_11440
Guideline(s):	European Union Council Directive 91/414/EEC of 15 July 1991, as amended by Commission Directive 96/68/EC of 21 October 1996. SANCO/7031/VI/95 (Livestock Feeding Studies - Appendix G). OECD guideline 50.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Analytical method GRM025.03A was used to determine the content of CGA192155 in egg and tissue samples. The method involved extraction of homogenised samples by refluxing with ammonium hydroxide/acetonitrile (80/20, v/v). Following filtration the aqueous phase was acidified and partitioned with the addition of salt and toluene. Fludioxonil and its metabolites were converted to CGA192155 by heating in the presence of potassium permanganate and aqueous sodium hydroxide. The oxidation was then quenched by the addition of sodium metabisulphite, with the extracts filtered and acidified prior to partition into dichloromethane/ethyl acetate (80/20, v/v). Following evaporation residues were dissolved in acetonitrile/water (50/50, v/v) with final quantification by Liquid Chromatography with tandem Mass Spectrometry detection (LC-MS/MS) in the multiple reaction monitoring (MRM) mode.

All results reported were quantified using the transition, m/z 200.9→91.0 with m/z 200.9→156.9 employed for confirmation.

The analytical procedure converts fludioxonil and structurally-related metabolites to the common moiety CGA192155. A molecular weight correction factor of 1.23 is applied when calculating procedural recovery values and quantifying residues of CGA192155.

The extraction and clean-up procedure was identical to the primary method GRM025.03A.

Results and discussions

Recovery of CGA192155 from each matrix fortified at the LOQ and expected residues levels was determined in quintuplicate.

Acceptable mean recoveries of between 70% and 110% with relative standard deviations (RSD) of <20% were found for both transitions in all matrices.

Table A 19: Recovery results from independent laboratory validation of total fludioxonil using the analytical method GRM025.03A

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range
Eggs	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	70	5	66-74
		0.6 (n=5)	77	3	73-79
		Overall	*74*	*6*	*66-79*

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	70	7	64-76
		0.6 (n=5)	77	3	74-80
		Overall	*74*	*7*	*64-80*
Poultry muscle	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	90	4	84-94
		0.1 (n=5)	73	2	72-75
		Overall	*82*	*11*	*72-94*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	95	5	88-99
		0.1 (n=5)	73	2	71-74
		Overall	*84*	*14*	*71-99*
Poultry fat	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	83	7	76-90
		0.1 (n=5)	76	4	73-80
		Overall	*80*	*7*	*73-90*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	91	6	86-99
		0.1 (n=5)	77	4	74-81
		Overall	*84*	*10*	*74-99*
Poultry liver	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	*94*	*11*	79-108
		0.1 (n=5)	*84*	*5*	77-88
		Overall	*89*	*10*	*77-108*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	102	10	88-114
		0.1 (n=5)	84	5	77-88
		Overall	*93*	*13*	*77-114*

Table A 20: Characteristics for the analytical method used for independent laboratory validation of fludioxonil residues in animal matrices

	Total fludioxonil
Specificity	Residues of fludioxonil as CGA192155 measured in control samples were <30% of the limit of quantification (LOQ) in any of the control or reagent blank samples.
Calibration (type, number of data points)	Standard solutions containing CGA192155 at concentrations ranging from 0.0005 to 0.05 µg/mL were analysed. The response of the LC-MS/MS system was shown to be linear for CGA192155 primary transition and the confirmatory transition over the concentration range tested. Correlation coefficients were > 0.99.
Calibration range	0.0005 to 0.05 µg/mL.
Assessment of matrix effects is presented	No significant interferences arising from animal matrices were observed and there was no significant enhancement or suppression of detector response.
Limit of determination/quantification	The limit of quantification for fludioxonil residues was established at 0.01 mg/kg.

Conclusion

The study is suitable as an independent laboratory validation study.

A 2.2.2.2.1.3 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.2.2.2.1.4 Extraction efficiency

The extraction procedures used in analytical methods GRM025.03 and AG-616B are very similar, so extractability efficiency of analytical method GRM02.03 can be demonstrated by reference to AG-616. Radio validation of analytical method AG-616 has been carried out and reported (Denmark, 2005).

A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted.

A 2.2.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted.

A 2.2.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

A 2.2.2.5.1 GRM025.01A

A 2.2.2.5.1.1 Independent laboratory validation

Reference:	KCP 5.2.5
Report	Fludioxonil- Independent Laboratory Validation (ILV) of Analytical Method GRM025.01A for the Determination of Residues of Fludioxonil (CGA173506) and its Metabolites CGA192155 and CGA339833 in Water. [redacted] (2016) Report Number CGA173506DW. Syngenta File No. CGA173506_11942.
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Analytical method GRM025.01A was independently validated in drinking water samples for fludioxonil and its metabolites CGA192155 and CGA339833, at the limit of quantification (LOQ) of the method (0.05 µg/L) and at 10 x LOQ (0.5 µg/L).

By following the method and washing the SPE column with 1 mL of water, the method was successfully validated for fludioxonil and CGA192155. However, CGA339833 failed the validation. The method procedure was slightly modified and the SPE column was washed with 2 mL of water. Using this modified procedure, the method was successfully validated for fludioxonil and CGA339833 but not for CGA192155. Therefore, two SPE columns should be prepared and post application of the water specimens, one SPE columns should be washed with 1 mL of water to enable fludioxonil and CGA192155 analysis and the other one should be washed with 2 mL of water to enable fludioxonil and CGA339833 analysis.

Results and discussions

Recoveries at the LOQ and at ten times the LOQ were determined in quintuplicate.

Acceptable mean recoveries of between 70% and 110% with relative standard deviations (RSD) of <20% were found for fludioxonil, CGA129155 and CGA339833 for both transitions.

