REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: A23109A

Product name: ORONDIS VIP

Chemical active substances:

Metalaxyl-M, 174.4 g/L
Oxathiapiprolin, 30 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorisation)

Applicant: Syngenta

Submission date: June 2022

MS Finalisation date: July 2023 (initial Core Assessment)
December 2023 (final Core Assessment)

updated July 2024

Version history

When	What
June 2022	Part B - Section 5 - Core Assessment - Central Zone
July 2023	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.
December 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.
July 2024	Final report - updated with regard to the provisions of COMMISSION IMPLEMENTING REGULATION (EU) 2024/1718 of 19 June 2024 amending Implementing Regulations (EU) 2020/617 and (EU) No 540/2011 as regards the conditions of approval of the active substance metalaxyl-M. Additional information/assessments included by the zRMS in the report are highlighted in green. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

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5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS conclusions:

Metalaxyl-M
EFSA concluded in EFSA Journal 2015;13(3):3999 – "Peer review of the pesticide risk assessment of the active substance metalaxyl-M" that "*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. 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. LC-MS/MS (liquid chromatography with tandem mass spectrometry) methods are available to monitor the compounds in the residue definitions for water, soil and air.*
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 submitted a number of methods for analysis of residues of ~~azoxystrobin~~ metalaxyl-M for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.
The details of the evaluation of new and additional studies are referred in Appendix 2.

Oxathiapiprolin
EFSA concluded in EFSA Journal 2016;14(7):4504 - "Peer review of the pesticide risk assessment of the active substance oxathiapiprolin" that: *Oxathiapiprolin residues can be monitored in food and feed of plant origin by the multi-residue method DFG S19 using LC-MS/MS in dry, high water content and acidic matrices with limits of quantification (LOQs) of 0.01 mg/kg, or by a single HPLC-MS/MS method with LOQs of 0.01 mg/kg for all plant commodity groups. Residues of oxathiapiprolin in food of animal origin can be monitored with the multi-residue method DFG S19 using LC-MS/MS in meat, fat, liver, milk and eggs with LOQs of 0.01 mg/kg or by a single HPLC-MS/MS method with LOQs of 0.01 mg/kg for all animal matrices.*
Residues of oxathiapiprolin in soil, water and air can be monitored by LC-MS/MS with LOQs of 1 µg/kg, 0.1 µg/L and 0.05 µg/m^3, respectively.
No analytical method is required for the determination of oxathiapiprolin in body fluids and tissues as oxathiapiprolin is not classified as toxic or very toxic.

The Applicant submitted a number of methods for analysis of residues of oxathiapiprolin for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.
The details of the evaluation of new and additional studies are referred in Appendix 2.

Applicant submitted additional information:
The Applicant would like to highlight that the Technical Guideline on the Evaluation of Extraction Efficiency, SANTE/2017/10632, states that 'for renewal of product authorisations or for new product authorisations or extension of uses for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. This means that no additional proof of extraction efficiency is required if it had not been required in the renewal of approval/approval procedure itself.' Both oxathiapiprolin and metalaxyl-M were last renewed prior to the implementation of the extraction efficiency guideline SANTE/2017/10632 (implementation date: 23 Nov 2019) and, hence, an assessment of extraction efficiency was not required during the renewal, the demonstration of extraction efficiency is not required to support this product submission.

No additional data is required to support this application.

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: None.

~~Impurity 2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester (coded CGA226048) was considered as non-relevant at the time of submission, due to ongoing discussions on EU level (EFSA). In case this impurity is considered as relevant, the availability of a validated method is considered as a data requirement. However as the discussion on the impurities on EU level is still ongoing the analytical method for impurity should be available. According to the information~~

~~provided by the applicant on the zRMS request, the relevant analytical method for CGA226048 is under development.~~

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

Noticed data gaps are: None

Commodity / crop	Supported / Not supported
Garlic	supported
Onion	supported
Shallots	supported
Spring onion, Green onion, Welsh onion	supported
Broccoli	supported
Cauliflower	supported
Brussels sprout	supported
Head cabbage	supported
Savoy cabbage	supported
Pe-tsai	supported
Kale and curly kale	supported
Lamb's lettuce	supported
Lettuce (open leaf) Iceberg lettuce	supported
Escarole	supported
Cress	supported
Rocket	supported
Red mustard	supported
Baby leaf	supported
Spinach	supported
Common purslane	supported
Chards and beet leaves	supported
Water cress	supported
Chicory / Endive	supported
Herbs and edible flowers	supported
Chives	supported
Leek	supported

5.2 Methods used for the generation of pre-authorisation 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 A23109A has not been reviewed at EU level as a consequence of the review of oxathiapiprolin or metalaxyl-M.

An overview on the acceptable methods and possible data gaps for the analysis of metalaxyl-M and oxathiapiprolin in plant protection product A23109A is provided as follows:

Comments of zRMS:	The proposed analytical method was successfully validated for the determination of active substances in plant protection product according to the requirements laid down by SANCO3030/99 rev.5.

Reference:	KCP 5.1.1/01
Report	SF-1027/2- Determination of Metalaxyl-M (achiral and chiral) and Oxathiapiprolin in A23109A by HPLC, Bradbury L. M., 2021, Method No. SF-1027/2, Syngenta File No. VV-903867
Guideline(s):	None (no guideline required)
Deviations:	None
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1/02
Report	A23109A – Validation of Analytical Method SF-1027/2, Khot S. B., 2021, Report No. SMG16622, Syngenta File No. VV-903871
Guideline(s):	SANCO/3030/99/rev.5 Validation of analytical methods for active constituents and agricultural products document dated 1 July 2014 by the Australian Pesticides and Veterinary Medicines Authority (APVMA)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

Oxathiapiprolin and metalaxyl-M (including its S-enantiomer) are determined simultaneously by analytical method SF-1027/2, a liquid chromatography method, using an Agilent HPLC system with a Kinetex C18 column (Length: 150 mm, internal diameter: 4.6 mm, particle size 2.6 µm, at 30°C), UV detection and an external standard. For separation, an acetonitrile/0.1 % v/v aqueous phosphoric acid in water/methanol gradient as mobile phase was used. Quantification was obtained by comparing peak areas of test samples with the areas from calibrated analytical reference solutions.

In addition, this analytical method includes a chiral procedure to determine the content of CGA329351 (R-enantiomer of metalaxyl-M) and CGA351920 (S-enantiomer of metalaxyl-M) simultaneously using a chiral CHIRALPAK IB phase (Length: 150 mm, internal diameter: 4.6 mm, particle size 5 µm, at 40°C), UV detection and an external standard. For separation, an acetonitrile/water gradient as mobile phase was used. Quantification was obtained by comparing peak areas of test samples with the areas from calibrated analytical reference solutions.

Table 5.2-1: Material and method of SF-1027/2 for the determination of active substances oxathiapiprolin, metalaxyl-M, CGA329351 (R-enantiomer of metalaxyl-M) and CGA351920 (S-enantiomer of metalaxyl-M) in plant protection product A23109A

	Non-chiral procedure	Chiral procedure
Instrument	Agilent HPLC system	Agilent HPLC system
Dwell Volume	1200 µL	1240 µL
Detector		
Wavelength	235 nm 360 nm (Reference)	230 nm
Bandwidth	4 nm 100 (Reference)	4 nm
Column Description		
Stationary Phase	Kinetex C18 phase	Chiralpak IB phase
Length	150 mm	150 mm
Internal Diameter	4.6 mm	4.6 mm
Particle Size	2.6 µm	5 µm
Column Temperature	30°C	40°C
Injection Volume	5 µL	10 µL
Total Run Time	16 min.	28 min.
Typical Backpressure	245 Bar (At start, just for information)	44 Bar (At start, just for information)
Mobile Phase	Gradient A 0.1% v/v Phosphoric Acid in water B Acetonitrile C Methanol **Time (min.) / %A / %B / %C** 0 / 50 / 40 / 10 8.0 / 30 / 60 / 10 9.0 / 10 / 90 / 0 12.0 / 10 / 90 / 0 12.1 / 50 / 40 / 10 16.0 / 50 / 40 / 10	Gradient A Water : Acetonitrile (65 : 35) B Acetonitrile **Time (min.) / %A / %B** 0 / 100 / 0 10.0 / 100 / 0 11.0 / 20 / 80 20.0 / 20 / 80 21.0 / 100 / 0 28.0 / 100 / 0
Retention time	Oxathiapiprolin: 7.0 min. Metalaxyl-M: 3.6 min.	CGA351920 (S-enantiomer): 7.2 min. CGA329351 (R-enantiomer): 8.4 min.

Validation - Results and discussions

The following validation of the analytical method for the determination of oxathiapiprolin and metalaxyl-M in formulation A23109A has not previously been reviewed and is provided in support of this assessment.

Full validation of the method SF-1027/2 has been conducted for A23109A. The details are summarized in the table below:

Table 5.2-2: Methods suitable for the determination of active substances oxathiapiprolin, metalaxyl-M, CGA329351 (R-enantiomer of metalaxyl-M) and CGA351920 (S-enantiomer of metalaxyl-M) in plant protection product A23109A

	Oxathiapiprolin	**Metalaxyl-M (R/S-enantiomer)**	**CGA329351 (R-enantiomer of Metalaxyl-M)**	**CGA351920 (S-enantiomer of Metalaxyl-M)**
Author(s), year	Khot S. B.			
Principle of method	HPLC, UV		chiral-HPLC, UV	
Linearity n = 6 (2 determinations each) (correlation coefficient, expressed as r)	Linear between 50 % - 150 % of the declared content. r = 0.99998 Y = 0.098*X + 0.214	Linear between 50 % - 150 % of the declared content. r = 0.99992 Y = 0.016*X + 0.577	Linear between 50 % - 150 % of the declared content of Metalaxyl-M. r = 0.99973 Y = 0.089*X + 3.053	Linear between 50 % - 150 % of the declared content of Metalaxyl-M. r = 0.99987 Y = 0.093*X – 0.031

	Oxathiapiprolin	**Metalaxyl-M (R/S-enantiomer)**	**CGA329351 (R-enantiomer of Metalaxyl-M)**	**CGA351920 (S-enantiomer of Metalaxyl-M)**
Precision – Repeatability Mean n = 6 (double injection)	Mean concentration: 2.79 % w/w RSD: 0.88 % RSD_r (mod. Horwitz): 2.30 % Horrat: 0.38	Mean concentration: 16.79 % w/w RSD: 0.90 % RSD_r (mod. Horwitz): 1.75 % Horrat:0.51	Mean concentration: 16.4 % w/w RSD: 0.013 % RSD_r (mod. Horwitz): 1.76 % Horrat: 0.007	Mean concentration: 0.40 % w/w RSD: 0.53 % RSD_r (mod. Horwitz): 3.08 % Horrat: 0.172
Accuracy - Recovery n = 4 (2 determinations each)	Mean: 100.7 % L70: 101.6 % L90: 100.6 % L110: 100.6 % L130: 100.1 %	Mean: 100.5 % L70: 101.3 % L90: 101.1 % L110: 100.5 % L130: 99.2 %	Mean: 100.0 % L70: 100.1 % L90: 100.0 % L110: 100.0 % L130: 99.9 %	Mean: 99.7 % L70: 97.8 % L90: 99.6 % L110: 100.3 % L130: 101.2 %
Interference/ Specificity	No significant co-elution			
Comment	The method is acceptably validated			

Conclusion
The method has shown to be specific for the determination of oxathiapiprolin and metalaxyl-M (and its enantiomers) in the product A23109A and no significant interference was observed. Based on the results for linearity, precision, accuracy and specificity the method is suitable for the specific, accurate and precise determination of oxathiapiprolin and metalaxyl-M (and its enantiomers) in product A23109A.

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

2,6-Dimethylphenylamine (CGA72649) [≤ 0,5 g/kg in active substance] , 4-Methoxy-5-methyl-5H-[1,2]oxathiole 2,2-dioxide (CGA363736) [≤ 1 g/kg in active substance] and 2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxy-carbonyl-ethyl ester (CGA226048)) [< 10 g/kg in active substance] are relevant impurities of the active ingredient metalaxyl-M as stated in Annex 1 of the metalaxyl-M implementing Regulation ~~(EU) 2020/617~~ (EU) 2024/1718 and thus introduced in the product A23109A.

~~An on-going EU evaluation of impurity CGA226048 under article 7 (submission of documentation 25th July 2020) is being finalised. The RMS Belgium concluded that impurity CGA226048 is shown to be non-genotoxic and non-relevant and an updated RAR has been made available in May 2021 for public commenting. Therefore, no analytical method will be provided for the determination of CGA226048 in product A23109A.~~

Analytical method SD-1751/1 has been developed for the determination of Metalaxyl-M Relevant Impurities CGA72649 and CGA363736 in formulation by GC/MS/MS, and originally fully validated for A9651D. This method has been reviewed and evaluated as valid also for A23109A.

The analytical method SD-2790/1 determines the relevant impurity **CGA226048** (consists of CGA226048 diastereomer A and CGA226048 diastereomer B) in formulations containing metalaxyl-M using standard addition sample preparation coupled with liquid chromatography with mass spectrometry detection.

The method validation was performed under GLP, study number CHMU240179, within the GLP Testing Facility WMU, 4333 Muenchwilen, Switzerland, for formulation A9642D (metalaxyl-M ES (350)). The validation has demonstrated accuracy (recovery, linearity), precision (repeatability), specificity and limit of quantification of the method.

Method SD-2790/1 will also be used for the determination of CGA226048 (both CGA226048 diastereomer A and CGA226048 diastereomer B) in A23109A. The different nominal concentrations of metalaxyl-M in formulations A9642D and A23109A are not of relevance, as the sample weighing is adapted accordingly. Thus, the concentration of metalaxyl-M and its relevant impurity remains the same in the test solutions.

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

Comments of zRMS:	The analytical method was successfully validated for the quantification of the impurites in the plant protection product according to the requirements laid down by SANCO3030/99 rev.5.

Reference:	KCP 5.1.1/03
Report	Analytical Method SD-1751/1 – Determination of Metalaxyl-M Relevant Impurities CGA72649 and CGA363736 in formulation by GC/MS/MS, Kouzoumis A., Burkhard R. and Heintz K., 2014, Method No. SD-1751/1, Syngenta File No. VV-128413
Guideline(s):	None (no guideline required)
Deviations:	None
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1/04
Report	Metalaxyl-M/mancozeb A9651D - Validation of Analytical Method SD-1751/1, Heintz K., 2014, Report No. CHMU140410, Syngenta File No. VV-411110
Guideline(s):	None
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.1/05
Report	Statement on Validation of the Analytical Method SD-1751/1 for the determination of CGA72649 and CGA363736 in A23109A oxathiapiprolin/metalaxyl-M DC (030/180), Heintz K., 2021, Syngenta File No. VV-910672
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Materials and methods

The analytical method SD-1751/1 determines relevant impurities 2,6-Dimethylphenylamine (CGA72649) and 4-Methoxy-5-methyl-5H-[1,2]oxathiole 2,2-dioxide (CGA363736) in formulations containing metalaxyl-M using standard addition sample preparation coupled with gas chromatography with MS detection.

The method validation was performed under GLP, study number CHMU140410, for the formulation A9651D (Metalaxyl-M/Mancozeb WG (4/64). Method SD-1751/1 will also be used for the determination of CGA72649 and CGA363736 in product A23109A. The different nominal concentrations of metalaxyl-M in formulations A9651D and A23109A are not of relevance, as the sample weighing is adapted accordingly. Thus, the concentration of metalaxyl-M and its relevant impurities remains the same in the test solutions.

Method SD-1751/1 uses the standard addition procedure, which implies that 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. 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 limit of quantification, can be accounted for. The fact that this method also utilizes mass spectroscopy detection (MS/MS), a highly specific detection method, allows the assumption that the interferences will be non-significant.

With SD-1751/1 being used for both these formulations, with a multiple point linear calibration conducted for every sample analysed combined with compensation for any formulation matrix effects, it was concluded that the repeatability data generated for A9651D provides the confidence that repeatability would also be acceptable for A23109A as the other measured parameters are identical.

Table 5.2-3: Material and method of SD-1751/1 for the determination of relevant impurities CGA72649 and CGA363736 in plant protection product A23109A

Instrument	**Thermo Trace GC ultra**	
Detector (mass spectrometer)	Thermo TSQ Quantum XLS	
	CGA72649	The selective reaction monitoring transition is *m/z* 121 to 106 using 15 V collision energy, segment start time 7 min., segment end time 9 min.
	CGA363736	The selective reaction monitoring transition is *m/z* 164 to 121 using 12 V collision energy, segment start time 9 min., segment end time 13 min.
Column Description		
Type	Fused silica	
Length	15 m	
Inside diameter	0.32 mm	
Stationary phase	DB-1701	
Film thickness	1 µm	
Column temperature	75°C, 1 min. isothermal 10°C/min. to 140°C 25°C/min. to 305°C 305°C, 10 min. 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/min., constant flow	
Size of sample	1 µL of test solution / spiked test solution	
Split ratio	20:1	
Total Run Time	Approx. 24 min.	

Validation - Results and discussions

The following validation parameters of the analytical method for the determination of CGA72649 and CGA363736 in formulation A23109A have not previously been reviewed and is provided in support of this assessment.

The statement contains results for linearity, precision, accuracy, specificity and limit of quantification of method SD-1751/1 for the determination of CGA72649 and CGA363736 in product A23109A. The results show that method SD-1751/1 is valid and can be used for the determination of product A23109A. The details are summarized in the Table 5.2-4 below:

Table 5.2-4: Method suitable for the determination of the relevant impurities CGA72649 and CGA363736 in plant protection product (PPP) A23109A

	2,6-Dimethylphenylamine (CGA72649) **≤ 0.09 g/kg**	**4-Methoxy-5-methyl-5H-[1,2]oxathiole 2,2-dioxide (CGA363736)** **≤ 0.18 g/kg**
Author(s), year	Heintz K.(2021)	
Principle of method	GC, MS/MS	
Linearity (A23109A) n = 6 (2 determinations each) (correlation coefficient, expressed as r)	Linear between 99 to 708 ppm relative to the content of Metalaxyl-M. r = 0.9997 Y = 774.41*X + 6335.22	Linear between 195 to 1399 ppm relative to the content of Metalaxyl-M. r = 0.9970 Y = 29.68*X + 1509.69
Precision – Repeatability Mean (A9651D) n = 6 (double injection)	Mean concentration: 291.39 ppm RSD: 4.22 % RSD_r (mod. Horwitz): 4.56 % Horrat: 0.92	Mean concentration: 549.57 ppm RSD: 2.94 % RSD_r (mod. Horwitz): 4.15 % Horrat: 0.71

	2,6-Dimethylphenylamine (CGA72649) **≤ 0.09 g/kg**	**4-Methoxy-5-methyl-5H-[1,2]oxathiole 2,2-dioxide (CGA363736)** **≤ 0.18 g/kg**
Accuracy – Recovery (A23109A) n = 5 (2 determinations each)	Mean: 101.0 % Level 98.83 ppm: 102.5 % Level 197.86 ppm: 100.2 % Level 304.68 ppm: 102.4 % Level 508.51 ppm: 101.2 % Level 707.81 ppm: 98.9 %	Mean: 102.4 % Level 195.35 ppm: 96.5 % Level 391.09 ppm: 109.3 % Level 602.23 ppm: 106.9 % Level 1005.13 ppm: 102.5 % Level 1399.05 ppm: 96.8 %
Limit of Quantification (LoQ) (A23109A)	100 ppm	200 ppm
Interference/ Specificity (A23109A)	The fact that this method also utilises mass spectroscopy detection (MS/MS), a highly specific detection method, allows the assumption that the interferences will be non-significant.	
Comment	The method is acceptably validated	

Conclusion

The method has been shown to be specific for the determination of CGA72649 and CGA363736 in the product A23109A and no significant interference was observed. Based on the results for linearity, precision, accuracy, specificity and limit of quantification the method is suitable for the specific, accurate and precise determination of CGA72649 and CGA363736 in product A23109A.

Reference:	KCP 5.1.1/06
Report	Statement on Validation of the Analytical Method SD-1751/1 for the determination of CGA72649 and CGA363736 in A23109A oxathiapiprolin/metalaxyl-M DC (030/180)
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

The analytical method **SD-1751/1** determines relevant impurities CGA72649 and CGA363736 in formulations containing metalaxyl-M using standard addition sample preparation coupled with gas chromatography with mass spectroscopy detection.
The method validation was performed under GLP, study number CHMU140410, within the GLP Testing Facility WMU, 4333 Muenchwilen, Switzerland, for formulation **A9651 D** (metalaxyl-M/mancozeb WG (4/64)). The validation has demonstrated accuracy (recovery, linearity), precision (repeatability), specificity and limit of quantification of the method.
Method **SD-1751/1** will also be used for the determination of CGA72649 and CGA363736 in **A23109A.** The different nominal concentrations of metalaxyl-M in formulations **A9651D** and **A23109A** are not of relevance, as the sample weighing is adapted accordingly. Thus, the concentration of metalaxyl-M and its relevant impurities remains the same in the test solutions.
The data (specificity, linearity, recovery and limit of quantification) demonstrating the validity of analytical method **SD-1751/1** for the determination of CGA72649 and CGA363736 in **A23109A** was presented in a previous statement dated 08-July-2021.

The chromatogramms corresponding to the data used to demonstrate the limit of quantification:

Limit of quantification

Figure 1: Chromatograms of A23109A, batch JHU003-044-001

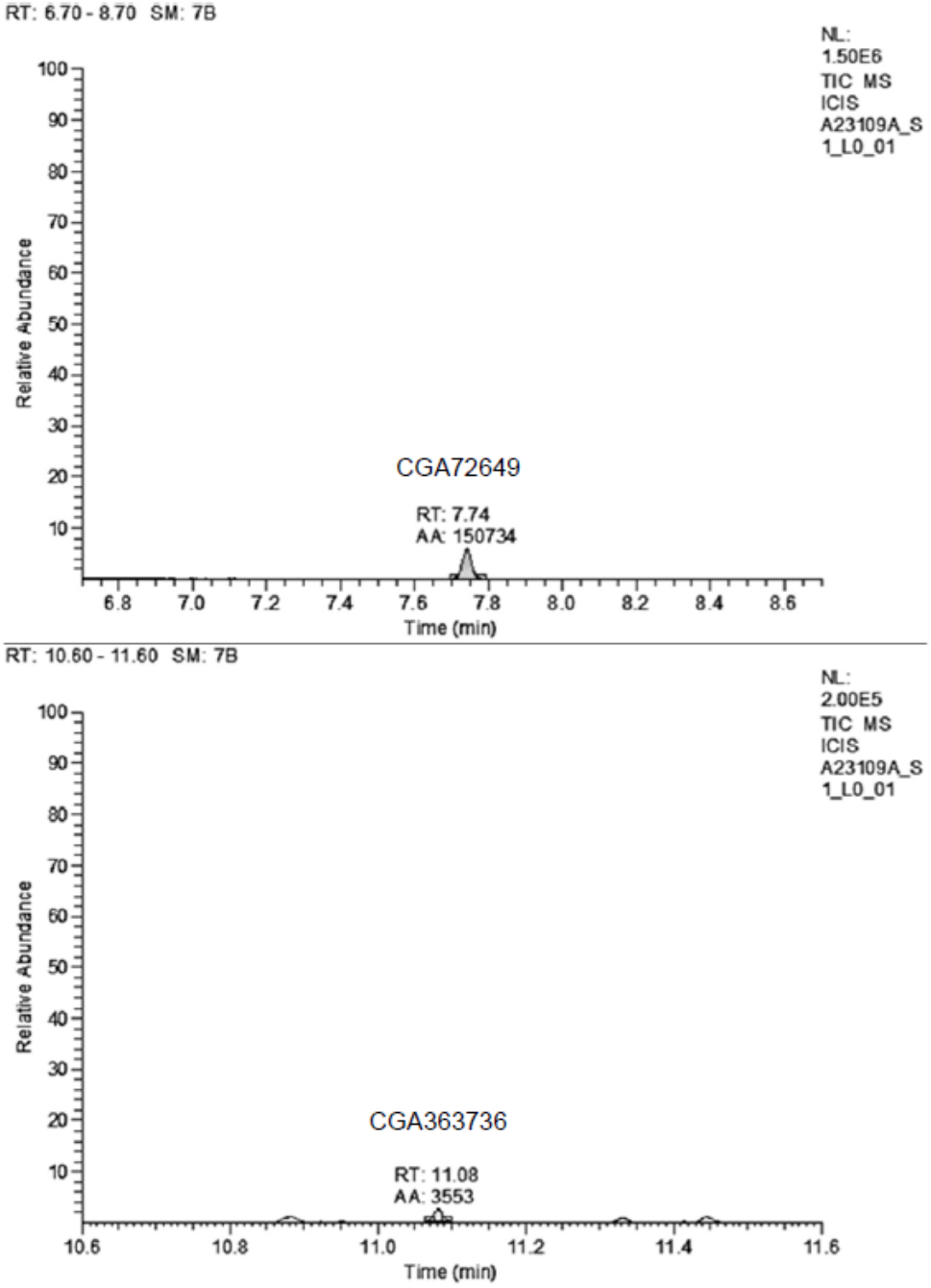
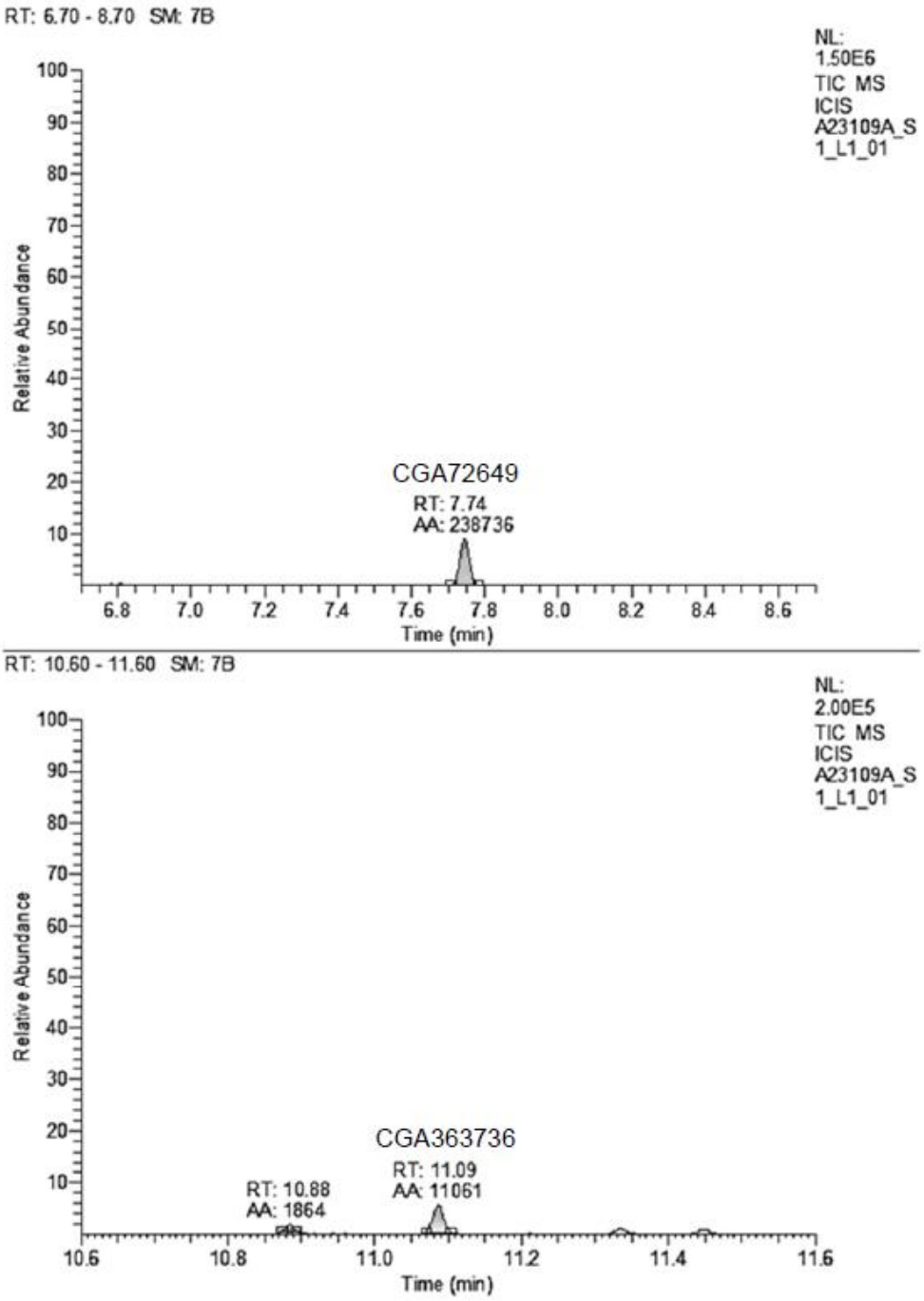


Figure 2: Chromatograms of A23109A batch JHU003-044-001 spiked with 99 ppm CGA72649 and 195 ppm CGA363736 relative to the amount of metalaxyl-M present in formulation



Reference:	KCP 5.1.1/07
Report	Analytical Method SD-2790/1– Metalaxyl-M, SD-2790/1- Determination of CGA226048 in technical material and formulations by LC/MS Analytical Method, Stephanie Sigel/Sandro Tamburello, 2024.
Guideline(s):	None (no guideline required)
Deviations:	None
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1/08
Report	Metalaxyl-M - A9642D - Validation of Analytical Method SD-2790/1, Final Report, Stephanie Sigel, 2024.
Guideline(s):	None
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.1/09
Report	Statement on Validation of the Analytical Method SD-2790/1 for the determination of CGA226048 in A23109A oxathiapiprolin/metalaxyl-M DC (030/180), Stephanie Sigel, 2024.
Guideline(s):	None
Deviations:	None
GLP:	No
Acceptability:	Yes

Materials and methods

Method SD-2790/1 uses the standard addition procedure, which implies that calibration solutions are prepared by adding known amounts of CGA226048 directly to formulation samples and diluting all samples to the same final volume. The resulting spiked solutions contain different known levels of CGA226048, ranging from between approx. 500 mg/kg to 6000 mg/kg for each CGA226048 diastereomer relative to metalaxyl-M, and by plotting the amounts of each CGA226048 diastereomer added against their respective instrument responses (area of CGA226048 diastereomer A or 8), the calibration curve is generated. One of the samples is prepared without the addition of CGA226048, as it is from this sample that the actual content of each CGA226048 diastereomer can be calculated using the calibration curve generated. Due to the fact, that the analytes of interest, in this case CGA226048 diastereomer A and B, 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 fact that this method also utilizes mass spectrometry detection, a highly specific detection method, allows the assumption that the interferences will be nonsignificant.

