REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: EF-243

Product name(s): Lontrel 300

Chemical active substances:

Clopyralid-olamine, 395 g/l (300 g ae/l)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Renewal of Authorization under Art.43)

Applicant: Corteva Agriscience

Submission date: 22/12/2021

Finalisation date: 05/12/2022

After commenting: 22/02/2023

zRMS - update of the report in terms of additional study on onion and honey study submitted by Applicant : 28/02/2024

Version history

When	What
December 2021	Article 43 submission for re-registration of EF-243 following Clopyralid Renewal of approval (Commission Implementing Regulation (EU) 2021/1191)
December 2022	First zRMS evaluation
February 2023	After commenting
July 2023	Corteva's amendment for onion use
February 2024	zRMS - update of the report in terms of additional study on onion and honey study submitted by Applicant

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5 Analytical methods

5.1 Conclusion and summary of assessment

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

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

- GAP rev. 1, date: 2021-December

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

- GAP rev. 1, date: 2021-December

Commodity/crop	Supported/ Not supported
Sugar beet	Supported
Oilseed rape	Supported
Maize	Supported
Honey	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of clopyralid (present as the olamine salt) in plant protection product is provided as follows:

Comments of zRMS:	Study is acceptable and used for the evaluation. Described HPLC-UV Method Validation for the Determination of the Active Ingredient (Clopyralid) in the SL formulation has been validated in accordance with SANCO/3030/99 rev. 4.. The method is acceptable for the determination of clopyralid.

Reference:	KCP 5.1.1
Report	EF-243: Analytical Method Validation for the Determination of the Active Ingredient (Clopyralid) Content, O'Connor, B.J., 2019, AM-191198

Guideline(s):	Yes, Commission Regulation (EU) No 284/2013 of 01 March 2013 implementing Regulation No 1107/2009 (meets requirements of SANCO 3030/99/rev.4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Clopyralid standard and the clopyralid 300 SL formulation are dissolved in acetonitrile and determined by HPLC on a YMC-Pack ODS-AQ column using an acetonitrile/water (90/ 10 w/w) mixture as eluent and UV detection at 275 nm. Quantitation is by external standard calibration using peak areas.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances clopyralid in plant protection product EF-243

	Clopyralid	**Internal Standard (dimethyl phthalate)**
Author(s), year	O'Connor, B.J., 2019	
Principle of method	The active ingredient content was determined by a reverse phase high performance liquid chromatography (HPLC) method, with ultraviolet (UV) detection, employing an internal standard procedure. The clopyralid content of the samples was quantified relative to bracketing standard solutions.	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	100 mg/L to 400 mg/L, equivalent to 13.2% w/w to 52.6% w/w in EF-243 r = 0.9999	200 mg/L to 1000 mg/L, equivalent to 40 wt% to 200 wt% of nominal internal standard concnetration r = 0.9999
Precision – Repeatability Mean n = 10 (%RSD)	1.62% RSD at average concentration of 26.1% w/w clopyralid	Not Applicable
Accuracy n = 7 (% Recovery)	Average recovery of 100.0% over a concentration range of 9.84% to 53.2% w/w clopyralid	Not Applicable
Interference/ Specificity	No interference was observed at the retention time of the internal standard or active ingredient on analysis of the formulation blank. The identity of the active ingredient was confirmed by the use of diode array analysis and compare to an external standard.	
Comment	The method is linear, precise, accurate and specific when used for the assay EF-243.	

Conclusion

The method is acceptable in accordance with the currently published guidance.

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

There are no relevant impurities in EF-243.

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

No methods are required as none of the co-formulants are defined as relevant for toxicity (environment, health).

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

No CIPAC methods are available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: Clopyralid , its salts and conjugates, expressed as clopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products, (wet crops, dry crops, oily crops, acidic crops)	Primary	0.01 mg/kg	LC-MS/MS	Vogel, E., 2012 DAS: 120610/EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
	Primary	0.01 mg/kg	GC-MS	[a]Hastings, M. 2002 DAS: GRM 01.16 /EU agreed
	Primary	0.2 mg/kg	GC-MS	[b]Clements.B, Harrington, R.; 1997 DAS: ERC 97.10
Plants, plant products, wet crops, dry crops, oily crops; canola	Primary	0.01 mg/kg	GC-MS	Hastings, M.J., 2003 DAS: 021200 or GRM 00.19
Animal products, food of animal origin,(muscle, fat, kidney, liver, milk, eggs)	Primary	0.01 mg/kg	LC-MS/MS	Shaffer, S., 2012 DAS: 120483 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
	Primary	0.01 mg/kg	GC-MS	Hastings, M.J., 2002, DAS: GRM 02.14/EU agreed

Component of residue definition: Clopyralid , its salts and conjugates, expressed as clopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Water, Soil, sediment (Ecotoxicology)	Primary	2 µg/L	LC-MS/MS	Banman, C.S., Moore, S. 2015 DAS: 150051
	Primary	0.165 mg/L	LC-MS/MS	Arnie, J.R., etl. 2020 DAS: 200843
	Primary	1.5 mg/L	LC-UV	Arnie, J.R., etl. 2020 DAS: 191747
	Primary	0.045 mg/L water 0.10 mg/kg sediment	LC-MS/MS	Gonsior, G. 2018 DAS: 170354
	Primary	0.165 mg/L	LC-MS/MS	Ross, T.L., etl. 2020 DAS: 200841
	Primary	0.165 mg/L	LC-MS/MS	Ross, T.L., etl. 2020 DAS: 200842
	Primary	1500 mg/L	LC-MS/MS	Tänzler, V., etl. 2019 DAS: 190300
	Primary	150 mg/L	LC-UV	Davies, C., 2019 DAS: 190287
	Primary	150 mg/L	LC-UV	Stead, A.; 2019 DAS: 190288
Soil (Environmental Fate)	Primary	0.5 µg/kg	LC-MS/MS	Vincent, T.P., 2013, DAS: 120612/EU agreed

(a): Method was concluded as fit for purpose in the RAR (Finland, 2018), as only 1 determination was performed for every crop at each fortification level (n=3, including LOQ). However, combining crops within commodity groups gave 11-13 recoveries for wet and dry crops at each fortification level. The method was used in wheat, grass, sugar beet, oilseed rape, onion and brassica supervised residue trials. Considering all the procedural recoveries conducted in the residue trials (see appendix 2.1) along with the existing recovery data accepted as fit for purpose in the RAR, this method can be considered suitable for the determination of clopyralid residues in high water, high oil and high protein/high starch content (dry) commodities, which accommodates the intended uses.
(b): Method ERC 97.10 was concluded as fit for purpose in the RAR (Finland, 2018), as only 4 determination were performed for each crop at fortification levels above the LOQ. However, 8 determinations were made at the LOQ fortification level. A modified version of this method was used in broccoli supervised residue trials (see appendix 2.1), this method and the modified version can be considered suitable for determination of clopyralid residues in high water and high protein/high starch content (dry) commodities, which accommodates the uses in brassica and cereals.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Clopyralid, its salts and conjugates, expressed as clopyralid	0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Plant, high acid content		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Plant, high oil content		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Muscle	Clopyralid and its salts	0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Milk		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Eggs		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Fat		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Liver, kidney		0.05 mg/kg	Reg. (EU) 2021/1807 (*lowest MRL)*
Soil (Ecotoxicology)	Clopyralid	NOEC = 2 mg/kg soil	EFSA 2018; NOEC = 1.97 mg/a.s./kg dsw, E. fetida
Drinking water (Human toxicology)	Clopyralid	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Clopyralid	ErC50 = 3 mg/L	EFSA 2018; EC50
Air	Clopyralid	15 µg/m^3	EFSA 2018 AOEL = 0.15 mg/kg bw/d
Body fluids (Urine and whole blood)	Clopyralid	0.05 mg/L	RAR (Finland, 2018)

5.3.2.2 Description of analytical methods for the determination of residues in plant

matrices (KCP 5.2)

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

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: Clopyralid, its salts and conjugates, expressed as clopyralid				
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	0.01 mg/kg	LC-MS/MS	Vogl, E., 2012 DAS: 120610 / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Austin, R., 2012 DAS: 120614 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Vogl, E., 2012 DAS: 120610 / EU agreed
	ILV	Not applicable	Not applicable	Not applicable
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Vogl, E., 2012 DAS: 120610 / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Austin, R., 2012 DAS: 120614 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Vogl, E., 2012 DAS: 120610 / EU agreed
	ILV	Not applicable	Not applicable	Not applicable
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Agricultural Commodities: High water content High acid content	Multi-Residue	0.01 mg/kg	LC-MS/MS 130729	Lindner, M., Giesau, A., 2013/EU agreed
Agricultural Commodities: High water content	Multi-Residue ILV	0.01 mg/kg	LC-MS/MS 130728	Austin, R., Turner, R., 2014/EU agreed

Component of residue definition: Clopyralid, its salts and conjugates, expressed as clopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High acid content				

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:	For clopyralid, the efficiency of the extraction process of the analytical method for the MOR alkaline extraction solution was successfully established under GLP conditions in the NOR metabolism study, Hall, Larry R. 2013, Study Number 130733, for the determination of residues of clopyralid in agricultural commodities for high water, dry, and high starch (EFSA 2018). An extraction efficiency of oily matrices was assessed in study, Sahvorost, N. 2020, DAS Study ID 200353, using incurred residues and bridging the analytical method, alkaline extraction solution, to the method in the NOR study GHE-P-9938 of oilseed rape matrices. The extraction efficiency is acceptable based on the results of the extraction efficiency studies which demonstrated that the MOR and NOR methods yielded results which satisfies the requirements defined in the guideline, SANTE 2017/10632 Rev. 3.

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

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

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

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Clopyralid and its salts				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Shaffer,S., 2012 DAS: 120483 / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Gemrot, F., 2012 DAS: 120484 / EU agreed
	Confirmatory	Same as the	Same as the primary	Same as the primary method

Component of residue definition: Clopyralid and its salts				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
		primary method	method	
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Shaffer,S., 2012 DAS: 120483 / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Gemrot, F., 2012 DAS: 120484 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Shaffer,S., 2012 DAS: 120483 / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Gemrot, F., 2012 DAS: 120484 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Fat	Primary	0.01 mg/kg	LC-MS/MS	Shaffer,S., 2012 DAS: 120483 / EU agreed
	ILV	Not applicable	Not applicable	Not applicable
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Liver, kidney	Primary	0.01 mg/kg	LC-MS/MS	Shaffer,S., 2012 DAS: 120483 / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Gemrot, F., 2012 DAS: 120484 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Milk	Primary	0.01 mg/kg	LC-MS/MS (QuEChERS multi-residue method)	Lindner, M.H., Giesau, A. 2013; DAS: 130729/ EU agreed
	ILV	0.01 mg/kg	LC-MS/MS (QuEChERS multi-residue method)	Austin, R., Turner, R. 2014; DAS: 130728 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Honey, Pollen, Nectar	Primary	0.001 mg/kg	GC-NCI/MS	Forbes, T. 2018; DAS: 171332
	ILV	0.001 mg/kg	GC-NCI/MS	Bendig, P., Przybylek, A., 2018; DAS: 180870
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method

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 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Assessment of the analytical extraction procedure for animal matrices[a], was conducted under GLP conditions, study 190543, Fears, S.L. 2019 . The study using incurred residues bridged the analytical extraction procedure, study 120483, with the procedure used in the NOR for animal matrices, study 130202. The extraction efficiency is acceptable based on the results of the extraction efficiency bridging study which demonstrated that the MOR and NOR methods yielded results which satisfies the requirements defined in the guideline, SANTE 2017/10632 Rev. 3.

[a] Incurred residues where only available for muscle, kidney, fat, liver and milk matrices. The extraction efficiency was completed for all the matrices that were available. Eggs is the only matrix that was not evaluated. The NOR for poultry determined the clopyralid residue to be less than the LOQ (0.01 mg/kg) in the daily analysis. We deemed it unnecessary to sacrifice additional animals to evaluate the extraction in eggs.

For the detailed evaluation of (additional) studies on extraction efficiency please refer to Appendix 2.

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

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

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: Clopyralid			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.5 µg/kg	LC-MS/MS	Vincent T., 2013 DAS: 120612 / EU agreed
Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method

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

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

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

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: Clopyralid				
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	Shaffer, S., 2012 DAS: 120611 / EU agreed
	ILV	0.05 µg/L	LC-MS/MS	Austin, R., Turner, R., 2013 DAS: 120613 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS	Shaffer, S., 2012 DAS: 120611 / EU agreed
	ILV	0.05 µg/L	LC-MS/MS	Austin, R., Turner, R., 2013 DAS: 120613 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS	Shaffer, S., 2012 DAS: 120611 / EU agreed
	ILV	0.05 µg/L	LC-MS/MS	Austin, R., Turner, R., 2013 DAS: 120613 / EU agreed
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method

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

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

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

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: Clopyralid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	4.5 µg/m^3	LC-MS/MS	Bacher, R. 2012 DAS: 120601 / EU agreed
Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method

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

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

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

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Clopyralid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L (blood and urine)	LC-MS/MS	Senciuc, M. 2014 DAS: 130727 / EU agreed
Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method

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

5.3.2.8 Other studies/ information

No new or additional studies have been submitted.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1.1	O'Connor, B.J.	2019	EF-243: Analytical Method Validation for the Determination of the Active Ingredient (Clopyralid) Content AM-191198 Covance CRS Research Limited GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.1	Skaggs, C.S., Penning, B.N.	2021	Storage Stability of Clopyralid for One Year in Dried Beans Corteva Report No. 191728 Study No. SGS-19-01-08 SGS North America, Inc GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.1	Teasdale, R.	1996	Frozen Storage Stability of Clopyralid Residues in Strawberries Corteva Report No. GHE-P-4832 Study No. CEMS-235 CEM Analytical Services Ltd. GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 5.1.2 KCA 6.1	Forbes, T., Cross, M	2021	Frozen Storage Stability of Clopyralid in Pollinator Matrices Corteva Report No. 180869 Study No. CEMS-8756 CEM Analytical Services (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.3.1/01	Delmotte, R.	2017	Magnitude of the Residues of Halauxifen-methyl and Clopyralid in Oilseed rape (RAC Whole Plant, Seed and Straw), following One Application of GF-3488, Northern Europe - 2015 DAS Report No. 150534 Study No. RDE-15-20400 Staphyt GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.3.3/01	Devine, C.	2021	Residues of Clopyralid in Maize at Intervals at Harvest Follow-ing a Single Application of GF-1966 – Northern Europe – 2020 Corteva Report No. 201513 Study No. CEMS-9387 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.3.3/02	Devine, H. C.	2003	Residues of Clopyralid in Maize at Intervals and At Harvest Following One or Two Applications of LONTREL 100 (EF-1136), Northern and Southern Europe – 2002 Study No. CEMS-1786; DAS Report No. GHE-P-10534 CEM Analytical Services Ltd GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA	Pirie, D.	2021	Magnitude and Decline of Residues of Clopyralid in Sugar Beet Following Applications of GF-1966 in Northern Europe and the UK, Initiated in 2020.	N	Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
6.3.4/01			DAS Study No. 200809 Study No. 684083 Charles River Laboratories Edinburgh Ltd. GLP Unpublished		