Table A 21: Recovery results from independent laboratory validation of fludioxonil using the analytical method – original procedure

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery range
Drinking water	Fludioxonil *m/z* 247→ 180 (quantification)	0.05 (n=5)	78	5	74-84
		0.5 (n=5)	78	4	75-83
		Overall	*78*	*4*	*74-84*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.05 (n=5)	78	5	73-82
		0.5 (n=5)	79	4	76-83
		Overall	*79*	*4*	*73-83*
	CGA192155 *m/z* 201→ 91 (quantification)	0.05 (n=5)	80	5	77-86
		0.5 (n=5)	76	5	70-79
		Overall	*78*	*6*	*70-86*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.05 (n=5)	81	6	76-88
		0.5 (n=5)	75	6	70-80
		Overall	*78*	*6*	*70-88*

Table A 22: Recovery results from independent laboratory validation of fludioxonil using the analytical method – modified procedure

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery range
Drinking water	Fludioxonil *m/z* 247→ 180 (quantification)	0.05 (n=5)	76	6	70-81
		0.5 (n=5)	77	5	71-80
		Overall	*77*	*5*	*70-81*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.05 (n=5)	80	9	69-87
		0.5 (n=5)	78	5	73-81
		Overall	*79*	*7*	*69-87*
	CGA339833 *m/z* 311→ 267 (quantification)	0.05 (n=5)	78	3	75-81
		0.5 (n=5)	84	5	77-87
		Overall	*81*	*5*	*75-87*
	CGA339833 *m/z* 311→ 66 (confirmation)	0.05 (n=5)	78	5	72-82
		0.5 (n=5)	83	6	75-88
		Overall	*81*	*6*	*72-88*

Table A 23: Characteristics for the analytical method used for independent laboratory validation of fludioxonil residues in drinking water

	Fludioxonil	**CGA129155**	**CGA339833**
Specificity	Residues in control samples and reagent blanks were less than 30% of the LOQ.		
Calibration (type, number of data points)	The detector response for fludioxonil, CGA192155 and CGA339833 was shown to be linear over the range 1 pg to 500 pg injected (n=9). Straight lines with correlation coefficients ranging from 0.9986 to 0.9999 were obtained.		
Calibration range	0.1 µg/L to 50 µg/L when using a 10 µL injection volume.		
Assessment of matrix effects is presented	No significant interferences, above 30% of the LOQ, arising from the drinking water matrix, the lab ware, reagents or solvents have been observed at the retention times of interest.		
Limit of determination/quantification	The limit of quantification for fludioxonil, CGA192155 and CGA339833 was established at 0.05 µg/L.		

Conclusion

Analytical method GRM025.01A has been independently validated for the determination of fludioxonil, CGA192155 and CGA339833 in drinking water with a limit of quantification of 0.05 µg/L.

A 2.2.2.5.1.2 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.2.2.5.1.3 Extraction efficiency

Not required for an ILV study.

A 2.2.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.3 Analytical methods for metalaxyl-M

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority: HSE Chemicals Regulation Division (CRD), UK

The following study was evaluated for the B6 DAR. The method (BFI089MS) has been validated in the box below.

Principle of the method

CGA226048 concentrations were determined using mouse blood and plasma diluted at a ratio of 1:3 v/v with 1 % (v/v) formic acid in acetonitrile. The samples are then analysed by HPLC-MS/MS using the following conditions:

HPLC-MS/MS conditions:

Analytical column:	50 x 2.1 mm Luna C18, 5µm		
Target column temperature:	Ambient		
Injection volume:	2-15 µL		
Mobile phase A: Mobile phase B:	0.1 % (v/v) Formic acid Methanol		
Flow rate:	250 µL/min		
Gradient	Time (min)	Phase A (%)	Phase B (%)
	0.00	40	60
	2.50	0	100
	4.00	0	100
	4.10	40	60
	5.2	40	60
Ionisation:	Electro Spray (ESI)		
Analyte:	Transitions	Polarity	Expected Retention Time
CGA 226048	352 → 220 352 → 192	Positive	approx. 1.1 min

Specificity/ Confirmation of analyte identity

Chromatograms were not provided; however, LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. Additionally, the method was only used for the purpose of detecting the presence of CGA 226048 in the samples – not quantification of the levels found. Additionally, the method utilised a reference standard for CGA 226048 and matrix matched standards; therefore, the method is considered sufficiently specific to detect the presence of CGA 226048 in the samples, despite lack of chromatograms.

Matrix effects

Matrix-matched standards were used.

Linearity

Linearity was demonstrated by the analysis of 8 standards of increasing concentration. Duplicate measurements were made. The linear range covered 2.5 – 1000 ng/mL (equivalent to 10 – 4000 ng/mL blood/plasma in the sample). The response was not linear; the equation of the curve was found to fit a polynomial regression, with a correlation coefficient (r^2) of at least 0.9997. The equations of the calibration curves are presented in the table below. Samples at higher concentrations were diluted, so as to remain within the calibration curve. This is acceptable.

Precision (repeatability)

Precision was determined from the accuracy recovery data. Four samples were prepared at each fortification level; the % RSD at each fortification level was < 20%.

Accuracy (recovery)

Recovery (quality control) samples were prepared by fortifying blank plasma/blood samples with CGA226048 and analysing them by the method described. The fortification levels were in the range 25 to 3200 ng/mL. Four replicates were prepared at each fortification level (2 for blood and 2 for plasma). Mean recovery levels for both transitions were within the range 93 – 95 % and are acceptable.