This method of analyte calibration therefore provides specificity, linearity, recovery and the limit of quantification for every individual sample.

With SD-2790/1 being used for both these formulations, with a multiple point linear calibration conducted for every sample analysed combined with compensation for any formulation matrix effects, it was concluded that the repeatability data generated for A9642D provides the confidence that repeatability would also be acceptable for A23109A as the other measured parameters are identical.

Table 5.2-5: Material and method of SD-2790/1 for the determination of relevant impurity CGA226048 in plant protection product A23109A

Instrument	**Thermo Vanquish UHPLC**
Detector (mass spectrometer)	Triple Quadrupole (e.g. Thermo Scientific TSQ Quantis Plus) Ionization mode: Pas. ESI Scan Type: SRM Precursor: 352.1 m/z Product: 160.1 m/z Resolution: Ql Resolution: 0.7 Q3 Resolution: 1.2 Collision Energy (CE): 30V Acquisition time: 11.5 - 15.5 min
Column Description	
Length	150 mm
Inside diameter	2.1 mm
Stationary phase	Phenyl-Hexyl (Kinetex)
Particle Size	1.7 µm
Column temperature	40°C
Injection Volume	2 µl
Total Run Time	25 min
Flow Rate	0.5 ml/min
Typical Backpressure	720 bar (at start, just for information)
Principle of the Method	The test substance is spiked with several levels of CGA226048, using a Reference solution, to obtain a multi-level standard addition calibration curve. A calibration curve for CGA226048 can be obtained using the following levels (the use of a minimum of three levels is recommended}. The levels refer to the sum of CGA226048 diastereomer A and B: 1000 mg/kg (0.1 %), 2000 mg/kg (0.2 %), 5000 mg/kg (0.5 %), 10000 mg/kg (1 %), 12000 mg/kg (1.2 %).
Retention time guide values:	CGA226O48 diastereomer B – 13.1 min CGA226O48 diastereomer A – 13.4 min

Validation - Results and discussions

The analytical method SD-2790/1 has been validated for the determination of CGA226048 in formulation A9642D (Metalaxyl-M ES (350)). Accuracy (specificity, recovery, linearity) and precision (repeatability) were acceptable. Specificity and interference were established using standard addition mode and LC/MS analysis. The details are summarized in the Table 5.2-4 below:

Table 5.2-6: Method suitable for the determination of the relevant impurity in plant protection product (PPP) A9642D

	2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxy-carbonyl-ethyl ester (CGA226048) < 10 g/kg in the active substance
Author(s), year	Stephanie Sigel (2024)
Principle of method	LC/MS
Linearity (A9642D) n = 6 (2 determinations each) (correlation coefficient, expressed as r)	CGA226048 diastereomer A: Linear between 500 - 6000 mg/kg relative to the content of Metalaxyl-M. r = 1.0000 Y´= 1.16 * X + 5.63 CGA226048 diastereomer B: Linear between 600 – 7000 mg/kg relative to the content of Metalaxyl-M. r = 1.0000 Y´= 1.04 * X + 12.48
Precision – Repeatability Mean (A9642D) n = 5 The repeatability was tested with 5	CGA226048 diastereomer A Mean concentration: 5320.18 mg/kg RSD: 1.57 %

independent determinations (5 sets of standard addition) of each CGA226048 diastereomer in A9642D, batch POR9K80245.
The calculation was performed using spiked level L4 (which corresponds to a level of approx. 10000 mg/kg of CGA226048, relative to Metalaxyl-M1) of each set.

Sample	Level spiked [mg/kg]	Level found [mg/kg]
S1-L4	5265.37	5263.36
S2-L4	5380.46	5398.25
S3-L4	5439.91	5375.26
S4-L4	5209.65	5202.99
S6-L4	5439.91	5361.03
	Mean [mg/kg]	5320.18
	SD	83.31
	RSD [%]	1.57
	RSDr mod. Hor [%]	2.95
	HorRat	0.53

CGA226048 diastereomer B
Mean concentration: 5983.67 mg/kg
RSD: 1.78 %

Sample	Level spiked [mg/kg]	Level found [mg/kg]
S1-L4	5914.52	5932.58
S2-L4	6043.80	6027.04
S3-L4	6110.59	6063.67
S4-L4	5851.94	5821.34
S6-L4	6110.59	6073.70
	Mean [mg/kg]	5983.67
	SD	106.47
	RSD [%]	1.78
	RSDr mod. Hor [%]	2.90
	HorRat	0.61

Accuracy – Recovery (A23109A)
n = 5 (2 injections each)

The recovery was tested with A9642D, batch POR9K80245, spiked using pure reference substance of CGA226048 to 5 levels over the range of
500 mg/kg to 6000 mg/kg for CGA226048 diastereomer A and
600 mg/kg to 7000 mg/kg for CGA226048 diastereomer B.

CGA226048 diastereomer A
Mean: 100.2%

Level	Level spiked [mg/kg]	Level found [mg/kg]	Recovery [%]
L1	534.71	532.72	99.6
L2	1069.41	1076.71	100.7
L3	2673.53	2691.23	100.7
L4	5347.06	5320.18	99.5
L5	6416.47	6450.97	100.5
		Mean Recovery :	100.2

CGA226048 diastereomer B
Mean: 100.4 %

Level	Level spiked [mg/kg]	Level found [mg/kg]	Recovery [%]
L1	600.63	602.97	100.4
L2	1201.26	1214.23	101.1
L3	3003.14	3021.01	100.6
L4	6006.29	5983.67	99.6
L5	7207.54	7242.19	100.5
		Mean Recovery :	100.4

Limit of Quantification (LoQ) (A9642D)
The limit of quantification for each CGA226048 diastereomer was tested with A9642D, batch POR9K80245 spiked with pure reference substance of CGA226048 to level 1 (corresponding to approx. 1000 mg/kg CGA226048, relative to Metalaxyl-M). Five determinations of Level 1 of 5 sets (2 injections each) were performed and the mean recovery was determined.

The limit of quantification for CGA226048 diastereomer A is below 500 mg/kg.

Sample	Level spiked [mg/kg]	Level found [mg/kg]	Recovery [%]
S1-L1	526.54	512.89	97.4
S2-L1	538.05	533.69	99.2
S3-L1	543.99	552.06	101.5
S4-L1	520.97	527.60	101.3
S6-L1	543.99	537.38	98.8
		Mean Recovery	99.6%
		Mean [mg/kg]	532.72
		SD	14.28
		RSD [%]	2.68
		RSDr mod. Hor [%]	4.17
		HorRat	0.64

The limit of quantification for CGA226048 diastereomer B is below 600 mg/kg.

Sample	Level spiked [mg/kg]	Level found [mg/kg]	Recovery [%]
S1-L1	591.45	583.09	98.6
S2-L1	604.38	618.35	102.3
S3-L1	611.06	624.73	102.2
S4-L1	585.19	578.86	98.9
S6-L1	611.06	609.84	99.8
		Mean Recovery	100.4%
		Mean [mg/kg]	602.97
		SD	20.82
		RSD [%]	3.45
		RSDr mod. Hor [%]	4.09
		HorRat	0.84

Limit of detection

The limit of detection for each CGA226048 diastereomer was tested at 250 mg/kg by diluting one test solution Level 1 used for the determination of the limit of quantification by a factor of 2 with acetonitrile.

Since a peak was visible for each CGA226048 diastereomer, it is proven that the respective limit of detection is below 250 mg/kg.

Interference/ Specificity

The specificity and interference are established using a LC/MS analysis and standard addition mode.
The specificity was tested by comparison of the retention time of each CGA226048 diastereomer in the chromatograms as well as the spectra obtained from the unspiked test solution Level 0 (L0) with the spiked test solution Level 4 (L4) of Set 1:

Figure 3: Chromatogram of A9642D, POR9K80245, unspiked (Level 0)

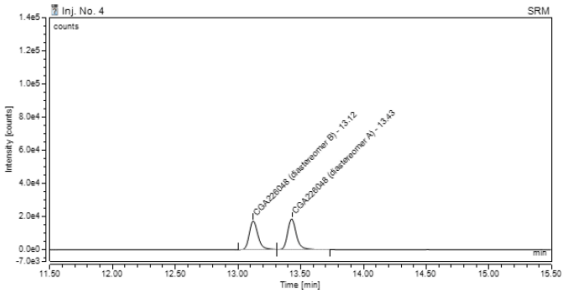


Figure 4: Chromatogram of A9642D, POR9K80245, spiked with approx. 10000 mg/kg of CGA226048 (Level 4)

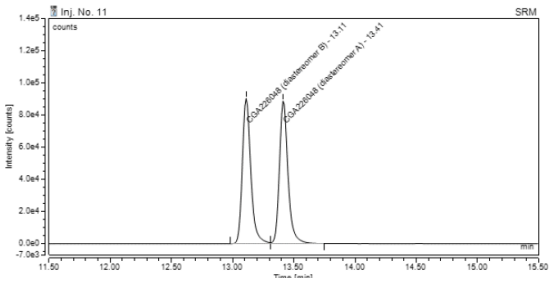
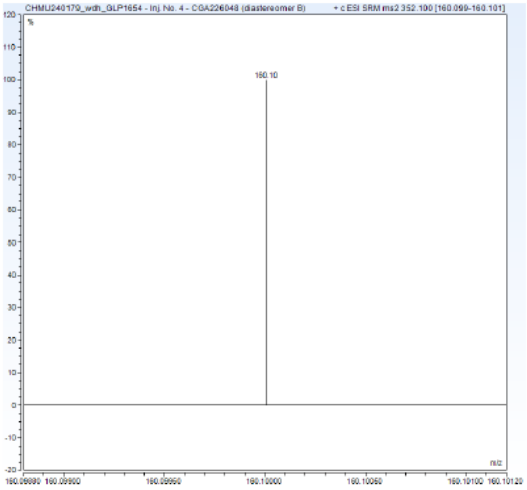


Figure 5: Spectra of A9642D, POR9K80245, unspiked (Level 0)

CGA226048 diastereomer B



CGA226048 diastereomer A

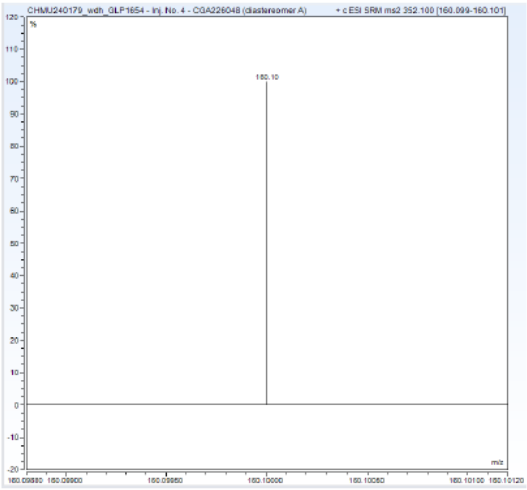
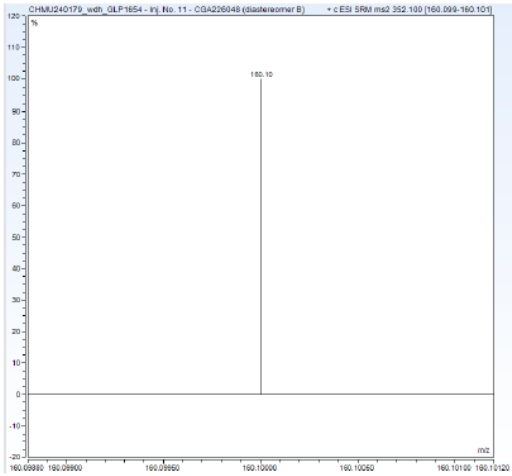
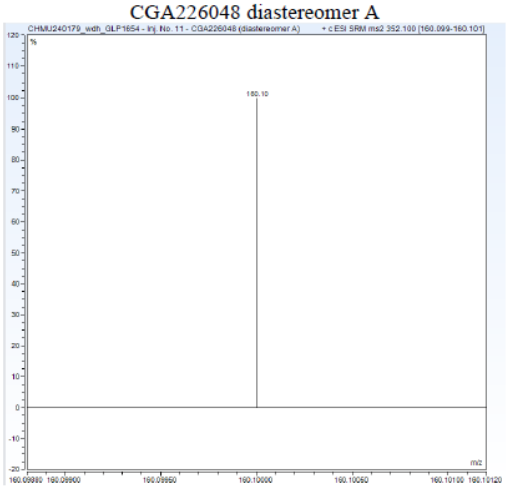


Figure 6: Spectra of A9642D, POR9K80245, spiked with approx. 10000 mg/kg of CGA226048 (Level 4)

CGA226048 diastereomer B



	CGA226048 diastereomer A  (See chromatograms in Figures 3-4 and spectra in Figures 5-6). Retention times did not deviate by more than 0.2 minutes. Additionally, the spectra of both solutions were qualitatively identical.
Comment	The method is acceptably validated. This validation has been performed according to the guidelines described in: - "SANCO/3030/99/rev.5" document dated 20 March 2019 by the European Commission Directorate General for Health and Consumer Protection - "Validation of analytical methods for active constituents and agricultural products" document dated 1 July 2014 by the Australian Pesticides and Veterinary Medicines Authority (APVMA) and the acceptance criteria therein. Based on the results, accuracy (recovery, linearity), precision (repeatability), specificity, limit of detection and limit of quantification of the method are established.

Linearity, recovery, limit of quantification and specificity of CGA226048 (max. content. < 1.8 g/kg in A23109A), determination in A23109A according to SD-2790/1:

The following data shows acceptable linearity and recovery for method SD-2790/1 when used for formulation A23109A. With the recovery at the lowest concentration levels (400 mg/kg for CGA226048 diastereomer A and 500 mg/kg for CGA226048 diastereomer B) being acceptable, a 400 mg/kg level for CGA226048 diastereomer A and a 500 mg/kg level for CGA226048 diastereomer B, each relative to metalaxyl-M are established as limits of quantification.

Linearity

The linearity was tested using 6 concentration levels (2 determinations each) of CGA226048 diastereomer A and CGA226048 diastereomer Bin formulation. The 6 levels correspond to 1 unspiked and 5 spiked levels (using pure reference substance of CGA226048) of GA226048 diastereomer A over the range of 400 mg/kg to 5000 mg/kg and CGA226048 diastereomer B over the range of 500 mg/kg to 6000 mg/kg each relative to the amount of metalaxyl-M in formulation A23109A. The results are illustrated both in tabular form and graphically below.

Sample	CGA226048 A		CGA226048 B	
	Level spiked [mg/kg]	Area found (average)	Level spiked [mg/kg]	Area found (average)
L0	0	0	0	0
L1	445	327	500	348
L2	891	644	1000	670
L3	2227	1618	2501	1704
L4	4453	3172	5002	3352
L5	5344	3745	6003	3953
	Slope	**0.70**	Slope	**0.66**
	Intercept	**18.41**	Intercept	**16.77**
	Correlation coefficient	**1.0000**	Correlation coefficient	**1.0000**

Recovery and limit of quantification

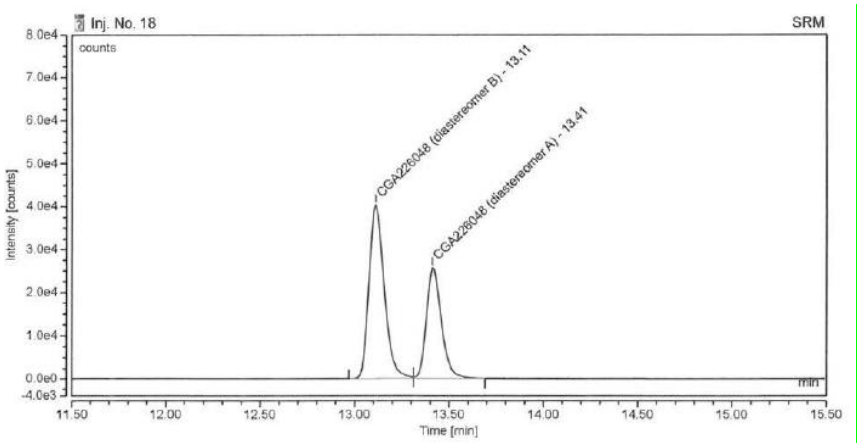
The recovery was tested with formulation spiked at 5 concentration levels (2 determinations each) using pure reference substance of CGA226048, over the range of 400 mg/kg to 5000 mg/kg of CGA226048 diastereomer A and over the range of 500 mg/kg to 6000 mg/kg of CGA226048 diastereomer B, both relative to the amount of metalaxyl-M in formulation A23109A. With the recovery at the lowest concentration levels (400 mg/kg and 500 mg/kg respectively) being acceptable, a 400 mg/kg level CGA226048 diastereomer A and a 500 mg/kg level CGA226048 diastereomer B each relative to the amount of metalaxyl-M are established as limits of quantification. The results are tabulated below.

Sample	CGA226048 A			CGA226048 B		
	Level spiked [mg/kg]	level found [mg/kg]	Recovery [%]	Level spiked [mg/kg]	level found [mg/kg]	Recovery [%]
L1	445.34	**439.62**	**98.70**	500.25	**498.28**	**99.60**
L2	890.68	**890.28**	**100.00**	1000.49	**985.77**	**98.50**
L3	2226.70	**2281.35**	**102.50**	2501.23	**2551.04**	**102.00**
L4	4453.41	**4494.23**	**100.90**	5002.45	**5040.30**	**100.80**
L5	5344.08	**5310.34**	**99.40**	6002.94	**5949.77**	**99.10**
		Mean:	**100.3**		**Mean:**	**100.0**

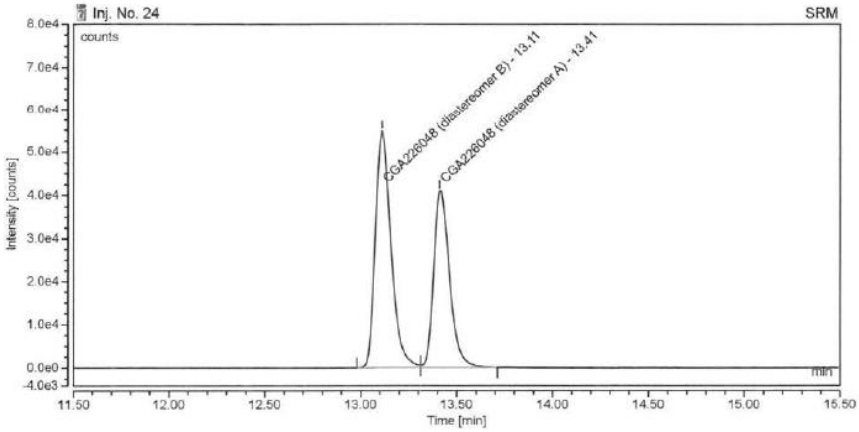
Specificity:

Using a specific detection technique and standard addition mode, the specificity is established and no significant interference was observed. The method has been shown to be specific for the determination of CGA226048 in formulation A23109A.

Chromatograms of A23109A, batch BSN2G0650:



Chromatograms of A23109A batch BSN2G0650 spiked with approx. 5000 mg/kg CGA226048 relative to the amount of metalaxyl-M present in formulation:



Conclusion
Method SD-2790/1 has been performed according to the guidelines described in "SANCO/3030/99/rev.5 and is valid for the determination of CGA226048 in formulation A23109A.

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

There are no relevant co-formulants in the product A23109A, therefore no methods are required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods for the determination of metalaxyl-M or oxathiapiprolin.

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

An overview on the acceptable methods and possible data gaps for analysis of residues of metalaxyl-M for the generation of pre-authorisation 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-authorisation data for metalaxyl-M in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

No specific analytical methods were used to support the environmental fate data generated on this

product.

Table 5.2-6: Validated methods for the generation of pre-authorisation data for metalaxyl-M in soil, water (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

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

Table 5.2-7: Validated methods for the generation of pre-authorisation data for metalaxyl-M in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

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

Table 5.2-8: Validated methods for the generation of pre-authorisation data for metalaxyl-M in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

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

Table 5.2-9: Validated methods for the generation of pre-authorisation 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 type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary (REM 181.01)	High water content *Tomato*	0.02 mg/kg	GC-NPD (original method) GC-MSD (confirmation) LC-MS/MS (updated method)	Method[(a)] and Validation: Kuehne, 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: Kuehne, 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: Kuehne, 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: Kuehne, 1999 Report No. 519/99 EU agreed (Belgium, 2014)
	High water content *Witloof chicory leaves*	0.01 mg/kg		Validation: Anderson, 2005 Report No. T004798-04

Component of residue definition for plant and animal products: metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	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, bean seeds, globe artichoke, leek, potato*	0.02 mg/kg		EU agreed (Belgium, 2014)
	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		

(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**
Primary (REM 181.13A)	High water content *Peach, tomato*	0.01 mg/kg	LC-MS/MS	Method[(a)]: Hill, 2005 Report REM 181.13 Validation: Hill, 2005 Report RJ3585B 04-S624 Hill, 2005 Report. 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	0.01 mg/kg		

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**
	Hops			
Primary (REM181.13A)	High water *Broccoli, cauliflower, cucumber, lettuce, melon, onion*	0.01 mg/kg	LC-MS/MS	Validation: Hillier K, 2021, Report GS12ND, (VV-901824) **New data**
	High acid *Grape*	0.01 mg/kg		
	High oil *Cotton seed*	0.01 mg/kg		
	High protein *Beans*	0.01 mg/kg		
	High starch *Barley, potato, sugar beet root*	0.01 mg/kg		
	Difficult commodities *Tobacco*	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.