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1.2 KCA 6.3.5/01	Devine, H.C.	2004	Residues of Clopyralid in Onions at Harvest and at Intervals Following Two Application of Lontrel 100 (EF-1136), UK - 2003 Study No. CEMS-2030 DAS Report No. GHE-P-10805 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.3.5/02	Devine, H.C.	2005	Residues of Clopyralid in Onions at Intervals Following Two Applications of Lontrel 100 (EF-1136), Northern Europe - 2004 Study No. CEMS-2346 DAS Report No. GHE-P-11080 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.3.5/03	Devine, H.C.	2006	Residues of Clopyralid in Onions at Intervals Following Two Applications of Lontrel 100 (EF-1136), Northern Europe-2005 Study No. CEMS-2696 DAS Report No. GHE-P-11272 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.3.5/04	Rawle, N. W.	2012	Residues of Clopyralid in Bulb Onions following Two Applications of EF-1136-Northern Europe-2011; Study No. CEMS-4969 DAS Report No. GHE-P-12680 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2	Appeltaur, A.	2021	Determination of Residues of Clopyralid in Nectar, Pollen, Plants and Honey of Winter Oilseed Rape after One Application of GF-1966 in a Semi-Field Residue Study in Germany, Romania, The Netherlands, Southern France and Spain in 2020 Study No. S20-00871	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			DAS Report No. 200098 Eurofins Agroscience Services EcoChem GmbH GLP unpublished		
KCP 5.1.2	Butler, R.E., Reynens, P.	1998	Determination of Residues of Clopyralid in Onions at Intervals following a Single application of Lontrel *100 (EF-1136), Belgium, 1997 DAS Report No. ~~GHE-P-728~~ GHE-P-7289 Dow AgroScience Letcombe Laboratory GLP Unpublished	N	Corteva Agriscience
KCP 5.12	Butler, R.E.	1999	Determination of Residues of Clopyralid in Onions DAS Report No. ERC 97.20 Dow AgroScience Letcombe Laboratory GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.5.3/01	Phillips, A. M.	1994	Determination of residues of clopyralid in sugar beet processed fractions DAS Report No. GH-C 3305 North American Environmental Chemistry Laboratory GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 6.5.3/02	Devine, H.C.	2020	Residues of Clopyralid in Sugar Beet and Process Fractions Following Multiple Applications of GF-1966 – Northern Europe – 2019 DAS Report No. 181493 Study No. CEMS-8908 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1.2 KCA 6.6.2/01	Devine, C.	2021	Determination of Residues of Clopyralid after One Application of GF-1966 (EC Formulation) on Bare Soil in Rotational Crops at 3 Sites in Northern Europe and 3 Sites in Southern Europe 2019-2020 Corteva Study No. 190557 Study No. CEMS-9009 CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 10.2	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L., Fierman, L.A.	2020	EF-243: A 72-Hour Toxicity Test with the Freshwater Alga (*Raphidocelis subcapitata*) DAS Study ID 200843 Eurofins EAG Agroscience LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 10.2	Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L	2020	GF-2895: A 72-Hour Toxicity Test with the Freshwater Alga (*Raphidocelis subcapitata*) DAS Study ID 191747 Eurofins EAG Agroscience LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 10.2	Banman, C. S. and S. Moore	2015	GF-1966: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*. DAS Study ID 150051 SynTech Research Laboratory Services GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 10.2	Gonsior G.	2018	GF-2895: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System DAS Study ID 170354 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2	[redacted]	2020	EF-243: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)	Y	Corteva

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 10.2	Zhang, L., Schneider, S.Z.;		[redacted] GLP Unpublished		Agriscience
KCP 5.1.2 KCP 10.2	Ross, T. L., Zhao, E., Zhang, L., Schneider, S.Z.;	2020	EF-243: A 48-Hour Static Acute Toxicity Test With the Cladoceran (*Daphnia magna*) DAS Study ID 200842 Eurofins EAG Agroscience LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCA 8.3.1	Tänzler, V., Kowalczyk, F.	2019	Clopyralid: Effects (Acute Contact and Oral) on Bumblebees (*Bombus terrestris* L.) in the Laboratory DAS Study ID 190300 ibacon GmbH GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 10.6	Stead, A.	2019	GF-1966: Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants DAS Study ID 190288 Stockbridge Technology Centre Ltd GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 10.6	Davies, C.	2019	GF-1966: Vegetative Vigour Test Terrestrial Non Target Plants DAS Study ID 190287 Stockbridge Technology Centre Ltd GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Sahvorost, N.	2020	Title: Extraction Efficiency Assessment of Clopyralid in High Oil Content Plants Study No.: 200353 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.2	Fears, S.L.	2019	Assessment of the Extraction Efficiency of the Analytical Method for Determining Residues of Clopyralid in Animal Matrices Study No.: 190543 Dow AgroSciences LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Forbes, T.	2018	Validation of an Analytical Method for the Determination of Clopyralid in Pollinator Matrices Study Number: 171332 CEM Analytical Services Ltd GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Bendig, P., Przybylek, A.	2018	Summary of Independent Laboratory Validation (ILV) of an Analytical Method for the Determination of Clopyralid in Honey and Pollen Matrices Study Number: 180870 EAG Laboratories GmbH GLP Unpublished	N	Corteva Agriscience

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	Verte-brate study Y/N	Owner
KCP 5.1.2	Hastings, M.	2002	Determination of Residues of Clopyralid on Agriculture Crops by Gas Chromatography with Negative-	N	Corteva

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Ion Chemical ionization Mass Spectrometry GRM 01.16 Study Number: GH-C-5439 Dow AgroSciences LLC GLP Unpublished		Agriscience
KCP 5.1.2	Clements, B. Harrington, R.	1997	Determination of Residues of MCPA, Clopyralid, and Fluroxypyr in Grass and Cereal Grain and Straw DAS Study No.: ERC 97.10 Dow AgroSciences, LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2	Hastings, M.J.	2003	Determination of Residues of Clopyralid and Picloram in Canola by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry DAS Study Number: 021200 (GRM 00.19) Dow AgroSciences, LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2	Hastings, M.J.	2002	Determination of Residues of Clopyralid in Animal Tissues by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry Study Number: GRM 02.14 Dow AgroSciences, LLC GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2	Vincent, T.	2013	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS Study Number: 120612 ABC Laboratories, Inc GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 5.1.2	Shaffer, S.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water and Surface Water by LC-MS/MS Study Number: 120611 GLP Unpublished	N	Corteva Agriscience
KCP 5.1.2 KCP 5.2	Vogl, E.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commondities by LC-MS/MS Study Number: 120610 ABC Laboratories, Inc GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Austin, R.	2012	Independent Laboratory Validation of Dow AgroSciences Method 120610, “Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS” Study Number: 120614 Battelle UK Ltd GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Hall, L.R.	2013	14C-Clopyralid: Metabolism in Confined Rotational Crops with a 30-Day Plant-back Interval Study Number: 130733 ABC Laboratories, Inc GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Shaffer, S.	2012	Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection Study Number: 120483 ABC Laboratories, Inc GLP Unpublished	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
KCP 5.2	Gemrot, F.	2012	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices Study Number: 120484 Eurofins Agroscience Services Chem GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Lindner, M., Giesau, A.	2013	Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin Study Number: 130729 Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Austin, R., Turner, R.	2014	Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin Study Number: 130728 Battelle UK Ltd GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Austin, R, Turner, R.	2014	Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS Study Number: 140079 Battelle UK Ltd. GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Shaffer, S.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water and Surface Water by LC-MS/MS Study Number: 120611 ABC Laboratories, Inc.	N	Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			GLP Unpublished		
KCP 5.2	Austin, R, Turner, R.	2013	Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS" Study Number: 120613 Battelle UK Ltd. GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Bacher, R.	2012	The Development and Validation of a Method for the Analysis of Clopyralid in Air Study Number: 120601 PTRL Europe GmbH GLP Unpublished	N	Corteva Agriscience
KCP 5.2	Senciuc, M.	2014	Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s) Study Number: 130727 PTRL Europe GmbH GLP Unpublished	N	Corteva Agriscience

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Clopyralid

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

A 2.1.1.1 Analytical method 1

Comments of zRMS:	Validation of an Analytical Method for the determination Clopyralid is acceptable and has been performed in compliance with SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were proposed at the initiation of the study at 0.003 mg/kg and 0.01 mg/kg, respectively. This submitted validated study has been performed in a proper manner.

Method Identifier No.:	120610
Performing Laboratory:	SGS North America, Inc; Brookings, South Dakota, USA
Reference:	KCA 6.1
Report:	Skaggs, C.S., Penning, B.N.; 2021; Storage Stability of Clopyralid for One Year in Dried Beans; SGS North America, Inc; Brookings, South Dakota, USA; Lab Study No. SGS-19-01-08; Corteva Study No. 191728
Guideline(s):	Yes, SANCO/3029/99 rev. 4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid are extracted from crop samples with 100:1 methanol:10N sodium hydroxide (NaOH) by blending for approximately 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to set ambient overnight. An aliquot of the extract is submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N NaOH. The cleanup for crops is affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract is acidified with hydrochloric acid (HCl) and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) cleanup and elution with DCM. After removal of the

DCM using nitrogen blow down, the sample is reconstituted in 10:90, methanol:0.1% formic acid. The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 1: Recovery results from method validation of clopyralid (m/z189.8/146.0Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Dried navy beans	0.01	90	2.9	5	N/A
Dried navy beans	0.10	84	3.5	5	N/A

Table 2: Procedural recovery results of clopyralid (m/z189.8/146.0Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Dried navy beans	0.01	90	6.5	21	N/A
Dried navy beans	0.10	83	8.7	21	N/A

Table 3: Characteristics for the analytical method used for determination of residues of clopyralid in dried navy beans

Analyte	Clopyralid
Matrix	Dried navy beans
Technique	LC-MS/MS
Specificity	*m/z* 189.8/146.0Q *m/z* 191.8/148.0C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points per set

Calibration range	Concentration range of 0.1-25.0 ng/mL (sample equivalence of 0.002 – 0.5 mg/kg)
Limit of quantitation	0.01 mg/kg
Validation Range	0.01 – 0.1 mg/kg

CONCLUSION

This method was successfully validated for the determination of clopyralid in navy beans.

A 2.1.1.2 Analytical validation 2

Comments of zRMS:	The analytical method is acceptable and suitable for the determination of clopyralid in Strawberries. The study has been performed in compliance with Good Laboratory Practice although not fully validated to meet the requirements set forth in SANTE/2020/12830/Rev.1, the method was considered to be fit for purpose for the determination of clopyralid in strawberries

Method Identifier No.: ERC 93.9

Performing Laboratory: CEM Analytical Services, Ltd.
Berkshire, UK

Reference: KCA 6.1

Report: Teasdale, R.; 1996; Frozen Storage Stability of Clopyralid Residues in Strawberries; CEM Analytical Services, Ltd.
Berkshire, UK; Lab Study No. CEMS-235; Corteva Study No. GHE-P-4832

Guideline(s): ~~Similar to SANCO/3029/99 rev. 4~~ –SANTE/2020/12830/Rev.1

Guideline Deviations: Yes, insufficient fortification levels

GLP: Yes

Acceptability: Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted strawberry samples by homogenising and shaking with caustic methanol. After centrifugation, an aliquot of the supernatant was acidified and the fluroxypyr

partitioned into dichloromethane. The dichloromethane layer was then partitioned into aqueous sodium bicarbonate and, after acidification, into diethyl ether. After removal of the diethyl ether by evaporation, the residue was treated with butylation reagent. The clopyralid (as the butyl ester) was partitioned into hexane in the presence of water prior to a silica Bond Elut clean-up procedure. The final sample was analysed for clopyralid by gas chromatography with electron capture (GC-ECD).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table:1 Procedural recovery results of Clopyralid using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Strawberries	0.1	80	9.7	7	

Table:2 Characteristics for the analytical method used for determination of residues of Clopyralid in Strawberries

Analyte	Clopyralid
Matrix	Strawberries
Technique	GC-ECD
Specificity	blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis without weighting r≥0.999 7 data points
Calibration range	Concentration range of 0 – 50.0 ng/mL (equivalent sample concentration 0 – 0.6 mg/kg)
Limit of quantitation	0.1 mg/kg
Validation Range	0.1 mg/kg

CONCLUSION

Although not fully validated to meet the requirements set forth in SANTE/2020/12830/Rev.1, the method was considered to be fit for purpose for the determination of clopyralid in strawberries.

A 2.1.1.3 Analytical validation 3

Comments of zRMS:	The analytical method of the study 180869 is acceptable and suitable for the determination of clopyralid in pollen, nectar and honey. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were at 0.0003 mg/kg and 0.001 mg/kg, respectively.

Method Identifier No.: 171332

Performing Laboratory: CEM Analytical Services, Ltd.
Berkshire, UK

Reference: KCA 6.1

Report: Forbes, T., Cross, M.; 2020; Frozen Storage Stability of Clopyralid in Pollinator Matrices; CEM Analytical Services, Ltd.
Berkshire, UK; Lab Study No. CEMS-8756; Corteva Study No. 180869

Guideline(s): SANCO/3029/99 rev. 4, EPA OPPTS 860.1380, EC Guideline 1607/VI/97 rev.2, Appendix H 7032/VI/95 rev.5, OECD Guideline 506 and OECD Regulation (EC) 1107/2009.