Table 127: Summary of validation data – blood/plasma

LOQ (ng/mL)	Recovery fortification level (ng/mL)	Matrix	Recoveries %		Repeatability % RSD (n)	Linearity	Equation of calibration curve
			Values	Mean			
25	25	Blood	106, 109	107	2.4 (4)	2.5 – 1000 ng/mL (equivalent to 10 – 4000 ng/mL blood/plasma) n=8	$y = -1.25x10^{-7} x^2 + 5.78x10^{-3} x + 4.15x10^{-3}$ r=0.9971
		Plasma	103, 108				
	200	Blood	106, 116	109	4.2 (4)		
		Plasma	107, 108				
	3200	Blood	107, 108	108	3.3 (4)		
		Plasma	105, 113				

Limit of Quantification

The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 25 ng/mL.

Conclusion

The method is not fully validated in accordance with SANCO/3029/99 rev.4 as only 4 determinations were made at each level and the lack of chromatograms (see specifity above); however, the method is suitable for the determination of CGA 226048 in blood/plasma samples with an LOQ of 25 ng/mL. Additionally, the method was only used in the range finding phase of the study to detect the presence of the analyte, the method is considered sufficiently validated for this purpose.

A 2.3.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.3.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

A 2.3.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted

A 2.3.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted

A 2.3.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new or additional studies have been submitted.

A 2.3.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted

A 2.3.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 2.3.2.1.1 Analytical Method QuEChERS (BS EN 15662:2008)

A 2.3.2.1.1.1 Independent Laboratory Validation (tomatoes and oilseed rape)

Reference:	KCP 5.2.1
Report	Metalaxyl-M – Independent Laboratory Validation (ILV) of an Analytical Method for Determination of Residues of Metalaxyl-M in Crops. [redacted] (2012). Report No S11-03712. Syngenta File No. CGA329351_11643 (Syngenta Task No. TK0055473)
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Principle of the method

The specimens were analysed for residues of Metalaxyl-M using QuEChERS Multiple Residue Method and detected by means of liquid chromatography with mass selective detection (module LC-MS/MS). The limit of quantitation (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.003 mg/kg.

Recovery Findings
Summaries of the results for metalaxyl-M are presented in the Tables below

Table A 24: Recovery results from method validation of metalaxyl- M in crops: primary transition m/z 280 → 192

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Tomato	0.01	97, 106, 104, 93, 97	5	99	5.0	93-106
	0.10	102, 103, 104, 105, 98	5	102	3.0	98-105
	Overall	-	10	101	4.0	93-106

Oilseed Rape	0.01	91, 97, 94, 88, 90	5	93	4.0	88-97
	0.10	93, 92, 93, 91, 88	5	91	2.0	88-93
	Overall	-	10	92	3.0	88-97

Table A 25: Recovery results from validation for metalaxyl-M in crops: confirmatory transition m/z 280 → 160

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Tomato	0.01	97, 106, 104, 93, 97	5	99	5.0	93-106
	0.10	102, 103, 104, 105, 98	5	102	3.0	98-105
	Overall	-	10	101	4.0	93-106
Oilseed Rape	0.01	91, 97, 94, 88, 90	5	93	4.0	88-97
	0.10	93, 92, 93, 91, 88	5	91	2.0	88-93
	Overall	-	10	92	3.0	88-97

RSD: relative standard deviation

Table A 26: Characteristics for the analytical method used for independent laboratory validation of metalaxtyl-M residues in tomatoes and oilseed rape

	Metalaxy-M
Specificity / Interferences	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required (SANCO/825/00 rev.8.1, 16/11/2010). No significant interferences arising from the matrices, the labware, reagents or solvents have been observed at the retention times of interest
Linearity / Calibration	The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.25 ng/mL to 50 ng/mL). Standards at seven different concentrations were injected and the response plotted against standard concentration for both primary and confirmatory transitions. Straight lines with coefficients of determination $R^2 \geq 0.99$ were obtained for metalaxyl-M..
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 87% and 112% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.
Repeatability	The relative standard deviations (RSDs) of metalaxyl-M recoveries at each fortification level and overall for each matrix tested during method validation were < 20% and therefore accord-ing to the EU guideline (SANCO 3029/99 rev. 4 11/7/00) demonstrate the method was satisfac-tory repeatability.
Limit of quantification	The limit of quantitation was 0.01 mg/kg for tomato and oilseed rape. No interfering peaks around the retention time of metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification.

	Metalaxy-M
Limit of detection	The limit of quantitation was calculated to be 0.003 mg/kg for the primary and confirmatory transition for the matrices tomato and oilseed rape.
Matrix effects	Significant matrix effects (suppression) were found in the crop matrices tested during method validation, therefore matrix matched linearity standards were used for quantification..

A 2.3.2.1.1.2 Method validation – Difficult commodities

Comments of HSE:	Study previously evaluated.

Reference: KCP 5.2.1

Report: Metalaxyl-M – Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans by LC-MS/MS.

[redacted] (2016).

Report No RES-00055. Syngenta File No. CGA329351_11643 (Syngenta Task No. TK0308525)

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Principle of the method

Metalaxyl-M was extracted from hops and cocoa beans by hydration of the matrix using water followed by mixing with acetonitrile. After addition of QuEChERS salts, samples were vortex mixed and centrifuged. Extracts were frozen overnight to freeze-out co-extracted fats and oils. Aliquots were then further purified by addition of QuEChERS dispersive SPE reagents followed by vortex mixing and centrifugation of the extracts. Supernatants were diluted with water. Extracts were analysed for metalaxyl-M residues by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (*m/z* 280→192) and the confirmatory transition (*m/z* 280→160).

Results and discussions

Summaries of the results for metalaxyl-M are presented below.