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**
Primary (DFG S19)	Tomato	0.02 mg/kg	GC-MSD (single residue)	Validation (tomato, spinach): Wiesner, Breyer, 2012 Report: S11-03698 (VV-401335) EU agreed (see Table 5.3-4) EU agreed (Belgium, 2014)
	Spinach	0.02 mg/kg	GC-MSD (single residue)	

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**
Primary (QuEChERS)	Milk	0.01 mg/kg	LC-MS/MS (single residue)	QuEChERS validation (milk, eggs, muscle, fat, liver, kidney and blood): xxxxxxx, 2011 Report No. S11-01732 (VV-400487) (see Table 5.3-4) **New data**
	Eggs	0.01 mg/kg	LC-MS/MS (single residue)	
	Muscle/meat	0.01 mg/kg	LC-MS/MS (single residue)	
	Fat	0.01 mg/kg	LC-MS/MS (single residue)	
	Liver	0.01 mg/kg	LC-MS/MS (single residue)	
	Kidney	0.01 mg/kg	LC-MS/MS (single residue)	

Component of residue definition: 2,6-dimethylaniline				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary (GRM031.06A)	Milk	0.01 mg/kg	LC-MS/MS (single residue)	GRM031.06A Method: xxxxxxxxxx, 2012 Report No. S11-03382 (VV-402332) (see in (see Table 5.3-5) Validation (milk, eggs, muscle, fat, liver, kidney): xxxxxxxxxx, 2012 Report No. S11-03382 (VV-402332) (see Table 5.3-5) EU agreed (Belgium, 2014)
	Eggs	0.01 mg/kg	LC-MS/MS (single residue)	
	Muscle/meat	0.01 mg/kg	LC-MS/MS (single residue)	
	Fat	0.01 mg/kg	LC-MS/MS (single residue)	
	Liver	0.01 mg/kg	LC-MS/MS (single residue)	
	Kidney	0.01 mg/kg	LC-MS/MS (single residue)	

Table 5.2-10: Methods and relationship to studies presented in document Part B, Section 7

Method	**Suppored study (Part B Section 7)**		
Identifier	Data Point	Report Reference	Matrix
REM 181.01	KCA 6.3.7	Magnitude of residues of Metalaxyl-M (CGA 329351), Chlorothalonil (ASF 41) and Mancozeb (ASF 21) in onions applied as formulations SC 537.5 and WP 65 in side-by-side trials in Spain and in the United Kingdom, Kühne R.O., 1998, report No 116/97, document No VV-376089	Onions
	KCA 6.7.3	Residue study with Metalaxyl-M (CGA 329351) + Chlorothanlonil (ASF 41) and Metalaxyl-M (CGA 329351) + Mancozeb (ASF 21) in or on onions in Switzerland, Kühne R.O., 1999, report No 2077/98, document No VV-312825	Onions
REM 181.13A	KCA 6.1.1	Metalaxyl-M – Storage Stability of Residues of Metalaxyl-M in Crop Matrices Stored Frozen for up to Two Years, Baumy. G, 2022, Report No. RNB19-00020, Syngenta document No. VV-894456	Oilseed rape seed, orange, lettuce, wheat grain
	KCA 6.1.1	Metalaxyl-M - Honey Residue Study on Spring Oilseed Rape in Northern Europe in 2020, Knäbe, S., 2021, Report No. S20-03602. Syngenta document No. VV-881362	Honey
	KCA 6.3.1	Oxathiapiprolin and Metalaxyl-M - Residue Study on Broccoli in Germany, Hungary, Poland and the United Kingdom 2020, Meyer M., Poperechna N., 2021, report No IF20-05335380, document No VV-908189	Brocolli
	KCA 6.3.1	Metalaxyl-M - Residue study on Cauliflower in the United Kingdom and Northern France in 2013, Yozgatli H.P., Breyer N. 2014, report No S13-03434, document No VV-407968	Cauliflower
	KCA 6.3.2	Oxathiapiprolin/Metalaxyl-M – Determination of residues of Oxathiapiprolin and Metalaxyl-M in Kale from Trials conducted in NEU in 2020, Brown S., 2021, report No RES-00256, document No VV-901782	Kale
	KCA 6.3.3	Oxathiapiprolin/Metalaxyl-M – Residue Study on Cabbage in Northern France, Austria, Hungary and Poland in 2020, Ford K., 2021, report No CEMR-9523, document No VV-901921	Cabbage

Method	**Suppored study (Part B Section 7)**		
Identifier	Data Point	Report Reference	Matrix
	KCA 6.3.4	Oxathiapiprolin/Metalaxyl-M – Determination of residues of Oxathia-piprolin and Metalaxyl-M in Brussels Sprouts from Trials conducted in NEU in 2020, Brown S., 2021, report No RES-00257, document No VV-901790	Brussels sprouts
	KCA 6.3.5	Oxathiapiprolin / Metalaxyl-M - Determination of residues of Oxathia-piprolin and Metalaxyl-M in Lettuce from trials conducted in NEU in 2020, Brown S., 2021, report No RES-00259, document No VV-901318	Lettuce
	KCA 6.3.5	Oxathiapiprolin / Metalaxyl-M - Determination of residues of Oxathia-piprolin and Metalaxyl-M in Lettuce from trials conducted in Southern Europe in 2020, Brown S., 2021, report No RES-00260, document No VV-901319	Lettuce
	KCA 6.3.1	Oxathiapiprolin/Metalaxyl-M – Determination of residues of Oxathia-piprolin and Metalaxyl-M in Protected Lettuce from Trials conducted in France, Poland, Italy and Spain in 2020, Brown S., 2021, report No RES-00258, document No VV-901190	Lettuce
	KCA 6.3.6	Oxathiapiprolin/Metalaxyl-M – Residue Study on Leek in Northern France, Poland, United Kingdom and Germany in 2020, Ford K., 2021, report No CEMR-9521, document No VV-901936	Leek
	KCA 6.3.6	Oxathiapiprolin/Metalaxyl-M – Residue Study on Leek in Southern France, Spain and Portugal in 2020, Ford K., 2021, report No CEMR-9522, document No VV-901926	Leek
DFG S19	KCA 6.3.7	Residue study with Metalaxyl-M (CGA 329351) and Mancozeb (ASF 21) in or on onions in Switzerland, Kühne R.O., 1998, report No 2338/97, document No VV-357405	Onions
	KCA 6.3.7	Residue study with Metalaxyl-M (CGA 329351) and Mancozeb (ASF 21) in or on onions in Switzerland, Kühne R.O., 1998, report No 2340/97, document No VV-357408	Onions

Table 5.2-11: Validated methods for the generation of pre-authorisation data for metalaxyl-M in soil, water (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition for soil and water: metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary (ECO_048_03A)	Royal jelly /ASS (50/50 w/w), 50% w/v sucrose containing 0.1% w/v xanthan, 0.5% v/v TritonX solution+	0.01 mg/kg	LC-MS/MS	Method: Lünsmann, V., 2021 Validation: Lünsmann, V., 2021 Report No. 21 35 CRB 0059 (VV-928043) **New data**
Primary (HPLC-UV)	Water	45.5 mg/L	HPLC-UV	Method: Eckert, J., 2016 Validation (water) Eckert, J., 2016 Report No. S15-02457, (A13947A_11455, VV-415529) **New data**

Table 5.2-12: Validated methods for the generation of pre-authorization data for A23109A in soil, water (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition for soil and water: A23109A				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary (GRM031.08A)	Fish water	0.258 mg/L	LC-MS/MS	Method: Schuler, L., 2021 Validation: Schuler, L., 2021 Report No. S20-06894 (VV-893371) **New data**
Primary (GRM031.08A)	APP medium	0.163 mg/L	LC-MS/MS	Method: Schuler, L., 2021 Validation: Schuler, L., 2021 Report No. S20-06896 (VV-898484) **New data**
Primary (GRM031.08A)	Elendt M4 medium	0.103 mg/L	LC-MS/MS	Method: Schuler, L., 2021 Validation: Schuler, L., 2021 Report No. S20-06895 (VV-893390) **New data**

Table 5.2-13: Methods and relationship to studies presented in document Part B, Section 9

Method	Supported Study (Part B Section 9)	
Identifier	**Data Point**	**Report Reference**
ECO_048_03A (Royal jelly /ASS (50/50 w/w), 50% w/v sucrose containing 0.1% w/v xanthan, 0.5% v/v TritonX solution+)	KCA1 8.3.1.1	Report Number 21 48 BBA 0011, VV-939735 (Acute toxicity to the bumblebee *Bombus terrestris* L. under laboratory conditions)
	KCA1 8.3.1.2	Report Number 21 48 BAC 0027, VV-932880 (Chronic toxicity to the honey bee, *Apis mellifera* L., in a 10-day continuous laboratory feeding study)
Water HPLC-UV	KCA1 8.3.1.3	Report No. S15-02457, VV-415529, A13947A_11455 (Honey Bee (*Apis mellifera L.*) Larval Toxicity Test (Repeated Exposure)
Fish water GRM031.08A	KCP 10.2.1	Report Number S20-06894, VV-893371 (Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions*)*
APP medium GRM031.08A	KCP 10.2.1	Report No. S20-06896, VV-898484 (Toxicity to the Single Cell Green Alga *Raphidocelis subcapitata* Korshikov under Laboratory Conditions)
Elendt M4 medium GRM031.08A	KCP 10.2.1	Report No. S20-06895, VV-893390 (Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions)

Table 5.2-14: Validated methods for the generation of pre-authorisation data for metalaxyl-M in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

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 oxathiapiprolin (KCP 5.1.2)

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

Table 5.2-15: Validated methods for the generation of pre-authorisation data for oxathiapiprolin in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

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

Table 5.2-16: Validated methods for the generation of pre-authorisation data for oxathiapiprolin in soil, water (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

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

Table 5.2-17: Validated methods for the generation of pre-authorisation data oxathiapiprolin in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

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

Table 5.2-18: Validated methods for the generation of pre-authorisation data for oxathiapiprolin in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

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

Table 5.2-19: Validated methods for the generation of pre-authorisation data for oxathiapiprolin in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Method type	Matrix type	Method LOQ	Principle of method (*i.e.*, GC-MS or HPLC-UV)	Author(s), year / Report No. / EU agreed
Component of residue definition for plant products: oxathiapiprolin				
Primary (DuPont-30422)	High protein/high starch content (dry) - *Wheat grain* - *Potatoes*	0.01 mg/kg	LC-MS/MS	Method: Henze R.M. & Stry J.J., 2011a, 2013 Report. DuPont-30422 EU agreed (Ireland, 2015) Validation: Brown D. & Woodmnansey L., 2012 Report: DuPont-31091 EU agreed (Ireland, 2015) Method and validation: Donald C. & Gibson R., 2020 Report: 231693 (VV-870136) (see Table 5.3-14) **New Data** Method and validation (lettuce): Lakaschus S. & Reinhardt R., 2020 Report: S19-02718 (VV-854039) **New Data**
	High water content - *Grapes* - *Tomatoes* - *Wheat forage* - *Pepper* - *Cucumber* - *Melon* - *Leek* - *Broccoli* - *Cauliflower* - *Cabbage* - *Kale* - *Brussel sprouts* - *Lettuce*	0.01 mg/kg		
	High oil content - *Canola seed* - *Soybean, hops*	0.01 mg/kg		
	No group - *Wheat straw*	0.01 mg/kg		
	Processed commodities - *Grapes pomace* - *Grape juice* - *Wine*	0.01 mg/kg		
Primary (DuPont-33818) (Supplement 1 to DuPont-30422)	High protein/high starch content (dry) - *Wheat grain* - *Potatoes*	0.01 mg/kg	LC-MS/MS	Method: Chapleo S., Inns L., 2013 Report:. Dupont-33818 Validation: Within in the report Dupont-33818 EU agreed (Ireland, 2015) Validation (honey): Ford, K., 2020 Report: CEMR-9533; DuPont-30422 Supplement No. 1 (VV-885771) (see Table 5.3-14) **New Data**
	High water content - *Grapes* - *Tomatoes* - *Ginseng* - *Wheat forage*	0.01 mg/kg		
	High oil content - *Canola seed* - *Soybean*	0.01 mg/kg		
	No group - *Wheat straw*	0.01 mg/kg		
	High acid content - *Oranges*[(c)]	0.01 mg/kg		
	Other - *Honey*	0.01 mg/kg		

Table 5.2-20: Methods and relationship to studies presented in document Part B, Section 7

Method	**Suppored study (Part B Section 7)**		
Identifier	Data Point	Report Reference	Matrix
DuPont-33818 (Supplement 1 to DuPont-30422)	KCA2 6.10	Oxathiapiprolin – Honey Residue Study on Winter Oilseed Rape in Northern and Southern Europe in 2021, Ford K (2021), Report number CEMR-9822 Syngenta File No. VV-924794	Honey
	KCA2 6.10	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020, Ford, K., 2020, CEMR-9533, VV-885771	Honey
	KCA2 6.3.1	Decline and magnitude of residues of DPX-QGU42 and its metabolites in dry bulb onions (Bulb vegetables) following foliar application of DPX-QGU42 100 g/L OD or DPX-QGU42 100 g/L SE - Europe 2012-2013, Spence, C. Brown, D., 2015, document No Dupont-31988.	Onions
DuPont-30422	KCA2 6.3.1	Oxathiapiprolin and Metalaxyl-M - Residue Study on Broccoli in Germany, Hungary, Poland and the United Kingdom 2020, Meyer M., 2021, report No IF20-05335380, document No VV-908189	Broccoli
	KCA2 6.3.1	Oxathiapiprolin and Metalaxyl-M - Residue Study on Cauliflower in Germany, Poland, the United Kingdom and Denmark 2020, Mahlo C., 2021, report No IF20-05336777, document No VV-901124	Cauliflower
	KCA 6.3.2	Oxathiapiprolin/Metalaxyl-M – Determination of residues of Oxathia-piprolin and Metalaxyl-M in Kale from Trials conducted in NEU in 2020, Brown S., 2021, report No RES-00256, document No VV-901782	Kale
	KCA 6.3.3	Oxathiapiprolin/Metalaxyl-M – Residue Study on Cabbage in Northern France, Austria, Hungary and Poland in 2020, Ford K., 2021, report No CEMR-9523, document No VV-901921	Cabbage
	KCA 6.3.4	Oxathiapiprolin/Metalaxyl-M – Determination of residues of Oxathiapiprolin and Metalaxyl-M in Brussels Sprouts from Trials conducted in NEU in 2020, Brown S., 2021, report No RES-00257, document No VV-901790	Brussels sprouts
	KCA 6.3.1	Decline and magnitude of residues of DPX-QGU42 and its metabolites in field lettuce (leafy vegetables) following foliar application of DPX-QGU42 100 g/L OD or DPX-QGU42 100 g/l SE – Europe, 2011-2012, Spence C., Brown D., 2011/12, report No 696296, document No Dupont-31734.	Lettuce
	KCA 6.3.1	Decline and magnitude of residues of DPX-QGU42 and its metabolites in field lettuce (leafy vegetables) following foliar application of DPX-QGU42 100 g/L OD or DPX-QGU42 100 g/l SE – Europe, 2011-2012, Spence C., Brown D., 2011/12, report No 696296, document No Dupont-31734.	Lettuce
	KCA 6.3.1	Oxathiapiprolin – Residue Study on Lettuce (protected) in Germany, Poland, Hungary, Denmark, Southern France and Italy 2020, Gabriel E.J., 2021, report No IF20-05334826, document No VV-895841	Lettuce
	KCA 6.3.6	Oxathiapiprolin/Metalaxyl-M – Residue Study on Leek in Northern France, Poland, United Kingdom and Germany in 2020, Ford K., 2021, report No CEMR-9521, document No VV-901936	Leek
	KCA 6.3.6	Oxathiapiprolin/Metalaxyl-M – Residue Study on Leek in Southern France, Spain and Portugal in	Leek

Method	**Suppored study (Part B Section 7)**		
Identifier	Data Point	Report Reference	Matrix
		2020, Ford K., 2021, report No CEMR-9522, document No VV-901926	

Table 5.2-21: Validated methods for the generation of pre-authorisation data for A23109A in soil, water (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition for soil and water: oxathiapiprolin				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary (ECO_052_03A)	Royal jelly/ASS 50% w/v sucrose solution	0.009 mg/kg	HPLC-MS/MS	Method and validation (royal jelly/ ASS and 50% sucrose solution) Lünsmann V., 2020 Report: 20 35 CRB 0103, (VV-884296) **New data**

Table 5.2-22: Validated methods for the generation of pre-authorization data for oxathiapiprolin in soil, water (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition for soil and water: A23109A				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary (DuPont-41989)	Feeding solution	3.2 mg/L	HPLC-UV	Method and validation (feeding solution): Tanzler, V., 2015 Report: 94441136 DuPont-41989 (VV-910995) **New data**
Primary (DuPont-48606)	Larval diet Test solution	0.5 mg/kg 150 mg/L	HPLC-MS/MS	Method and validation (larval diet, test solution) Oberrauch, S., 2017 Report: S17-01639, DuPont-48606 (VV-911004) **New data**

Table 5.2-23: Methods and relationship to studies presented in document Part B, Section 9

Method	**Supported Study (Part B Section 9)**	
Identifier	**Data Point**	**Report Reference**
ECO_052_03A (Royal jelly/ASS 50% w/v sucrose solution)	KCP 10.3.1.1.1	Report Number 21 48 BBA 0033, VV-936483 (Acute toxicity to the bumblebee *Bombus terrestris* L. under laboratory conditions)
	KCP 10.3.1.2	Report Number 20 48 BAC 0044, VV-896929 (– Chronic toxicity to the honey bee *Apis mellifera* L. in a 10-day continuous laboratory feeding study)
Feeding solution DuPont-41989	KCA2 8.3.1.2	Report: 94441136, DuPont-41989, VV-910995, (Chronic oral toxicity to the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae)
Test solution DuPont-48606	KCA2 8.3.1.3	Report: S17-01639, DuPont-48606 (VV-911004) (Honey bee (*Apis mellifera* L.) 22 day larval toxicity test (repeated exposure))

Table 5.2-24: Validated methods for the generation of pre-authorisation data for oxathiapiprolin in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

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

5.3 Methods for post-authorisation 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 metalaxyl-M (KCP 5.2)

5.3.2.1 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.

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	Metalaxyl and metalaxyl-M (metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers))	~~0.05~~ 0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Plant, high acid content		~~0.05~~ 0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Plant, high protein/high starch content (dry commodities)		~~0.05~~ 0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Plant, high oil content		~~0.05~~ 0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Plant, difficult matrices (hops, spices, tea)		~~0.1~~ 0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Muscle	Sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6-dimethylaniline moiety, expressed as metalaxyl	0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Milk		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Eggs		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Fat		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Liver, kidney		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164 (lowest MRL)
Soil (Ecotoxicology)	Metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)	0.05 mg/kg	Common limit

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Drinking water (Human toxicology)	Metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)	0.1 µg/L	General limit for drinking water SANTE/2020/12830, Rev.1
Surface water (Ecotoxicology)	Metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)	1.0 mg/L	NOEC for Daphnia (EFSA, 2015a)
Air	Metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)	24 µg/m^3	AOEL sys: 0.08 mg/kg bw/d (EFSA, 2015a)
Tissue (meat or liver)	Sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6-dimethylaniline moiety, expressed as metalaxyl	0.01 mg/kg	GRM031.06A (blood) (LOQ) SANTE/2020/12830, Rev.1
Body fluids		0.01 mg/L	

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

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 metalaxyl-M studies it is referred to Appendix 2.

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

Component of residue definition: metalaxyl[(a)]				
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	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS validation (tomato, potato, orange oilseed rape seed, dried bean): Weber & Gizler, 2011 Report: S11-01731 (VV-400486) (2 ion transitions validated) EU agreed (Belgium, 2014) ILV (tomatoes and oilseed rape) : Mewis, A, 2012 (Amendment 2014) Report: S11-03712 (VV-407367) **New data**
High acid content	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (multi-residue)	
High oil content	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (multi-residue)	
High protein/high starch content (dry)	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (multi-residue)	
Difficult matrices (if required,	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Validation (hop, cocoa bean):

Component of residue definition: metalaxyl[(a)]				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
depends on intended use) (Hops, cocoa beans, peppercorns)	ILV (QuEChERS)	0.01 mg/kg		Brown D, 2016 Report: RES-00055 (VV-465727) **New data** ILV (hop, cocoa bean): Burton D, 2016 Report: YB27DB (VV-465743) (2 ion transitions validated) **New data** QuEChERS Vaidation (honey, peppercorns) Mechelke, J., 2022 Report:20210433 VV-936304 **New data**
Component of residue definition: metalaxyl-M (enantiomer specific)				
High water content	Primary (REM 181.06)	0.02 mg/kg	GC-MSD (single residue)	REM 181.06 Method: Kühne, 2001 Validation (tomato, potato, orange oilseed rape seed, wheat grain): Kühne, 2001a Report: 212/00 EU agreed (Belgium, 2014) ILV (tomato, orange oilseed rape seed): Pointurier, 2001 Report: NOV/MET00111 EU agreed (Belgium, 2014) ---------- DFG S19 Validation (tomato, spinach): Wiesner, Breyer, 2012 Report: S11-03698 (VV-401335) EU agreed (Belgium, 2014)
	ILV (REM 181.06)	0.02 mg/kg		
	Primary (DFG S19)	0.01 mg/kg	LC-MS/MS (multi-residue)	
High acid content	Primary (REM 181.06)	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg		
High oil content	Primary (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)	Primary (REM 181.06)	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg		

(a) Residue definition for enforcement: metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers).

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
Required, available from:	**Extraction Efficiency (SANTE 2017/10632 Rev. 3)** Based on SANTE 2017/10632, 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. 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 any case some further data is provided below. The extraction efficiency for the crop analytical method REM181.01 (validation reports No. REM181.01, 517/99, 518/99, 519/99 and T004798-04) and REM181.13A

	(validation reports No. REM181.13, RJ3585B.04-S624 and REM181.13A[(a)]), can be demonstrated from radiolabelled extraction data available for metalayl/metalaxyl-M in the submitted metabolism study reports: Spring Wheat - Report No's 02JS37 and 02JS38 Tomato - report No 026135-1 Lettuce report No. 98JS30). In these studies, metalaxyl /metalaxyl-M residues are extracted using methanol: water (80:20) compared to the analytical methods, REM181.15 and REM181.13A in which the extraction solvent is acetonitrile: water (90:10). Syngenta believes that for these two methods, the two extraction solvents are similar and therefore extraction efficiency has been demonstrated in the radiolabelled metabolism studies. Additionally, the procedural recoveries utilised in the analytical methods are a good indication of extractability. A new study is available, to demonstrate extraction efficiency for the crop multi-residue analytical method QuEChERS (validation report No. S21-03444, see Appendix 2). A cross validation study was conducted where four crop matrices treated with metalaxyl-M were analysed for incurred residues. The field samples were analysed using methanol/ water (80/20 v/v) as used in metabolism studies and acetonitrile for QuEChERS method. The residue levels determined via the solvent system (acetonitrile) used in the monitoring analytical method QuEChERS demonstrated that comparable results were obtained to solvent systems used in metabolism studies (i.e. <30% difference in measured residues). Therefore cross validation is acceptable.
Not required, because:	-

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

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

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

Table 5.3-4: 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	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	QuEChERS Validation (milk, eggs, muscle, fat, liver, kidney and blood): xxxxxxxx, 2011 Report: S11-01732 VV-400487 **New data** ILV (milk, eggs, muscle, liver, and fat) : xxxxxxxx, 2018 Report: MM87YQ (VV-470901) **New data**
	ILV (QuEChERS)	0.01 mg/kg		
Eggs	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (QuEChERS)	0.01 mg/kg		
Muscle/meat	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (QuEChERS)	--		
Fat	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (QuEChERS)	0.01 mg/kg		
Liver	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (QuEChERS)	0.01 mg/kg		
Kidney	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (QuEChERS)	0.01 mg/kg		
Honey	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	QuEChERS Vaidation (honey, peppercorns) Mechelke, J., 2022 Report:20210433 VV-936304 **New data**
	ILV (QuEChERS)	0.01 mg/kg		QuEChERS Vaidation (honey, peppercorns) Jooß, S., Leibing F., Tussetschläger, S., 2022 Report number S21-08274 VV-948185 **New data**

Table 5.3-5: Validated methods for food and feed of animal origin

Component of residue definition: 2,6-dimethylaniline				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	Primary (GRM031.06A)	0.01 mg/kg	LC-MS/MS (single residue)	GRM031.06A Method: xxxxxxxxxx, 2012 Report: S11-03382 (VV-402332) Validation (milk, eggs, muscle, fat, liver, kidney): xxxxxxxxxx, 2012 Report: S11-03382 (VV-402332)
	ILV (GRM031.06A)	0.01 mg/kg		
Eggs	Primary (GRM031.06A)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (GRM031.06A)	0.01 mg/kg		
Muscle/meat	Primary (GRM031.06A)	0.01 mg/kg	LC-MS/MS (single residue)	

Component of residue definition: 2,6-dimethylaniline				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	ILV (GRM031.06A)	--		EU agreed (Belgium, 2014) ILV (milk, eggs, liver, kidney) : Amic, 2012 Report: S12-03412 (VV-402378) EU agreed (Belgium, 2014) ILV (fat): xxxxxxxx, 2016 Report: S16-00573 (VV-463097) **New data**
Fat	Primary (GRM031.06A)	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (GRM031.06A)	0.01 mg/kg		
Liver, Kidney	Primary (GRM031.06A)	0.01 mg/kg	LC-MS/MS (single residue)	
	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 5.3-6: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	**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. 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. The extraction efficiency for analytical method GRM031.06A, can be demonstrated from radiolabelled extraction data available in the submitted goat metabolism study report: ABR-90078. In this study milk samples were extracted with acetonitrile, and liver, kidney, perirenal fat and muscle were extracted with methanol: water (80:20). The analytical method GRM031.06A uses acetonitrile: water (80:20). Syngenta believes the polarity of methanol: water (80:20) and acetonitrile: water (80:20) are very similar, therefore extractability of residues has been demonstrated. An additional perirenal fat sample in the metabolism study was extracted with acetonitrile: hexane (80:20). The hexane was included to dissolve and remove the fat solids and the acetonitrile efficiently extracted the metalaxyl residues from the fat matrix. In method GRM031.06A, the fat samples are dissolved in a mixture of ethyl acetate: cyclohexane (1:1) with heating to solubilise the fat. Acetonitrile is then added to extract the metalaxyl-M residues. The subsequent freezing of the solvent mixture solidifies and separates the fat. Syngenta maintain extractability of metalaxyl-M residues in all animal commodities has been demonstrated using GRM031.06A.
Not required, because:	See above justification

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

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

Table 5.3-7: 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**
Blood	Primary (QuEChERS)	0.01 mg/kg	LC-MS/MS (single residue)	QuEChERS Validation (milk, eggs, muscle, fat, liver, kidney and blood): xxxxxxxxx, 2011 Report: S11-01732 (VV-400487) **New data**

Table 5.3-8: Methods for body fluids and tissues

Component of residue definition: 2,6-dimethylaniline				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing**
Blood	Primary and ILV (GRM031.06A)	0.01 mg/L	LC-MS/MS	GRM31.06A Method: xxxxxxxxx 2012 Report S11-03382 (VV-402332) Validation: xxxxxxxxxx, 2012 Report: S11-03382 (VV-402332) ILV: Amic, 2012 Report: S12-03412 (VV-402378) EU agreed (Belgium, 2014)

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

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

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

Table 5.3-9: Validated methods for soil

Component of residue definition: Metalaxyl-M 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 with 2 mass transitions (*non-enantiospecific*)	GRM031.03A Method and validation: Crook, 2008a Report: GRM031.03A EU agreed (Belgium, 2014)
Confirmatory	-	-	Not required: 2 ion transi-tions validated in primary method

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

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/additional studies it is referred to Appendix 2.