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

MATERIAL AND METHODS

Method Principle

Residues are extracted from pollen, nectar and honey by shaking with a mixture of methanol containing 10 N sodium hydroxide. The extracts are left overnight at room temperature. Extracts are neutralised with 10 N sulfuric acid before a clean-up step on Oasis WAX (6 mL, 120 mg).

The eluates are concentrated to near dryness before a propylation reagent is added. The samples are then incubated for at least 30 minutes at 105°C. The 1-propanol is then evaporated off and sodium chloride and hexane are added along with the clopyralid butyl ester internal standard at a concentration of 2 ng/mL. The sample extracts are concentrated further before residue levels are determined by gas chromatography with negative ion electrospray ionization mass spectrometry (GC/NCI-MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table:1 ***Recovery results from method validation of clopyralid (m/z 233) using the analytical method***

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.001	86	13.0	18	
Pollen	0.01	83	7.4	18	
Nectar	0.001	97	9.1	18	
Nectar	0.01	90	5.8	18	
Honey	0.001	90	7.0	18	
Honey	0.01	90	6.2	18	

Table:2 Characteristics for the analytical method used for determination of residues of clopyralid in pollen, nectar and honey

Analyte	clopyralid
Matrix	Pollen, nectar and honey
Technique	GC/NCI-MS
Specificity	*m/z* 233 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.3-30 ng/mL (equivalent sample concentration 0.0003 – 0.03 mg/kg)
Limit of quantitation	0.001 mg/kg
Validation Range	0.001-0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of clopyralid in pollen, nectar and honey.

A 2.1.1.4 Analytical validation 4

Comments of zRMS:	The analytical phase of residues study of the study 150534 (RDE-15-20400) is acceptable and suitable for the determination of clopyralid in Oilseed rape (RAC

	Whole Plant, Seed and Straw). The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4

Method Identifier No.:	120610
Performing Laboratory:	CEM Analytical Services Ltd (CEMAS)
Reference:	KCA 6.3.1/01
Report:	Delmotte, R., 2017; Magnitude of the Residues of Halauxifen-methyl and Clopyralid in Oilseed rape (RAC Whole Plant, Seed and Straw), following One Application of GF-3488, Northern Europe - 2015; CEM Analytical Services, Ltd; Wokingham, Berkshire, UK; Study No. CEMS-6840; DAS Report No. 150534 (RDE-15-20400): DAS Internal Report Code 2037895.
Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	Yes, insufficient number of fortification samples at each level
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of clopyralid were extracted from crop samples with 100:1 methanol:10N sodium hydroxide by blending for approximately 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts were allowed to settle at ambient overnight. An aliquot of the extract was submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N sodium hydroxide. The clean-up for crops was performed by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract was acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with DCM. After removal of the DCM using nitrogen blow down, the sample was reconstituted in 10:90, methanol:0.1% formic acid. The final extract was filtered through a 0.2-µm PTFE syringe filter and then analysed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table A 1: Recovery results from method validation of clopyralid (*m/z* 189.9/145.9) using the analytical method 120610

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Seeds	clopyralid	0.01	94	12	9	
	clopyralid	0.10	93	5	3	
	clopyralid	5.0	108	-	2	
	clopyralid	10	93	-	1	
Straw	clopyralid	0.01	88	7	9	
	clopyralid	0.10	87	20	3	
	clopyralid	5.0	100	5	2	
	clopyralid	10.0	78	-	1	
Whole plants	clopyralid	0.01	100	8	9	
	clopyralid	0.10	98	-	2	
	clopyralid	1.0	100	-	1	
	clopyralid	5.0	108	-	2	
	clopyralid	10	103	-	1	

Table A 2: Characteristics for the analytical method used for validation of clopyralid residues in oilseed rape

	Clopyralid
Specificity	*m/z* 189.9/145.9 quantification blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with a 1/x weighting. $r^2 \geq 0.9997$ 8 data points
Calibration range	Concentration range of 0.5-50 ng/mL; equivalent sample range of 0.0025-0.25 mg/kg
Limit of determination/quantification	LOQ=0.01 mg/kg

CONCLUSION

This method was considered fit for purpose for the determination of clopyralid in oilseed rape matrices.

A 2.1.1.5 Analytical method 5

Comments of zRMS:	The method is acceptable validated in accordance with SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were proposed at the initiation of the study at 0.003 mg/kg and 0.01 mg/kg, respectively.

Method Identifier No.:	120610
Performing Laboratory:	CEM Analytical Services Ltd (CEMAS) Wokingham, Berkshire, UK
Reference:	KCA 6.3.3/01
Report:	Devine, C.; Residues of Clopyralid in Maize at Intervals and at Harvest Following a Single Application of GF-1966 – Northern Europe – 2020; CEM Analytical Services Ltd (CEMAS), Wokingham, Berkshire, UK; Lab Study No. CEMS-9387; DAS Study No. 201513; 2021; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted from the crop by homogenising and shaking with a methanol:10 N sodium hydroxide (100:1) solution for one hour. After overnight storage, the methanol was removed from an aliquot under nitrogen and made to volume with 1N sodium hydroxide. The extract was shaken with dichloromethane and an aliquot was acidified with hydrochloric acid. Clean-up was performed on a polymeric reversed-phase HLB solid-phase extraction column and the residue was eluted with dichloromethane. After removal of the dichloromethane under nitrogen, the extract was reconstituted in a methanol:0.1% formic acid (10:90) solution and analysed by high performance liquid chromatography with negative-ion electrospray (ESI) tandem mass spectrometry (LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table:1 ***Recovery results from method validation of clopyralid (m/z190/146) using the analytical method***

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Maize Forage	0.01	89	6.2	5	
Maize Forage	10	92	9.2	5	
Maize Grain	0.01	97	4.2	5	
Maize Grain	1.0	90	5.0	5	
Maize Stover	0.01	100	7.1	5	
Maize Stover	10	89	5.2	5	

Table:2 ***Characteristics for the analytical method used for determination of residues of clopyralid in maize forage, grain and stover***

Analyte	clopyralid
Matrix	Maize forage, grain, stover
Technique	LC-MS/MS
Specificity	*m/z* 190/146Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 # data points
Calibration range	Concentration range of 0.5-50 ng/mL(equivalent sample concentration 0.0025 – 0.25 mg/kg)
Limit of quantitation	0.01 mg/kg
Validation Range	0.01-10 mg/kg

CONCLUSION

This method was successfully validated to meet the requirements set forth in SANTE/2020/12830/Rev.1, for the determination of clopyralid in maize forage, grain and stover.

A 2.1.1.6 Analytical method 6

Comments of zRMS:	The method is acceptable validated and fit for purpose for the determination of

	clopyralid in maize matrices.

Method Identifier No.:	GRM 01.16
Performing Laboratory:	CEM Analytical Services Ltd (CEMAS)
Reference:	KCA 6.3.3/02
Report:	Devine, H. C., (2003); Residues of Clopyralid in Maize at Intervals and At Harvest Following One or Two Applications of LONTREL 100 (EF-1136), Northern and Southern Europe - 2002; Study No. CEMS-1786; DAS Report No. GHE-P-10534; DAS Internal Report Code 137088.
Guideline(s):	Commission Directive 96/68/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market and is designed to comply with the FAO Guidelines on Producing Pesticide Residue Data from Supervised Trials, Rome 1990.
Guideline Deviations:	Yes, insufficient number of fortification samples at each level
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Clopyralid was extracted from the samples by homogenising with a mixture of methanol and sodium hydroxide solution. After centrifugation, an aliquot was diluted with hydrochloric acid and purified on an HLB solid-phase extraction column. The residue was eluted with dichloromethane, evaporated to dryness and derivatised with a mixture of 1-propanol and sulphuric acid solution. The derivatising agent was evaporated and the clopyralid propyl ester partitioned into hexane containing 0.01 µg/mL, clopyralid butyl ester as internal standard. The extract was then analysed by gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table A 3: Recovery results from method validation of clopyralid (*m/z* 232.7/233.7) using the analytical method GRM 01.16

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Cobs	Clopyralid	0.01	111	-	1	
	Clopyralid	0.1	89	-	1	
Rest of Plant	Clopyralid	0.01	110	-	1	
	Clopyralid	0.1	90	-	1	
Whole plant	Clopyralid	0.01	108	-	2	
	Clopyralid	0.1	94	-	2	

Table A 4: Characteristics for the analytical method used for validation of clopyralid residues in maize

	Clopyralid
Specificity	GS-MS: *m/z* 232.7/233.7 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis (forced through origin) r≥0.9999 8 data points
Calibration range	Concentration range of 0.0001 to 0.05 µg/mL
Limit of determination/quantification	LOQ=0.01 mg/kg

CONCLUSION

This method was considered fit for purpose for the determination of clopyralid in maize matrices.

A 2.1.1.7 Analytical method 7

Comments of zRMS:	The method is acceptable validated in accordance with SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were proposed at the initiation of the study at 0.003 mg/kg and 0.010 mg/kg, respectively.

Method Identifier No.:	120610
Performing Laboratory:	CEM Analytical Services Ltd (CEMAS) Wokingham, Berkshire, UK
Reference:	KCA 6.3.4/01
Report:	Devine, C.; Magnitude and Decline of Residues of Clopyralid in Sugar Beet Following Applications of GF-1966 in Northern Europe and the UK,

	Initiated in 2020; CEM Analytical Services Ltd (CEMAS), Wokingham, Berkshire, UK; Lab Study No. CEMS-9487; DAS Study No. 200809; 2021; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted from crop samples with 100:1 methanol:10N sodium hydroxide by blending for approximately of 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts were allowed to set ambient overnight. An aliquot of the extract was submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N sodium hydroxide. The clean-up was affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract was acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with DCM. After removal of the DCM using nitrogen blow down, the sample was reconstituted in 10:90, methanol:0.1% formic acid. The final extract was filtered through a 0.2- µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table:1 ***Recovery results from method validation of clopyralid (m/z190/146Q) using the analytical method***

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Sugar Beet Top	0.01	93	6.1	6	97, 97, 99, 90, 84, 91
Sugar Beet Top	5.0	86	9.9	6	92, 81, 95, 94, 75, 80
Sugar Beet Root	0.01	92	8.6	8	89, 92, 93, 79, 91, 85, 100, 104
Sugar Beet Root	5.0	92	7.5	8	88, 90, 108, 89, 87, 87, 93, 92

Table:2 ***Characteristics for the analytical method used for determination of residues of clopyralid in sugar beet top and sugar beet root***

Analyte	Clopyralid
Matrix	sugar beet top and sugar beet root
Technique	LC-MS/MS
Specificity	*m/z* 190/146Q *m/z* 192/148C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Concentration range of 0.5-50 ng/mL (equivalent sample concentration 0.0025 – 0.25 mg/kg)
Limit of quantitation	0.01 mg/kg
Validation Range	0..01 – 5.0 mg/kg

CONCLUSION

This method was successfully validated for the determination of clopyralid in sugar beet top and sugar beet root.

A 2.1.1.8 Analytical method 8

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

Method Identifier No.: GRM 01.16

Performing Laboratory: CEM Analytical Services Ltd (CEMAS)

Reference: KCA 6.3.5/01

Report: Devine, H.C., (2004); Residues of Clopyralid in Onions at Harvest and at Intervals Following Two Application of Lontrel 100 (EF-1136), UK - 2003; DAS Study No. CEMS-2030; DAS Report No. GHE-P-10805; DAS Internal Report Code 146451.

Guideline(s): Commission Directive 96/68/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market and is designed to comply with the FAO Guidelines on Producing Pesticide Residue Data from Supervised Trials, Rome 1990.

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

Study No. CEMS-2030/GHE-P-10805 was submitted, evaluated and deemed acceptable by RMS Finland as part of an MRL evaluation (Finland, 2008) but was not reviewed at EU level because two additional N-EU trials were required. The data also supports the intended critical GAP for onion in C-EU. This study can be deemed as previously evaluated by a competent authority. Therefore, does not require re-evaluation.

MATERIALS AND METHODS

Method Principle

Clopyralid was extracted from the samples by homogenising with a mixture of methanol and sodium hydroxide solution. After centrifugation, an aliquot was diluted with hydrochloric acid and purified on an HLB solid-phase extraction column. The residue was eluted with dichloromethane, evaporated to dryness and derivatised with a mixture of l-propanol and sulphuric acid solution. The derivatising agent was evaporated and the clopyralid propyl ester partitioned into hexane containing 0.01 µg/ml, clopyralid butyl ester as internal standard. The extract was then analysed by gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table A 5: Recovery results from method validation of clopyralid (*m/z* 232.7/233.7) using the analytical method GRM 01.16

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole plant	clopyralid	0.01	87	-	1	
	clopyralid	0.1	91	-	1	
	clopyralid	1.0	83	-	1	
Mature bulbs	clopyralid	0.01	90	-	1	
	clopyralid	1.0	83	-	1	

Table A 6: Characteristics for the analytical method used for validation of clopyralid residues in onions

	Clopyralid
Specificity	*m/z* 232.7/233.7 quantification blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis (forced through origin) r≥0.9997 8 data points
Calibration range	Concentration range of 0.0001-0.05 µg/mL
Limit of determination/quantification	LOQ=0.01 mg/kg

CONCLUSION

This method was considered acceptable for the determination of clopyralid in onion matrices.

A 2.1.1.9 Analytical method 9

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

Method Identifier No.:	GRM 01.16
Performing Laboratory:	CEMAS Berkshire, UK
Reference:	KCA 6.3.5/02
Report:	Devine, H.C.; 2005; Residues of Clopyralid in Onions at Intervals Following Two Applications of Lontrel 100 (EF-1136), Northern Europe - 2004; CEMAS, Berkshire, UK; Lab Study No. CEMS-2346; DAS Study No. GHE-P-11080; 21 July 2005; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev. 4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	The top point was removed from the calibration curve for one batch, as the system was not linear up to this point. There was no impact on the study as the linear range used was sufficient to quantify the samples.

Study No. CEMS-2346/GHE-P-11080 was submitted, evaluated and deemed acceptable by RMS Finland as part of an MRL evaluation (Finland, 2008) but was not reviewed at EU level because two additional N-EU trials were required. The data also supports the intended critical GAP for onion in C-EU. This study can be deemed as previously evaluated by a competent authority. Therefore, does not require re-evaluation.