Table A 27: Recovery results from method validation of metalaxyl-M using the QuEChERS analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Hops	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	94, 94, 91, 88, 92	92	2.9	88 – 94
		0.1 (n=5)	83, 83, 85, 82, 84	83	1.5	82 – 85
		Overall	-	*88*	*5.5*	*82 – 94*
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	101, 99, 94, 90, 98	96	4.5	90 – 101
		0.1 (n=5)	83, 81, 85, 82, 84	83	2.0	81 – 85
		Overall	-	*90*	*8.5*	*81 – 101*
Cocoa beans	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	95, 99, 96, 92, 95	95	2.7	92 – 99
		0.1 (n=5)	97, 92, 87, 87, 86	90	4.9	86 – 97
		Overall	-	92	4.8	86 – 99
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	94, 97, 93, 90, 97	94	3.2	90 – 97
		0.1 (n=5)	94, 92, 86, 89, 87	90	3.7	86 – 94
		Overall	-	92	4.0	86 – 97

Table A 28: Characteristics for the analytical method used for validation of metalaxyl-M residues in hops and cocoa bean

	Metalaxyl-M
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. The method includes two MS/MS transitions for metalaxyl-M, both of which have been validated. No significant interferences arising from the crop matrix, the lab ware, reagents or solvents have been observed at the retention time of interest.
Calibration (type, number of data points)	The linearity was tested using matrix matched standard solutions for all MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9991 to 0.9997 were obtained.
Calibration range	0.06 - 10 ng/ml
Assessment of matrix effects is presented	Significant matrix effects (i.e. suppression ≥ 20%) were observed for hops during method validation, therefore matrix matched linearity standards were used for quantification. Insignificant matrix effects (i.e. suppression ≤ 20%) were observed for cocoa beans during method validation, however matrix matched linearity standards were used for quantification.
Limit of determination/quantification	The limit of quantification for metalaxyl-M residues in the matrix tested using the QuEChERS method was established

	at 0.01 mg/kg. No interfering peaks around the retention time of metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification.

Stability of Final Extracts

The stability of final sample extracts fortified with metalaxyl-M at the LOQ level (0.01 mg/kg) was checked after a storage period of 12 days in a refrigerator at 4-8ºC against freshly prepared calibration standards. The results proved that metalaxyl-M residues in the stored fortified samples were stable. The mean recovery values for hops at the LOQ level were 95% with a RSD of ≤ 20% when re-analysed, and were found to be within 20% of the original result when re-analysed. The mean recovery values for cocoa beans at the LOQ level were 96% with a RSD of ≤ 20% when re-analysed, and were found to be within 20% of the original result when re-analysed.

Stability of Standard Solutions

The stability of stored working standard solutions of metalaxyl-M at 0.0002 µg/mL was assessed after a storage period of 15 days in a refrigerator at 4-8ºC against freshly prepared calibration standards. The mean peak area of the stored standard solution was found to be within ± 10% of the mean peak area of the freshly prepared standard solution for metalaxyl-M, demonstrating that the standard solutions were stable for the storage period assessed when stored under the described conditions.

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of metalaxyl-M in hops and cocoa beans to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.2.1.1.3 Independent laboratory validation

Comments of HSE:	Study previously evaluated.

Reference:	KCP 5.2.1
Report	Metalaxyl-M - Independent Laboratory Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans. [redacted] (2016). Report No YB27DB. Syngenta File No. CGA329351_11745.
Guideline(s):	European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 revision 8.1 (16 Nov 2010). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007). OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.
Deviations:	No.

GLP: Yes.

Acceptability: Yes.

Principle of the method

1 g sub-samples were extracted by the multi-residue QuEChERS method with extraction by homogenisation.

Samples were extracted by homogenisation with acetonitrile in the presence of buffering salts and cleaned-up by dispersive solid phase extraction. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (m/z 280 → 192) and the confirmatory transition (m/z 280 → 160). The limit of quantification of the method was 0.01 mg/kg (0.01 ppm, 10 ppb).

The QuEChERS analytical method was independently validated in two crop types; dried hops and cocoa beans.

Results and discussions

Summaries of the results for metalaxyl-M are presented below.

Table A 29: Recovery results from independent laboratory validation of metalaxyl-M using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Hops	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	102, 98, 90, 97, 93	96	4.8	90 – 102
		0.1 (n=5)	102, 95, 99, 98, 95	98	3.0	95 - 102
		Overall	-	97	3.9	90 - 102
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	105, 99, 83, 86, 89	92	10.0	83 - 105
		0.1 (n=5)	104, 100, 101, 102, 96	101	2.9	96 - 104
		Overall	-	97	8.1	83 - 105
Cocoa beans	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	91, 94, 99, 98, 94	95	3.4	91 - 99
		0.1 (n=5)	104, 102, 98, 106, 102	102	2.9	98 - 106
		Overall	-	99	4.9	91 - 106
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	90, 96, 99, 95, 99	96	3.9	90 – 99
		0.1 (n=5)	105, 102, 98, 105, 102	102	2.8	98 - 105
		Overall	-	99	4.7	90 - 105

Table A 30: Characteristics for the analytical method used for independent laboratory validation of metalaxyl-M residues in hops and cocoa

	Metalaxyl-M
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. The method includes two MS/MS transitions for metalaxyl-M, both of which have been validated. No significant interferences arising from the crop matrix, the lab ware, reagents or solvents have been observed at the retention time of interest.
Calibration (type, number of data points)	The linearity was tested using matrix matched standard solutions for all MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9973 to 0.9996 were obtained.
Calibration range	0.05 - 5 ng/ml
Assessment of matrix effects is presented	No significant matrix effects were observed in the crop matrices tested during method validation, however matrix matched linearity standards were used for quantification.
Limit of determination/quantification	The limit of quantification for metalaxyl-M residues in the matrix tested using the QuEChERS method was established at 0.01 mg/kg. No interfering peaks around the retention time of metalaxyl-M were found in any of the control samples at levels above 25% of the limit of quantification

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of metalaxyl-M in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.2.1.1.4 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions for metalaxyl-M, both of which have been validated.

A 2.3.2.1.1.5 Extraction efficiency

No extraction efficiency required for an ILV study.