Table 5.3-10: Validated methods for water

Component of residue definition: Metalaxyl-M 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 with 2 mass transitions *(non-enantiospecific)*	GRM031.02A Crook, 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	ILV	0.05 µg/L	LC-MS/MS with 2 mass transitions *(non-enantiospecific)*	Link, 2016 Report: IF-15/03469803-TK (VV-415481) **New data**
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS with 2 mass transitions *(non-enantiospecific)*	GRM031.02A Crook, 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS with 2 mass transitions *(non-enantiospecific)*	GRM031.02A Crook, 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method

* CGA108906 is the racemic form of metalaxyl diacid metabolite, the R enantiomer of which is SYN546520.

Table 5.3-11: Validated methods for water

Component of residue definition: Metabolite 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 with 2 mass transitions *(non-enantiospecific)*	GRM031.08A Crook & Tessier, 2015 Report: GRM031.08A (VV-132583) KCP 5.2.5 **New data** Validation: Tessier, 2015 Report: TK0222545 (VV-412805) KCP 5.2.5 **New data**
	ILV	0.05 µg/L	LC-MS/MS with 2 mass transitions *(non-enantiospecific)*	Link, 2016 Report: IF-15/03469803-TK (VV-415481) KCP 5.2.5 **New data**
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS with 2 mass transitions	GRM031.08A Crook & Tessier, 2015

Component of residue definition: Metabolite CGA67868				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing**
			(non-enantiospecific)	Report: GRM031.08A (VV-132583) KCP 5.2.5 **New data** Validation: Tessier, 2015 Report: TK0222545 (VV-412805) KCP 5.2.5 **New data**
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS with 2 mass transitions *(non-enantiospecific)*	GRM031.08A Crook & Tessier, 2015 Report: GRM031.08A (VV-132583) KCP 5.2.5 **New data** Validation: Tessier, 2015 Report: TK0222545 (VV-412805) KCP 5.2.5 **New data**
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method

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 for the analysis of metalaxyl-M in air (KCP 5.2.6)

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

Table 5.3-12: Validated methods for air

Component of residue definition: Metalaxyl-M			
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 Evans, 2006 Report: T003619-05-REG EU agreed (Belgium, 2014)
Confirmatory	-	-	Not required: 2 ion transitions validated in primary method

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

5.3.2.8 Other studies/ information

No new or additional studies have been submitted.

5.3.3 Description of analytical methods for the determination of residues of

oxathiapiprolin (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels of oxathiapiprolin 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 5.3-13: Relevant residue definitions for monitoring/enforcement and levels oxathiapiprolin for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Oxathiapiprolin	0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Plant, high acid content		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Plant, high oil content		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Muscle	Oxathiapiprolin	0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Milk		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Eggs		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Fat		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Liver, kidney		0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Soil (Ecotoxicology)	Oxathiapiprolin	0.05 mg/kg Method LOQ: 0.001 mg/kg	Common limit NOEC for other soil macro-organisms (*Hypoaspis aculeifer*): 6.25 mg/kg dw soil (EFSA Journal 2016;14(7):4504)
Drinking water (Human toxicology)	Oxathiapiprolin	0.1 µg/L	General limit for drinking water SANTE/2020/12830, Rev.1
Surface water (Ecotoxicology)	Oxathiapiprolin	0.058 mg/L Method LOQ: 0.1 µg/L	NOEC for aquatic invertebrate (*Americamysis bahia*), reproduction, 32-day flow-through study (EFSA Journal 2016;14(7):4504)
Air	Oxathiapiprolin	0.05 µg/m^3	AOEL sys: 0.04 mg/kg bw/d
Tissue (meat or liver)	Oxathiapiprolin	0.01 mg/kg (LOQ)	MRL Regulation (EU) No ~~2017/1016~~ 2023/163
Body fluids		0.01 mg/L	Default LOQ SANTE/2020/12830, Rev.1

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

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-14: Validated methods for food and feed of plant origin

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Single residue method DuPont-30422				
High water content - *Ginseng* - *Tomatoes* - *Carrot roots* - *Spinach leaves* - *Wheat forage* - *Spinach* - *Broccoli* - *Whole pepper* - *Peppers* - *Cucumbers* - *Melon* - *Leek* - *Broccoli* - *Cauliflower* - *Cabbage* - *Kale* - *Brussels Sprouts*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2011b, 2013 Report DuPont-30422, Supplement No. 1 and 2 *Validation*: Brown D. & Woodmnansey L., 2012 Report DuPont-31091 Cairns S., et al., 2013 / Report DuPont-31091 Supplement No.1 Vincent T., 2013 / Report DuPont-31545 EU agreed (Ireland, 2015) *Validation*: Donald C. & Gibson R., 2020 / Report No. 231693 (VV-870136) **New Data**
	ILV	0.01 mg/kg	LC-MS/MS	*ILV:* Lissemore L., Li P., 2013 / Report DuPont-37739 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High acid content - *Oranges* - *Grapes*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2011b, 2013 / Reports DuPont-30422, Supplement No. 1 and 2 *Validation*: Brown D., & Woodmnansey L., 2012 / Report DuPont-31091 Cairns S., et al., 2013 / Report DuPont-31091 Supplement No.1 Vincent T., 2013 / Report DuPont-31545 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV:* Lissemore L., Li P., 2013 / Report DuPont-37739 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High oil content - *Canola, dried* - *Soybean seed*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2 *Validation*: Brown D., & Woodmnansey L., 2012 / Report: DuPont-31091 Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1 Vincent T., 2013 / Report: DuPont-31545 EU agreed (Ireland,2015)

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) *- Wheat grain* *- Potatoes* *- Dried beans* *- Dried tobacco leaves* *- Dried ginseng roots* *- Dried bulb onion*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2 *Validation*: Brown D., & Woodmnansey L., 2012 / Report: DuPont-31091 Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1 Vincent T., 2013 / Report: DuPont-31545 EU agreed (Ireland,2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV:* Lissemore L., Li P., 2013 / Report DuPont-37739 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Difficult to analyse *- Hops*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M. & Stry J.J., 2011a, 2013 / Report No. DuPont-30422 *Validation*: Brown D. & Woodmnansey L., 2012 / Report No. DuPont-31091 EU agreed (Ireland, 2015) *Validation*: Donald C. & Gibson R., 2020 / Report. 231693 (VV-870136) **New Data**
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
No group *- Wheat straw* *- Honey*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2 *Validation*: Brown D., & Woodmnansey L., 2012 / Report: DuPont-3109 Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1 Vincent T., 2013 / Report: DuPont-31545 EU agreed (Ireland,2015) *Validation (honey)*: Ford, K., 2020 / Report No. CEMR-9533 (VV-885771) **New Data**
	ILV	-	-	-

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Processed commodities - *Potato chips* - *Dry grape pomace* - *Tomato juice*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2011b, 2013 / Reports: DuPont-30422, Supplement No. 1 and 2 *Validation*: Brown D., & Woodmnansey L., 2012 / Report: DuPont-3109 Cairns S., et al., 2013 / Report: DuPont-31091 Supplement No.1 Vincent T., 2013 / Report: DuPont-31545 EU agreed (Ireland,2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Multi-residue method DFG S19				
High water content - *Apples* - *Tomatoes*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Weber H., 2012a / Report DuPont-31140 *Validation*: Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV:* Richter S., 2013 / Report DuPont-37477 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High acid content - *Citrus*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Weber H., 2012a / Report DuPont-31140 *Validation*: Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High oil content - *Oilseed rape*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Weber H., 2012a / Report DuPont-31140 *Validation*: Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV:* Richter S., 2013 / Report DuPont-37477 EU agreed (Ireland, 2015)

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) - *Barley grain*	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Weber H., 2012a / Report DuPont-31140 *Validation*: Within the report DuPont-31140 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV:* Richter S., 2013 / Report DuPont-37477 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Multi-residue method QuEChERS				
High water content - *Lettuce*	Primary	0.01 mg/kg	LC-MS/MS	*Method:* Schwarz T., 2009 / Report DuPont-28696 *Validation*: Within the report DuPont-28696 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High acid content - *Oranges*	Primary	0.01 mg/kg	LC-MS/MS	*Method:* Schwarz T., 2009 / Report DuPont-28696 *Validation*: Within the report DuPont-28696 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
High protein/high starch content (dry) - *Maize grain* - *Wheat grain*	Primary	0.01 mg/kg	LC-MS/MS	*Method:* Schwarz T., 2009 / Report DuPont-28696 *Validation*: Within the report DuPont-28696 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

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 5.3-15: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency was demonstrated for all EU crop groups for which incurred radiolabelled residues were generated during the radiolabelled

	Method for products of plant origin
	metabolism studies (Chapleo. S., Inns, L., 2013, Dupont-33818; Inns, L., 2012, DuPont-36106). Extraction efficiency was demonstrated for the watery (foliage), acidic (grape berries), and dry (wheat grain) EU crop groups. The radiolabelled metabolism studies conducted did not generate any incurred residues samples that could be classified as an oily crop matrices. In addition, the MOR studies did not generate any samples with incurred residues greater than 0.01 mg/kg in oily crops that could be used to demonstrate extraction efficiency. Due to the lack of oily crop incurred residue samples extraction efficiency was tested on only three of the four EU crop groups. The residue method extraction procedure removed between 98-113% (for method: Henze, R.M., Stry, J.J., 2011b, DuPont-30422), 81.3-103.3% (for method Weber, H., 2012a; DuPont-31140), 77.5-81.2% (for method Schwarz, T., 2009, DuPont-28696) of the incurred residue removed by the metabolism extraction procedure. For the crop matrices tested the residue method demonstrated the ability to extract incurred residues. EU agreed (Ireland, 2015)
Not required, because:	-

zRMS comments:

It should be noted that an ILV method for hop is required. Applicant submitted additional information that an ILV for hops was submitted as part of the evaluation of the MRL for oxathiapiprolin in hops: (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5759)
(…) the applicant (Du Pont de NemoursGmbH) submitted validation data (including confirmatory data) and an ILV for themulti residue DFG-19 method which was proposed as enforcement method by the peer review.Validation data were provided for different matrices that are considered as complex: coffee beans,hops (dried), black tea (leaves) and dried tobacco (Ireland, 2017a). The validation data demonstratethat DFG-19 method is acceptable to enforce residues of oxathiapiprolin in coffee beans, hops, blacktea and dried tobacco at the LOQ of 0.01 mg/kg.

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

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.*, GC-MS or HPLC-UV)**	**Author(s), year / missing**
Single residue method DuPont-31138				
Milk	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.*, GC-MS or HPLC-UV)**	**Author(s), year / missing**
Eggs	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Muscle	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Fat	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Kidney	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Liver	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.*, GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Harris J.A., 2012 / Report No: DuPont-32355 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Heavy cream	Primary	0.01 mg/kg	LC-MS/MS	*Method*: Henze R.M., Stry J.J., 2012 / Report No: DuPont-31138 *Validation*: Within the report DuPont-31138 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Multi-residue method DFG S19				
Milk	Primary	0.01 mg/kg	LC-MS/MS	*Method*: xxxxxxxxx 2012b / Report No: DuPont-31951 *Validation*: Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Eggs	Primary	0.01 mg/kg	LC-MS/MS	*Method*: xxxxxxxxx 2012b / Report No: DuPont-31951 *Validation*: Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Muscle	Primary	0.01 mg/kg	LC-MS/MS	*Method*: xxxxxxx., 2012b / Report No: DuPont-31951 *Validation*: Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.*, GC-MS or HPLC-UV)**	**Author(s), year / missing**
Fat	Primary	0.01 mg/kg	LC-MS/MS	*Method*: xxxxxxxx., 2012b / Report No: DuPont-31951 *Validation*: Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	0.01 mg/kg	LC-MS/MS	*ILV*: Asekunowo J., Bacher R., 2013 / Report DuPont-37476 EU agreed (Ireland, 2015)
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Liver	Primary	0.01 mg/kg	LC-MS/MS	*Method*: xxxxxxx., 2012b / Report No: DuPont-31951 *Validation*: Within the report DuPont-31951 EU agreed (Ireland, 2015)
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific
Single residue method DuPont-30422				
Honey	Primary	0.01 mg/kg	LV-MS/MS	*Validation (honey)*: Ford, K., 2020 / Report No. CEMR-9533 (VV-885771) **New Data**
	ILV	-	-	-
	Confirmatory (if required)	-	-	Not required, primary method is highly specific

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 5.3-17: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	The residue analytical method (Henze, R.M., Stry, J.J., 2012 Report No: DuPont-31138 uses the same solvents (acetonitrile and hexane) as in the goat and hen metabolism studies (DuPont-28213 and DuPont-28244). The only difference is that the metabolism extraction protocol used a homogenizing probe while in the analytical residue method extraction, samples are extracted using a Genogrinder. Since the extraction protocols are similar, it can be concluded that Method DuPont-31138 provides acceptable extraction efficiency. The extraction efficiency of DFG S19 method (Weber, H., 2012b Report No: DuPont-31951) was examined in DuPont-36106 using samples of muscle, kidney, fat and milk containing incurred residues of radiolabelled test substance. The residue profiles obtained using DFG S19 extraction procedures were compared with those obtained for the same samples in the goat metabolism study. Both extraction procedures showed similar distribution and levels of metabolites. The levels of oxathiapiprolin and metabolites obtained using the residue method for animal matrices were within 95 to 135% of those found in the metabolism study, indicating that the residue method provides acceptable extraction efficiency. EU agreed (Ireland, 2015)
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in body fluids is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-18: Validated methods for body fluids

Component of residue definition: Oxathiapiprolin			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/L	LC-MS/MS oxathiapiprolin	Method: xxxxxxxx.; 2022 Report No. S22-02422; Corteva Study No. 220385 **New study**

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

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-19: Validated methods for soil

Component of residue definition: Oxathiapiprolin			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.001 mg/kg	LC-MS/MS oxathiapiprolin	Method: Henze, Stry & McCorquodale, 2010 (revised 2013) Report No. DuPont-28806 & DuPont-29443, Revision 1 EU agreed (2015)
Primary	0.001 mg/kg	LC-MS/MS oxathiapiprolin	Method: Henze, R.M., Stry, J.J., & Henze, R.M., Stry, J.J., & 2010, 2013, 2013 Report No. DuPont-31005, DuPont-31005 (Supplement No. 1), & DuPont-29443, Revision No. 1 ILV: Ju, L., & McCorquodale, G, 2011, DuPont-31141 EU agreed (2015)

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

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in surface and drinking water is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-20: Validated methods for water

Component of residue definition: Oxathiapiprolin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e., GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water/ Surface water	Primary	Potable, groundwater and surface water: 0.0001 mg/kg	LC-MS/MS Oxathiapiprolin	Method: Henze, Stry, 2011, 2013 Report No. DuPont-32124, DuPont-32124, Supplement No. 1 EU agreed (2015)
	ILV	Potable, groundwater and surface water: 0.0001 mg/kg	LC-MS/MS Oxathiapiprolin	ILV: Xu, 2012 Report No. DuPont-32693 EU agreed (2015)
Drinking water/ Surface water	Primary	Groundwater, surface water 0.01 µg/mL	LC/UV Oxathiapiprolin	Henze, Stry, 2012 Report No. DuPont-32692 EU agreed (2015)

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

An overview on the acceptable methods and possible data gaps for analysis of oxathiapiprolin in air is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-21: Validated methods for air

Component of residue definition: Oxathiapiprolin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 µg/m^3	HPLC-MS/MS oxathiapiprolin	Method: Traub, 2012 Report No. DuPont-32356 EU agreed (2015)

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 other studies are required.

5.4 References

Metalaxyl-M

Belgium, 1999. Draft assessment report on the active substance metalaxyl-M prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, July 1999.

Belgium, 2001. Addendum to the draft assessment report on the active substance metalaxyl-M prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, September 2001.

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.

Oxathiapiprolin

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance oxathiapiprolin. EFSA Journal 2016;14(7):4504, [89 pp.] doi:10.2903/j.efsa.2016.4504.

Ireland, 2015. Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009, December 2015.

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	Previously evaluated
A23109A						
KCP 5.1.1/01	Bradbury, L.	20/04/2021	SF-1027/2- Determination of Metalaxyl-M (achiral and chiral) and Oxathiapiprolin in A23109A by HPLC Report No. N/A Document No. VV-903867 Test Facility N/A Not GLP Unpublished	N	SYN	N
KCP 5.1.1/02	Khot, S.	05/04/2021	A23109A – Validation of Analytical Method SF-1027/2 Report No. SMG16622 Document No. VV-903871 Test Facility Syngenta Biosciences Pvt., Ltd. - GLP Testing Facility GOA GLP Unpublished	N	SYN	N
KCP 5.1.1/03	Heintz, K.	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	N
KCP 5.1.1/04	Heintz, K.	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	N

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	Previously evaluated
KCP 5.1.1/05	Heintz, K.	08/07/2021	Statement on Validation of the Analytical Method SD-1751/1 for the Determination of CGA72649 and CGA363736 in A23109A Oxathiapiprolin/metalaxyl-M DC (030/180) Report No. N/A Document No. VV-910672 Test Facility Syngenta Crop Protection AG, GLP Testing Facility WMU Not GLP Unpublished	Y	SYN	N
KCP 5.1.1/06	Heintz, K.	28/11/2023	Statement on Validation of the Analytical Method SD-1751/1 for the determination of CGA72649 and CGA363736 in A23109A oxathiapiprolin/metalaxyl-M DC (030/180) Report No. N/A Syngenta Crop Protection AG Not GLP Unpublished	Y	SYN	N
KCP 5.1.1/07	Stephanie Sigel/Sandro Tamburello	11/07/2024	Analytical Method SD-2790/1– Metalaxyl-M, SD-2790/1- Determination of CGA226048 in technical material and formulations by LC/MS Analytical Method. Syngenta Crop Protection AG, Report No. not available Document No. VV-1043801 Not GLP Unpublished	Y	SYN	N
KCP 5.1.1/08	Stephanie Sigel	02/07/2024	Metalaxyl-M - A9642D - Validation of Analytical Method SD-2790/1, Final Report. Syngenta Crop Protection AG, Report No. CHMU240179 Document No. VV-1043800 GLP Unpublished	Y	SYN	N
KCP 5.1.1/09	Stephanie Sigel	11/07/2024	Statement on Validation of the Analytical Method SD-2790/1 for the determination of CGA226048 in A23109A oxathiapiprolin/metalaxyl-M DC (030/180). Syngenta Crop Protection AG, Report No. not available Document No. VV-1043799 Not GLP Unpublished	Y	SYN	N

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	Previously evaluated
Metalaxyl-M						
KCP 5.1.2	Hillier, K.	05/05/2021	Metalaxyl-M - Validation of Analytical Method REM181.13A for the Determination of Residues of Metalaxyl-M in Crop and Processed Matrices by LC-MS/MS Report No. GS12ND Document No. VV-901824 Test Facility Covance Laboratories Ltd. GLP Unpublished	N	SYN	N
KCP 5.1.2	xxxxxxxx	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 xxxxxxxx GLP Unpublished	N	SYN	N
KCP 5.1.2	xxxxxxxx	17/08/2012	Metalaxyl-M – Validation of Analytical Method GRM031.06A for the Determination of Residues of Metalaxyl-M and Structurally Related Metabolites as the Common Moiety 2,6-Dimethylaniline (CGA72649) in Animal Matrices Report No. S11-03382 Document No. VV-402332 , CGA329351_11524 Test Facility xxxxxxxx GLP Unpublished	N	SYN	N
KCP 5.1.2	Eckert, J.	14/01/2016	Metalaxyl-M SL (A13947A) – Honey Bee (Apis mellifera L.) Larval Toxicity Test (Repeated Exposure) Report No. S15-02457 Document No. VV-415529 , A13947A_11455 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	SYN	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)

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	Previously evaluated
KCP 5.1.2	Schuler, L.	26/02/2021	Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Static) Report No. S20-06894 Document No. VV-893371 Test Facility Eurofins Agroscience Services EcoTox GmbH GLP Unpublished	Y	SYN	N
KCP 5.1.2	Schuler, L.	12/04/2021	Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Single Cell Green Alga Raphidocelis subcapitata Korshikov under Laboratory Conditions Report No. S20-06896 Document No. VV-898484 Test Facility Eurofins Agroscience Services EcoTox GmbH GLP Unpublished	N	SYN	N
KCP 5.1.2	Schuler, L.	26/02/2021	Oxathiapiprolin/metalaxyl-M DC (A23109A) - Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test – Static) Report No. S20-06895 Document No. VV-893390 Test Facility Eurofins Agroscience Services EcoTox GmbH GLP Unpublished	N	SYN	N
KCP 5.1.2	Lünsmann, V.	20/10/2021	Metalaxyl-M – Analytical Method ECO_048_03A and Validation for the Determination of Metalaxyl-M in Honey Bee Larvae Diets, Adult Honey Bee Feeding Solutions and Bumble Bee Contact Test Solutions Report No.:21 35 CRB 0059, Document No.: VV-928043 Test Facility BioChem Agrar, Germany GLP Unpublished	N	SYN	N
KCP 5.1.2	Mewis, A.	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	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)

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	Previously evaluated
KCP 5.2.1	Brown, D.	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	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)
KCP 5.2.1	Burton, D.	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 GLP Unpublished	N	SYN	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)
KCP 5.2.1	Fritzsch S, Mohaupt R	2022	Metalaxyl-M – Extraction Efficiency Study on Leaf Lettuce, Grapes, Pepper Corn and Dried Hops in the Germany and Italy in 2021 Report No S21-03444. Syngenta File No. VV-951401 GLP Unpublished	N	SYN	N
KCP 5.2.1	xxxxxxxx	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 xxxxxxxxx GLP Unpublished	N	SYN	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)
KCP 5.2.1	xxxxxxxx	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 xxxxxxxx GLP Unpublished	N	SYN	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)

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	Previously evaluated
KCP 5.2.1	Link, T.	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	Y Study already submitted, but evaluation ongoing (A15605D - Ridomil Gold R)
KCP 5.2.2	Mechelke, J.	11/01/2022	Metalaxyl-M (CGA329351) – Validation of Analytical QuEChERS Method for the Determination of Residues of Metalaxyl-M in Peppercorn and Honey by LC-MS/MS, J. Mechelke (2022) Report number 20210433 Document No. VV-936304 Test Facility IES, Switzerland GLP Unpublished	N	SYN	N
KCP 5.2.2	Jooß, S., Leibing F., Tussetschläger, S.	27/04/2022	Metalaxyl-M - ILV of Analytical QuEChERS Method for the Determination of Residues of Metalaxyl-M in Honey and Peppercorn by LC-MS/MS Report number S21-08274 Document No. VV-948185 Test Facility Eurofins Agroscience Services, Germay GLP Unpublished	N	SYN	N
KCP 5.2.5	Crook, S. Tessier, V.	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 Not GLP Unpublished	N	SYN	N

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	Previously evaluated
Oxathiapiprolin						
KCP 5.1.2	Donald, C. Gibson, R.	27/08/2020	Oxathiapiprolin (SYN546539): Validation of the Analytical Method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS Report No. 231693 Document No. VV-870136 Test Facility Charles River Laboratories Edinburgh, Ltd. GLP Unpublished	N	SYN	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.1.2	Reinhardt, R. Lakaschus, S.	27/04/2020	Oxathiapiprolin - Residue Study on Protected Lettuce in Northern France, Germany, Italy, Spain and the United Kingdom in 2019 Report No. S19-02718 Document No. VV-854039 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	SYN	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.1.2	Ford, K.	15/12/2020	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020 Report No. CEMR-9533 Document No. VV-885771 Test Facility CEM Analytical Services Limited (CEMAS) GLP Unpublished	N	SYN	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.1.2	Tanzler, V.	2015	Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, Apis mellifera L. (Hymenoptera, Apidae) Report No. 94441136, DuPont-41989 Document No.: VV-910995 Test Facility: Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished ⇒ KCA2 8.3.1.2	N	~~DuPont~~ Corteva (SYN LoA)	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)

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	Previously evaluated
KCP 5.1.2	Oberrauch, S.	2017	Oxathiapiprolin (DPX-QGU42) technical: Honey bee (Apis mellifera L.) 22 day larval toxicity test (repeated exposure) Report No. S17-01639, DuPont-48606 Document No. VV-911004 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eu-tinger Str. 24, 75223 Niefern-Öschelbronn, Germany GLP Unpublished ⇒ KCA2 8.3.1.3	N	~~DuPont~~ Corteva (SYN LoA)	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.1.2	Lunsmann, V.	07/12/2020	Oxathiapiprolin - Analytical Method ECO_052_03A and Validation for the Determination of Oxathiapiprolin in Honey Bee Larvae Diets and Adult Honey Bee Feeding Solutions Report No. 20 35 CRB 0103 Document No. VV-884296 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.2.1	Donald, C. Gibson, R.	27/08/2020	Oxathiapiprolin (SYN546539): Validation of the Analytical Method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS Report No. 231693 Document No. VV-870136 Test Facility Charles River Laboratories Edinburgh, Ltd. GLP Unpublished	N	SYN	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.2.1	Ford, K.	15/12/2020	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020 Report No. CEMR-9533 Document No. VV-885771 Test Facility CEM Analytical Services Limited (CEMAS) GLP Unpublished	N	SYN	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)
KCP 5.2.2	xxxxxxxx	2022	Method Validation of Oxathiapiprolin in Body Fluids Report No. S22-02422 Document No. 220385 xxxxxxxxx GLP Unpublished	N	Corteva	Yes, in RR, Part B5 for A22773A/ Orondis Evo (2023)

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
-	-	-	-	-	-

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for metalaxyl-M

A 2.1.1 Methods used for the generation of pre-authorisation 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 Analytical method REM181.13A

A 2.1.1.5.1.1 Method validation

Comments of zRMS:	The analytical method REM 181.13A was successfully validated for the determination of metalaxyl-M in crop and processed matrices at a limit of quantitation (LOQ) of 0.01 mg/kg. Matrix types used to validate the methodology were: Beans (dry and fresh), broccoli, cauliflower, cereal grain (barley), cotton seed, cucumber, grape and processed fractions (juice, wine, dry pomace and raisin), lettuce, melon, onion, potato, sugarbeet root and tobacco (dry and fresh). The mean recoveries for metalaxyl-M at each fortification level, and overall, for each of the matrices tested were within the acceptable range of 70-110% with the relative standard deviation (RSD) within the acceptable range of ≤ 20%. The method is acceptable.

Reference: KCP 5.1.2

Report: Metalaxyl-M - Validation of Analytical Method REM181.13A for the Determination of Residues of Metalaxyl-M in Crop and Processed Matrices by LC-MS/MS, Hillier K, (2021), Report number GS12ND Syngenta File No. VV-901824

Guideline(s):	Yes: OECD ENV/JM/MONO (2007)17 EPA OPPTS 860.1340 (1996) SANCO/3029/99 Rev.4 (2000) Regulation (EC) No 1107/2009
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of crop matrices are extracted twice with methanol using a high-speed homogeniser. The extracts are combined and centrifuged to remove solid material. All extracts are further cleaned-up by solid phase extraction (SPE) using Oasis HLB cartridges. The eluates from the SPE cartridges are evaporated to dryness and reconstituted in 0.2% formic acid (aq) / methanol (70/30 v/v). Metalaxyl-M is determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS).