MATERIALS AND METHODS

Method Principle

Clopyralid was extracted from the samples by homogenizing with a mixture of methanol and sodium hydroxide solution. After centrifugation, an aliquot was diluted with hydrochloric acid and purified on a HLB solid-phase extraction column. The residue was eluted with dichloromethane, evaporated to dryness and derivatized with a mixture of 1-propanol and sulphuric acid solution. The derivatizing agent was evaporated and the clopyralid propyl ester partitioned into hexane containing 0.01µg/mL clopyralid butyl ester as internal standard. The extract was then analysed by gas chromatography with negative-ion chemical ionization mass spectrometry (GC-NCI-MS).

RESULTS AND DISCUSSION

Mean recovery values of clopyralid at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are presented in the tables below.

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

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole plant	clopyralid	0.01	106	NA	2	
		0.1	105	NA	2	
Mature Bulb	clopyralid	0.01	74	NA	1	
		0.1	109	NA	1	

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

	Clopyralid
Specificity	*m/z* 233 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.9996 8 data points
Calibration range	Concentration range of 0.0001-0.05 µg/mL
Limit of quantification	LOQ=0.01 mg/kg

CONCLUSION

Although, this method was not fully validated according to the current Sanco 3029/99 rev.4 Guidelines, fit for purpose has been demonstrated for the determination of clopyralid in onion matrices.

A 2.1.1.10 Analytical method 10

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

Method Identifier No.: GRM 01.16

Performing Laboratory: CEMAS
Berkshire, UK

Reference:	KCA 6.3.5/03
Report:	Devine, H.C.; 2006; Residues of Clopyralid in Onions at Intervals Following Two Applications of Lontrel 100 (EF-1136), Northern Europe - 2005; CEMAS, Berkshire, UK; Lab Study No. CEMS-2696; DAS Study No. GHE-P-11272; 14 March 2006; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev. 4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	The top point was removed from the calibration curve, as the system was not linear up to this point. There was no impact on the study as the linear range used was sufficient to quantify the samples.

Study No. CEMS-2696/GHE-P-11272 was submitted, evaluated and deemed acceptable by RMS Finland as part of an MRL evaluation (Finland, 2008) but was not reviewed at EU level because two additional N-EU trials were required. The data also supports the intended critical GAP for onion in C-EU. This study can be deemed as previously evaluated by a competent authority. Therefore, does not require re-evaluation.

MATERIALS AND METHODS

Method Principle

Clopyralid was extracted from the samples by homogenizing with a mixture of methanol and sodium hydroxide solution. After centrifugation, an aliquot was diluted with hydrochloric acid and purified on a HLB solid-phase extraction column. The residue was eluted with dichloromethane, evaporated to dryness and derivatized with a mixture of 1-propanol and sulphuric acid solution. The derivatizing agent was evaporated and the clopyralid propyl ester partitioned into hexane containing 0.01µg/mL clopyralid butyl ester as internal standard. The extract was then analysed by gas chromatography with negative-ion chemical ionization mass spectrometry (GC-NCI-MS).

RESULTS AND DISCUSSION

Mean recovery values of clopyralid at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are presented in the tables below.

Table A 8: Recovery results from method validation of clopyralid using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole plant	clopyralid	0.01	105	NA	1	
		1.0	104	NA	1	
Mature Bulb	clopyralid	0.01	96	NA	1	

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
		0.1	99	NA	1	

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

	Clopyralid
Specificity	*m/z* 233 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.9990 7 data points
Calibration range	Concentration range of 0.0001-0.03 µg/mL
Limit of quantification	LOQ=0.01 mg/kg

CONCLUSION

Although, this method was not fully validated according to the current Sanco 3029/99 rev.4 Guidelines, fit for purpose has been demonstrated for the determination of clopyralid in onion matrices.

A 2.1.1.11 Analytical method 11

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information

Method Identifier No.: GRM 01.16

Performing Laboratory: CEMAS
Berkshire, UK

Reference: KCA 6.3.5/04

Report: Rawle, N.W.; 2012; Residues of Clopyralid in Bulb Onions Following Two Applications of EF-1136, Northern Europe - 2011; CEMAS, Berkshire, UK; Lab Study No. CEMS-4969; DAS Study No. GHE-P-12680; 26 January 2012; Unpublished

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

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

Method Alterations:

Study No. CEMS-4969/GHE-P-12680 was submitted, evaluated and deemed acceptable by RMS Finland as part of an MRL evaluation (Finland, 2008) but was not reviewed at EU level because two additional N-EU trials were required. The data also supports the intended critical GAP for onion in C-EU. This study can be deemed as previously evaluated by a competent authority. Therefore, does not require re-evaluation.

MATERIALS AND METHODS

Method Principle

Residues of clopyralid were extracted from the crop by homogenising with a mixture of methanol and ION sodium hydroxide solution (99: I). Following centrifugation, an aliquot was diluted with 1.0 N hydrochloric acid and purified using an HLB solid-phase extraction (SPE) column. After elution with dichloromethane, the eluate was evaporated to dryness and derivatised with a solution of I-propanol and 4% concentrated hydrochloric acid solution (96:4). The derivatising reagent was removed by evaporation and the clopyralid ester was partitioned into hexane containing clopyralid butyl ester as internal standard. The hexane extract was then analysed by capillary gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-MS).

RESULTS AND DISCUSSION

Mean recovery values of clopyralid at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are presented in the tables below.

Table A 9: Recovery results from method validation of clopyralid using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Bulbs	clopyralid	0.01	96	NA	2	

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

	Clopyralid
Specificity	*m/z* 233 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis no weighting r≥0.999 7 Data points
Calibration range	Concentration range of 0.0001-0.05 µg/mL (sample equivalence of 0.002-1.0 mg/kg)
Limit of quantification	0.01 mg/kg

CONCLUSION

Although, this method was not fully validated according to the current SANTE/2020/12830 Rev. 1 Guidelines, fit for purpose has been demonstrated for the determination of clopyralid in onion matrices.

A 2.1.1.12 Analytical method 12

Comments of zRMS:	The method is acceptable validated in accordance with Environmental Protection Agency under Section 171-4(c)(2)(iv), Subdivision 0 of the EPA Pesticide Assessment Guideline and fit for purpose for the determination of clopyralid Residues of Clopyralid in Sugar Beet. The limits of detection (LOD) 0.05 mg/kg.

Method Identifier No.:	GRM 94.04
Performing Laboratory:	North American Environmental Chemistry Laboratory, DowElanco
Reference:	KCA 6.5.3/01
Report:	Phillips, A.M., (1994); Determination of Residues of Clopyralid in Sugar Beet Processed Fractions; Study No. RES93011; DAS Report No. GH-C 3305; DAS Internal Report Code 21350.
Guideline(s):	Environmental Protection Agency under Section 171-4(c)(2)(iv), Subdivision 0 of the EPA Pesticide Assessment Guideline.
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of clopyralid were extracted from sugar beet processed fractions by shaking for at least 1 hour with a solution of 0.5% sodium hydroxide in water. An aliquot of the sample was extacted into methyl t-butyl ether (MTBE) and then into 0.25M sodium bicarbonate. The sample was acidified and again extracted into MTBE. After evaporating the MTBE, the sample was derivatized with 14% boron trifluoride in methanol to form the methyl derivative. The sample was then treated with potassium permanganate and allowed to react for 30 minutes. Excess potassium

permanganate was reduced with sodium sulfite and then the sample was extracted into 20:80 diethyl ether:hexane. An aliquot of the sample in diethyl ether:hexane was solvent exchanged into toluene and internal standard was added. The final sample in toluene was then transferred to an auto sampler vial for analysis by gas chromatography (GC).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table A 10: Recovery results from method validation of clopyralid using the analytical method GRM 94.04

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sugar beet (RAC)	clopyralid	0.05	82	5	8	
	clopyralid	0.1	75	-	2	
	clopyralid	0.2	74	-	2	
	clopyralid	0.5	76	-	2	
	clopyralid	0.75	83	-	2	
	clopyralid	1.0	80	-	2	
Pulp	clopyralid	0.05	79	7	8	
	clopyralid	0.1	80	-	2	
	clopyralid	0.2	93	-	2	
	clopyralid	0.5	96	-	2	
	clopyralid	0.75	97	-	2	
	clopyralid	1.0	88	-	2	
Molasses	clopyralid	0.05	101	4	8	
	clopyralid	0.2	76	-	2	
	clopyralid	0.5	76	-	2	
	clopyralid	0.75	87	-	2	
	clopyralid	1.0	100	-	2	
	clopyralid	5.0	89	-	2	
Sugar	clopyralid	0.05	93	6	8	
	clopyralid	0.1	77	-	2	
	clopyralid	0.2	88	-	2	
	clopyralid	0.5	85	-	2	
	clopyralid	0.75	97	-	2	
	clopyralid	1.0	96	-	2	

Table A 11: Characteristics for the analytical method used for validation of clopyralid

residues in sugar beet RAC and processed fractions

	Clopyralid
Specificity	GC/MSD in SIM: *m/z* 110 and 205 blank value <30% LOQ
Calibration (type, number of data points)	Power regression analysis (y= constant x $X^{exponent}$) r≥0.9992 7 data points
Calibration range	Concentration range of 0.01-0.5 µg/mL
Limit of determination/quantification	LOQ=0.05 mg/kg

CONCLUSION

This method was considered fit for purpose for the determination of clopyralid in sugar beet and processed matrices.

A 2.1.1.13 Analytical method 13

Comments of zRMS:	The method is acceptable validated in accordance with SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were at 0.003 mg/kg and 0.01 mg/kg, respectively.

Method Identifier No.:	120610
Performing Laboratory:	CEM Analytical Services Ltd. (CEMAS) Wokingham, Berkshire, RG41 2FD, UK
Reference:	KCA 6.5.3/02
Report:	Devine, C.; 2020; Residues of Clopyralid in Sugar Beet and Process Fractions Following Multiple Applications of GF-1966 – Northern Europe – 2019 CEM Analytical Services Limited (CEMAS, Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire RG41 2FD, UK; Lab Study No. EMS-8908; DAS Study No. 181493 ; 15 July 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of clopyralid were extracted from crop samples with 100:1 methanol:10N sodium hydroxide by blending for approximately of 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts were allowed to set ambient overnight. An aliquot of the extract was submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N sodium hydroxide. The clean-up was affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract was acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with DCM. After removal of the DCM using nitrogen blow down, the sample was reconstituted in 10:90, methanol:0.1% formic acid. The final extract was filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20). The results obtained are summarised in the following tables.

Table A 12: Recovery results from method validation of clopyralid (*m/z* 196/146) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Individual Recoveries
Tops with leaves	Clopyralid	0.01	84	3.4	5	82, 82, 83, 87, 88
Tops with leaves	Clopyralid	1.0	96	5.6	5	91, 97, 94, 105, 94
Roots	Clopyralid	0.01	81	7.7	7	80, 78, 76, 93, 74, 80, 83
Roots	Clopyralid	1.0	80	5.9	5	83, 82, 84, 74, 75
Roots	Clopyralid	5.0	74	4.5	5	70, 78, 71, 74, 76
Juice	Clopyralid	0.01	78	5.9	5	79, 82, 79, 80, 70
Juice	Clopyralid	1.0	83	14.3	5	75, 97, 94, 79, 70
Molasses	Clopyralid	0.01	89	5.2	7	86, 85, 86, 92, 85, 95, 95
Molasses	Clopyralid	1.0	84	3.3	5	82, 80, 85, 85, 87
Molasses	Clopyralid	5.0	84	3.7	5	81, 83, 82, 88, 87
Non-refined sugar	Clopyralid	0.01	74	3.1	5	74, 78, 73, 72, 74
Non-refined sugar	Clopyralid	5.0	73	3.9	5	73, 77, 69, 74, 73
Pulp	Clopyralid	0.01	79	4.6	5	84, 80, 78, 76, 75
Pulp	Clopyralid	5.0	74	6.5	5	70, 71, 70, 80, 78
Brown sugar	Clopyralid	0.01	78	3.3	5	77, 75, 78, 81, 81
Brown sugar	Clopyralid	5.0	83	7.2	5	83, 84, 83, 92, 75
White sugar	Clopyralid	0.01	81	3.9	5	80, 83, 82, 84, 76
White sugar	Clopyralid	5.0	86	10.2	5	73, 81, 91, 95, 89

Table A 13: Characteristics for the analytical method used for validation of clopyralid residues in roots, tops with leaves and process fractions

	Clopyralid
Specificity	*m/z* 196/146 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 8 data points
Calibration range	Concentration range of 0.5-25 ng/mL equivalent to 0.0025-0.125 mg/kg
Limit of determination/quantification	LOQ= 0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of clopyralid residues in roots, tops with leaves and process fractions.

A 2.1.1.14 Analytical method 14

Comments of zRMS:	The method is acceptable validated in accordance with SANCO/3029/99 rev. 4. The limits of quantitation 0.01 mg/kg.