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

A 2.3.2.2.1 QuEChERS – Validation (milk, egg, fat, liver, kidney and blood)

Study not required.

Reference:	KCP 5.2.2
Report	Metalaxyl-M - Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices . [redacted] (2011) Report No. S11-01732. Syngenta document No. CGA329351_11472.
Guideline(s):	OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996. EU Directive 91/414/EC (as amended by 96/46/EC 4.2) Guidance document SANCO/825/00 rev. 8.1 of 16/11/2010 of the European Commission, BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Principle of the method

5 g homogenised sub-samples were extracted by the multi-residue QuEChERS method with extraction by shaking.

Samples were extracted by homogenisation with acetonitrile and water, followed by a buffer salt mixture and cleaned-up by dispersive solid phase extraction. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (m/z 280 → 160) and the confirmatory transition (m/z 280 → 192). The limit of quantification of the method was 0.01 mg/kg (0.01 ppm, 10 ppb).

The QuEChERS analytical method was validated in seven animal matrices (milk, eggs, meat, fat, liver, kidney and blood).

Recovery Findings

Summaries of the results for Metalaxyl-M are presented below.

Table A 31: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: primary transition m/z 280 160

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery (%)	RSD (%)	Recovery Range

						(%)
Milk	0.01	97, 107, 108, 108, 106	5	105	4.4	97 – 108
	0.10	91, 107, 107, 110, 108	5	105	7.4	91 - 110
	Overall	-	10	105	5.7	91 - 110
Eggs	0.01	103, 104, 96, 105, 108	5	103	4.3	96 -108
	0.10	91, 107, 101, 105, 105	5	102	6.3	91 – 107
	Overall	-	10	103	5.1	91 – 108
Meat	0.01	88, 105, 104, 101, 110	5	102	8.1	88 -110
	0.10	92, 107, 106, 105, 104	5	103	6.0	92 – 107
	Overall	-	10	102	6.7	88 - 110
Fat	0.01	94, 109, 108, 106, 110	5	105	6.2	94 – 110
	0.10	92, 107, 108, 104, 104	5	103	6.2	92 – 108
	Overall	-	10	104	6.0	92 – 110
Liver	0.01	93, 103, 108, 105, 107	5	103	5.8	93 – 108
	0.10	93, 106, 103, 105, 108	5	103	5.7	93 – 108
	Overall	-	10	103	5.4	93 - 108
Kidney	0.01	101, 102, 105, 103, 105	5	103	1.7	101 - 105
	0.10	110, 104, 103, 103, 100	5	104	3.5	100 - 110
	Overall	-	10	104	2.7	100 - 110
Blood	0.01	96, 105, 103, 104, 10	5	103	3.7	96 - 105
	0.10	92, 109, 105, 107, 108	5	104	6.7	92 - 109
	Overall		10	103	5.2	92 - 109

Table A 32: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: confirmatory transition m/z 280-192

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Milk	0.01	95, 103, 105, 106, 105	5	103	4.4	95 -106
	0.10	87, 108, 107, 108, 106	5	103	8.8	87 – 108
	Overall	-	10	96	8.7	80 - 104
Eggs	0.01	97, 106, 103, 107, 112	5	105	5.3	97 - 112
	0.10	93, 107, 104, 103, 105,	5	102	5.3	93 – 107
	Overall	-	10	104	5.2	93 – 112
Meat	0.01	96, 110, 102, 107, 104	5	104	5.1	96 – 107
	0.10	93, 107, 103, 108, 107	5	104	6.0	93 – 108
	Overall	-	10	104	5.3	93 - 110
Fat	0.01	91, 105, 100,105, 108	5	102	6.6	91 - 108
	0.10	94, 106, 105, 104, 100	5	102	4.8	94 – 106
	Overall	-	10	102	5.4	91 – 108
Liver	0.01	98, 94, 107, 107, 100	5	101	5.7	94 – 107
	0.10	95, 101, 102, 101, 105	5	101	3.6	95 – 105
	Overall	-	10	95	12.6	78 - 114
Kidney	0.01	99, 100, 105, 107, 105	5	103	3.4	99 - 107
	0.10	106, 104, 102, 104, 101	5	103	1.9	101 - 106
	Overall	-	10	103	2.6	99 - 107
Blood	0.01	95, 104, 106, 106, 108	5	104	4.9	95 - 108
	0.10	94, 109, 108, 109, 106	5	105	6.1	94 - 109
	Overall	-	10	105	5.3	94 - 109

Table A 33: Characteristics for the analytical method used for validation of metalaxyl-M residues in animal commodities

	Metalaxy-M
Specificity / Interferences	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. The method includes two MS/MS transitions for metalaxyl-M, both of which have been validated. No significant interferences arising from the crop matrix, the lab ware, reagents or solvents have been observed at the retention time of interest.
Linearity / Calibration	The linearity was tested using matrix matched standard solutions for all MS/MS transitions. Standards at nine different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients were found to be ≥ 0.9962 to 0.9988 were obtained for Metalaxyl-M.
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 87% and 112% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.

	Metalaxy-M
Repeatability	The relative standard deviations (RSDs) of Metalaxyl-M recoveries at each fortification level and overall for each animal commodity tested during method validation were <20% and there-fore according to the EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory repeatability.
Limit of quantification	The limit of quantification for Metalaxyl-M residues in animal commodities using The QuEChERS analytical method was established at 0.01 mg/kg. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification
Limit of detection	Limit of Detection The limit of detection (LOD) was defined in this study as the lowest prepared instrument cali-bration solution that gave rise to a measureable chromatographic response. The LOD for this study is 0.003 mg/kg.
Matrix effects	No significant matrix effects were observed in the animal commodities tested during method validation, therefore non-matrix matched linearity standards were used for quantification.