HPLC-MS/MS Conditions

HPLC system: Waters Acquity system (vacuum solvent degasser, binary HPLC pump, column oven)

Detector: AB Sciex API 4000
Autosampler: Waters Acquity Autosampler
Column: Acquity UPLC® BEH C_{18} (2.1 mm x 50 mm, 1.7 µm)
Mobile phase: A: 0.2% formic acid in water
B: 0.2% formic acid in water: methanol (70:30 ,v:v)

Time	%A	%B
0	70	30
2.0	10	90
3.0	10	90
3.01	70	30
5.0	70	30

Flow rate: 0.5 ml/min
Column oven temperature 40ºC
Injection volume: 10 µL
Retention time: Metalaxyl-M: 1.0 min

API 4000

Ionisation mode	ESI
Source polarity:	Positive
Curtain gas (CUR):	30 (arbitrary units)
Gas 1 (GSI):	40 (arbitrary units)
Gas 2 (GSI):	40 (arbitrary units)
Temperature (TEM):	400ºC
Interface heater (IHC):	On
Ion spray voltage (IS):	5000V
Collision gas setting (CAD):	6
Entrance potential (EP):	10 V
Dwell time	100 msec
Resolution Q1 and Q2	Low

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	Fragment ions (m/z)	
Metalaxyl-M	280	21 20	60 60	18 22	220 192	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

Quantification: Peak areas of fragment ion at m/z = 220, external standards in matrix
Confirmation: Peak areas of fragment ion at m/z = 192, external standards in matrix

Results and discussions

Recoveries of Metalaxyl-M obtained from beans (dry and fresh), broccoli, cauliflower, cereal grain (barley), cotton seed, cucumber, grape and processed fractions (juice, wine, dry pomace and raisin), lettuce, melon, onion, potato, sugar beet root and tobacco (dry and fresh) at each fortification level using method REM181.13A are presented in the table below.

Table A 1: Recovery results from method validation of metalaxyl-M using the analytical method

Matrix	Fortification Level (mg/kg)*	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Primary ion transition 280 m/z> 220 m/z						
Beans (Dry)	0.01*	81, 94, 83, 91, 60	5	82	16.3	60-94
	0.1	92, 74, 68, 84, 72	5	78	12.6	68-92
	Overall	-	-	80	14.0	60-94
Beans (Fresh)	0.01*	79, 97, 83, 87, 71	5	83	11.6	71-97
	0.1	97, 87, 85, 99, 89	5	91	6.8	85-99
	Overall	-	-	87	10.0	71-99
Broccoli	0.01*	74, 68, 78, 79, 91	5	78	10.8	68-91
	0.1	106, 105, 105, 104, 103	5	105	1.1	103-106
	Overall	-	-	91	16.6	68-106
Cauliflower	0.01*	101, 94, 99, 95, 87	5	95	5.7	87-101
	0.1	86, 94, 84, 88, 90	5	88	4.4	84-94
	Overall	-	-	92	6.2	84-101
Cereal Grain (Barley)	0.01*	86, 87, 93, 89, 97	5	90	5.0	86-97
	0.1	96, 86, 94, 99, 100	5	95	5.9	86-100
	Overall	-	-	93	5.8	86-100
Cotton Seed	0.01*	86, 85, 84, 86, 110	5	90	12.3	84-110
	0.1	99, 99, 102, 95, 102	5	99	2.9	95-102
	Overall	-	-	95	9.5	84-110
Cucumber	0.01*	95, 97, 107, 90, 103	5	98	6.8	90-107
	0.1	87, 91, 86, 91, 95	5	90	4.0	86-95
	Overall	-	-	94	7.1	86-107
Grape (Fruit)	0.01*	66, 65, 79, 82, 84	5	75	12.0	65-84
	0.1	85, 97, 101, 98, 98	5	96	6.5	85-101
	Overall	-	-	86	15.3	65-101
Grape (Dry Pomace)	0.01*	82, 72, 77, 81, 96	5	82	11.0	72-96
	0.1	107, 107, 100, 80, 104	5	100	11.3	80-107
	Overall	-	-		14.9	72-107
Grape (Juice)	0.01*	109, 94, 98, 99, 92	5	98	6.7	92-109
	0.1	86, 84, 82, 93, 84	5	86	5.0	82-93
	Overall	-	-	92	9.2	82-109
Grape (Raisin)	0.01*	94, 98, 96, 107, 97	5	98	5.1	94-107
	0.1	99, 93, 108, 88, 88	5	95	8.9	88-108
	Overall	-	-	97	7.0	88-108
Grape (Wine)	0.01*	101, 96, 88, 99, 110	5	99	8.1	88-110
	0.1	93, 68, 84, 74, 89	5	82	12.8	68-93
	Overall	-	-	90	14.0	68-110
Lettuce	0.01*	88, 95, 93, 95, 104	5	95	6.1	88-104
	0.1	91, 91, 91, 91, 88	5	90	1.5	88-91
	Overall	-	-	93	5.0	88-104
Melon	0.01*	105, 109, 107, 98, 122	5	108	8.1	98-122
	0.1	97, 97, 98, 93, 89	5	95	4.0	89-98
	Overall	-	-	102	9.4	89-122
Onion	0.01*	93, 100, 97, 117, 116	5	105	10.7	93-117
	0.1	93, 98, 97, 94, 96	5	96	2.2	93-98
	Overall	-	-	100	8.9	93-117
Potato	0.01*	87, 103, 84, 85, 92	5	90	8.6	84-103
	0.1	105, 97, 96, 97, 87	5	96	6.6	87-105
	Overall	-	-	93	8.0	84-105

Sugar beet Root	0.01*	91, 95, 82, 79, 102	5	90	10.5	79-102
	0.1	96, 102, 90, 74, 73	5	87	15.0	73-102
	Overall	-	-	88	12.2	73-102
Tobacco (Dry)	0.01*	97, 109, 106, 96, 93	5	100	6.9	93-109
	0.1	94, 102, 99, 107, 94	5	99	5.6	94-107
	Overall	-	-	100	5.9	93-109
Tobacco (Fresh)	0.01*	92, 88, 88, 101, 111	5	96	10.3	88-111
	0.1	107, 102, 99, 93, 93	5	99	6.1	93-107
	Overall	-	-	97	8.1	88-111
Confirmatory ion transition 280 m/z > 192 m/z						
Beans (Dry)	0.01*	78, 86, 93, 87, 80	5	85	7.0	78-93
	0.1	91, 73, 68, 82, 70	5	77	12.5	68-91
	Overall	-	-	81	10.7	68-93
Beans (Fresh)	0.01*	102, 102, 102, 99, 101	5	101	1.3	99-102
	0.1	92, 84, 85, 96, 91	5	90	5.6	84-96
	Overall	-	-	95	7.4	84-102
Broccoli	0.01*	100, 81, 83, 80, 85	5	86	9.5	80-100
	0.1	108, 108, 101, 102, 102	5	104	3.4	101-108
	Overall	-	-	95	12.0	80-108
Cauliflower	0.01*	83, 73, 80, 65, 61	5	72	13.0	61-83
	0.1	86, 96, 82, 85, 92	5	88	6.4	82-96
	Overall	-	-	80	13.8	61-96
Cereal Grain (Barley)	0.01*	88, 85, 78, 75, 90	5	83	7.8	75-90
	0.1	93, 81, 90, 93, 100	5	91	7.5	81-100
	Overall	-	-	87	8.7	75-100
Cotton Seed	0.01*	115, 79, 118, 91, 120	5	105	17.7	79-120
	0.1	96, 97, 98, 91, 95	5	95	2.8	91-98
	Overall	-	-	100	13.4	79-120
Cucumber	0.01*	89, 94, 86, 84, 112	5	93	12.1	84-112
	0.1	88, 91, 90, 94, 95	5	92	3.1	88-95
	Overall	-	-	92	8.4	84-112
Grape (Fruit)	0.01*	91, 88, 94, 77, 84	5	87	7.6	77-94
	0.1	84, 97, 105, 98, 102	5	97	8.3	84-105
	Overall	-	-	92	9.6	77-105
Grape (Dry Pomace)	0.01*	80, 75, 90, 81, 97	5	85	10.4	75-97
	0.1	97, 97, 95, 72, 97	5	92	12.0	72-97
	Overall	-	-	88	11.4	72-97
Grape (Juice)	0.01*	115, 93, 103, 93, 101	5	101	9.0	93-115
	0.1	87, 85, 84, 95, 86	5	87	5.0	84-95
	Overall	-	-	94	10.4	84-115
Grape (Raisin)	0.01*	64, 79, 68, 85, 85	5	76	12.8	64-85
	0.1	104, 100, 115, 92, 92	5	101	9.5	92-115
	Overall	-	-	88	17.8	64-115
Grape (Wine)	0.01*	96, 100, 102, 99, 108	5	101	4.4	96-108
	0.1	94, 68, 83, 77, 86	5	82	12.0	68-94
	Overall	-	-	91	13.7	68-108
Lettuce	0.01*	103, 99, 91, 83, 113	5	98	11.7	83-113
	0.1	92, 96, 93, 93, 92	5	93	1.8	92-96
	Overall	-	-	96	8.5	83-113
Melon	0.01*	82, 117, 118, 104, 115	5	107	14.1	82-118
	0.1	92, 97, 93, 90, 86	5	92	4.4	86-97
	Overall	-	-	99	13.4	82-118
Onion	0.01*	85, 101, 97, 107, 114	5	101	10.8	85-114
	0.1	88, 97, 96, 90, 97	5	94	4.6	88-97
	Overall	-	-	97	8.9	85-114
Potato	0.01*	87, 95, 87, 98, 100	5	93	6.5	87-100
	0.1	103, 100, 94, 98, 83	5	96	8.1	83-103
	Overall	-	-	95	7.1	83-103
Sugar beet Root	0.01*	90, 91, 111, 93, 89	5	95	9.7	89-111
	0.1	97, 95, 88, 71, 68	5	84	16.1	68-97
	Overall	-	-	89	13.8	68-111
Tobacco (Dry)	0.01*	87, 89, 122, 72, 96	5	93	19.7	72-122
	0.1	99, 103, 101, 112, 94	5	102	6.5	94-112
	Overall	-	-	98	14.1	72-122
Tobacco	0.01*	89, 98, 93, 102, 118	5	100	11.2	89-118

(Fresh)	0.1	104, 93, 96, 91, 95	5	96	5.2	91-104
	Overall	-	-	98	8.6	89-118
*Limit of quantitation is defined by the lowest validated fortification level. Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.						

Table A 2: Characteristics for the analytical method used for validation of metalaxyl-M residues in plant matrices

	Metalaxyl-M
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required. No peaks 30% of LOQ were found in control samples.
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented Calibration performed with 9 single external calibration standards.
Calibration range	0.075 to 5 ng/mL (equivalent to 0.003 – 0.2 mg/kg in matrix) **All crops except dried tobacco** Quantification - y= -189.458 x^2 + 22480.7 x + 0.0787411 (r = 0.9995) Confirmation - y = -50.6776 x^2 + 7937.3 x + 591.781 (r = 0.9998) **Dried tobacco** Quantification – y = -37.8457 x^2 + 18272.2 x + 6991.09 (r = 0.9989) Confirmation - y = -3.81876 x^2 + 6523.74 x + 2021.29 (r = 0.9989)
Assessment of matrix effects is presented	Yes, No significant enhancement or suppression (i.e. $\geq \pm 20\%$) of the detector response was observed for metalaxyl-M in the final sample extracts in all but dried tobacco for which matrix matched calibration standards were used.
Limit of determination/quantification	LOQ = 0.01mg/kg LOD = 0.003 mg/kg

Conclusion
Method REM181.13A has been successfully validated for the determination of residues of Metalaxyl-M in beans (dry and fresh), broccoli, cauliflower, cereal grain (barley), cotton seed, cucumber, grape and processed fractions (juice, wine, dry pomace and raisin), lettuce, melon, onion, potato, sugar beet root and tobacco (dry and fresh) with a limit of quantification (LOQ) of 0.01 mg/kg.

A 2.1.1.5.1.2 Confirmatory method (if required)

No confirmatory method is required, two mass transitions validated.

A 2.1.1.5.2 QuEChERS method

Comments of zRMS:	The QuEChERS Multiple Residue Method was successfully validated for the determination of Metalaxyl-M in animal matrices: milk, egg, muscle, fat, liver, kidney and blood at a limit of quantitation (LOQ) of 0.01 mg/kg. Acceptable mean recoveries between 70% and 120% with a relative standard deviation lower than 30% were found using quantification and confirmatory transitions for each matrix tested. The method is acceptable.

Reference	KCP 5.1.2
Report	Metalaxyl-M - Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices . xxxxxxx. 2011.

	Report No. S11-01732. Syngenta document No. VV-400487.
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

The QuEChERS analytical method for the determination of metalaxyl-M in animal matrices is also used as a monitoring method. Refer to KCP 5.2.1 (see in A 2.1.2.2.1) for full method summary.

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

A 2.1.1.6.1 Analytical method: honey bee larval toxicity

A 2.1.1.6.1.1 Method validation

Comments of zRMS:	The analytical method was sufficiently validated for the quantification of metalaxyl-M in water samples with a limit of quantification of 45.5 mg/L. The method is acceptable for risk assessment.

Reference:	KCP 5.1.2
Report	Eckert J. (2016) Metalaxyl-M SL (A13947A) - Honey bee (Apis mellifera L.) Larval Toxicity Test (Repeated Exposure). Report No: S15-02457. Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany (Syngenta File No. A13947A_11455, VV-415529)
Guideline(s):	SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (PART a; Section 5) of directive 91/414.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Water samples are analysed using Liquid Chromatography (LC) coupled with UV-Vis detection. The limit of quantification (LOQ) is 45.5 mg metalaxyl-M/L. The analytical method was validated in water as a representative complex aqueous matrix that would demonstrate suitability of the analytical method for analysis of metalaxyl-M.
Detailed information on the analytical method validation can be found in appendix 2, page 44 of the report.

Results and discussions

Table A 3: Recovery results from method validation of metalaxyl-M using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	Metalaxyl-M	45.7 (n=5)	103	0	102%-103%
		59410 (n=5)	102	2	100%-102%

Table A 4: Characteristics for the analytical method used for validation of metalaxyl-M residues in water

	metalaxyl-M
Specificity	LC-UV provides high specificity for the analysis and detection of metalaxyl-M for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. Chromatograms of low and high (45.7 and 59410 mg/L respectively) samples exhibit a peak at approximately 4.1 minutes with no co-eluting peaks arising from the aqueous matrices, the labware, reagents or solvents.
Calibration (type, number of data points)	individual calibration data presented calibration line equation presented 6 calibration points
Calibration range	10-100 mg/L
Assessment of matrix effects is presented	No significant matrix effect (suppression or enhancement) was observed
Limit of determination/quantification	LOQ = 45.5 mg/L

Conclusion
The analytical method has been demonstrated to be accurate and reliable for the analysis metalaxyl-M according to SANTE/2020/12830, Rev.1 with a limit of quantification of 45.5 mg metalaxyl-M/L.

A 2.1.1.6.1.2 Confirmatory method (if required)

No confirmatory method is required, method used is specific.

A 2.1.1.6.2 Analytical method: fish water

Comments of zRMS:	The analytical method GRM031.08A was sufficiently validated for the quantification of metalaxyl-M in fish water samples with a limit of quantification of 0.258 mg/L (1.5 mg/L test item). The method is acceptable for risk assessment.

Reference:	KCP 5.1.2
Report	Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test – Static), Schuler, L., 2021, Report Number: S20-06894, Document No.: VV-893371
Guideline(s):	Yes, SANCO/3029/99/rev.4.
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical method GRM031.08A: Aqueous samples were thawed to ambient temperature and shaken. A sample portion (10 mL) was taken and formic acid (200 uL) was added. The sample was shaken and pH checked until pH<2. The sample was cleaned up with Phenomenex Strata-X-SPE cartridge, eluting with methanol (2 mL) and further rinsed with water and water containing 2% formic acid (2 mL). Metalaxyl-M residues were eluted with methanol (2 mL). The extract was evaporated to dryness and reconstituted with acetonitrile (200 uL) and water (1.8 mL). The sample was further diluted if necessary using acetonitrile: water (10: 90 v/v). The sample was analysed by LC-MS/MS using Phenomenex Luna C18 column.

HPLC-MS/MS conditions

Instrument	SCIEX API 5500	
Detector (mass spectrometer)	ESI Positive ion mode	
	Metalaxyl-M	The selective reaction quantifier transition is *m/z* 280 to 220 using 17 V collision energy, dwell time 150 ms
		The selective reaction monitoring transition is *m/z* 280 to 192 using 23 V collision energy, dwell time 150 ms
Column:	Phenomenex Luna C18, 50 mm x 2.0 mm i.d., 5 µm mean particle size (No. 00B-4252-B0)	
Column oven temperature	40 °C	
Injection volume	30 µL	
Mobile phase	Eluent A: water + 0.2% formic acid Eluent B: acetonitrile	
Gradient	**Time (min.)** / **%A** / **%B** / **Flow (µL/min)** 0.0 / 95 / 5 / 1000 3.0 / 10 / 90 / 1000 3.5 / 10 / 90 / 1000 3.6 / 95 / 5 / 1000 5.0 / 95 / 5 / 1000	
Retention time	Approx 1.8 min	

Results and discussions

Full validation for the determination of metalaxyl-M in aqueous media is available and summarised below.

Table A 5: Recovery results from method validation of metalaxyl-M using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
m/z 280 →220					
Fish water	Metalaxyl-M	0.258 (n=5)	99	5	Range 92% – 105%
		25.8 (n=5)	101	4	Range 96% – 108%
m/z 280 →192					
Fish water	Metalaxyl-M	0.258 (n=5)	97	6	Range 90% to 104%
		25.8 (n=5)	100	5	Range 94% to 107%

Table A 6: Characteristics for the analytical method used for validation of metalaxyl-M residues in fish water

	Metalaxyl-M
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented 7 levels (single determinations)
Calibration range	0.1 to 10 ng/mL

	Metalaxyl-M
Specificity	blank value < 30 % LOQ
Assessment of matrix effects is presented	No specific assessment of matrix effects was presented in the report. However, the assessment of matrix effects involves comparison of the response of the analyte in matrix compared to that of the response of the analyte in solvent, to consider if solvent standards can be used throughout analysis. However, as matrix matched standards were used during the analytical method, the assessment of matrix effects was not considered necessary. ~~however matrix matched standards used~~
Limit of determination/quantification	LOQ = 0.258 mg/L (1.5 mg/L test item)

Conclusion
The method has been successfully validated for the determination of metalaxyl-M in fish water.

A 2.1.1.6.2.1 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.6.3 Analytical method: APP medium

Comments of zRMS:	The analytical method GRM031.08A was sufficiently validated for the quantification of metalaxyl-M in APP medium with a limit of quantification of 0.163 mg/L (0.95 mg/L test item). The method is acceptable for risk assessment.

Reference:	KCP 5.1.2
Report	Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Single Cell Green Alga *Raphidocelis subcapitata* Korshikov under Laboratory Conditions, Schuler, L., 2021, Report Number: S20-06896, Document No.: VV-898484
Guideline(s):	Yes, SANCO/3029/99/rev.4.
Deviations:	None, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods
Analytical method GRM031.08A: Aqueous samples were thawed to ambient temperature and shaken. A sample portion (10 mL) was taken and formic acid (200 uL) was added. The sample was shaken and pH checked until pH<2. The sample was cleaned up with Phenomenex Strata-X-SPE cartridge, eluting with methanol (2 mL) and further rinsed with water and water containing 2% formic acid (2 mL). Metalaxyl-M residues were eluted with methanol (2 mL). The extract was evaporated to dryness and reconstituted with acetonitrile (200 uL) and water (1.8 mL). The sample was further diluted if necessary using acetonitrile: water (10: 90 v/v). The sample was analysed by LC-MS/MS using Phenomenex Luna C18 column.

HPLC-MS/MS conditions:

Instrument	SCIEX API 5500	
Detector (mass spectrometer)	ESI Positive ion mode	
	Metalaxyl-M	The selective reaction quantifier transition is *m/z* 280 to 220 using 17 V collision energy, dwell time 150 ms
		The selective reaction monitoring transition is *m/z* 280 to 192 using 23 V collision energy, dwell time 150 ms

Column:	Phenomenex Luna C18, 50 mm x 2.0 mm i.d., 5 µm mean particle size (No. 00B-4252-B0)
Column oven temperature	40 °C
Injection volume	30 µL
Mobile phase	Eluent A: water + 0.2% formic acid Eluent B: acetonitrile
Gradient	**Time (min.) / %A / %B / Flow (µL/min)** 0.0 / 95 / 5 / 1000 3.0 / 10 / 90 / 1000 3.5 / 10 / 90 / 1000 3.6 / 95 / 5 / 1000 5.0 / 95 / 5 / 1000
Retention time	Approx 1.8 min

Results and discussions
Full validation for the determination of metalaxyl-M in aqueous media is available and summarised below.

Table A 7: Recovery results from method validation of metalaxyl-M using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
m/z 280 →220					
APP medium	Metalaxyl-M	0.163 (n=5)	105	8	Range 94% – 112%
		25.8 (n=5)	97	1	Range 95% – 98%
m/z 280 →192					
APP medium	Metalaxyl-M	0.163 (n=5)	101	8	Range 89% to 108%
		25.8 (n=5)	95	1	Range 93% to 96%

Table A 8: Characteristics for the analytical method used for validation of metalaxyl-M residues in APP medium

	Metalaxyl-M
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented 6 levels (single determinations)
Calibration range	0.25 to 10 ng/mL
Assessment of matrix effects is presented	No, however matrix is aqueous
Limit of determination/quantification	LOQ = 0.163 mg/L (0.95 mg/L test item)

Conclusion
The method has been successfully validated for the determination of metalaxyl-M in APP medium.

A 2.1.1.6.3.1 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.6.4 Analytical method: Elendt M4 medium

Comments of zRMS:	The analytical method GRM031.08A was validated for the quantification of metalaxyl-M in water samples (Elendt M4 medium) with a limit of quantification of 0.103 mg/L (0.60 mg/L test item). The method is considered as fit for purpose.

Reference:	KCP 5.1.2
Report	Oxathiapiprolin/metalaxyl-M DC (A23109A) - Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Static), Schuler, L., 2021, Report Number: S20-06895, Document No.: VV-893390
Guideline(s):	Yes, SANCO/3029/99/rev.4.
Deviations:	None, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical method GRM031.08A: Aqueous samples were thawed to ambient temperature and shaken. A sample portion (10 mL) was taken and formic acid (200 uL) was added. The sample was shaken and pH checked until pH<2. The sample was cleaned up with Phenomenex Strata-X-SPE cartridge, eluting with methanol (2 mL) and further rinsed with water and water containing 2% formic acid (2 mL). Metalaxyl-M residues were eluted with methanol (2 mL). The extract was evaporated to dryness and reconstituted with acetonitrile (200 uL) and water (1.8 mL). The sample was further diluted if necessary using acetonitrile: water (10: 90 v/v). The sample was analysed by LC-MS/MS using Phenomenex Luna C18 column.

HPLC-MS/MS conditions:

Instrument	SCIEX API 5500	
Detector (mass spectrometer)	ESI Positive ion mode	
	Metalaxyl-M	The selective reaction quantifier transition is *m/z* 280 to 220 using 17 V collision energy, dwell time 150 ms
		The selective reaction monitoring transition is *m/z* 280 to 192 using 23 V collision energy, dwell time 150 ms
Column:	Phenomenex Luna C18, 50 mm x 2.0 mm i.d., 5 µm mean particle size (No. 00B-4252-B0)	
Column oven temperature	40 °C	
Injection volume	30 µL	
Mobile phase	Eluent A: water + 0.2% formic acid Eluent B: acetonitrile	
Gradient	**Time (min.)** / **%A** / **%B** / **Flow (µL/min)** 0.0 / 95 / 5 / 1000 3.0 / 10 / 90 / 1000 3.5 / 10 / 90 / 1000 3.6 / 95 / 5 / 1000 5.0 / 95 / 5 / 1000	
Retention time	Approx 1.8 min	

Results and discussions

Full validation for the determination of metalaxyl-M in aqueous media is available and summarised below.

Table A 9: Recovery results from method validation of metalaxyl-M using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
m/z 280 →220					
Elendt M4 medium	Metalaxyl-M	0.103 (n=5)	106	3	Range 102% – 110%
		25.8 (n=5)	100	2	Range 97% – 102%

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
m/z 280 →192					
Elendt M4 medium	Metalaxyl-M	0.103 (n=5)	105	2	Range 101% to 108%
		25.8 (n=5)	100	2	Range 98% to 102%

Table A 10: Characteristics for the analytical method used for validation of metalaxyl-M residues in Elendt M4 medium

	Metalaxyl-M
Specificity	blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented 7 levels (single determinations)
Calibration range	0.1 to 10 ng/mL
Assessment of matrix effects is presented	No, however matrix matched standards used
Limit of determination/quantification	LOQ = 0.103 mg/L (0.60 mg/L test item)

Conclusion
The method has been successfully validated for the determination of metalaxyl-M in Elendt M4 medium.

A 2.1.1.6.4.1 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.6.5 Analytical method: ECO_048_03A

Comments of zRMS:	The analytical method ECO_048_03A was validated for the quantification of metalaxyl-M in honey bee and bumble bee matrices with a limit of quantification of 0.01 mg/kg. Acceptable mean accuracy values of between 70% and 120% were found in all matrices with RSDs <20%. The method is acceptable.

Reference:	KCP 5.1.2
Report	Metalaxyl-M – Analytical Method ECO_048_03A and Validation for the Determination of Metalaxyl-M in Honey Bee Larvae Diets, Adult Honey Bee Feeding Solutions and Bumble Bee Contact Test Solutions, Lünsmann, V., 2021, Report No.:21 35 CRB 0059, document No. VV-928043
Guideline(s):	EPA OCSPP 850.6100 SANTE/2020/12830 Rev. 1 (2021)
Deviations:	None, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods
Honey bee or bumble bee diet samples are extracted by a QuPPE approach prior to quantification by LC-MS/MS, monitoring for two transitions (m/z = 280.2 → 220.1 and m/z = 280.2 → 192.1). The limit of quantification (LOQ) of the method was 0.01 mg/kg for all matrices.
Detailed information on the analytical method can be found in appendix 3, page 65 of the report.