Method Identifier No.:	120610
Performing Laboratory:	CEM Analytical Services Ltd (CEMAS) Wokingham, Berkshire, UK
Reference:	KCA 6.6.2/01
Report:	Devine, C.; Determination of Residues of Clopyralid after One Application of GF-1966 (EC Formulation) on Bare Soil in Rotational Crops at 3 Sites in Northern Europe and 3 Sites in Southern Europe 2019-2020; CEM Analytical Services Ltd (CEMAS), Wokingham, Berkshire, UK; Lab Study No. CEMS-9009; DAS Study No. 190557; 2021; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes

Acceptability: Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted from crop samples with 100:1 methanol:10N sodium hydroxide by blending for approximately of 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts were allowed to set ambient overnight. An aliquot of the extract was submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N sodium hydroxide. The clean-up was affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract was acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with DCM. After removal of the DCM using nitrogen blow down, the sample was reconstituted in 10:90, methanol:0.1% formic acid. The final extract was filtered through a 0.2- µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table:1 ***Recovery results from method validation of clopyralid (m/z190/146) using the analytical method***

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Radish Roots	0.01	86	10.8	11	
Radish Roots	1.0	87	8.6	11	
Radish Tops	0.01	90	11.7	11	
Radish Tops	0.1	92	7.9	5	
Radish Tops	1.0	89	13.6	7	
Cabbage	0.01	85	7.8	11	
Cabbage	0.1	80	10.2	11	
Barley whole plant	0.01	93	9.5	9	
Barley whole plant	1.0	92	10	9	
Barley Grain	0.01	85	8.1	9	
Barley Grain	1.0	82	11.9	9	
Barley Straw	0.01	87	12.8	9	
Barley Straw	1.0	89	11.1	9	
Wheat Whole plant	0.01	95	6.9	5	
Wheat Whole plant	1.0	89	4.7	5	

Wheat Grain	0.01	96	7.3	7	
Wheat Grain	0.1	89	4.7	5	
Wheat Grain	1.0	85	5.5	5	
Wheat Straw	0.01	96	16.2	7	
Wheat Straw	1.0	93	3.1	7	
Oilseed Rape Whole Plant	0.01	88	9.1	13	
Oilseed Rape Whole Plant	0.1	85	9.6	9	
Oilseed Rape Whole Plant	1.0	79	4.2	5	
Oilseed Rape Seed	0.01	86	13.3	13	
Oilseed Rape Seed	0.1	82	9.5	13	
Oilseed Rape Rest of Plant	0.01	89	13.5	13	
Oilseed Rape Rest of Plant	0.1	81	11.7	13	
Sunflower Whole Plant	0.01	80	8.1	5	

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Sunflower Whole Plant	1.0	82	9.1	10	
Sunflower Seed	0.01	93	3.1	5	
Sunflower Seed	0.1	78	6.9	5	
Sunflower Rest of Plant	0.01	83	5.4	5	
Sunflower Rest of Plant	0.1	85	7.0	5	

Table:2 Characteristics for the analytical method used for determination of residues of clopyralid in radish, cabbage, barley, wheat, oilseed rape, sunflower matrices

Analyte	Clopyralid
Matrix	Radish, cabbage, barley, wheat, oilseed rape and sunflower matrices
Technique	LC-MS/MS
Specificity	*m/z* 190/146Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 7 data points
Calibration range	Concentration range of 0.5-50 ng/mL (equivalent sample concentration 0.0025 – 0.25 mg/kg)

Limit of quantitation	0.01 mg/kg
Validation Range	0.01-1.0 mg/kg

CONCLUSION

This method was successfully validated to meet the requirements set forth in SANTE/2020/12830/Rev.1, for the determination of clopyralid in radish, cabbage, barley, wheat, oilseed rape and sunflower matrices.

A 2.1.1.15 Analytical method 15

Comments of zRMS:	The analytical phase of the study 379P-176 is acceptable and suitable for the determination of the active substance Clopyralid in the test freshwater. LOQ was 0.165 mg a.e./L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.: 200843

Performing Laboratory: Eurofins EAG Agroscience, LLC
Easton, MD 21601 USA

Reference: KCP 10.2

Report: Arnie, J.R., Zhao, J., Aufderheide, J.A., Zhang, L., Fierman, L.A.; 2020; EF-243: A 72-Hour Toxicity Test with the Freshwater Alga (Raphidocelis subcapitata); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive Easton, MD 21601US; Lab Study No. 379P-176; DAS Study No. 200843; 24 July 2020; Unpublished

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

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

Method Alterations:

MATERIALS AND METHODS

Method Principle

Residues of clopyralid, active ingredient of EF-243, are determined from samples of freshwater AAP medium by diluting samples into calibration curves as necessary with freshwater AAP medium. The final sample is analysed for clopyralid by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 14: Recovery results from method validation of clopyralid (*m/z* 192.0/147.8) using the analytical method

Matrix	Analyte	Fortification level (mg a.e. /L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP Medium	clopyralid	0.165 (0.630 mg EF-243/L)	104	1.01	6	Standard Deviation: 1.05 (103,103,104,104,105,102)
Freshwater AAP Medium	clopyralid	30.0 (114 mg EF-243/L)	103	1.34	6	Standard Deviation: 1.38 (103,104,102,101,104,101)

Table A 2: Characteristics for the analytical method used for validation of EF-243 residues in freshwater AAP medium

	Clopyralid
Specificity	*m/z 192.0/147.8*Q blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.998 12 data points
Calibration range	Concentration range of 0.0500 to 1.25 mg a.e./L (0.191 to 4.77 mg EF-243/L)
Limit of determination/quantification	LOQ= 0.165 mg a.e./L (0.630 mg EF-243/L)

CONCLUSION

This method was successfully validated for the determination of clopyralid residues from EF-243 in freshwater AAP medium.

A 2.1.1.16 Analytical method 16

Comments of zRMS:	The analytical phase of the study 379P-168 is acceptable and suitable for the determination of the active substance Clopyralid in the test freshwater medium. LOQ was 1.50 mg a.e./L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.:	191747
Performing Laboratory:	Eurofins EAG Agroscience, LLC, Easton, Maryland, USA
Reference:	KCP 10.2
Report:	Arnie, J.R., Zhao, E., Aufderheide, J.A., Zhang, L.; 2020; GF-2895: A 72-Hour Toxicity Test with the Freshwater Alga (*Raphidocelis subcapitata*); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive,Easton, MD 21601, USA; Lab Study No. 379P-168; DAS Study No. 191747 ; 22 May 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4; OECD Guideline 201 EU Directive 92/69/EEC, Method C.3.
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-2895 (clopyralid) are determined from samples of freshwater AAP medium by diluting samples into calibration curve with freshwater AAP medium, and analysed by high performance liquid chromatography with ultraviolet absorbance detection (HPLC-UV) at a wavelength of 280 nm.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 15: Analytical Verification of GF-2895 (Clopyralid) in Freshwater AAP Medium

Matrix	Analyte	Fortification level (mg a.e./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP	GF-2895 (Clopyralid)	1.50	102	0.737%	6	Recoveries range from 101-103%
Freshwater AAP	GF-2895 (Clopyralid)	50.0	101	0.744%	6	Recoveries range from 100-102%

Table A 2: Characteristics for the Analytical Method Used for Verification of GF-2895 (Clopyralid) Residues in Freshwater AAP Medium

	GF-2895 (Clopyralid)
Specificity	blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 12 data points
Calibration range	Concentration range of 0.250-2.50 mg a.e./L
Limit of quantification	LOQ=1.50 mg a.e./L

CONCLUSION

This method was successfully validated for the determination of GF-2895 (Clopyralid) residues in freshwater AAP medium.

A 2.1.1.17 Analytical method 17

Comments of zRMS:	The analytical phase of the study 150051 is acceptable and suitable for the determination of the active substance Clopyralid in water. LOQ was for active substance in water 2.0 µg/L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.:	150051 Appendix 3
Performing Laboratory:	SynTech Research Laboratory Services LLC Ecotoxicology Stilwell, Kansas USA
Reference:	KCP 10.2
Report:	Banman, C.S.; Moore, S.; 2015; GF-1966: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum*; SynTech Research

	Laboratory Services LLC, Ecotoxicology, Stilwell, Kansas, USA; Lab Study No. 014SRLS15C01; DAS Study No. 150051 ; 01 July 2015; Unpublished
Guideline(s):	SANCO 3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle

Residues of clopyralid were extracted from samples of hard water by acidifying an aliquot with hydrochloric acid to adjust pH below 2, capturing the clopyralid on an HLB SPE column and eluting with dichloromethane, evaporating to dryness and reconstituting with methanol and formic acid solution. The final samples were analysed to determine the concentrations of clopyralid using liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS). The limit of quantification (LOQ) was 2.0 µg/L.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70--110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table A 16: Recovery results from method validation of clopyralid (*m/z* 189.88/146.00) using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
hard water	clopyralid	2.0	92.4	1.23	5	
hard water	clopyralid	10	96.5	NA	2	
hard water	clopyralid	5000	94.4	1.21	5	

Table A 2: Characteristics for the analytical method used for validation of clopyralid residues in water

	clopyralid
Specificity	*m/z* 189.88/146.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis y = 897534x + 138321 with 1/x weighting r≥0.999 6 data points
Calibration range	Concentration range of 1.0-20.0 ng/mL
Limit of determination/quantification	LOQ=2 µg/L

CONCLUSION

This method was successfully validated for the determination of clopyralid in hard water.

A 2.1.1.18 Analytical method 18

Comments of zRMS:	The analytical phase of the study 200841 is acceptable and suitable for the determination of the active substance Clopyralid in the water and sediments. LOQ was for active substance in water 0.045 mg/L and for sediment 0.1 mg/kg. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.:	170354 Appendix G
Performing Laboratory:	Eurofins EAG Agroscience, LLC Niefern-Öschelbronn, Germany
Reference:	KCP 10.2
Report:	Gonsior, G.; 2018; GF-2895: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System; Eurofins Agroscience Services EcoChem GmbH, Eutinger Straße 24, Niefern-Öschelbronn, Germany; Lab Study No. S17-01552; DAS Study No. 170354; 21 February 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes

Acceptability: Yes

Method Alterations:

MATERIALS AND METHODS

Method Principle

Residues of clopyralid are determined in samples of test medium by diluting samples 1:1 with acetonitrile then if needed diluted further into calibration curves using 1:1 acetonitrile/test medium solution. The final sample is analysed for clopyralid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Residues of clopyralid are determined in samples of sediment by extracting samples with acetonitrile/water (1:1, v/v), shaking, centrifuging and decanting. If needed, diluted further into calibration curves using sediment blank extract. The final sample is analysed for clopyralid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 17: Concurrent Recovery results of clopyralid (*m/z* 192/146)

Matrix	Fortification level Medium (mg/L) Sediment (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Test medium	0.045	109	1	5	
Test medium	65.0	109	1	5	
Sediment	0.1	105	2	5	
Sediment	50.0	102	5	5	

Table A 2: Characteristics for the analytical method used for validation of EF-243 residues in freshwater AAP medium

	Clopyralid
Specificity	*m/z 192/146* blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 12 data points

Calibration range	Concentration range of Test medium 3-100 ng /mL; sample equivalent 0.006 to 0.20 mg /L Sediment 2-100 ng/mL; sample equivalent 0.02 mg/kg to 1 mg/kg
Limit of determination/quantification	LOQ= water 0.045 mg/L; sediment 0.1 mg/kg

CONCLUSION

This method was successfully validated for the determination of clopyralid residues in test medium and sediment.

A 2.1.1.19 Analytical method 19

Comments of zRMS:	The analytical phase of the study 200841 is acceptable and suitable for the determination of the active substance Clopyralid in the test freshwater medium. LOQ was 0.630 mg/L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.: 200841

Performing Laboratory: Eurofins EAG Agroscience, LLC,
8598 Commerce Drive,Easton, MD 21601, USA

Reference: KCP 10.2

Report: [redacted] 2020; EF-243: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*); Eurofins EAG Agroscience, [redacted] 30 July 2020; Unpublished

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

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

Method Alterations: None

MATERIALS AND METHODS

Method Principle

Residues of clopyralid, the active ingredient in EF-243 are determined from samples of freshwater by diluting sample with freshwater, as necessary, into the range of the calibration curve and analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarized in the following tables.

Table A 18: Recovery results from method validation of EF-243 (Clopyralid) (*m/z* 192.0/147.8) using the analytical method

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	EF-243 (Clopyralid)	0.630 mg/L or 0.165 mg a.e./L	100	0.853	6	QC sample recoveries ranged from 99.0-101%
Freshwater	EF-243 (Clopyralid)	381mg/L or 100 mg a.e./L	101	2.29	6	QC sample recoveries ranged from 97.2-104%

Table A 2: Characteristics for the analytical method used for validation of EF-243 (Clopyralid) residues in Freshwater

	EF-243 (Clopyralid)
Specificity	*m/z* 192.0/147.8 *m/z* 190.0/146.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 5data points
Calibration range	Concentration range of 0.0500-1.25 mg a.e./L
Limit of determination/quantification	LOQ=0.630 mg/L or 0.165 mg a.e./L

CONCLUSION

This method was successfully validated for the determination of EF-243 (Clopyralid) in freshwater.

A 2.1.1.20 Analytical method 20

Comments of zRMS:	The analytical phase of the study 200841 is acceptable and suitable for the determination of the active substance Clopyralid in the water. LOQ was for active substance in water 0.630 mg/L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.:	200842
Performing Laboratory:	Eurofins EAG Agroscience, LLC, 8598 Commerce Drive,Easton, MD 21601, USA
Reference:	KCP 10.2
Report:	Ross, T.L., Zhao, J., Zhang, L., Schneider, S.Z.; 2020; EF-243: A 48-Hour Static Acute Toxicity Test with the Cladoceran (Daphnia magna); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive,Easton, MD 21601, USA; Lab Study No. 379A-334; DAS Study No. 200842 ; 03 September 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of clopyralid, the active ingredient in EF-243 are determined from samples of freshwater by diluting sample with freshwater, as necessary, into the range of the calibration curve and analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 19: Recovery results from method validation of EF-243 (Clopyralid) (*m/z* 192.0/147.8) using the analytical method

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	EF-243 (Clopyralid)	0.630 mg/L or 0.165 mg a.e./L	103	0.790	6	QC sample recoveries ranged from 102-104%
Freshwater	EF-243 (Clopyralid)	381mg/L or 100 mg a.e./L	105	3.91	6	QC sample recoveries ranged from 98.8-111%

Table A 2: Characteristics for the analytical method used for validation of EF-243 (Clopyralid) residues in Freshwater

	EF-243 (Clopyralid)
Specificity	*m/z* 192.0/147.8 *m/z* 190.0/146.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 5data points
Calibration range	Concentration range of 0.0500-1.25 mg a.e./L
Limit of determination/quantification	LOQ=0.630 mg/L or 0.165 mg a.e./L

CONCLUSION

This method was successfully validated for the determination of EF-243 (Clopyralid) in freshwater.

A 2.1.1.21 Analytical method 21

Comments of zRMS:	The analytical phase of the study 190300 is acceptable and suitable for the determination of the active substance Clopyralid in the test solutions. LOQ was 1500 mg /L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.: 190300

Performing Laboratory: ibacon GmbH
Rossdorf

Hessen / Germany

Reference:	KCA 8.3.1
Report:	Tänzler V., Kowalczyk, F.; 2019; Clopyralid: Effects (Acute Contact and Oral) on Bumblebees (Bombus terrestris L.) in the Laboratory; ibacon GmbH, Rossdorf (Hessen)/Germany; Lab Study No. 141721105; DAS Study No. 190300 ; 30 October 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes

Method Alterations:

MATERIALS AND METHODS

Method Principle

Residues of clopyralid are determined from samples of acetone (contact test solution) and in 95% (sucrose 50%) / 5 % acetone (feeding solution) by diluting the samples into calibration range with acetonitrile / pure water (50/50). The final sample is analysed for clopyralid by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following table.