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of Metalaxyl-M in animal commodities to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.2.2.2 QuEChERS – Independent laboratory validation (milk, egg, fat, liver and kidney)

Reference: KCP 5.2.2

Report: Metalaxyl-M - Independent Laboratory Validation of Analytical Method QuEChERS for the Determination of Residues of Metalaxyl-M in Animal Matrices by LC-MS/MS.

[redacted] (2018)

Report No. MM87YQ. Syngenta document No. CGA329351_11851.

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Principle of the method
5 g homogenised sub-samples were extracted by the multi-residue QuEChERS method with extraction by shaking.

Samples were extracted by homogenisation with acetonitrile and water, followed by a buffer salt mixture and cleaned-up by dispersive solid phase extraction. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (m/z 280 → 160) and the confirmatory transition (m/z 280 → 192). The limit of quantification of the method was 0.01 mg/kg (0.01 ppm, 10 ppb).

The QuEChERS analytical method was independently validated in five animal matrices (milk, eggs, meat, fat, liver).

Recovery Findings
Summaries of the results for Metalaxyl-M are presented below.

Table A 34: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: primary transition m/z 280 160

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Milk	0.01	99, 96, 97, 103, 102	5	99	3.1	96 – 103
	0.10	93, 85, 98, 88, 79	5	89	8.2	79 - 98
	Overall	-	10	94	8.3	79 - 103
Eggs	0.01	90, 88, 110, 103, 100	5	98	9.3	88 – 110
	0.10	100, 102, 109, 105, 90	5	101	7.0	90 – 109
	Overall	-	10	100	7.9	88 – 110
Meat	0.01	91, 82, 88, 87, 94	5	88	5.1	82 – 94
	0.10	106, 96, 116, 116, 102	5	107	8.2	96 – 116
	Overall	-	10	98	12.2	82 - 116
Fat	0.01	105, 93, 110, 100, 106	5	103	6.4	93 – 110
	0.10	96, 95, 105, 111, 103	5	102	6.5	95 – 111
	Overall	-	10	102	6.1	93 – 111
Liver	0.01	96, 83, 90, 76, 90	5	87	8.8	76 – 96
	0.10	73, 107, 100, 107, 97	5	97	14.5	73 – 107
	Overall	-	10	92	12.9	73 - 107

Table A 35: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: confirmatory transition m/z 280-192

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery(%)	RSD (%)	Recovery Range (%)

Milk	0.01	103, 96, 104, 104, 99	5	101	3.5	96 – 104
	0.10	94, 86, 101, 89, 80	5	90	8.9	80 – 101
	Overall	-	10	96	8.7	80 - 104
Eggs	0.01	85, 82, 106, 90, 81	5	89	11.5	82 – 106
	0.10	98, 100, 101, 105, 91	5	99	5.2	91 – 105
	Overall	-	10	94	9.9	82 – 106
Meat	0.01	110, 80, 77, 98, 106	5	94	15.9	77 – 110
	0.10	108, 97, 118, 118, 104	5	109	8.4	97 – 118
	Overall	-	10	102	13.8	77 - 118
Fat	0.01	93, 96, 94, 82, 87	5	90	6.4	82 – 96
	0.10	97, 98, 108, 110, 104	5	103	5.6	97 – 110
	Overall	-	10	97	9.0	82 – 110
Liver	0.01	87, 83, 95, 88, 91	5	89	5.1	83 – 95
	0.10	78, 114, 105, 109, 104	5	102	13.7	78 – 114
	Overall	-	10	95	12.6	78 - 114

Table A 36: Characteristics for the analytical method used for independent laboratory validation of metalaxyl-M residues in animal commodities

	Metalaxy-M
Specificity / Interferences	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) no further confirma-tory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the animal matrices, the labware, rea-gents or solvents have been observed at the re-tention times of interest.
Linearity / Calibration	The linearity of the LC-MS/MS detector was tested using solvent standard solutions (0.25 ng/ml to 100 ng/ml). Linearity was tested for both MS/MS transitions. Standards at nine different concentrations were injected and the response plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9962 to 0.9988 were obtained for Metalaxyl-M.
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.
Repeatability	The relative standard deviations (RSDs) of Metalaxyl-M recoveries at each fortification level and overall for each animal commodity tested during method validation were <20% and there-fore according to the EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory repeatability.
Limit of quantification	The limit of quantification for Metalaxyl-M residues in animal commodities using The QuEChERS analytical method was established at 0.01 mg/kg. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification

	Metalaxy-M
Limit of detection	The limit of detection (LOD) was defined in this study as the lowest prepared instrument cali-bration solution that gave rise to a measureable chromatographic response. For this study, it was shown to be 0.25 ng/mL (equivalent to 0.0025 mg/kg in sample matrix).
Matrix effects	No significant matrix effects were observed in the animal commodities tested during method validation, therefore non-matrix matched linearity standards were used for quantification.

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate proce-dure for the determination of Metalaxyl-M in animal commodities to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.2.2.2.1 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions, both of which have been validated.

A 2.3.2.2.3 Analytical Method GRM031.06A

A 2.3.2.2.3.1 Independent laboratory validation

Study not required.

Reference: KCP 5.2.2

Report: Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.06A for the Determination of Metalaxyl-M and Structurally Related Metabolites as the Common Moiety 2,6-Dimethylaniline (CGA72649) in Animal Fat.

[redacted] (2016.)

Report No. TK0261461. Syngenta document No. CGA329351_11737.

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Analytical method GRM031.06A was independently validated in animal fat.