HPLC-MS/MS Conditions

HPLC system:	Agilent 1200
Pumps:	G1312B
Degasser:	G4225A
Column Oven:	G1316C
Detector:	Agilent 6470 Triple Quadropole with Software Agilent Mass Hunter Version B.06.00
Autosampler:	G7167A
Column:	ACE Excel Super C_{18} (75 x 2.1 mm, 3 µm; Article No.: EXL-1111-7502U)
Mobile phase:	A: HPLC grade water with 0.1 % formic acid and 5 mM ammonium formate B: HPLC grade methanol with 0.1 % formic acid

Time	%A	%B	Gradient
0.0	70	30	-
4.0	0	100	Linear
6.0	0	100	-
6.1	70	30	Linear
9.0	70	30	-

Flow rate:	0.4 ml/min
Column oven temperature	40 °C
Injection volume:	1 µL
Retention time:	Metalaxyl-M: 4.1 min

Detector	Agilent 6470	
	Ionisation mode	Jetstream ESI
	Source polarity:	Positive
	Gas flow (L/min):	12
	Gas temperature (°C):	200
	Nebulizer (psi):	60
	Sheath gas heater (°C):	250
	Sheath gas flow (L/min)	11
	Capillary voltage (V):	4500
	Collision gas setting (CAD):	Nitrogen
	Resolution Q1 and Q2	Unit
	Scan Type	MRM

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	Collision energy (V)	Fragmentor	Cell accelerator voltage (V)	Fragment ions (m/z)	
Metalaxyl-M	280.2	11 19	78 78	4 4	220.1 192.1	Quantification Confirmation

Quantification: Peak areas of fragment ion at m/z = 220.1, external standards in matrix
Confirmation: Peak areas of fragment ion at m/z = 192.1, external standards in matrix

Results and discussions

Recovery and precision data of Metalaxyl-M obtained from honey bee and bumble diets at each fortification level using method ECO_048_03A are presented in the table below

Table A 11: Accuracy and precision results from validation of ECO_048_03A for Metalaxyl-M in honey bee and bumble bee matrices.

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean recovery (%)	RSD (%)	Range (%)
Mass transition 280.2 → 220.1 m/z (Primary)						
Royal jelly /ASS (50/50 w/w)	101.5	97, 98, 100, 99, 97	5	98	1.1	97-100
	0.010*	92, 92, 93, 101, 93	5	94	4.1	92-101
	0.000	-	2	-	-	-
	Overall		**12**	**96**	**3.6**	**92-101**
50% w/v sucrose containing 0.1% w/v xanthan	101.5	98, 94, 104, 104, 105	5	101	4.7	94-105
	0.010*	85, 88, 84, 86, 86	5	86	1.5	84-88
	0.000	-	2	-	-	-
	Overall		**12**	**93**	**9.3**	**84-105**
0.5% v/v TritonX solution^{+}	101.5	103, 105, 104, 108, 105	5	105	1.9	103-108
	0.010*	92, 83, 88, 87, 82	5	86	4.8	82-92
	0.000	-	2	-	-	-
	Overall		**12**	**96**	**10.8**	**82-108**
Mass transition 280.2 → 192.1 m/z (Confirmatory)						
Royal jelly /ASS (50/50 w/w)	101.5	96, 99, 101, 100, 98	5	99	1.8	96-101
	0.010*	92, 90, 91, 98, 87	5	92	4.4	87-92
	0.000	-	2	-	-	-
	Overall		**12**	**95**	**5**	**87-101**
50% w/v sucrose containing 0.1% w/v xanthan	101.5	98, 96, 103, 102, 104	5	101	3.4	96-104
	0.010*	87, 87, 87, 86, 90	5	87	1.9	86-90
	0.000	-	2	-	-	-
	Overall		**12**	**94**	**7.9**	**86-104**
0.5% v/v TritonX solution^{+}	101.5	104, 103,103, 107, 104	5	104	1.6	103-107
	0.010*	88, 79, 88, 85, 81	5	84	4.8	79-88
	0.000	-	2	-	-	-
	Overall		**12**	**94**	**11.6**	**79-103**

*Limit of quantitation, defined by the lowest validated fortification level

Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 12: Characteristics of the analytical method used for the quantification of Metalaxyl-M in honey bee and bumble bee matrices

Analyte	Metalaxyl-M
Equipment/ Chromatographic method	HPLC-MS/MS
Accuracy/ Precision (repeatability)	79-108% recovery across all matrices and both transitions. Fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.010 mg/kg, and at 101.5 mg/kg. Acceptable mean accuracy values of between 70 % and 120 % were found in all matrices and therefore according to EU guidance demonstrate the method has satisfactory accuracy.
Precision (reproducibility)	The relative standard deviations (RSDs) of analyte(s) recovery values at each fortification level and overall during method validation were <20 % and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.
Specificity	No peaks in controls above 30% of LOQ. LC-MS/MS provides high specificity for the analysis and detection of analyte(s) for the purpose of ecotoxicity studies i.e. clean, well described test matrices analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to analyte(s) in any of the control samples tested.

Confirmatory method	-
Assessment of matrix effects is presented	yes. Matrix effects were not significant (< ± 5%); for royal jelly and 0.5% triton, suppression was observed; for 50% w/v sucrose, enhancement was observed. Matrix-matched standards are routinely used. No significant matrix effects were observed for Metalaxyl-M in honey bee and bumble bee matrices during the method validation. Matrix-matched standards were used throughout the method.
Calibration/Linearity	Calibration was performed with 8 levels in duplicate. The calibration range was from 0.065 to 6.47 µg/L, corresponding to 0.0027 to 135 mg/kg (applying the total dilution factor of the LOQ (=41.8) for the lower calibration level and the total dilution factor of the high validation level (=20885) for the upper calibration end) The linear range was from 27% of the LOQ to 33% above the highest fortification level measured, or similar. The linearity of the LC-MS/MS detector was tested for Metalaxyl-M using matrix-matched standard solutions from 0.065 to 6.47 µg/L. This range is equivalent to 0.0027 to 135 mg/kg in samples. Standards at eight different concentrations were injected in duplicate and the signal area plotted against concentration for all calibration points. A correlation coefficient of >0.99 was obtained for Metalaxyl-M (primary transition: 280.2 → 220.1, confirmatory transition: 280.2 → 192.1) Matrix: Royal jelly/ASS (50/50 w/w) Quantification - $y= 133.38 x^2 + 5495.97 x + 99.71$ ($r = 0.9997$) Confirmation - $y = 79.89 x^2 + 4083.41 x + 90.85$ ($r = 0.9998$) Matrix: 50% w/v sucrose containing 0.1% w/v xanthan Quantification - $y= 7022.37 x + 262.06$ ($r = 0.9994$) Confirmation - $y = 5091.06 x + 189.43$ ($r = 0.9996$) Matrix: 0.5% v/v TritonX Quantification - $y= 108.35 x^2 + 5815.04 x + 604.46$ ($r = 0.9998$) Confirmation - $y = 66.50 x^2 + 4257.17 x + 445.69$ ($r = 0.9999$)
Limit of quantification (LOQ)	Limit of quantification representing the lowest validated level with acceptable recovery and precision The LOQ for Metalaxyl-M in honey bee and bumble bee matrices using method ECO_048_03A was established at 0.010 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 LOQ.

Conclusion:
Analytical method ECO_048_03A has been demonstrated to be a reliable and accurate procedure for the determination of Metalaxyl-M in honey bee and bumble bee matrices with a limit of quantification (LOQ) of 0.010 mg/kg in accordance with to SANTE/2020/12830, Rev. 1, using commercially available laboratory equipment and reagents.

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-authorisation 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 QuEChERS (BS EN 15662:2008)

A 2.1.2.1.1.1 Independent Laboratory Validation (tomatoes and oilseed rape)

Comments of zRMS:	The analytical method was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M in crop matrices at the LOQ of 0.01 mg/kg. The method is acceptable.

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.
Mewis A (2012).
Report No S11-03712. Syngenta File No. CGA329351_11643 (Syngenta Task No. TK0055473) (VV-407367)

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, SANTE/2020/12830 rev.1

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 13: Recovery results from validation for 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	96, 105, 104, 89, 95	5	98	7.0	95-106
	0.10	103, 103, 103, 106, 97	5	102	3.0	97-106
	Overall	-	10	100	6.0	95-106
Oilseed Rape	0.01	91, 97, 91, 91, 90	5	92	3.0	90-97
	0.10	94, 91, 93, 93, 89	5	92	2.0	89-94
	Overall	-	10	92	3.0	89-97

RSD: relative standard deviation

Table A 14: 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

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. No significant interferences arising from the matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity
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
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 120% were found for both transitions on all matrices tested and therefore according to EU guidance (SANTE/2020/12830 rev.1) 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 according to the EU guideline (SANTE/2020/12830 rev.1) demonstrate the method was satisfactory 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.

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 Extract
Significant matrix effects (suppression) were found in the crop matrices tested during method validation, therefore matrix matched linearity standards were used for quantification.

Conclusion
The repeatability and specificity of the method have been independently demonstrated, and the original validation (Weber 2011) is therefore considered valid for the determination of residues of metalaxyl-M in crop matrices at the LOQ of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.1.2.1.1.2 Method validation – Difficult commodities (hops and cocoa beans)

Comments of zRMS:	The QuEChERS analytical method was validated for the quantification of metalaxyl-M in hops and cocoa beans with a limit of quantification of 0.01 mg/kg. Acceptable mean accuracy values of between 70% and 120% were found in all matrices with RSDs <20%. The method is acceptable.

Reference: KCP 5.2.1

Report: Metalaxyl-M – Validation of the QuEChERS multiple residue method in hops and cocoa beans by LC-MS/MS.
Brown D (2016).
Report No RES-00055. Syngenta File No. CGA329351_11743 (Syngenta Task No. TK0308525) (VV-465727)

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, SANTE/2020/12830 rev.1

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 15: 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*

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
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 16: 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 (SANTE/2020/12830 rev.1), 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 2.1.2.1.1.3 Independent method validation (hops and cocoa)

Comments of zRMS:	The analytical method was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M in hops and cocoa beans at the LOQ of 0.01 mg/kg. The method is acceptable.

Reference:	KCP 5.2.1
Report	Metalaxyl-M - Independent laboratory validation of the QuEChERS multiple residue method in hops and cocoa beans. Burton D (2016). Report No YB27DB. Syngenta File No. CGA329351_11745. (VV-465743)
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, SANTE/2020/12830 rev.1
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 $\rightarrow$ 192) and the confirmatory transition (m/z 280 $\rightarrow$ 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 17: 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	0.01***** (n=5)	105, 99, 83, 86, 89	92	10.0	83 - 105

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
	m/z 280→160 (confirmatory)	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 18: 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 (SANTE/2020/12830 rev.1), 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 2.1.2.1.1.4 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 2.1.2.1.1.5 Extraction efficiency

Comments of zRMS:	The residue levels determined *via* the solvent system (acetonitrile) used in the monitoring analytical method QuEChERS demonstrated that comparable results were obtained to solvent systems used in metabolism studies for lettuce, grape, hops (dried cones) and dried pepper corn crop matrices (i.e. <30% difference in measured residues).

	Acceptable.

Reference:	KCP 5.2.1
Report	Metalaxyl-M – Extraction Efficiency Study on Leaf Lettuce, Grapes, Pepper Corn and Dried Hops in the Germany and Italy in 2021 Fritzsch S, Mohaupt R (2022) Report No S21-03444. Syngenta File No. VV-951401
Guideline(s):	OECD 509 OECD (2007) No. 72 / 39 OECD (2009) No. 64 / 32 EC 1107/2009 EC 7029/VI/95 SANTE/2019/12752 SANTE/2020/12830, Rev.1
Deviations:	No, SANTE/2017/10632 rev. 4
GLP:	Yes.
Acceptability:	Yes.

Principle of the method
The extractability of active substance relevant residues from plant matrices can be demonstrated via ^{14}C radiolabelled primary crop metabolism studies if samples are available. If, as is the case with metalaxyl-M, no suitable radiolabelled crop samples are available, the extraction efficiency of a method can be demonstrated via cross validation using samples containing incurred residues. In accordance with SANTE 2017/10632, cross validation is defined as "the comparison of amounts of relevant residues extracted from samples with incurred residues using the solvent system of the monitoring method (QuEChERs) and the solvent system under the conditions applied during the metabolism studies".

The existing analytical method for the determination of residues of metalaxyl-M in crop matrices by LC-MS/MS (e.g. REM181.13A) extract metalaxyl-M residues by homogenisation with methanol. The QuEChERS procedure uses an extraction based on manual shaking with acetonitrile. No radiolabelled metabolism data are available to positively confirm that the QuEChERs extraction system is efficient in extracting incurred metalaxyl-M residues. However, shaking with methanol/water (80/20, v/v) has been shown to be efficient from metabolism studies. In order to confirm that the exact systems employed in these methods are efficient, a cross validation study was undertaken.

Cross Validation Study
A cross validation study was conducted where four crop matrices treated with metalaxyl-M were analysed for incurred residues. The field samples were analysed using the data generation method REM181.13A, the samples were then selected with an appropriate residue for the cross validation. Methodology based on the different extraction systems (i.e. acetonitrile with water added if necessary and the metabolism extraction system methanol/water 80/20 v/v) in order to determine the measured metalaxyl-M residues so that direct comparison between each extraction system could be undertaken. Four crop matrices - lettuce, grape, hops (dried cones) and pepper (dried corns) aged residue samples were analysed with at least five replicates alongside duplicate fortified samples, a control sample and a reagent blank sample.

Results and discussions
The residue levels determined via the solvent system (acetonitrile) used in the monitoring analytical method QuEChERS demonstrated that comparable results were obtained to solvent systems used in metabolism studies (i.e. <30% difference in measured residues).

A summary of the data is presented below:

Table A 19: Summary of Extraction Efficiency Assessments

	Extraction Solvent:	**Acetonitrile***	**Methanol/Water (80/20, v/v)**
	Method:	**QuEChERS**	**Metabolism Studies**
Matrix		**Metalaxyl-M**	**Metalaxyl-M**
Lettuce	**Mean Residue Uncorrected** (mg/kg)**	0.30	0.30
	Procedural Recoveries Mean (%)	94	110
	Mean Residue Corrected (mg/kg)**	**0.32**	**0.27**
	%Difference# (%)	**19**	
Grape	**Mean Residue Uncorrected** (mg/kg)**	0.06	0.06
	Procedural Recoveries Mean (%)	103	112
	Mean Residue Corrected (mg/kg)**	**0.06**	**0.05**
	%Difference[#] (%)	**20**	
Hops	**Mean Residue Uncorrected** (mg/kg)**	0.70	0.70
	Procedural Recoveries Mean (%)	74	74
	Mean Residue Corrected (mg/kg)**	**0.95**	**0.95**
	%Difference[#] (%)	**0**	
Pepper	**Mean Residue Uncorrected** (mg/kg)**	1.1	0.90
	Procedural Recoveries Mean (%)	86	87
	Mean Residue Corrected (mg/kg)**	**1.28**	**1.03**
	%Difference[#] (%)	**24**	

Residues in control samples and reagent blanks were less than 30% of the LOQ.
*Extraction with acetonitrile for QuEChERS (with water if needed).
**Mean calculated using un-rounded values.
[#]%Difference compared to the mean corrected residue recovered using the metabolism study extraction systems, calculated using rounded values. The following equation was used for calculation of %Difference:-

$$\%\text{Difference} = \left(\frac{\text{Mean Residue Corrected}}{\text{Mean Residue Corrected (metabolism study)}} \times 100\right) - 100$$

Analytical Method Suitability

As part of the cross-validation study sufficient fortification recovery experiments were undertaken to demonstrate that all of the analytical procedures utilised were functioning as expected; a summary is provided below:

Calibration Data

At least five non-zero calibration points were used with each matrix prepared using each extraction technique. Matrix effects were significant and therefore matrix matched standards were used. In all cases the residual plots were deemed to be randomly distributed visually.

Precision and Repeatability

At each fortification level for each matrix/method combination, acceptable mean recoveries of between 70% and 110% were found.

Repeatability was also tested with the incurred residue samples. At least five replicate samples of each matrix were analysed by each of the separate methods. The relative standard deviations of these replicates were in the range <10 % RSD for all of the methods.

Selectivity

In all analytical procedures used and across all matrices, residues in control samples and reagent blank samples were shown to be less than 30% of the LOQ (0.003 mg/kg).

Confirmation
In all analytical procedures, data were collected from two LC-MS/MS MRM transitions, however as each sample was known to contain metalaxyl-M residues and that the study was specifically a data generation exercise, it was not deemed necessary to report the confirmatory MRM information. Hence only data from the primary transition were reported.

Conclusion
The data generated in the cross validation study clearly demonstrated that the analytical procedures used met the method validation criteria as set out in the EC Guidance for data generation methods (EC, 2000). QuEChERs analytical procedures are considered fit for purpose.
The current EU Technical Guideline for the evaluation of extraction efficiency (EC, 2017), sets an acceptance criteria of less than a 30% difference in measured residues determined by different methods.
The residue levels determined via the solvent system (acetonitrile) used in the monitoring analytical method QuEChERs demonstrated that comparable results were obtained to solvent systems used in metabolism studies (i.e. <30% difference in measured residues).

It is concluded that the extraction system used in the QuEChERs residue analytical methods is comparable to the extraction systems used in the radiolabelled crop metabolism studies (within 30%) for lettuce, grape, hops (dried cones) and dried pepper corn crop matrices. These are therefore considered efficient and meet the requirements set out in the European Technical Guideline (EC, 2017).

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

A 2.1.2.2.1 QuEChERS – Validation (milk, egg, fat, liver, kidney and blood)

Comments of zRMS:	The QuEChERS Multiple Residue Method was successfully validated for the determination of Metalaxyl-M in animal matrices: milk, egg, muscle, fat, liver, kidney and blood at a limit of quantitation (LOQ) of 0.01 mg/kg. Acceptable mean recoveries between 70% and 120% with a relative standard deviation lower than 30% were found using quantification and confirmatory transitions for each matrix tested. The method is acceptable.

Reference: KCP 5.2.2

Report: Metalaxyl-M - Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices .
xxxxxxxx. 2011.
Report No. S11-01732. Syngenta document No. CGA329351_11472 (VV-400487)

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, SANTE/2020/12830 rev.1

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 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 20: 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 21: 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

Specificity
LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830 rev.1)no further confirmatory 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, reagents or solvents have been observed at the retention times of interest.

Linearity
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 were found to be ≥ 0.9962 to 0.9988 were obtained for Metalaxyl-M.

Accuracy
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 (SANTE/2020/12830 rev.1) 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 therefore according to the EU guidance (SANTE/2020/12830 rev.1) 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
The limit of detection (LOD) was defined in this study as the lowest prepared instrument calibration solution that gave rise to a measureable chromatographic response. The LOD for this study is 0.003 mg/kg.

Matrix Extract
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 2.1.2.2.2 QuEChERS - Independent laboratory validation (milk, egg, fat, liver and kidney)

Comments of zRMS:	The QuEChERS analytical method was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M in animal matrices with the LOQ of 0.01 mg/kg. The method is acceptable.

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. xxxxxxxxxx. 2018.
Report No. MM87YQ. Syngenta document No. CGA329351_11851. (VV-470901)

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, SANTE/2020/12830 rev.1

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 22: 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

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery (%)	RSD (%)	Recovery Range (%)
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 23: 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

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830 rev.1) no further confirmatory 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, reagents or solvents have been observed at the retention times of interest.

Linearity

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

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 (SANTE/2020/12830 rev.1) 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 therefore according to the EU guidance (SANTE/2020/12830 rev.1) 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
The limit of detection (LOD) was defined in this study as the lowest prepared instrument calibration 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 Extract
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 2.1.2.2.3 QuEChERS – honey

Comments of zRMS:	The QuEChERS analytical method was validated for the quantification of metalaxyl-M in peppercorn and honey with a limit of quantification of 0.01 mg/kg. Acceptable mean accuracy values of between 70% and 120% were found in all matrices with RSDs <20%. The method is acceptable.

Reference: KCP 5.2.2

Report: Metalaxyl-M (CGA329351) – Validation of Analytical QuEChERS Method for the Determination of Residues of Metalaxyl-M in Peppercorn and Honey by LC-MS/MS, J. Mechelke (2022), Report number 20210433
Syngenta File No. VV-936304

Guideline(s): OECD ENV/JM/MONO(2007)17
SANTE/2020/12830, Rev.1
EPA OPPTS 860.1340 (1996)
Regulation (EC) No 1107/2009

Deviations: No, SANTE/2020/12830 rev.1

GLP: Yes.

Acceptability: Yes.

Materials

Test Material	Metalaxyl-M (CGA329351)
Lot/Batch #:	AMS 758/5
Purity (%):	99.3
IUPAC name:	(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester
CAS number:	70630-17-0

Test commodities

Crop Group	Commodity	Commodity type	Source
Honey	*Bee Honey*	*Multi-flower honey*	*Online Shop*
Difficult/unique commodities	*Peppercorn*	*Peppercorn*	*Local Supermarket*

Study Design and Methods
Test facility: Innovative Environmental Services (IES) Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland
Study start date: 3 Nov 2021
Study end date: 11 Jan 2022
Homogenised sub-samples of the test commodity (5.0 g for honey and 2.0 g for peppercorn) were fortified with standard solutions of metalaxyl-M in acetonitrile. Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.01 mg/kg) and five at 10x LOQ (0.1 mg/kg). Matrices used were multi-flower honey and peppercorn, representative of difficult/unique commodities (peppercorn). The fortified samples were analysed alongside untreated control samples.

Principle of the method
Samples of honey (5.0 g) and peppercorn (2.0 g) are extracted with acetonitrile and water. After addition of QuEChERS extraction salt kit (magnesium sulphate, sodium chloride and buffering citrate salts), the mixture is shaken intensively and centrifuged for phase separation. The organic extract is cleaned up by dispersive SPE (d-SPE) using magnesium sulphate and PSA. For honey matrix, an aliquot of the cleaned-up extract is transferred into an autosampler vial and diluted with methanol/water (1/1) to minimize matrix effects. For peppercorn matrix, an aliquot of the cleaned-up extract is transferred into an autosampler vial and diluted with water to minimize matrix effects.
Metalaxyl-M was determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS).

HPLC-MS/MS Conditions
The final extracts were analysed for metalaxyl-M using an HPLC (Agilent Technologies) coupled to an AB Sciex 6500 or 6500+ QTrap mass spectrometer with electrospray nebuliser. Typical HPLC and mass spectral operating conditions are summarized below.

Instrumentation

HPLC:	Agilent 1290 Infinity II HPLC pump
Detector:	AB Sciex 6500 or 6500+ QTrap mass spectrometer
Autosampler:	Agilent 1290 Infinity II autosampler

<u>Experimental conditions for metalaxyl-M (CGA329351)</u>

Column Phase	:	Phenomenex Kinetex C18
Column Dimension	:	50 mm x 2.1 mm, 2.6 µm particle size
Column Oven Temperature	:	40 °C
Injection volume	:	3 µL
Injection protocol	:	Calibration standards injected after 3 to 4 samples
Mobile phase	:	A: 0.1% formic acid in water B: methanol

Mobile Phase Composition:

Time (mins)	% A	% B	Flow rate (µL/min)
0.00	80	20	500
3.00	10	90	500
4.00	10	90	500
4.01	80	20	500
6.00	80	20	500

Divert Valve Switching Programme:

Time (mins)	Position
0	To waste

1.8	To mass spectrometer
3.2	To waste

Expected Retention Times:

Time (mins)	Analyte
2.5	Metalaxyl-M

Mass Spectrometer Conditions

Interface : ESI (ElectroSpray Ionisation)
Polarity: Positive
Curtain Gas: 30 psi
Ion Source Gas 1: 50 psi
Ion Source Gas 2: 50 psi
Collision Gas: Medium
Source Temperature: 500 °C
Ion Spray Voltage: 5000 V
Entrance Potential: 10 V
Scan Type: Multiple Reaction Monitoring (MRM)
Dwell Time: 50 ms
Resolution Q1 and Q3: unit

	Metalaxyl-M Primary Transition	*Metalaxyl-M Confirmatory Transition*
Q1 mass:	280	280
Q3 mass:	160	192
Declustering Potential (V):	46	46
Collision Energy (V):	31	25
Collision Cell Exit Potential (V):	16	18

Results

Recoveries of metalaxyl-M obtained from honey and peppercorn at each fortification level using the described QuEChERS analytical method are presented in the table below. Other validation parameters of the method are presented in the following table.

Table A 24 Recovery results from method validation of metalaxyl-M using the QuEChERS analytical method in honey and peppercorn.

Matrix	Analyte	Fortification Level (mg/kg)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean Recovery (%)	RSD (%)
Honey	Metalaxyl-M	*Mass transition m/z = 280 → 160 (quantification)*				
		0.010*	103, 104, 103,115, 106	103 - 115 (n = 5)	107	4.7
		0.10	109, 106, 106, 108, 106	106 - 109 (n = 5)	107	1.4
		Overall		103 - 115 (n = 10)	107	3.3
		Mass transition m/z = 280 → 192 (confirmation)				
		0.010*	110, 108, 108, 118, 111	108 - 118 (n = 5)	111	3.6
		0.10	109, 107, 106, 106, 105,	105 – 109 (n = 5)	107	1.4
		Overall		105 - 118 (n = 10)	109	3.4
Pepper-corn	Metalaxyl-M	*Mass transition m/z = 280 → 160 (quantification)*				
		0.010*	93, 87, 92, 87, 88	87 – 93 (n = 5)	89	3.1
		0.10	114, 108, 107, 99, 100	99 – 114 (n = 5)	106	5.6
		Overall		87 – 114 (n = 10)	98	9.9
		Mass transition m/z = 280 → 192 (confirmation)				

		0.010*	94, 89, 90, 90, 89	89 – 94 (n = 5)	90	2.4
		0.10	114, 107, 107, 100, 99	99 – 114 (n = 5)	105	5.7
		Overall		89 – 114 (n = 10)	98	9.2

*Limit of quantitation, defined by the lowest validated fortification level

Table A 25 Characteristics of the data collection/enforcement analytical method used for the quantification of metalaxyl-M residues in honey and peppercorn

Analyte	Metalaxyl-M
Equipment/ Chromatographic method	HPLC-MS/MS
Accuracy/ Precision (repeatability)	For all fortification levels in honey and peppercorn acceptable mean recoveries in the range of 70 - 120% with relative standard deviations of ≤20% for LOQ (0.010 mg/kg) and 10x LOQ (0.10 mg/kg) fortification level were found for metalaxyl-M for both the quantification and confirmation mass transitions.
Specificity	HPLC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANTE/2020/12830, Rev.1), no further confirmatory technique is required. No residues of metalaxyl-M were measured above 30% of the LOQ in any of the control and reagent blank samples used in this study, indicating that no interferences were present at the retention times of the analyte in the test system.
Assessment of matrix effects is presented	No significant matrix effects (i.e. ≥20% suppression or enhancement) on the LC-MS/MS detector response were observed for metalaxyl-M in honey matrix. Nevertheless, honey sample extracts were evaluated with a multi-point calibration based on matrix-matched calibration standards. Significant matrix effects (>20% suppression) on the LC-MS/MS detector response were observed for metalaxyl-M in peppercorn matrix. Therefore, peppercorn sample extracts were evaluated with a multi-point calibration based on matrix-matched calibration standards.
Calibration/Linearity	The linearity of the detector response was confirmed by injecting at least five solvent calibration standards.
	Matrix-matched calibration standards covered the working range of 0.30 ng/mL to 25 ng/mL (equivalent to 0.0003 to 0.25 mg/kg). The lower margin of the linearity test was 30% of the LOQ and the upper margin was at least 20% above the highest concentration in the final measured extracts. These margins cover the range as demanded in SANTE/2020/12830, Rev.1.
	The detector response was linear: **Quantification of metalaxyl-M in honey** Quantification - y = 2.6696e+005 x + 3502.7 (r = 0.999594) Confirmation - y = 3.7387e+005 x + 6437 (r = 0.999761) **Quantification of metalaxyl-M in peppercorn** Quantification – y = 1.9752e+005 x + 25624 (r = 0.999300) Confirmation - y = 2.7412e+005 x + 16976 (r = 0.999377)
Limit of quantification (LOQ)	0.010 mg/kg = limit of quantification, representing the lowest validated fortification level with acceptable recovery and precision
Limit of detection (LOD)	The detected LOD for honey and peppercorn matrix was set at 0.003 mg/kg for both the quantification and confirmation mass transition for metalaxyl-M.
Extract Stability	Refrigerated (2 – 8°C) stability of metalaxyl-M in honey and peppercorn final sample extracts following 7 days and 8 days of storage for honey and peppercorn, respectively, was confirmed.