Table A 20: Recovery results from method validation of clopyralid (*m/z* 192 to 148 Q) using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
acetone (contact test solution)	clopyralid	30000	90	3	5	94 / 89 / 88 / 88 /89
acetone (contact test solution)	clopyralid	120000	104	5	5	97 / 105 / 100 / 108 / 104
95% (sucrose 50%) / 5 % acetone (feeding	clopyralid	1500	96	14	5	100 / 118 / 90 / 87 / 96

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
solution)						
95% (sucrose 50%) / 5 % acetone (feeding solution)	clopyralid	3000	100	8	5	110 / 98 / 91 / 96 / 105

Table A 21: Characteristics for the analytical method used for validation of clopyralid residues in acetone (contact test solution) and in 95% (sucrose 50%) / 5 % acetone (feeding solution)

	clopyralid
Specificity	*m/z* 192 to 148 Q *m/z* 190 to 146 C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis r ≥ 0.9993 9 data points
Calibration range	Concentration range of 0.0025 to 0.050 mg/L
Limit of quantification	LOQ = 1500 mg /L (diluted by factor 100000)

CONCLUSION

This method was successfully validated for the determination of clopyralid in acetone and in 95% (sucrose 50%) / 5 % acetone.

A 2.1.1.22 Analytical method 22

Comments of zRMS:	The analytical phase of the study 190300 is acceptable and suitable for the determination of the active substance Clopyralid in the test solutions. LOQ was 150 mg /L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.: 190287

Performing Laboratory: Envigo CRS Limited, Eye, Suffolk, UK

Reference: KCP 10.6

Report:	Davies, C.; 2019; GF-1966 Vegetative Vigour Test Terrestrial Non Target Plants; Stockbridge Technology Centre Ltd, Cawood, North Yorkshire, UK; Lab Study No. STC/19/E1261; DAS Study No. 190287 ; 10 October 2019; Unpublished
Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

The active substance in the spray solutions were determined by dilution of the supplied samples with acetonitrile. Triplicate samples (10 mL) of the supplied spray solution are pipetted into separate 100 mL volumetric flasks and diluted to volume with acetonitrile. The final samples were analysed for clopyralid by high performance liquid chromatography (HPLC) with UV detection. The samples are analysed relative to a bracketing standard solution containing the active standard in diluent comprising acetonitrile:water (9:1 v/v).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

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

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Aqueous spray solution	clopyralid	1650	100	0.5	5	-
		150	104	1.2	5	-

Table A 2: Characteristics for the analytical method used for validation of clopyralid spray solutions

	Clopyralid
Specificity	UV detector = 275 nm No interference from reagents
Calibration (type, number of data points)	linear regression analysis r = 0.9999 9 data points
Calibration range	Concentration range of 6 – 250 mg /L Samples quantified by reference to bracketing standard solutions (approximately 151 mg/L)
Limit of determination/quantification	150 mg/L

CONCLUSION

The method was considered acceptable for the determination of clopyralid in the aqueous spray solutions of GF-1966 because of the good precision and accuracy.

A 2.1.1.23 Analytical method 23

Comments of zRMS:	The analytical phase of the study 190288 is acceptable and suitable for the determination of the active substance Clopyralid in the test solutions. LOQ was 150 mg /L. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev.4.

Method Identifier No.: 190288

Performing Laboratory: Envigo CRS Limited, Eye, Suffolk, UK

Reference: KCP 10.6

Report: Stead, A.; 2019; GF-1966 Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants; Stockbridge Technology Centre Ltd, Cawood, North Yorkshire, UK; Lab Study No. STC/19/E1262; DAS Study No. 190288 ; 11 October 2019; Unpublished

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

Guideline Deviations: No

GLP: Yes

Acceptability: Yes

Method Alterations: None

MATERIALS AND METHODS

Method Principle

The active substance in the spray solutions were determined by dilution of the supplied samples with acetonitrile. Triplicate samples (10 mL) of the supplied spray solution are pipetted into separate 100 mL volumetric flasks and diluted to volume with acetonitrile. The final samples were analysed for clopyralid by high performance liquid chromatography (HPLC) with UV detection. The samples are analysed relative to a bracketing standard solution containing the active standard in diluent comprising acetonitrile:water (9:1 v/v).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table A 22: Recovery results from method validation of clopyralid using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Aqueous spray solution	clopyralid	1650	100	0.5	5	-
		150	104	1.2	5	-

Table A 2: Characteristics for the analytical method used for validation of clopyralid spray solutions

	Clopyralid
Specificity	UV detector = 275 nm No interference from reagents
Calibration (type, number of data points)	linear regression analysis r = 0.9999 9 data points
Calibration range	Concentration range of 6 – 250 mg /L Samples quantified by reference to bracketing standard solutions (approximately 151 mg/L)
Limit of determination/quantification	150 mg/L

CONCLUSION

The method was considered acceptable for the determination of clopyralid in the aqueous spray solutions of GF-1966 because of the good precision and accuracy.

A 2.1.1.24 Analytical method 24

Comments of zRMS:	The analytical method of the study 200098 is acceptable and suitable for the determination of clopyralid in pollen, nectar and honey. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for all matrices with a limit of detection (LOD) set at 0.003 mg/kg (30 % of the LOQ).

Reference Report:	KCP 5.2 Appeltauer, A. (2021) Determination of Residues of Clopyralid in Nectar, Pollen, Plants and Honey of Winter Oilseed Rape after One Application of GF-1966 in a Semi-Field Residue Study in Germany, Romania, The Netherlands, Southern France and Spain in 2020. Niefern-Öschelbronn, Germany, Eurofins Agroscience Services EcoChem GmbH. Lab Study no. S20-00871. Corteva study no. 200098
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP	Yes
Accaptability	Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted with methanol/10 N sodium hydroxide (100/1, v/v) by blending for approximately 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to set ambient overnight. An aliquot of the extract is submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1 N sodium hydroxide. The extracts are cleaned up by partitioning the basic extract with dichloromethane (DCM). An aliquot of the basic extract is acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) cleanup and elution with DCM. After removal of the DCM using nitrogen blow down, the sample is reconstituted in 10/90, methanol/0.1% formic acid. The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 1: Recovery results from method validation of clopyralid (m/z 192/110) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.01	85	4	5	
Pollen	0.1	88	2	5	
Pollen	5	101	5	5	
Nectar	0.01	104	6	5	
Nectar	0.1	99	2	5	
Nectar	5	109	2	5	
Whole plant	0.01	101	4	5	
Whole plant	0.1	97	4	5	
Whole plant	5	92	4	5	
Honey	0.01	86	6	5	
Honey	0.1	85	13	5	

Table 2: Recovery results from method validation of clopyralid (m/z 194/112) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.01	84	8	5	
Pollen	0.1	88	3	5	
Pollen	5	105	6	5	
Nectar	0.01	102	7	5	
Nectar	0.1	94	3	5	
Nectar	5	109	3	5	
Whole plant	0.01	101	6	5	
Whole plant	0.1	96	3	5	
Whole plant	5	92	6	5	
Honey	0.01	87	7	5	
Honey	0.1	84	10	5	

Table 3: Procedural recovery results of clopyralid (m/z 192/110Q) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	0.01	91	14	10	
Pollen	0.1	92	10	11	
Nectar	0.01	100	7	5	
Nectar	0.1	102	8	5	
Whole plant	0.01	91	7	10	
Whole plant	0.1	92	8	10	
Honey	0.01	105	5	5	
Honey	0.1	103	2	5	

Table 4: Characteristics for the analytical method used for determination of residues of clopyralid in honey, nectar, whole plant and pollen

Analyte	Clopyralid
Matrix	Honey. Nectar, whole plant and pollen

Technique	LC-MS/MS
Specificity	*m/z* 192/110Q *m/z* 194/112C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.06-10 ng/mL (equivalent sample concentration 0.003 – 0.5 mg/kg)
Limit of quantitation	0.01 mg/kg
Validation Range	0.01 – 5 mg/kg

CONCLUSION

This method was successfully validated for the determination of clopyralid in pollen, nectar, whole plant and honey.

A 2.1.1.25 Analytical method 25

Comments of zRMS:	The analytical phase of the study GHE-P-7289 Determination of Residues of Clopyralid in Onions at Intervals following a Single application of Lontrel has been performed in compliance with Good Laboratory Practice and not fully validated to meet the requirements of SANTE/2020/12830/Rev.2. However, in our point of view the method can be considered acceptable as confirmatory method.

Reference	KCP 5.2
Report:	Butler, R.E., Reynens, P. (1998): Determination of Residues of Clopyralid in Onions at Intervals following a Single application of Lontrel *100 (EF-1136), Belgium, 1997. Wantage, Oxon, UK, Dow AgroScience Letcombe Laboratory. Corteva study no. GHE-P-7289
Guideline(s):	Similar to SANCO/3029/99 rev. 4 / ERC-97.20
Deviations:	No
GLP	Yes
Accaptability	Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted with methanol/10 N sodium hydroxide (100/1, v/v) by blending for approximately 1 minute and shaking for 2 hour on a reciprocal shaker. The extracts are allowed to set ambient overnight. An aliquot of the extract is combined with sodium chloride and sulphuric acid and MTBE. The aliquot is then shaken and centrifuged, and the MTBE layer removed and combined with a second extraction using MTBE. The MTBE extracts were combined with sodium bicarbonate, shake, centrifuge. The bicarbonate layer is removed, saved and

combined with sodium chloride and sulphuric acid, shake and then add MTBE, shake, centrifuge and repeat with MTBE. The combined MTBE extracts are evaporated to dryness. Samples and standards are then derivatized using a butylation reagent. The samples and standards are then extracted into hexane and analyzed using gas chromatography coupled with a mass selective detector (GC-MSD).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 1: Procedural recovery results of clopyralid (m/z 174) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Onion whole plant	0.1	101	NA	2	Results 98 & 104%
Onion whole plant	0.5	98	NA	2	Results 99 & 97%
Onion whole plant	1.0	96	NA	2	Results 95 & 96%
Onion bulb	0.1	100	NA	2	Results 99 & 101%

Table 2: Characteristics for the analytical method used for determination of residues of clopyralid in onion whole plant and bulb

Analyte	Clopyralid
Matrix	Onion whole plant and bulb
Technique	GC-MSD
Specificity	*m/z* 174 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with no weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0025-0.12 µg/mL (equivalent sample concentration 0.0074 – 0.35 mg/kg)
Limit of quantitation	0.1 mg/kg
Validation Range	0.1 – 1 mg/kg

CONCLUSION

Although not fully validated to meet the requirements set forth in SANTE/2020/12830/Rev.2, the method was considered to be fit for purpose for the determination of clopyralid in onion whole plant and bulb.

A 2.1.1.26 Analytical method 2

Comments of zRMS:	The analytical phase of the study RV98-019 Determination of Residues of Clopyralid in Onions has been performed in compliance with Good Laboratory Practice and not fully validated to meet the requirements of SANTE/2020/12830/Rev.2. However, in our point of view the method can be considered acceptable as confirmatory method.

Reference	KCP 5.2
Report:	Bulter, R.E (1999): Determination of Residues of Clopyralid in Onions, Dow AgroScience Letcombe Laboratory, Wantage, Oxon, UK Lab study no RV98-019
Guideline(s):	Similar to SANCO/3029/99 rev. 4 / ERC-97.20
Deviations:	No
GLP	Yes
Accaptability	Yes

MATERIAL AND METHODS

Method Principle

Residues of clopyralid were extracted with methanol/10 N sodium hydroxide (100/1, v/v) by blending for approximately 1 minute and shaking for 2 hour on a reciprocal shaker. The extracts are allowed to set ambient overnight. An aliquot of the extract is combined with sodium chloride and sulphuric acid and MTBE. The aliquot is then shaken and centrifuged, and the MTBE layer removed and combined with a second extraction using MTBE. The MTBE extracts were combined with sodium bicarbonate, shake, centrifuge. The bicarbonate layer is removed, saved and combined with sodium chloride and sulphuric acid, shake and then add MTBE, shake, centrifuge and repeat with MTBE. The combined MTBE extracts are evaporated to dryness. Samples and standards are then derivatized using a butylation reagent. The samples and standards are then extracted into hexane and analyzed using gas chromatography coupled with a mass selective detector (GC-MSD).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 1: Recovery results from method validation of clopyralid (m/z 174) using the analytical method

Matrix	Fortification level mg/kg	Mean Recovery (%)	RSD (%)	n	Comments
Onion immature plant	0.02	103	1.7	4	
Onion immature plant	0.1	101	NA	2	Results 100 & 102%
Onion immature plant	0.5	99	NA	2	Results 99 & 98%
Onion immature plant	5.0	95	2.3	4	
Onion bulb	0.02	101	2.3	8	

Onion bulb	0.1	97	6.5	4	
Onion bulb	0.5	95	3.8	4	
Onion bulb	5.0	92	3.9	4	
Spring onion	0.02	109	NA	2	Results 116 & 101%
Spring onion	0.1	102	NA	2	Results 103 & 101%
Spring onion	5.0	93	NA	2	Results 93 & 92%

Table 2: Characteristics for the analytical method used for determination of residues of clopyralid in onion immature plant, bulb and spring onion

Analyte	Clopyralid
Matrix	Onion immature plant, bulb, and spring onion
Technique	GC-MSD
Specificity	*m/z* 174 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with no weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.0025-0.12 µg/mL (equivalent sample concentration 0.0074 – 0.35 mg/kg)
Limit of quantitation	0.02 mg/kg
Validation Range	0.02 – 5 mg/kg

CONCLUSION

Although not fully validated to meet the requirements set forth in SANTE/2020/12830/Rev.2, the method was considered to be fit for purpose for the determination of clopyralid in onion immature plant, bulb, and spring onion.