Residues of metalaxyl-M were extracted from animal fat by adding ethyl acetate/cyclohexane (1:1, v/v) and dissolving the fat at 40°C in a water bath. Acetonitrile was added and the samples were stored for 1 h at -20°C. The precipitating fat was separated from the extract by filtration. Water was added to the extract and the solution was evaporated to near dryness. The remainder was heated under reflux in methane sulfonic acid for 20 minutes. The extract was diluted with water, a solution of sodium hydroxide and methanol. Final determination of metalaxyl-M (analysed as 2,6-dimethylaniline) was done by LC-MS/MS, monitoring for the primary transition (*m/z* 122-105) and the confirmatory transition (*m/z* 122-103). The limit of quantification of the method was 0.01 mg/kg.

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore demonstrate the method has satisfactory accuracy.

The relative standard deviations (RSDs) of metalaxyl-M recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore demonstrate the method has satisfactory repeatability.

Table A 37: Recovery results from the independent laboratory validation of metalaxyl-M (as 2,6-dimethylaniline) using the analytical method GRM031.06A

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Animal fat	2,6-dimethylaniline *m/z* 122→105 (primary)	0.01 (n=5)	117, 73, 91, 117, 100	100	19	73 – 117
		0.1 (n=5)	91, 84, 87, 88, 86	87	3	84 – 91
		Overall	-	*93*	*15*	*73 – 117*
	2,6-dimethylaniline *m/z* 122→103 (confirmatory)	0.01 (n=5)	113, 72, 88, 115, 100	98	18	72 – 115
		0.1 (n=5)	91, 84, 84, 88, 86	87	3	84 – 91
		Overall	-	*92*	*15*	*72 – 115*

Table A 38: Characteristics for the analytical method used for the independent laboratory validation of metalaxyl-M (as 2,6-dimethylaniline) residues in animal fat

	2,6-dimethylaniline
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention time of interest. No interfering peaks around the retention time of metalaxyl-M (analysed as 2,6-dimethylaniline) were found in any of the control samples at levels above 30% of the limit of quantification.
Calibration (type, number of data points)	The linearity of the LC-MS/MS detector was tested using standard solutions and matrix matched standard solutions. Linearity was tested in both solvent mixtures used and for

	2,6-dimethylaniline
	both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients 0.9999 were obtained for metalaxyl-M (analysed as 2,6-dimethylaniline).
Calibration range	0.025 µg/ml to 10 µg/ml.
Assessment of matrix effects is presented	No significant matrix effects were observed in the matrices tested during method validation.
Limit of determination/quantification	The limit of quantification for metalaxyl-M residues in animal matrices using method GRM031.06A was established at 0.01 mg/kg.

Conclusion

The repeatability and specificity of the method have been independently demonstrated, and GRM031.06A is therefore considered valid for the determination of residues of metalaxyl-M in animal fat at the LOQ of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.2.2.3.2 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and there-fore no confirmatory method is required.

A 2.3.2.2.3.3 Extraction efficiency

Not applicable for an ILV study.

A 2.3.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted.

A 2.3.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted.

A 2.3.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

A 2.3.2.5.1 Analytical method GRM031.08A

A 2.3.2.5.1.1 Method validation

Reference: KCP 5.2.5

Report	Metalaxyl-M - Residue Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and Metabolites NOA409045, CGA108906 and CGA67868 in water. Non-enantiospecific Method. Final Determination by LC-MS/MS. [redacted] & [redacted] (2015) Report No. TK0222544. Syngenta document No. CGA329351_11693.
Guideline(s):	None (method description only).
Deviations:	No (method description only).
GLP:	No (method description only).
Acceptability:	Yes.

Reference:	KC 5.2.5/02
Report	Metalaxyl-M - Validation of an Analytical Method for the Determination of the Metalaxyl-M Metabolite CGA67868 in Water. [redacted] (2015). Report No. TK0222545. Syngenta document No. CGA092370_10006.
Guideline(s):	Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712C-96-174, August 1996. EPA Field Test Data Reporting Guideline, Environmental Chemistry Methods and Associated Independent Laboratory Validation, OCSPP 850.6100. Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Water samples were acidified and passed through Phenomenex Strata-X solid phase extraction cartridges. The columns were dried under vacuum and eluted from the columns with methanol. The column eluates were evaporated to dryness and the residual material re-dissolved in acetonitrile/ultra-pure water (10/90, v/v) solution. The samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS), monitoring for the primary transition *m/z* 194.1-134.2 and the confirmatory transition *m/z* 194.1-91.1 for CGA67868.

The analytical method GRM031.08A was validated for the determination of CGA67868 in surface water

and groundwater matrices. GRM031.08A was based on GRM031.02A with the inclusion of CGA67868.

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (0.05 µg/L) and at ten times the LOQ (0.5 µg/L) for surface water and ground water matrices. Acceptable mean recoveries of between 70% and 110% were found for both transitions. The relative standard deviations (RSDs) at each fortification level for both transitions and overall for each water matrix tested were <20%. The method has satisfactory accuracy and repeatability.

Table A 39: Recovery results from the method validation of CGA67868 using the analytical method GRM031.08A

Matrix	Analyte	Fortification level (µg/L) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Surface water	CGA67868 *m/z* 194→134 (primary)	0.05 (n=5)	94, 95, 96, 92, 108	97	7	92-108
		0.5 (n=5)	105, 95, 88, 98, 99	97	6	88-105
		Overall	-	*97*	*6*	*88-108*
	CGA67868 *m/z* 194→91 (confirmatory)	0.05 (n=5)	99, 88, 94, 90, 99	94	5	88-99
		0.5 (n=5)	99, 90, 83, 91, 93	91	6	83-99
		Overall	-	*93*	*5*	*83-99*
Ground water	CGA67868 *m/z* 194→134 (primary)	0.05 (n=5)	102, 98, 92, 107, 106	101	6	92-107
		0.5 (n=5)	101, 94, 93, 106, 75	94	13	75-106
		Overall	-	*97*	*10*	*75-107*
	CGA67868 *m/z* 194→91 (confirmatory)	0.05 (n=5)	96, 90, 95, 111, 110	100	9	90-111
		0.5 (n=5)	98, 91, 89, 102, 74	91	12	74-102
		Overall	-	*96*	*11*	*74-111*