Conclusion
QuEChERS analytical method as described has been successfully validated for the determination of residues of metalaxyl-M in honey and peppercorn with a limit of quantification (LOQ) of 0.010 mg/kg.

A 2.1.2.2.3.1 Independent laboratory validation

Comments of zRMS:	The QuEChERS analytical method was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M in honey and peppercorn with the LOQ of 0.01 mg/kg. The method is acceptable.

Reference: KCP 5.2.2

Report Metalaxyl-M - ILV of Analytical QuEChERS Method for the Determination

of Residues of Metalaxyl-M in Honey and Peppercorn by LC-MS/MS, Jooß, S., Leibing, F., Tussetschläger, S. (2022),
Report number S21-08274
Syngenta File No. VV-948185

Guideline(s): Yes:
ENV/JM/MONO(2007)17
EPA 860.1340
EC 1107/2009
SANTE/2020/12830, rev.1

Deviations: No, SANTE/2020/12830 rev.1

GLP: Yes.

Acceptability: Yes.

Materials

Test Material	**Metalaxyl-M**
Lot/Batch #:	**AMS 758/5**
Purity (%):	**99.3 %**
IUPAC name:	**(R)-2-[(2,6-dimethylphenyl)methoxyacetylamino]-propionic acid methyl ester**
CAS number:	**70630-17-0**

Test commodities			
Crop	**Commodity**	**Commodity type**	**Source**
-	*Honey (multi-flower)*	*Difficult to analyse*	*Local supermarket*
-	*Peppercorn*	*Difficult to analyse*	*Local supermarket*

Study Design and Methods
Test facility: Eurofins Agroscience Services EAG Laboratories GmbH
Study start date: 23 Feb 2022
Study end date: 27 Apr 2022
Biological phase dates: -
Analytical phase dates: 14 Feb to 04 Mar 2022
Homogenised sub-samples of the test commodity (5.0 g for honey, 2.0 g for peppercorn) were fortified with standard solutions of metalaxyl-M in acetonitrile. Five samples of honey and peppercorn were fortified at the limit of quantification (LOQ; 0.01 mg/kg) and five at a level of 10x LOQ. Matrices used were multi-flower honey and black peppercorn. The fortified samples were analysed alongside untreated control samples.

Principle of the method
Samples of honey and peppercorn are treated with water and extracted with acetonitrile using a mechanical shaker. For phase separation, the content of a citrate extraction tube (4 g magnesium sulfate, 1 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate, and 1 g sodium citrate tribasic dehydrate) is added and the sample is shaken. After centrifugation an aliquot of the supernatant is further cleaned-up by transferring it into a PSA clean-up tube and shaking it. After centrifugation an aliquot of the supernatant is further diluted in methanol/water (1/1, v/v) (honey) or water (peppercorn). Metalaxyl-M is determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS).

HPLC-MS/MS Conditions

HPLC-System:	PAL system HTS-xt autosampler, Agilent Infinity II 1290 Series binary gradient pump and MayLab MistraSwitch column oven
Tandem Mass Spectrometer	Applied Biosystems API 6500+ Triple Quad Mass Spectrometer
Column:	Phenomenex Kinetex C18, 50 × 4.6 mm, 2.6 µm particle size (Pre-Column: Phenomenex AJO-4287 4x3 mm, C18)
Column Temperature:	40 °C

Injection Volume:	10 µL			
Mobile Phase Conditions:	A: Water + 0.1% formic acid B: Methanol			
	Time (min)	%A	%B	Flow (mL/min)
	0.00	80	20	0.500
	3.00	10	90	0.500
	4.00	10	90	0.500
	4.01	80	20	0.500
	6.00	80	20	0.500
Retention Times (approx.):	Metalaxyl-M: approx. 3.6 min			
Valco Valve	to MS: 0.0 – 6.0 min			

Mass Spectrometer Conditions:

MS System:	Applied Biosystems API 6500+ Mass Spectrometer				
Ionisation type:	Electrospray (ESI, TurboIon Spray)				
Polarity:	Positive ion mode				
Scan type:	MS/MS, Multiple Reaction Monitoring (MRM)				
Analyte Monitored	Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Collision Cell Exit Potential (CXP)	Dwell Time
Metalaxyl-M	280 → 160*	36 V	31 eV	18 V	0.75 s
	280 → 192**	36 V	25 eV	10 V	0.75 s

* used for quantification, ** used for confirmation

Results

Recoveries of metalaxyl-M obtained from multi-flower honey and black peppercorn at each fortification level using method multi-residue method QuEChERS are presented in the table below. Other validation parameters of the method are presented in the following table.

Table A 26: Recovery results from method validation of metalaxyl-M using the multi-residue method QuEChERS in honey and peppercorn

Matrix	Analyte	Fortification level (mg/kg)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%)	Comments
Honey	Metalaxyl-M	Mass transition m/z = 280 → 160 (quantitation)					
		0.01	68*, 102, 94, 105, 101	94 – 105 (n=4)	101	4.4	*Identified outlier
		0.1	110, 109, 108, 110, 109	108 – 110 (n=5)	109	0.8	-
		Overall		94 – 110 (n=9)	105	5.1	-
		Mass transition m/z = 280 → 192 (confirmation)					
		0.01	68*, 102, 95, 105, 101	95 – 105 (n=4)	101	4.3	*Identified outlier
		0.1	111, 109, 108, 110, 109	108 – 111 (n=5)	109	0.9	-
		Overall		95 – 111 (n=9)	105	5.0	-
Peppercorn	Metalaxyl-M	Mass transition m/z = 280 → 160 (quantification)					
		0.01	105, 100, 114, 106, 106	100 – 114 (n=5)	106	4.8	-
		0.1	97, 80, 85, 95, 92	80 – 97 (n=5)	90	8.0	-
		Overall		80 – 114 (n=10)	97	9.7	-
		Mass transition m/z = 280 → 192 (confirmation)					
		0.01	103, 99, 113, 104, 101	99 – 113 (n=5)	104	5.1	
		0.1	97, 80, 86, 95, 92	80 – 97 (n=5)	90	7.6	
		Overall		80 – 113 (n=10)	97	9.7	

Table A 27: Characteristics of the analytical method used for the quantification of metalaxyl-M residues in honey and peppercorn

Analyte	Metalaxyl-M
Equipment/ Chromatographic method	LC-MS/MS
Accuracy/ Precision (repeatability)	The mean recoveries at the LOQ (0.01 mg/kg) and at the higher fortification level (0.1 mg/kg) were in the range 70 – 120%, with relative standard deviations (RSDs) of < 20%.
Precision (reproducibility)	This independent laboratory validation [ILV] study was conducted to verify the reliability of QuEChERS method for the determination of metalaxyl-M residues in honey and peppercorn. The results indicate that the method is reproducible.
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance SANTE/2020/12830, rev.1, no further confirmatory technique is required. No significant interferences (> 30% of LOQ) from the sample matrix were detected in the LC-MS/MS chromatograms at the retention time corresponding to metalaxyl-M in any of the control samples.
Confirmatory method	-
Assessment of matrix effects is presented	yes. Matrix effects on detector response caused by honey for metalaxyl-M were considered insignificant, nevertheless matrix-matched standards were used for quantification. Matrix effects on detector response caused by peppercorn matrices for metalaxyl-M were considered significant and therefore matrix-matched standards were used for quantification
Calibration/Linearity	The linearity of the detector response was confirmed for both matrices by injecting at least five matrix-matched standard solutions covering the working range of 0.30 ng/mL to 10 ng/mL. The lower margin of the linearity was 30 % of the LOQ, and the upper margin was at least 20 % above the highest concentration in the final extracts. These margins cover the range as demanded in SANTE/2020/12830, rev.1.
	The detector response was linear: Quantification (honey) - y= 2.85e+006 x + 2.75e+005 (r = 0.9999) Confirmation (honey) - y = 474e+006 x + 4.62e+005 (r = 0.9997) Quantification (peppercorn) - y= 6.49e+005 x + 4.94e+004 (r = 0.9996) Confirmation (peppercorn) - y = 1.04e+006 x + 8.15e+004 (r = 0.9994)
Limit of quantification (LOQ)	0.01 mg/kg
Limit of detection (LOD)	0.003 mg/kg (30% of LOQ)

Conclusion
The QuEChERS method has been successfully validated for the determination of residues of metalaxyl-M in honey and peppercorn with a limit of quantification (LOQ) of 0.01 mg/kg.

A 2.1.2.2.4 Analytical method GRM031.06A

A 2.1.2.2.4.1 Independent laboratory validation

Comments of zRMS:	The GRM031.06A analytical method was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M (as 2,6-dimethylaniline) residues in animal fat with the LOQ of 0.01 mg/kg. The method is acceptable.

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.
xxxxxxxxx 2016.

	Report No. TK0261461, S16-00573. Syngenta document No. CGA329351_11737, VV-463097
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, SANTE/2020/12830 rev.1
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 28: 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 29: 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

	2,6-dimethylaniline
	(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 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 2.1.2.2.4.2 Confirmatory method

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

A 2.1.2.2.4.3 Extraction efficiency

Not applicable for an ILV study.

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

See KCP 5.2.2 study xxxxxxx. (2011 (see A 2.1.2.2.1).

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 2.1.2.5.1.1 Method GRM031.08A

Comments of zRMS:	The GRM031.08A analytical method was successfully validated for the analysis of residues of metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 in water with an LOQ of 0.05 µg/L for each analyte. The method is acceptable.

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. Crook S and Tessier V (2015). Report No. TK0222544. Syngenta document No. CGA329351_11693. (VV-

	132583)
Guideline(s):	None (method description only).
Deviations:	No.
GLP:	No (method description only).
Acceptability:	Yes.
Reference:	KCP 5.2.5
Report	Metalaxyl-M - Validation of an Analytical Method for the Determination of the Metalaxyl-M Metabolite CGA67868 in Water. Tessier V (2015). Report No. TK0222545. Syngenta document No. CGA092370_10006. (VV-412805)
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 is effectively identical to GRM031.02A and that validation for metalaxyl and the other two metabolites are covered by data agreed at EU level.

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 30: 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 31: 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.
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 2.1.2.5.1.2 Independent laboratory validation

Comments of zRMS:	The GRM031.08A analytical method 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.

	The method is acceptable.

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. Link T (2016). Report No. IF-15/03469803-TK. Syngenta document No. CGA329351_11732, VV-415481

Guideline(s): EPA OCSPP 850.6100 (2012). SANCO/3029/99 Rev. 4 (2000). SANCO/825/00 Rev. 8.1 (2010).

Deviations: No, SANTE/2020/12830 rev.1

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 32: 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	0.05 (n=5)	93	10	87-110
		0.5 (n=5)	96	2	93-97

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
	(primary)	*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 33: 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 presented	Matrix effects (either enhancement or suppression) were not considered to 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 2.1.2.5.1.3 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 applicable.

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 the oxathiapiprolin

A 2.2.1 Methods used for the generation of pre-authorisation 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 toxicological studies (KCP 5.1.2.3)

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 operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

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 residues studies (KCP 5.1.2.5)

A 2.2.1.5.1 Analytical method 1: DuPont-30422

A 2.2.1.5.1.1 Method validation (231693)

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The analytical residue method DuPont-30422 was successfully validated for the determination of residues of oxathiapiprolin in crop matrices (peppers, cucumbers, melon, leek, broccoli, cauliflower, cabbage, kale, brussel sprouts and hops) at a limit of quantification (LOQ) of 0.01 mg/kg. For all fortification levels (0.01 mg/kg, 0.10 mg/kg), acceptable mean recoveries in the range of 70 - 110% with a relative standard deviation (RSD) of ≤ 20% were found for oxathiapiprolin for both the quantification and confirmation mass transitions, in all matrices. The method has therefore been successfully validated according to the EU guidelines SANCO/3029/99 rev.4. The study is acceptable.

Reference:	KCP 5.1.2
Report	Oxathiapiprolin (SYN546539) – Validation of the Analytical method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS Donald C., Gibson R. (2020) Report No. 231693, Syngenta File No. VV-870136
Guideline(s):	Yes

	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 Analyti-cal Method, EPA 712-C-96-174, August 1996.
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Analytical method DuPont-30422 is also used for the generation of post-authorisation data. The validation of this method (231693) is summarised in A 3.1.2.1.1.1.

A 2.2.1.5.1.2 Method & Validation (S19-02718)

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The method DuPont-30422 has been successfully validated for determination of oxathiapiprolin residues in lettuce. The specificity, linearity, accuracy, precision and repeatability has been demonstrated for both the quantification and confirmatory transitions by taking one reagent blank, two control samples, five samples fortified at the LOQ and five at 10 x LOQ through the method using LC-MS/MS detection. Recovery values at the LOQ were in the range 103 – 106% with a mean recovery of 105% and 1.1% relative standard deviation (RSD) for the primary transition (*m/z* 540/500) and were in the range 97 – 108% with a mean recovery of 104% and 4.1% relative standard deviation (RSD) for the confirmatory mass transition (*m/z* 500/163). No interferences were present at or above 30% of the LOQ in any of the control samples and reagent blanks. The LOD and the matrix effect were assessed. LOQ = 0.01 mg/kg The method DuPont-30422 has been validated according to the EU guidelines SANCO/3029/99 rev.4. The method is acceptable.

Reference:	KCP 5.1.2
Report	Oxathiapiprolin - Residue Study on Protected Lettuce in Northern France, Germany, Italy, Spain and the United Kingdom in 2019 Lakaschus S., Reinhardt, R. (2020) Report No. S19-02718, Syngenta File No. VV-854039
Guideline(s):	SANCO/3029/99 rev. 4 (2000) ENV/JM/MONO(2007)17 EPA OPPTS 860.1340 (1996) Regulation (EC) No 1107/2009
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Principle of the method
5 g sample of lettuce was extracted three times by homogenising in a genogrinder using a solution of formic acid/water/acetonitrile. An aliquot of the extract was diluted and analysed by LC-MS/MS giving a final sample concentration of 0.0071 g/mL for high water content crops.
Final extracts were analysed for oxathiapiprolin (SYN546539) by high performance liquid chromatography with mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (*m/z* 540→500) and the confirmatory transition (*m/z* 540→163).

HPLC-MS/MS Conditions

HPLC system:	1260 Infinity Binary LC System, Agilent Technologies (HPLC, ≤ 600 bar)
Detector:	TripleQuad 5500 System, SCIEX (Triple quadrupole mass spectrometer)
Column:	Ascentis Express C18 (100 mm x 2.1 mm, 2.7 µm, Supelco, Art. No. 53823-U)
Mobile phase (High water content samples):	A: Methanol + 0.1 % formic acid + 5 mM ammonium formate B: Water + 0.1 % formic acid + 5 mM ammonium formate

Time	%A	%B	Gradient
0.00	40	60	
3.5	95	5	
4.50	95	5	
4.60	40	60	
6.00	40	60	

Flow rate:	0.5 ml/min
Column oven temperature	40°C
Injection volume:	15 µL
Retention time:	Oxathiapiprolin (SYN546539): approx. 3.6 min

Detector	AB Sciex API 6500 equipped with a TurboIonSpray source	
	Ionisation mode:	Electrospray (ESI, TurboIonSpray)
	Source polarity:	Positive
	Dwell time	0.1 s

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	
Oxathiapiprolin (SYN546539)	540 → 500 540 → 163	45 35	140 180	10 12	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

The analytical method, DuPont-30422 was validated in lettuce.

Recovery Findings
Summaries of the results for oxathiapiprolin (SYN546539) are presented in the tables below.

Table A 34: Recovery results from validation for oxathiapiprolin (SYN546539) in crops: Primary transition *m*/z 539.8-499.9

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Lettuce	0.01*	106, 105, 105, 103, 104	5	105	1.2	103 - 106
	0.1	101, 99, 103, 109, 107	5	104	4.0	99 - 109
	Overall		10	104	2.8	99 - 109

*Limit of quantitation, defined by the lowest validated fortification level

Table A 35: Recovery results from validation for oxathiapiprolin (SYN546539) in crops: Confirmatory transition *m*/z 539.8-163.0

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Lettuce	0.01*	97, 106, 103, 108, 105	5	104	4.1	97 - 108
	0.1	102, 100, 102, 105, 105	5	103	2.1	100 - 105
	Overall		10	103	3.1	97 - 108

*Limit of quantitation, defined by the lowest validated fortification level

Specificity
LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830 rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity
The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.02 ng/mL to 2.5 ng/mL for lettuce) for both transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. The response factor was calculated for each standard in the calibration line and the mean response factor for each calibration line was calculated. Lines with a coefficient of variation ≤20% for all calibration lines were obtained for oxathiapiprolin (SYN546539).
The detector response was linear.

Lettuce
Quantification – $y = 338445.6981x – 103.5261$, $R^2 = 0.9998$
Confirmation – $y = 70060.3627x – 8.5290$, $R^2 = 0.9990$

Accuracy
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 (SANTE/2020/12830 rev.1) demonstrate the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of oxathiapiprolin (SYN546539) recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore according to the EU guidance (SANTE/2020/12830 rev.1) demonstrate the method has satisfactory repeatability.

Limit of Quantification
The limit of quantification for oxathiapiprolin (SYN546539) residues in crop matrices using method DuPont-30422 was established at 0.01 mg/kg. No interfering peaks around the retention time of oxathiapiprolin (SYN546539) were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects
No significant matrix effects (suppression and enhancement) were observed in lettuce matrix during method validation, therefore matrix matched linearity standards were used for all matrices.

Stability of Final Extracts
The stability of sample extracts fortified with oxathiapiprolin (SYN546539) at the LOQ level was checked after a storage period of at least 9 days in a refrigerator set to 5 ºC against freshly prepared calibration standards. The results proved that the oxathiapiprolin (SYN546539) residues in the stored fortified samples were stable. The mean recovery values at the LOQ level were between 70 % and 110 %, with a RSD of ≤ 20 % and within ±20% of the initial values when re-analysed.

Conclusion
Analytical method DuPont-30422 has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin (SYN546539) in lettuce to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.1.5.1.3 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 2.2.1.5.2 Analytical method 2: DuPont-30422 (Supplement 1)

A 2.2.1.5.2.1 Method validation (CEMR-9533)

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The method DuPont-30422 – Supplement No.1 has been successfully validated for determination of oxathiapiprolin residues in honey. The specificity, linearity, accuracy, precision and repeatability was demonstrated for both the primary and confirmatory transitions by taking one reagent blank, two control samples, five samples fortified at the LOQ and five at 10 × LOQ through the method using LC-MS/MS detection. No interferences were present at or above 30% of the LOQ in any of the control samples and reagent blanks. LOQ=0.01 mg/kg The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20%. The analytical method DuPont-30422 Supplement No.1 was successfully validated for the determination of oxathiapiprolin in honey according to the EU guideline SANCO/3029/99 rev.4. The sugar content of the honey samples was assessed by performing BRIX analysis. The method is acceptable.

Reference:	KCP 5.1.2
Report	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020 Ford K. (2020) Report No. CEMR-9533, Syngenta File No. VV-885771
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)SANCO/3029/99 rev. 4 (2000) ENV/JM/MONO(2007)17 EPA OPPTS 860.1340 (1996) SANTE/11956/2016 rev. 9
Deviations:	No, SANTE/2020/12830 rev.1

GLP: Yes

Acceptability: Yes

Analytical method DuPont-30422 (Supplement 1) is also used for the generation of post-authorisation data. The validation of this method for honey (CEMR-9533) is summarised in A 3.1.2.1.2.1.

A 2.2.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

A 2.2.1.6.1 Analytical method: DuPont-41989

A 2.2.1.6.1.1 Method validation

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). This method was successfully validated for the determination of oxathiapiprolin in test solution in accordance with SANCO/3029/99 rev.4. LOQ = 0.6 g/L The mean recovery values were between 70% and 110%, with a RSD of ≤ 20%. The study is acceptable.

Reference: KCP 5.1.2

Report: Tanzler, V., 2015, Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic Oral Toxicity to The Honey Bee, *Apis Mellifera* L. (Hymenoptera, Apidac) Report No.: DuPont-41989, 94441136 (VV-910995)

Guideline(s): SANCO/3029/99 rev. 4

Deviations: No, SANTE/2020/12830 rev.1

GLP: Yes

Acceptability: Yes

Materials and methods
Residues of oxathiapiprolin were extracted from samples of feeding solutions by diluting with methanol/pure water (50/50 v/v). The final sample was analysed for oxathiapiprolin by high performance liquid chromatography with UV detection (HPLC-UV).

Chromatographic conditions
HPLC system: VW Hitachi
Column: US ES Phen 2 (250mm*3mm)
Oven temperature: 40 ºC
Detector: UV-Vis at 250 nm
Mobile phase:
Solvent A: methanol + 0.1% formic acid
Solvent B: pure water + 0.05% formic acid
Gradient:

Time (min)	A (%)	B (%)
0	30	70
0.5	30	70
3	80	20
7	80	20
7.1	30	70
12	30	70

Flow rate: 0.6 mL/min
Injection volume: 25 µL

Results and discussions

Table A 36: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Feding solution	oxathiapiprolin	3.2 (n=5)	108	8	600 mg test item/L
		9.1 (n=5)	80	6	1700 mg test item/L
		Overall (n=10)	94	17	

Table A 37: Characteristics for the analytical method used for validation of oxathiapiprolin residues in feeding solution

	oxathiapiprolin
Specificity	Wavelength = 250 nm Blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| Linear regression, calibration curve prepared in solvent y = 276877x + 41442 (r=1.0000) 9 data points
Calibration range	0.5 - 25 mg/L that covers 30% LOQ and at least + 20% highest concentration level
Assessment of matrix effects is presented	No
Limit of determination/quantification	0.6 g test item/L (diluted by factor 20) equivalent to approx. 3.2 mg a.s./L (diluted by factor 20)

Conclusion
This method was successfully validated for the determination of oxathiapiprolin in feeding solutions.

A 2.2.1.6.1.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.2.1.6.2 Analytical method: DuPont-48606

A 2.2.1.6.2.1 Method validation

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). This method was successfully validated for the determination of oxathiapiprolin in larval diet sample in accordance with SANCO/3029/99 rev.4. LOQ = 0.5 mg/kg for larval diet (Diet C) LOQ =150 mg/L for acetone The mean recovery values were between 70% and 110%, with a RSD of ≤ 20%. The study is acceptable.

Reference: KCP 5.1.2

Report: Oberrauch, S., 2017, Oxathiapiprolin (DPX-QGU42) Technical: Honey Bee (*Apis Mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure) Report No.: DuPont-48606, S17-01639 (VV-911004)

Guideline(s): SANCO/3029/99 rev. 4

Deviations: No, SANTE/2020/12830 rev.1

GLP: Yes

Acceptability: Yes

Materials and methods

Residues of oxathiapiprolin were determined from samples of larval diet by spiking acetonitrile/water (8:2, v/v). The recovery samples were homogenised on a shaker and then centrifuged. After phase separation, the organic phase was diluted with acetonitrile/water (8:2, v/v). The final sample was analysed for oxathiapiprolin by high performance liquid chromatography with mass spectrometry (HPLC-MS/MS). Residues of oxathiapiprolin were determined from untreated samples of acetone and were diluted with acetonitrile/water (8:2, v/v). If necessary, further diluted with acetonitrile/water (8:2, v/v) and analysed by high performance liquid chromatography with mass spectrometry (HPLC-MS/MS).

Chromatographic conditions

HPLC system: Agilent 1290 infinity HPLC system
Column Phenomenex Synergi Fusion-RP 80A, 50 mm x 2.0 mm ID; 4.0 µm mean particle size (No. 00B-4424-B0) with 4 mm Fusion guard column
Column oven temperature: 40 °C
Injection volume: 10 µL
Mobile phase:
Eluent A: Water + 0.1 % (v/v) formic acid
Eluent B: Methanol + 0.1 % (v/v) formic acid
Gradient:

Time (min)	% Eluent A	% Eluent B	Flow (µL/min)
0.00	70	30	500
3.50	10	90	500
4.50	10	90	500
4.51	70	30	500
6.00	70	30	500

Mass spectrometric conditions

MS system: SCIEX API 6500
Ionisation type: Electrospray ionization (ESI)
Polarity: Positive ion mode
Scan type: MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS): 5500 V
Ionspray turbo heater (TEM): 500 °C
Curtain gas (CUR): 40 (arbitrary units)
Collision gas (CAD): 9 (arbitrary units)
Gas flow 1 (GS1) 60 (arbitrary units)
Gas flow 2 (GS2) 50 (arbitrary units)

Analyte	Mass (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)	Dwell time (ms)
Oxathiapiprolin	540 → 522*	141	10	35	20	150
	540 → 500	141	10	35	20	150
	540 → 350	141	10	41	20	150

*used as quantifier

Results and discussions

Table A 38: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Larval diet	oxathiapiprolin	0.5 (n=5)	87	3	
		400 (n=5)	103	6	
		Overall (n=10)	95	11.9	

Table A 39: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Test solution	oxathiapiprolin	150 (n=4)	101	2	179 was outlier, hence not used in calculations
		82000 (n=5)	93	2	
		Overall (n=10)	97	5.8	

Table A 40: Characteristics for the analytical method used for validation of oxathiapiprolin residues in test solution and larval diet

	oxathiapiprolin
Specificity	m/z 540/350Q Blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented| Linear regression, 1/x weighting y = 2.69e+005x – 3.23e+004 (r=0.9986) – larval diet y = 1.85e+005x – 8.85e+003 (r=0.9997) – in acetone Matrix matched calibration curve used for larval diet Solvent calibration curve used for test solution
Calibration range	0.5 – 15 ng/mL that covers 30% LOQ and at least + 20% highest concentration level (equivalent sample concentration 0.15 - 18 mg/kg)
Assessment of matrix effects is presented	Matrix matched solutions used for larval diet
Limit of determination/quantification	0.5 mg/kg for larval diet and 150 mg/L for test solutions

Conclusion
This method was successfully validated for the determination of oxathiapiprolin in larval diet and test solutions.