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

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

A 2.1.2.1.1 Extraction efficiency

Reference:	KCP 5.2
Report	Sahvorost, N.; 2020; Extraction Efficiency Assessment of Clopyralid in High Oil Content Plants; Eurofins Agroscience Services EcoChem GmbH, Eutinger Str 24, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S20-00238; DAS Study No. 200353 ; 22 June 2020
Guideline(s):	Yes, SANCO 825/00 Rev. 8.1, SANCO 3029/99 Rev.4
Deviations:	No
GLP:	Yes

Acceptability: Yes

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Clopyralid Technical
Purity:	95.9 (% w/w)
Description (physical state):	solid / white to tan
Lot/batch no.:	TSN100167

Method Scope

Both methods are applicable for the quantitative determination of residues clopyralid in oilseed rape (whole plant, seeds and straw). The methods were validated over the concentration range of 0.01-1.0 mg/kg (for seeds and straw) or 10 mg/kg (for whole plant) with a validated limit of quantitation of 0.01 mg/kg for all matrix types.

Method Principle

a) DAS study 120610

Residues of clopyralid are extracted from crop samples with methanol/10N sodium hydroxide (100/1, v/v) by blending for approximately of 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to set ambient overnight. An aliquot of the extract is submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N sodium hydroxide. The clean-up for crops is affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract is acidified with hydrochloric acid (HCl) and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with DCM. After evaporation of the DCM using nitrogen blow down, the sample is reconstituted in methanol/0.1% formic acid (10/90, v/v). The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

b) GHE-P-9938

Residues of clopyralid are extracted from crop samples with acetonitrile/water (1/1, v/v) and methanol/0.125N sodium hydroxide by blending several times for approximately of 5 minutes. An aliquot of the extract is submitted to a nitrogen stream to remove the organic solvent and then brought back to volume with water. The clean-up for crops is affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract is acidified with hydrochloric acid (HCl) and incubated at ca. 60 °C for 1 h. The samples are brought to room temperature and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with DCM. After evaporation of the DCM using nitrogen blow down, the samples were reconstituted in methanol/0.1% formic acid (10/90, v/v). The final extract is filtered through a 0.2 µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

Linearity

For DAS study 120610, the linearity of the detector response was demonstrated by single determination of solvent/matrix-matched calibration standards at least six (6) concentration levels ranging from 0.6 to 30 ng/mL. This range is equivalent to final concentration of calibration standard from 0.003 to 0.15 mg/kg, and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a (diluted) sample extract.

For GHE-P-9938, the linearity of the detector response was demonstrated by single determination of solvent/matrix-matched calibration standards at least eight (8) concentration levels ranging from 0.75 ng/mL (for seed and whole plant) and 0.82 ng/mL (for straw) to 40 ng/mL. This range is equivalent to final concentration of calibration standard from 0.003 mg/kg to 0.16 mg/kg (for seed and whole plant) and 0.15 mg/kg (for straw), and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a (diluted) sample extract.

The calibration curves obtained for both mass transitions were linear since correlation coefficients (R) were ≥ 0.9996. Linear regression was performed with 1/x weighting.

Selectivity

Table 10: Transitions monitored

Clopyralid	m/z 190/146 (quantitative)
Clopyralid	m/z 192/148 (confirmatory)

RESULTS AND DISCUSSION

Accuracy and Precision

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table* 2*: Procedural Recoveries of Clopyralid, Method Described in Dow AgroSciences Study ID 120610

Clopyralid							
Matrix	**Fortification Level (mg/kg)**	**Individual Recoveries (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition *m/z* 190→146 (Quantification)							
Seed	0.01 (LOQ)	80, 102, 89, 91, 99	92	9.4	5	89	7.7
	1.0	81, 89, 88, 88, 87	87	3.7	5		
Whole Plant	0.01 (LOQ)	(100, 104=102*), 95, 100, 104, 90	98	5.8	5	90	11
	10	79, 78, 81, 80, 86	81	3.9	5		

Clopyralid							
Matrix	**Fortification Level (mg/kg)**	**Individual Recoveries (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Straw	0.01 (LOQ)	79, 72, 78, 72, 81	76	5.4	5	76	5.0
	1.0	72, 73, 76, (81, 80=81*), (72, 72=72*)	75	4.9	5		
Mass Transition *m/z* 192→148 (Confirmation)							
Seed	0.01 (LOQ)	83, 98, 99, 91, 106	95	9.2	5	91	8.6
	1.0	81, 89, 88, 88, 86	86	3.7	5		
Whole Plant	0.01 (LOQ)	(99, 99=99*), 91, 92, 104, 89	95	6.6	5	87	11
	10	77, 76, 79, 79, 83	79	3.4	5		
Straw	0.01 (LOQ)	79, 81, 87, 86, 89	84	5.0	5	80	7.8
	1.0	72, 74, 76, (81, 81=81*), (72, 72=72*)	75	5.0	5		

***: Mean of two injections.**

Table 3: ***Procedural Recoveries of Clopyralid, Method Described in Dow AgroSciences Study ID GHE-P-9938***

Clopyralid							
Matrix	**Fortification Level (mg/kg)**	**Individual Recoveries (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition *m/z* 190→146 (Quantification)							
Seed	0.01 (LOQ)	84, 86, 85, 86, 86	85	1.0	5	84	2.0
	1.0	81, 83, 83, 83, 83	83	1.1	5		
Whole Plant	0.01 (LOQ)	82, 70, 86, 82, 82	80	8.0	5	78	7.0
	10	72, 78, 82, 72, 78	76	5.7	5		
Straw	0.01 (LOQ)	70, 73, 89, 88, 89	82	12	5	81	12
	1.0	84, 84, 85, 85, 85	85	0.6	5		
Mass Transition *m/z* 192→148 (Confirmation)							
Seed	0.01 (LOQ)	82, 78, 79, 86, 84	82	4.1	5	82	2.8
	1.0	82, 82, 82, 82, 83	82	0.5	5		
Whole Plant	0.01 (LOQ)	87, 74, 87, 84, 84	83	6.7	5	80	7.0
	10	72, 78, 82, 73, 78	77	5.4	5		
Straw	0.01 (LOQ)	70, 70, 87, 85, 87	80	11	5	84	9.3
	1.0	85, 85, 84, 83, 85	84	1.1	5		

Extraction Efficiency

Results obtained were describe relation of residue method results to NOR method results. The results obtained are summarised in the following table.

Table 4: Extraction efficiency data for Clopyralid (m/z 190/146)

Matrix	**Residue Analytical Method DAS study 120610**	**NOR Method GHE-P-9938**	**%NOR Findings**	n
	Mean (mg/kg)	**Mean (mg/kg)**	**(%)**	
Seed	0.372	0.343	**108**	3
Whole Plant	8.65	8.04	**108**	3
Straw	1.04	0.988	**105**	3

CONCLUSION

Extraction efficiency is acceptable based on current guidelines: SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as as well as SANTE 2017/10632, rev. 3.

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

A 2.1.2.2.1 Extraction efficiency

Reference:	KCP 5.2
Report	Summary of Assessment of the Extraction Efficiency of the Analytical Method for Determining Residues of Clopyralid in Animal Matrices , Fears, S.L., 2019, 190543
Guideline(s):	Yes, SANCO 825/00 Rev. 8.1, SANCO 3029/99 Rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Clopyralid
Purity:	99.9%
Description (physical state):	Powder
Lot/batch no.:	TSN301194

Test item (Common name):	[14]C-Clopyralid
Purity:	99.9%
Specific Activity:	51.2 mCi/mmol
Lot/batch no.:	INV304597

Method Scope

This method is applicable for the quantitative determination of residues of clopyralid in animal matrices (muscle, fat, kidney, liver and milk). The method was validated over the concentration range of 0.003-1.0 mg/kg with a validated limit of quantitation of 0.01 mg/kg.

Method Principle

Residues of clopyralid are extracted from samples by hydrolyzing with a 2.5 N sodium hydroxide solution. An aliquot of extraction solution was acidified with HCl prior to purification by HLB solid phase extraction. Following elution, samples were concentrated to dryness, reconstituted and filtered prior to analysis. The final sample is analysed for clopyralid by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

Within the nature of residue study, residues of clopyralid are extracted from tissue samples using the Dionex Accelerated Solvent Extractor, model 350, using hexane and acetonitrile/water (80/20, v/v). After the acetonitrile/water extracts were pooled and evaporated and an aliquot is removed, acidified with HCl prior to purification by HLB solid phase extraction. Following elution, samples were concentrated to dryness, reconstituted and filtered prior to analysis. The final sample is analysed for clopyralid by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

Within the nature of residue study, residues of clopyralid were extracted from milk using acetone. Samples were shaken, centrifuged and the supernatant decanted. This extraction was repeated, and the supernatants combined. One aliquot was acidified prior to purification by HLB solid phase extraction, while a second aliquot was hydrolyzed using 5 N NaOH. Following hydrolysis, the samples were acidified and purified using the same HLB solid phase extraction procedure. Following elution, both sets of samples were concentrated to dryness, reconstituted and filtered prior to analysis. The final sample is analysed for clopyralid by liquid chromatography coupled with negative-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 quadratic regression analysis with 1/x weighting. Calibration curves resulting from the injection of nine standards over the concentration range of 0.3-50.0 ng/mL (or the sample equivalent range of 0.003-0.50 mg/kg) with correlation coefficients (r) of at least 0.995.

Selectivity

Table 11: Transitions monitored

Clopyralid	m/z Q1/Q3 190/146 (quantitative)
Clopyralid	m/z Q1/Q3 192/148 (confirmatory)

RESULTS AND DISCUSSION

Accuracy and Precision

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 2: Procedural Recoveries of Clopyralid (m/z 190/146), Method Described in Dow AgroSciences Study ID 120483

Matrix	Fortification Level (mg/kg)	Recovery (%)		SD (σ_{n-1})	RSD (%)	n
		Mean	Range			
Muscle	0.01	84	81-87	2.3	2.7	5
	0.1	84	83-86	1.2	1.5	5
Liver	0.01	88	85-91	2.3	2.6	5
	0.1	88	86-89	1.1	1.3	5
Kidney	0.01	84	82-87	1.9	2.3	5
	1.0	86	85-88	1.3	1.5	5
Fat	0.01	91	89-93	1.5	1.7	5
	0.1	89	87-90	1.3	1.5	5
Milk	0.01	88	85-90	1.8	2.1	5
	0.01	85	83-88	2.2	2.5	5

Table 3: Procedural Recoveries of Clopyralid (m/z 190/146), Method Described in Dow AgroSciences Study ID 130202

Matrix	Fortification Level (mg/kg)	Recovery (%)		SD (σ_{n-1})	RSD (%)	n
		Mean	Range			

Muscle	0.01	89	86-93	2.7	3.0	5
	0.1	87	83-90	2.5	2.9	5
Liver	0.01	82	75-89	5.4	6.7	5
	0.1	88	86-90	1.8	2.0	5
Kidney	0.01	90	87-92	2.1	2.3	5
	1.0	91	88-93	1.9	2.1	5
Fat	0.01	91	80-98	7.7	8.5	4
	0.1	90	86-93	2.9	3.2	5
Milk Pre-Hydrolysis	0.01	103	100-104	1.7	1.6	5
	0.1	99	97-101	1.7	1.7	5
Milk Post Hydrolysis	0.01	89	86-90	1.5	1.7	5
	0.1	86	84-88	1.8	2.1	5

Table 4: Procedural Recoveries of Clopyralid (m/z 192/148), Method Described in Dow AgroSciences Study ID 120483

Matrix	Fortification Level (mg/kg)	Recovery (%)		SD (σ_{n-1})	RSD (%)	n
		Mean	Range			
Muscle	0.01	89	88-91	1.6	1.8	5
	0.1	86	85-88	1.1	1.3	5
Liver	0.01	113	108-116	3.1	2.8	5
	0.1	91	88-95	2.7	3.0	5
Kidney	0.01	89	87-90	1.1	1.2	5
	1.0	85	84-87	1.3	1.5	5
Fat	0.01	89	86-90	1.6	1.9	5
	0.1	86	84-87	1.3	1.5	5
Milk	0.01	87	84-89	1.8	2.1	5
	0.1	85	83-87	1.6	1.9	5

Table 5: Procedural Recoveries of Clopyralid (m/z 192/148), Method Described in Dow AgroSciences Study ID 130202

Matrix	Fortification Level (mg/kg)	Recovery (%)		SD (σ_{n-1})	RSD (%)	n
		Mean	Range			

Muscle	0.01	90	88-92	1.9	2.1	5
	0.1	87	85-90	2.0	2.3	5
Liver	0.01	77	72-83	5.1	6.6	5
	0.1	84	82-86	1.8	2.2	5
Kidney	0.01	86	85-89	1.9	2.3	5
	1.0	90	87-92	1.9	2.1	5
Fat	0.01	92	81-96	7.0	7.7	4
	0.1	91	89-93	1.8	2.0	5
Milk Pre-Hydrolysis	0.01	103	101-105	1.4	1.4	5
	0.1	98	95-100	2.2	2.2	5
Milk Post Hydrolysis	0.01	84	83-85	1.0	1.2	5
	0.1	85	83-87	1.6	1.9	5

Extraction Efficiency

Results obtained were comparable between the analytical and NOR extraction methods. The results obtained are summarised in the following tables.

Table 6: Extraction efficiency data for clopyralid (m/z 190/146)

Matrix	Residue Analytical Method	NOR Method	%NOR Findings	n
	mean (mg/kg)	mean (mg/kg)	(%)	
Muscle	0.03	0.03	100	3
Fat	0.06	0.06	100	3
Kidney	0.77	0.78	99	3
Liver	0.06	0.05	120	3
Milk	0.003	0.003[1]	100	3

[1] NOR extraction method only resulted in 0.001 mg/kg, a hydrolysis step was added after the extraction to hydrolyze known clopyralid-conjugates to clopyralid

Table 7: Extraction efficiency data for clopyralid (m/z 192/148)

Matrix	Residue Analytical Method	NOR Method	%NOR Findings	n
	mean (mg/kg)	mean (mg/kg)	(%)	
Muscle	0.03	0.03	100	3
Fat	0.06	0.06	100	3
Kidney	0.78	0.81	96	3
Liver	0.07	0.05	140	3
Milk	0.003	0.003[1]	100	3

[1] NOR extraction method only resulted in 0.001 mg/kg, a hydrolysis step was added after the extraction to hydrolyze known clopyralid-conjugates to clopyralid.