Table A 40: Characteristics for the analytical method used for validation of CGA67868 residues in surface and ground water

	CGA67868
Specificity	No interfering peaks around the retention time of CGA67868 were found in any of the control samples at levels above 30% of the limit of quantification.
Calibration (type, number of data points)	Linearity was assessed using matrix matched standard solutions for both MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9960 to 0.9994 were obtained.
Calibration range	0.075 to 10 µg/L.
Assessment of matrix effects is presented	No significant matrix effects (i.e. suppression or enhancement of the detector response ≤ ± 20%) were observed in the ground water matrix tested for the primary and confirmatory transitions. Significant matrix effects (i.e. suppression or enhancement of the detector response ≥ ± 20%) were observed in the surface water matrix tested. Matrix matched linearity standards were used for the quantification of CGA67868 during this study.

	CGA67868
Limit of determination/quantification	The limit of quantification for CGA67868 residues in water matrices was 0.05 µg/L. The limits of detection (LODs) were calculated in each matrix type and ranged from 0.0003 to 0.0005 mg/kg for the primary transition and 0.0002 to 0.0143 mg/kg for the confirmatory transition.

Conclusion

Analytical method GRM031.08A has been demonstrated to be a reliable and accurate procedure for the determination of CGA67868 in surface water and ground water to a limit of quantification of 0.05 µg/L using commercially available laboratory equipment and reagents.

A 2.3.2.5.1.2 Independent laboratory validation

Reference: KCP 5.2.5

Report: Metalaxyl-M - Independent Laboratory Validation of Analytical Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and its Metabolites NOA409045, CGA108906 and CGA67868 in Drinking Water.

[redacted] (2016)

Report No. IF-15/03469803-TK. Syngenta document No. CGA329351_11732.

Guideline(s): EPA OCSPP 850.6100 (2012).

SANCO/3029/99 Rev. 4 (2000).

SANCO/825/00 Rev. 8.1 (2010).

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

In summary, acidified drinking water samples are concentrated using solid phase extraction (SPE). After elution with methanol, samples are evaporated to dryness and dissolved in acetonitrile/ultra-pure water and analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.05 µg/L for all analytes.

Results and discussions

The mean metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 recoveries for both primary and confirmatory ion transitions at each fortification level and overall were in the range 70% to 102%. The relative standard deviations (RSDs) of recoveries for all analytes for both primary and confirmatory ion transitions at each fortification level and overall were in the range 1 to 12%. These results demonstrate the method has satisfactory accuracy and repeatability.

Table A 41: Recovery results from the independent laboratory validation of metalaxyl-M residues using the analytical method GRM031.08A

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Metalaxyl-M *m/z* 280→220 (primary)	0.05 (n=5)	74	8	69-84
		0.5 (n=5)	82	1	80-82
		Overall	*78*	*7*	*69-84*
	Metalaxyl-M *m/z* 280→192 (confirmatory)	0.05 (n=5)	80	11	74-94
		0.5 (n=5)	82	3	80-86
		Overall	*81*	*7*	*74-94*
Drinking water	CGA62826 (NOA409045) *m/z* 266→192 (primary)	0.05 (n=5)	97	10	89-113
		0.5 (n=5)	96	2	94-98
		Overall	*97*	*7*	*89-113*
	CGA62826 (NOA409045) *m/z* 266→160 (confirmatory)	0.05 (n=5)	102	8	90-110
		0.5 (n=5)	96	3	93-99
		Overall	*99*	*6*	*90-110*
Drinking water	CGA108906 *m/z* 296→160 (primary)	0.05 (n=5)	93	10	87-110
		0.5 (n=5)	96	2	93-97
		Overall	*95*	*7*	*87-110*
	CGA108906 *m/z* 296→178 (confirmatory)	0.05 (n=5)	89	8	82-101
		0.5 (n=5)	96	2	94-98
		Overall	*92*	*7*	*82-101*
Drinking water	CGA67868 *m/z* 194→134 (primary)	0.05 (n=5)	72	9	64-80
		0.5 (n=5)	71	1	70-72
		Overall	*71*	*6*	*64-80*
	CGA67868 *m/z* 194→91 (confirmatory)	0.05 (n=5)	74	12	66-88
		0.5 (n=5)	70	3	68-73
		Overall	*72*	*9*	*66-88*

Table A 42: Characteristics for the analytical method used for independent laboratory validation of metalaxyl-M residues in water

	Metalaxyl-M	CGA62826 (NOA409045)	CGA108906	CGA67868
Specificity	Residues of all analytes measured in the control samples were always below 30% of the LOQ during method validation.			
Calibration (type, number of data points)	A minimum of 5 standard solutions were injected, the lowest concentration injected was at 30% of the LOQ of the method and the upper margin was higher by at least 20% above the highest concentrations in the final extracts. The LC-MS/MS detector response for metalaxyl-M, NOA409045, CGA108906 and CGA67868 was found to be linear.			
Calibration range	0.07 to 4.3 ng/mL			
Assessment of matrix effects is pre-	Matrix effects (either enhancement or suppression) were not considered to			

	Metalaxyl-M	**CGA62826 (NOA409045)**	**CGA108906**	**CGA67868**
sented	be significant for metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 and as such non-matrix calibration standards could be used if necessary.			
Limit of determination/quantification	The LOQ for metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 residues was confirmed at 0.05 µg/L in drinking water.			

Conclusion

Method GRM031.08A was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 in drinking water with an LOQ of 0.05 µg/L for each analyte.

A 2.3.2.5.1.3 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.3.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 2.3.2.7 Other Studies/ Information

No new or additional studies have been submitted.