A 2.2.1.6.2.2 Confirmatory method (if required)

No confirmatory method is required, method is specific.

A 2.2.1.6.3 Analytical method: ECO_052_03A

A 2.2.1.6.3.1 Method validation

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The method ECO_052_03A has been successfully validated for determination of

	oxathiapiprolin in honey bee larvae diets (royal jelly/ASS (50/50 w/w)) and adult honey bee feeding solutions (50% w/v sucrose containing 0.1% xanthan). The limit of quantification has been set at 0.009 mg/kg. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20%. This method satisfies the EC Guidance Document SANCO/3029/99 rev. 4. The method is acceptable.

Reference:	KCP 5.1.2
Report	Lünsmann V., 2020 Oxathiapiprolin - Analytical Method ECO_052_03A and Validation for the Determination of Oxathiapiprolin in Honey Bee Larvae Diets and Adult Honey Bee Feeding Solutions, Report No. 20 35 CRB 0103, (Syngenta File No. VV-884296)
Guideline(s):	EPA OCSPP 850.6100 SANCO/3029/99 rev. 4
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test facility: BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Study start date: 15 June 2020
Study end date: 18 June 2020
Homogenised sub-samples of royal jelly/ASS and 50% sucrose samples were fortified with standard solutions of oxathiapiprolin. Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.0090 mg/kg) and five at the highest fortification level (100.3 mg/kg). The fortified samples were analysed alongside untreated control samples.

Principle of the Method
Royal jelly/ASS and 50% sucrose solution samples with concentrations are extracted by solid phase extraction (SPE) prior to quantification by LC-MS/MS, monitoring for two transitions (primary transition m/z = 540.2 → 500.1 and confirmatory transition m/z = 540.2 → 163). The limit of quantification (LOQ) of the method was 0.0090 mg/kg.

HPLC-MS/MS Conditions

HPLC-System:	**Agilent 1200**
Pump:	G1312B
Degasser:	G4225A
Column compartment:	G1316C
Multisampler:	G7167A

Mass spectrometer:	**Agilent 6470 Triple Quadropole**
Vacuum pump:	Edwards, GI969-80230
Gas supply:	Nitrogen, in-house; Parker Balston N2-22
Column:	Ace Excel Super C_{18} (100 x 2.1mm, 3µm; Article No.: EXL-1111-1002U)
Column oven temperature:	35 °C
Injection volume:	2 µL
Stop time:	6.5 min (+ 3 min post time)
Injection protocol:	Re-analyse calibration standards after 5 -10 sample injections
Mobile phase:	A: Ultrapure water containing 0.1 % formic acid and 5 mM ammonium formiate B: Methanol containing 0.1% formic acid

Appendix 3 Mobile Phase Composition

Time (min)	% solvent A	% solvent B	Flow rate (mL/min)
0.0	60	40	0.35
5.0	0	100	
6.5	0	100	
6.51	60	40	
9.51	60	40	

Mass Spectrometer Conditions for Oxathiapiprolin

Interface	Agilent Jet Stream	
Polarity	Positive	
Gas flow (L/min)	Nitrogen set at 8	
Gas temperature	320 °C	
Capillary voltage	3500 V	
Collision gas setting (CAD)	Nitrogen	
Scan type	MRM	
MRM Conditions	**Oxathiapiprolin primary transition**	**Oxathiapiprolin confirmatory transition**
Precursor ion Q1 *m/z*	540.2	540.2
Product ion Q3 *m/z*	500.1	163
Dwell time (ms)	200	200
Resolution Q1	Unit	Unit
Resolution Q3	Unit	Unit
Fragmentor (V)	134	134
Cell accelerator voltage (V)	4	4
Collision energy (V)	29	50

Results and discussions

Table A 41: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
m/z 540.2 → 500.1					
Royal jelly/ASS	oxathiapiprolin	0.009 (n=5)	91	2.3	88-93
		100.3 (n=5)	99	3.3	94-103
		Overall (n=10)	95	5.0	88-103
m/z 540.2 → 163					
Royal	oxathiapiprolin	0.009 (n=5)	93	4.9	87-98

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
jelly/ASS		100.3 (n=5)	100	3.2	95-103
		Overall (n=10)	96	5.0	87-103

Table A 42: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
m/z 540.2 → 500.1					
50% w/v sucrose	oxathiapiprolin	0.009 (n=5)	86	3.2	83-89
		100.3 (n=5)	98	1.5	95-99
		Overall (n=10)	92	7	83-99
m/z 540.2 → 163					
50% w/v sucrose	oxathiapiprolin	0.009 (n=5)	84	2.7	81-87
		100.3 (n=5)	98	1.5	95-99
		Overall (n=10)	91	8	81-99

Table A 43: Characteristics for the analytical method used for validation of oxathiapiprolin residues in royal jelly and 50% w/v sucrose solution

	oxathiapiprolin
Specificity	LC-MS/MS provides high specificity for the analysis and detection of oxathiapiprolin for the purpose of ecotoxicity studies i.e. clean, well described test matrix analysing a pre-defined quantity of test item. No significant interferences from the sample matrix, the labware, reagents or solvents were detected in the LC-MS/MS chromatograms at the retention time corresponding to oxathiapiprolin in any of the control samples tested.
Calibration (type, number of data points)	Standards at 8 different concentrations were injected and the signal area plotted against concentration for all calibration points. The equation of the line for the royal jelly/ASS samples is y=1260.547124x + 24.098173 (primary transition) and y=945.987521x + 23.012326 (confirmatory transition). For the 50% w/v sucrose samples, this was calculated at y=1048.976068x +29.075644 (primary transition) and y=785.561616x + 23584345 (confirmatory transition). The correlation coefficient for both matrices is >0.999
Calibration range	0.054 µg/L to 77.75 µg/L
Assessment of matrix effects is presented	No significant matrix effects were observed for oxathiapiprolin in royal jelly/ASS and 50% w/v sucrose solution. Matrix-matched standards were used throughout the method.
Limit of determination/quantification	0.009 mg/kg

Conclusion
This method was successfully validated for the determination of oxathiapiprolin in royal jelly/ ASS and 50% w/v sucrose solutions.

A 3.1.1.1.1.1 Confirmatory method (if required)

No confirmatory method is required, method is specific.

A 3.1.1.2 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 3.1.2 Methods for post-authorisation control and monitoring purposes (KCP 5.2)

A 3.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 3.1.2.1.1 Analytical method: DuPont-30422

A 3.1.2.1.1.1 Method validation (231693)

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The analytical residue method DuPont-30422 was successfully validated for the determination of residues of oxathiapiprolin in crop matrices (peppers, cucumbers, melon, leek, broccoli, cauliflower, cabbage, kale, brussel sprouts and hops) at a limit of quantification (LOQ) of 0.01 mg/kg. For all fortification levels (0.01 mg/kg, 0.10 mg/kg), acceptable mean recoveries in the range of 70 - 110 % with a relative standard deviation (RSD) of ≤ 20 % were found for oxathiapiprolin for both the quantification and confirmation mass transitions, in all matrices. The method has therefore been successfully validated according to the EU guidelines SANCO/3029/99 rev.4. The study is acceptable.

Reference:	KCP 5.2.1
Report	Oxathiapiprolin (SYN546539) – Validation of the Analytical method DuPont-30422 for the Determination of Residues of Oxathiapiprolin in Crop Matrices by LC-MS/MS Donald C., Gibson R. (2020) Report No. 231693, Syngenta File No. VV-870136
Guideline(s):	Yes 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 Analyti-cal Method, EPA 712-C-96-174, August 1996.
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods

Five gram sample of high water content crop (2.5 g for hops) was extracted three times by homogenising in a genogrinder using a solution of formic acid/water/acetonitrile. For all matrices except hops, an aliquot of the extract was diluted and analysed by LC-MS/MS. For hops, a solid phase extraction (SPE) clean-up using graphitized carbon cartridges giving a final sample concentration of 0.0071 g/mL for high water content crops and 0.01 g/mL for hops.

Final extracts were analysed for oxathiapiprolin (SYN546539) by high performance liquid chromatography with mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 539.8-499.9) and the confirmatory transition (m/z 539.8-163.0).

HPLC-MS/MS Conditions

HPLC system:	Shimadzu Nexera X2 UPLC
Detector:	AB Sciex API 6500 equipped with a TurboIonSpray source
Column:	ACE 3 C18-PFP, 15 cm x 3.0 mm
Mobile phase (High water content samples):	A: 0.05% aqueous Formic Acid B: 0.01% Formic Acid in Methanol

Time	%A	%B	Gradient
0.00	90	10	
11.00	1	99	
13.00	1	99	
13.50	90	10	
21.00	90	10	

Mobile (Hops): A: 0.05% aqueous Formic Acid
B: 0.01% Formic Acid in Methanol

Time	%A	%B	Gradient
0.00	40	60	
0.50	40	60	
4.50	1	99	
5.50	1	99	
6.00	40	60	
8.00	40	60	

Flow rate:	0.5 ml/min
Column oven temperature	40°C
Injection volume:	20 µL
Retention time:	High water samples: Oxathiapiprolin (SYN546539): approx. 10.7 min Hops: Oxathiapiprolin (SYN546539): approx. 4.7 min
Detector	AB Sciex API 6500 equipped with a TurboIonSpray source

Ionisation mode:	TurboIonSpray
Source polarity:	Positive
Dwell time	200 msec

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	
Oxathiapiprolin (SYN546539)	539.8 → 499.9 539.8 → 163.0	33 49	82 82	40 19	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

The analytical method, DuPont-30422 was validated in peppers, cucumbers, melon, leek, broccoli, cauliflower, cabbage, kale, brussel sprouts and hops.

Results and discussions

The analytical method was validated in peppers, cucumber, melons, leek, broccoli, cauliflower, cabbage, kale, Brussels sprouts and hops, fortified with oxathiapiprolin.
The recoveries obtained for oxathiapiprolin are detailed in the table below.

Table A 44: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Primary transition m/z 539.8 – 499.9						
Peppers	Oxathiapiprolin	0.01	5	99	4	93 – 103
		0.10	5	95	8	83 – 102
		Overall	10	97	6	83 – 103
Cucumbers	Oxathiapiprolin	0.01	5	94	7	83 – 99
		0.10	5	96	6	88 – 103
		Overall	10	95	6	83 – 103
Melon	Oxathiapiprolin	0.01	5	86	14	67 – 95
		0.10	5	95	13	73 – 105
		Overall	10	90	14	67 – 105
Leek	Oxathiapiprolin	0.01	5	93	6	84 – 97
		0.10	5	97	3	94 – 100
		Overall	10	95	5	84 – 100
Broccoli	Oxathiapiprolin	0.01	5	91	6	86 – 97
		0.10	5	91	2	88 – 92
		Overall	10	91	4	86 – 97
Cauliflower	Oxathiapiprolin	0.01	5	95	8	87 – 103
		0.10	5	98	8	85 – 107
		Overall	10	97	8	85 – 107
Cabbage	Oxathiapiprolin	0.01	5	91	2	88 – 92
		0.10	5	100	2	98 – 102
		Overall	10	95	5	88 – 102
Kale	Oxathiapiprolin	0.01	5	85	3	83 – 89
		0.10	5	96	5	87 – 100
		Overall	10	90	7	83 – 100
Brussels Sprouts	Oxathiapiprolin	0.01	5	78	7	72 – 85
		0.10	5	84	4	80 – 88
		Overall	10	81	6	72 – 88

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Hops	Oxathiapiprolin	0.01	5	90	12	80 – 108
		0.10	5	85	5	81 – 92
		Overall	10	88	9	80 – 108
Confirmatory transition m/z 539.8 – 163.0						
Peppers	Oxathiapiprolin	0.01	5	95	6	88 – 101
		0.10	5	95	8	82 – 102
		Overall	10	95	7	82 – 102
Cucumbers	Oxathiapiprolin	0.01	5	97	9	84 – 110
		0.10	5	98	6	90 – 105
		Overall	10	97	7	84 – 110
Melon	Oxathiapiprolin	0.01	5	87	14	67 – 100
		0.10	5	98	14	74 – 110
		Overall	10	93	15	67 – 110
Leek	Oxathiapiprolin	0.01	5	92	7	81 – 98
		0.10	5	97	4	92 – 101
		Overall	10	94	6	81 – 101
Broccoli	Oxathiapiprolin	0.01	5	87	7	78 – 94
		0.10	5	91	2	89 – 94
		Overall	10	89	6	78 – 94
Cauliflower	Oxathiapiprolin	0.01	5	103	9	92 – 113
		0.10	5	99	8	86 – 108
		Overall	10	101	8	86 – 113
Cabbage	Oxathiapiprolin	0.01	5	91	6	85 – 98
		0.10	5	101	1	99 – 102
		Overall	10	96	7	85 – 102
Kale	Oxathiapiprolin	0.01	5	82	7	75 – 88
		0.10	5	97	5	88 – 102
		Overall	10	89	10	75 – 102
Brussels Sprouts	Oxathiapiprolin	0.01	5	79	6	74 – 85
		0.10	5	79	4	76 – 82
		Overall	10	79	4	74 – 85
Hops	Oxathiapiprolin	0.01	5	93	8	85 – 102
		0.10	5	84	8	79 – 96
		Overall	10	89	9	79 – 102

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity
The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.021 ng/mL to 1.0 ng/mL for high water content crops and 0.03 ng/mL to 2 ng/mL for hops) for both transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. The response factor was calculated for each standard in the calibration line and the mean response factor for each calibration line was calculated. Lines with a coefficient of variation ≤20% for all calibration lines were obtained for oxathiapiprolin (SYN546539).

The linear range is equivalent to 30% LOQ to 1400% LOQ (0.003 mg/kg to 0.14 mg/kg).
The detector response was linear. Linear equations and correlation coefficients are reported for the following matrices:

Pepper
Quantification – y = 4.33e+005 x
Confirmation – y = 8.73e+004 x

Cucumber
Quantification – y = 5.35e+005 x
Confirmation – y = 1.02e+005 x

Melon
Quantification – y = 5.73e+005 x
Confirmation – y = 1.05e+005 x

Leek
Quantification – y = 5.09e+005 x
Confirmation – y = 1e+005 x

Broccoli
Quantification – y = 5.17e+005 x
Confirmation – y = 1.01e+005 x

Cauliflower
Quantification – y = 5.07e+005 x
Confirmation – y = 9.7e+004 x

Cabbage
Quantification – y = 5.36e+005 x
Confirmation – y = 1.03e+005 x

Kale
Quantification – y = 4.26e+005 x
Confirmation – y = 8.3e+004 x

Brussel sprouts
Quantification – y = 4.08e+005 x
Confirmation – y = 8.48e+004 x

Hops
Quantification – y = 1.92e+005 x
Confirmation – y = 3.71e+004 x

Accuracy
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 (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of oxathiapiprolin (SYN546539) recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

Limit of Quantification
The limit of quantification for oxathiapiprolin (SYN546539) residues in crop matrices using method DuPont-30422 was established at 0.01 mg/kg. No interfering peaks around the retention time of compoundname were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects
Significant matrix effects (suppression and enhancement) were observed in some of the crop matrices tested during method validation, therefore matrix matched linearity standards were used for all matrices.

Stability of Final Extracts
The stability of sample extracts fortified with oxathiapiprolin (SYN546539) at the LOQ level was checked after a storage period of at least 7 days in a refrigerator set to 4 °C against freshly prepared calibration standards. The results proved that the oxathiapiprolin (SYN546539) residues in the stored fortified samples were stable except for brussel sprouts and hops, where it is recommended these are analysed immediately after extraction. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤20% and within ±20% of the initial values when re-analysed.

Stability of Standard Solutions
The stability of the stored working standard solutions of oxathiapiprolin (SYN546539) in acetonitrile at 100 µg/mL and working solutions of oxathiapiprolin (SYN546539) in 70/30 acetonitrile/water ranging from 10-1000 ng/mL were checked after a storage period of 82 days in a freezer at -20°C against freshly prepared standard solutions. All standard solutions were diluted to 1 ng/mL prior to analysis. The results demonstrated that oxathiapiprolin (SYN546539) residues in the stored working standard solutions were stable. The concentrations were within ±10% of the initial values. The standard solutions can thus be considered as stable.

Conclusion
Analytical method DuPont-30422 has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin (SYN546539) in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 3.1.2.1.1.2 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 3.1.2.1.1.3 Independent laboratory validation

No new or additional studies have been submitted.

A 3.1.2.1.1.4 Confirmatory method

Please refer to primary method.

A 3.1.2.1.1.5 Extraction efficiency

No new or additional studies have been submitted.

A 3.1.2.1.2 Analytical method 2: DuPont-30422 (Supplement 1)

A 3.1.2.1.2.1 Method validation (CEMR-9533)

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The method DuPont-30422 – Supplement No.1 has been successfully validated for determination of oxathiapiprolin residues in honey. The specificity, linearity, accuracy, precision and repeatability was demonstrated for both the primary and confirmatory transitions by taking one reagent blank, two control samples, five samples fortified at the LOQ and five at 10 × LOQ through the method using LC-MS/MS detection. No interferences were present at or above 30% of the LOQ in any of the control samples and reagent blanks. LOQ=0.01 mg/kg The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤ 20%. The analytical method DuPont-30422 Supplement No.1 was successfully validated for the determination of oxathiapiprolin in honey according to the EU guideline SANCO/3029/99 rev.4. The sugar content of the honey samples was assessed by performing BRIX analysis. The method is acceptable.

Reference:	KCP 5.2.1
Report	Oxathiapiprolin – Honey Residue Study on Spring Oilseed Rape in Northern and Southern Europe in 2020 Ford K. (2020) Report No. CEMR-9533, Syngenta File No. VV-885771
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)SANCO/3029/99 rev. 4 (2000) ENV/JM/MONO(2007)17 EPA OPPTS 860.1340 (1996) SANTE/11956/2016 rev. 9
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and methods
DuPont-30422 Supplement No. 1:
Residues of oxathiapiprolin are extracted by homogenizing in a genogrinder with acetonitrile/water and formic acid three times. Extracts are combined and mixed and 0.5 mL aliquots are diluted with 2 mL of methanol and 4.5 mL of 1% formic acid in water. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 540.2 –500.2) and the confirmatory transition (m/z 540.2 – 163.2).

Analytical method DuPont-30422 Supplement No. 1 was validated in honey.

HPLC-MS/MS Conditions

HPLC system:	Agilent 1100 LC system
Detector:	AB Sciex 5000 Triple Quad Mass Spectrometer
Column:	ACE 3 C18-PFP, 150 mm x 3.0 mm

Mobile phase: A: 0.05% aqueous Formic Acid
B: 0.01% Formic Acid in Methanol

Time	%A	%B	Gradient
0.00	40	60	
0.50	40	60	
4.50	1	99	
5.50	1	99	
6.00	40	60	
8.00	40	60	

Flow rate: 500 µL/minute
Column oven temperature 40°C
Injection volume: 20 µL
Retention time: Oxathiapiprolin (SYN546539): approx. 5 min

Ionisation mode: Positive
Ionspray voltage: 4500 V

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	
Oxathiapiprolin (SYN546539)	540.2→ 500.2 540.2 → 163.2	34 60	81 81	36 16	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential ; DP : Declustering Potential

Results and discussions
The analytical method was validated in honey, fortified with oxathiapiprolin.
The recoveries obtained for oxathiapiprolin are detailed in the table below.

Table A 45: Recovery results from method validation of oxathiapiprolin using the analytical method

Matrix	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Primary transition m/z 540.2 – 500.2						
Honey	Oxathiapiprolin	0.01	5	102	5.1	95 – 108
		0.1	5	102	2.5	98 – 105
		Overall	10	102	3.8	95 – 108
Confirmatory transition m/z 540.2 – 163.2						
Honey	Oxathiapiprolin	0.01	5	104	3.6	99 – 108
		0.1	5	104	3.4	99 – 108
		Overall	10	104	3.3	99 – 108

Specificity
LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANTE/2020/12830, Rev.1) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the honey matrix, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity
The linearity of the LC-MS/MS detector was tested using standard solutions (0.02 ng/mL to 2 ng/mL). Matrix-matched linearity was used for 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 all ≥0.995 were obtained for oxathiapiprolin.

The linear range is equivalent to 30% LOQ to 2800% LOQ (0.003 mg/kg to 0.28 mg/kg).
The detector response was linear. Linear equations and correlation coefficients are reported for the following matrices:
Honey
Quantification – $y = 47346x - 119$ ($r^2 = 0.9984$)
Confirmation – $y = 18599.3x – 57.8$ ($r^2 = 0.9981$)

Accuracy
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 the honey matrix tested and therefore according to EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of oxathiapiprolin recoveries at each fortification level and overall during method validation were <20% and therefore according to the EU guidance (SANTE/2020/12830, Rev.1) demonstrate the method has satisfactory repeatability.

Limit of Quantification
The limit of quantification for oxathiapiprolin residues in honey matrix using method DuPont-30422 Supplement No. 1 was established at 0.01 mg/kg. No interfering peaks around the retention time of oxathiapiprolin were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Effects
No significant matrix effects were observed in the honey matrix tested during method validation however matrix-matched linearity standards were used for quantification.

Stability of Final Extracts
The stability of sample extracts fortified with oxathiapiprolin at the LOQ level was checked after a storage period of 6 days in a refrigerator at 2-8°C against freshly prepared calibration standards. The results proved that the oxathiapiprolin residues in the stored fortified samples were stable. The mean recovery values at the LOQ level were between 70% and 110%, with a RSD of ≤20% and within ±20% of the initial values when re-analysed.

Conclusion
Analytical method DuPont-30422 Supplement No. 1 has been demonstrated to be a reliable and accurate procedure for the determination of oxathiapiprolin in honey to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 3.1.2.1.2.2 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 3.1.2.1.2.3 Independent laboratory validation

No new or additional studies have been submitted.

A 3.1.2.1.2.4 Confirmatory method

Please refer to primary method.

A 3.1.2.1.2.5 Extraction efficiency

No new or additional studies have been submitted.

A 3.1.2.2 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 3.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

A 3.1.2.3.1.1 Method validation

Comments of zRMS:	The study has been evaluated and accepted by zRMS-PL in RR – Part B5 for A22773A/ Orondis Evo (2023). The method has been validated for the determination of oxathiapiprolin in body fluids (in urine) in accordance to guidance document SANTE/2020/12830, rev.1 for risk assessment and/or monitoring. The limit of quantification is 0.01 mg/L. All mean recovery values at fortification levels of 0.01 mg/L for two mass transitions are within 70% - 120% with relative standard deviations ≤ 20% and thereby comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 1. The method is acceptable.

Reference:	KCP 5.2.1
Report	Method Validation of Oxathiapiprolin in Body Fluids xxxxxxxx; 2022 Report No. S22-02422, Corteva Study No. 220385
Guideline(s):	SANTE/2020/12830 rev. 1
Deviations:	No, SANTE/2020/12830 rev.1
GLP:	Yes
Acceptability:	Yes

Materials and Methods

Test Item(s)

Test item (common name):	Oxathiapiprolin
Purity:	98.9 %
Description (physical state):	powder
Lot/batch no.:	E105317-115 (TSN315458)

Method Scope

This method is applicable for the quantitative determination of residues of Oxathiapiprolin in body fluids. The method was validated in urine at LOQ level of 0.01 mg/L with a validated limit of quantitation of` 0.01 mg/L.

Method Principle

Residues of Oxathiapiprolin are extracted from samples by shaking with acetonitrile. An aliquot of the supernatant is diluted with water. The final sample is analyzed for Oxathiapiprolin by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Linearity
For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting, coefficients of determination (R^2) obtained for both transitions were ≥ 0.999. Eight non-matrix matched calibration standards were injected over a concentration range of 0.09 – 9.0 ng/mL (equivalent to 0.003 – 0.3 mg/L). The lowest calibration standard was equivalent to 30% of the LOQ and the highest standard was 30% above the highest fortification level in final sample extracts. The calibration range covered a maximum of two orders of magnitude, as required by SANTE/2020/12830 rev. 1.

Selectivity
The LC-MS/MS method is highly selective for both the quantitation and confirmation of Oxathiapiprolin. Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

Oxathiapiprolin	m/z 540/500 Q (quantitative)
Oxathiapiprolin	m/z 540/522 C (confirmatory)

Confirmation
Confirmation of the presence of Oxathiapiprolin was by comparison of retention times liquid chromatography of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transition met the same acceptance criteria as the validation data generated using the quantitative MS/MS transition, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

Limits of Detection and Quantitation
The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/L.
The limit of detection, defined as 30% of the LOQ, is 0.003 mg/L.

Results and Discussion

Summary of Recovery
Results obtained were within guideline requirements (mean recovery 70-120%; RSD ≤ 20%). The two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarized in the following tables.

Table A 46: Summary of quantitative recovery of Oxathiapiprolin (m/z 540/500)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		mg/L	mean	range	(%)	(%)	
Body fluids	Urine	0.01	98	94 – 103	3.4	3.5	5

Table A 47: Summary of confirmatory recovery of Oxathiapiprolin (m/z 500/522)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		mg/L	mean	range	(%)	(%)	
Body fluids	Urine	0.01	99	95 – 104	3.2	3.2	5

Working Solution Stability
Stock solutions of Oxathiapiprolin prepared in acetonitrile were tested after 361 days of storage at cooled (typically 1 °C-10 °C) and were found to be stable.
Calibration standard solutions of Oxathiapiprolin prepared in acetonitrile/water (1:5, v/v) were tested after

12 days of storage at typically 1 °C-10 °C and were found to be stable.

Sample Extract Stability
Sample extracts of Oxathiapiprolin in acetonitrile/water were tested after 9 days of storage at cooled (typically 1 °C-10 °C) and were found to be stable.

Matrix Effects
Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within ±20%.
Matrix matched standards were used for quantification for this study.

Conclusion
Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340 and SANTE/2020/12830 Rev.1, as well as PMRA Regulatory Directive Dir98 02.

A 3.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted.

A 3.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

No new or additional studies have been submitted.

A 3.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 3.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.