CONCLUSION

Extraction efficiency is acceptable based on current guidelines: SANCO/3029/99 rev.4, SANCO/825/00 rev.8.1 and EPA Residue Chemistry Test Guidelines OPPTS 860.1340.

Incurred residues where only available for muscle, kidney, fat, liver and milk matrices. The extraction efficiency was completed for all the matrices that were available. Eggs is the only matrix that was not evaluated. The NOR for poultry determined the clopyralid residue to be less than the LOQ (0.01 mg/kg) in the daily analysis. We deemed it unnecessary to sacrifice additional animals to evaluate the extraction in eggs.

A 2.1.2.2.2 Analytical method 1

Comments of zRMS:	The analytical method of the study 180869 is acceptable and suitable for the determination of clopyralid in pollen, nectar and honey. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were at 0.0003 mg/kg and 0.001 mg/kg, respectively.

CITATION

Forbes, T; 2018; Validation of an Analytical Method for the Determination of Clopyralid in Pollinator Matrices; CEM Analytical Services Ltd (CEMAS), Wokingham, Berkshire, RG41 2FD, UK; Lab Study No. CEMS-8336; DAS Study No. 171332 ; 17 April 2018; Unpublished

COMPLIANCE

Guideline(s):	SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1 PMRA Regulatory Directive Dir98-02
US EPA Guideline(s):	OPPTS 860.1340
Deviations:	None
Dates of work:	01 February 2018 to 16 March 2018
GLP status:	Yes
Number of pages in final report:	76

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Clopyralid
Purity:	99.9 (% w/w)
Description (physical state):	White solid
Lot/batch no.:	YC2-106153-68 (TSN301194)

Method Scope

This method is applicable for the quantitative determination of residues of clopyralid in pollinator matrices. The method was validated over the concentration range of 0.001-10 mg/kg with a validated limit of quantitation of 0.001 mg/kg.

Method Principle

Clopyralid is extracted from the pollinator matrix by shaking with a mixture of methanol containing 10 N sodium hydroxide. The extracts are left overnight at room temperature.

Extracts are neutralised with 10 N sulfuric acid before a clean-up step on Oasis WAX (6 mL, 120 mg). The eluates are concentrated to near dryness before a propylation reagent is added. The samples are then incubated for at least 30 minutes at 105°C. The 1-propanol is then evaporated off and sodium chloride and hexane are added along with the clopyralid butyl ester internal standard at a concentration of 2 ng/mL.

The samples are concentrated further before analysis by gas chromatography with negative ion electrospray ionization mass spectrometry (GC/NCI-MS).

Linearity

The linearity of detector response was evaluated using matrix-matched standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of seven standards over the concentration range of 0.3-30 ng/mL (or

the sample equivalent range of 0.0003-0.03 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.9994.

Selectivity

The method is selective for the determination of clopyralid by virtue of the chromatographic separation and MS detection. 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 the observation of a precursor ion plus a structurally significant product ion observed at the same retention time.

Table 12: Transitions monitored

Clopyralid-propyl ester	m/z 233.0 (quantitative)
Clopyralid-propyl ester	m/z 235.0 (confirmatory)
Clopyralid-propyl ester	m/z 175.0 (confirmatory)
Clopyralid-butyl ester (internal standard)	m/z 247.0 (internal standard)

Confirmation

Confirmation of the presence of clopyralid was by comparison of retention times (gas chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring three structurally characteristic MS transitions for clopyralid by mass spectrometry. Validation data obtained using the confirmatory MS transitions met the same acceptance criteria as the validation data generated using the quantitative MS transition, therefore demonstrating that the analyte signal of the quantitative 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 0.001 mg/kg for clopyralid in all tested matrices. The limit of detection, defined as three times the standard deviation of the lowest fortified recovery, is summarised below:

Table 13: Limits of detection

Analyte	Matrix	Limit of detection	Limit of quantitation
Clopyralid Quantitation transition (m/z 233.0)	Nectar	0.00009 mg/kg	0.001 mg/kg
	Honey	0.00005 mg/kg	0.001 mg/kg
	Pollen	0.00009 mg/kg	0.001 mg/kg
Clopyralid Confirmatory transition (m/z 235.0)	Nectar	0.00006 mg/kg	0.001 mg/kg
	Honey	0.00003 mg/kg	0.001 mg/kg
	Pollen	0.00011 mg/kg	0.001 mg/kg
Clopyralid Confirmatory transition (m/z 175.0)	Nectar	0.00009 mg/kg	0.001 mg/kg
	Honey	0.00007 mg/kg	0.001 mg/kg
	Pollen	0.00013 mg/kg	0.001 mg/kg

RESULTS AND DISCUSSION

Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

Table 14: Summary of quantitative recovery of clopyralid (m/z 233.0)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Pollinator	Nectar	0.001	88	83 - 90	2.84	3.21	5
Pollinator	Nectar	0.01	88	84 - 90	2.36	2.68	5
Pollinator	Nectar	0.5	92	89 - 93	1.54	1.69	5
Pollinator	Nectar	10	89	88 - 91	1.15	1.29	5
Pollinator	Honey	0.001	91	90 - 94	1.60	1.76	5
Pollinator	Honey	0.01	90	84 - 93	3.33	3.72	5
Pollinator	Honey	0.5	91	89 - 93	2.30	2.52	5
Pollinator	Honey	10	91	91 - 92	0.44	0.48	5
Pollinator	Honey	0.001	83	79 - 87	3.04	3.68	5
Pollinator	Pollen	0.01	80	77 - 85	3.68	4.60	5
Pollinator	Pollen	0.5	80	73 - 86	4.77	5.94	5
Pollinator	Pollen	10	80	78 - 81	1.24	1.56	5

Table 15: Summary of confirmatory recovery of clopyralid (m/z 235.0)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Pollinator	Nectar	0.001	90	87 - 93	2.15	2.38	5
Pollinator	Nectar	0.01	89	85 - 91	2.23	2.51	5
Pollinator	Nectar	0.5	92	90 - 93	1.44	1.57	5
Pollinator	Nectar	10	90	88 - 91	1.19	1.32	5
Pollinator	Honey	0.001	92	91 - 93	1.13	1.22	5
Pollinator	Honey	0.01	90	85 - 94	3.35	3.72	5
Pollinator	Honey	0.5	93	91 - 94	1.24	1.34	5
Pollinator	Honey	10	92	91 - 93	0.58	0.63	5
Pollinator	Honey	0.001	81	77 - 86	3.81	4.71	5
Pollinator	Pollen	0.01	80	77 - 85	3.72	4.64	5
Pollinator	Pollen	0.5	80	72 - 85	4.82	6.02	5
Pollinator	Pollen	10	79	78 - 81	1.25	1.57	5

Table 16: Summary of confirmatory recovery of clopyralid (m/z 175.0)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Pollinator	Nectar	0.001	89	84 - 91	2.87	3.23	5
Pollinator	Nectar	0.01	88	85 - 90	2.02	2.29	5
Pollinator	Nectar	0.5	92	90 - 93	1.47	1.60	5
Pollinator	Nectar	10	89	88 - 90	0.81	0.90	5
Pollinator	Honey	0.001	94	92 - 96	2.28	2.42	5
Pollinator	Honey	0.01	90	84 - 93	3.39	3.78	5
Pollinator	Honey	0.5	92	90 - 93	1.40	1.53	5
Pollinator	Honey	10	91	91 - 92	0.38	0.42	5
Pollinator	Honey	0.001	83	79 - 90	4.44	5.35	5
Pollinator	Pollen	0.01	81	77 - 86	3.79	4.68	5
Pollinator	Pollen	0.5	83	76 - 89	4.73	5.72	5
Pollinator	Pollen	10	81	78 - 83	1.65	2.04	5

Repeatability

Not applicable

Working Solution Stability

The stability of working solutions (fortification and calibration standards) was evaluated by comparing the peak area ratio of stored working solutions with a freshly prepared working solution from a new stock at the same level. The following levels were tested:

5 ng/mL clopyralid propyl ester
1.5 ng/mL clopyralid propyl ester
10 ng/mL clopyralid butyl ester (internal standard)
2 ng/mL clopyralid butyl ester (internal standard)

Clopyralid propyl ester was proven stable at 1.5 and 5 ng/mL for 10 days when stored at approximately 4°C in hexane.

Clopyralid butyl ester was proven stable at 2 and 10 ng/mL for 10 days when stored at approximately 4°C in hexane.

A clopyralid solution stored in methanol was used to prepare a fresh 5 ng/mL clopyralid propyl ester solution. This was compared to a clopyralid propyl ester solution made from a freshly prepared clopyralid solution at the same concentration. Clopyralid was proven stable at 100 µg/mL for 62 days when stored at approximately 4°C in methanol.

Sample Extract Stability

Final sample extracts containing clopyralid were tested after at least 7 days of storage at 4ºC and were determined to be stable.

Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing (for each matrix type) to the response of the analyte fortified in neat solvent. The results for clopyralid demonstrate that matrix effects are below 20% for nectar and honey and are above 20% for pollen (based on peak area ratio). Matrix effects are therefore considered to be significant for pollen but not significant for nectar and honey, according to SANCO guidelines.

Extraction Efficiency

The extraction conditions are the same as those used in NOR studies. Extraction efficiency was not determined in this study.

CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guideline OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

A 2.1.2.2.2.1 Independent laboratory validation

Comments of zRMS:	The analytical method of the study 180869 is acceptable and suitable for the determination of clopyralid in pollen, nectar and honey. The study has been performed in compliance with Good Laboratory Practice and SANCO/3029/99 rev. 4. The limits of detection (LOD) and quantitation (LOQ) were at 0.0003 mg/kg and 0.001 mg/kg, respectively.

CITATION

T. Forbes, April 17, 2018 “Validation of an Analytical Method for Determination of Clopyralid in Pollinator Matrices”

Dow AgroSciences Study ID: 171332

Paul Bendig, Agnieszka Przybylek; 2019; Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Clopyralid in Honey Matrix; EAG Laboratories GmbH, Eiselauer Weg 4, Geb./Bldg. 5, D-89081 Ulm, Germany; Lab Study No. P 5220 G; DAS Study No. 180870 ; 12 April 2019; Unpublished

COMPLIANCE

Guideline(s):	SANCO/825/00 rev.8.1
US EPA Guideline(s):	OPPTS 860.1340
Deviations:	None
Dates of work:	05 July 2018 to 21 February 2019
GLP status:	Yes
Number of pages in final report:	151

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Clopyralid
Purity:	99.9%
Description (physical state):	White solid
Lot/batch no.:	YC2-106153-68

Method Scope

An independent laboratory validation (ILV) was successfully performed in a study at the test facility (EAG Laboratories ID P 4932 G) for honey and pollen. Due to the long storage period of honey extracts the ILV for honey was repeated within the current study (EAG Laboratories ID P 5220 G). The analytical method was developed and validated within Dow AgroSciences Study ID 171332, CEMS-8336.

This method is applicable for the quantitative determination of residues of clopyralid independently validated in pollinator matrices (honey and pollen).

The method was validated over the concentration range of 0.001 – 10 mg/kg with a validated limit of quantitation of 0.001 mg/kg.

Method Principle

Clopyralid is extracted from the pollinator matrices by shaking with a mixture of methanol containing 10 N sodium hydroxide. The extracts are then left overnight at room temperature.

Extracts are neutralized with 10 N sulfuric acid before a clean-up step on Oasis WAX cartridge. The eluates are concentrated to dryness before a propylation reagent is added. The samples are then incubated for at least 30 minutes at 105°C. The 1-propanol is then evaporated off and sodium chloride and hexane are added along with the clopyralid butyl ester internal standard at a concentration of 2.0 ng/mL. Additionally the higher level fortification (100xLOQ and 10000xLOQ) extracts were diluted (10-fold and 50-fold) using the clopyralid butyl ester internal standard solution.

The samples are concentrated further before analysis by gas chromatography with negative chemical ionization mass spectrometry (GC/NCI-MS).

L**inearity**

Linear regression analysis with 1/x weighting was used to describe the detection response as a function of the calibration standard concentration. The correlation coefficient (r) and coefficient of determination (R^2) were greater than or equal to 0.98, respectively, for all of the calibration curve determinations during the independent laboratory method validation. The resulting indicate linearity of the detector response as a function of the standard concentration.

For each analyte, the linearity of detector response was evaluated using internal calibration solutions in solvent (for honey and high level fortifications in pollen matrix) or in matrix (for evaluation of pollen samples fortified with clopyralid at 0.001 mg/kg level). Calibration functions using linear regression with 1/x weighting (performed by the Xcalibur software) at ≥ 5 different concentrations ranging from 0.0003 to 0.30 mg/kg or 0.10 mg/kg (only for pollen at LOQ level) were used to evaluate the extracts.

Selectivity

GC-NCI/MS with three fragment ions is considered to be a highly specific detection technique and therefore, according to EU guidance (***SANCO/825/00 rev.8.1, 16/11/2010***), no further confirmatory technique is required. Significant peak response (>20% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention time of the analyte. Unambiguous identification is ensured by monitoring tree fragment ions characteristic of each analyte as follows in the table below.

Table 17 Transitions monitored

Clopyralid-propyl ester	*m/z* 233Q (quantitative)
Clopyralid-propyl ester	*m/z* 235C (confirmatory)
Clopyralid-propyl ester	*m/z* 175C (confirmatory)
Clopyralid-butyl ester	*m/z* 247I (internal standard)

Confirmation

The method is selective for the determination of clopyralid by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, two additional ions were monitored.

There were no analytes or other components present in the control or reagent blank that interfered with the analysis at levels above 30 % of the limit of quantification.

Validation data obtained using the confirmatory GC-NCI/MS fragment ions met the same acceptance criteria as the validation data generated using the quantitative GC-NCI/MS fragment ion, therefore demonstrating that the analyte signal of the quantitative GC-NCI/MS fragment ion 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.

The limits of detection (LOD) and quantitation (LOQ) were proposed at the initiation of the study at 0.0003 mg/kg and 0.0010 mg/kg, respectively.

A chromatographic peak was detected in the sample fortified at the LOD. The peak at the LOD could be distinguished from an unfortified control sample.

RESULTS AND DISCUSSION

Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). For each analyte, the three fragment ions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables

Table 2: Summary of quantitative recovery of Clopyralid (m/z 233)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Honey	Honey	0.001*	79	72 – 90	8	10	5
Honey	Honey	0.1	93	89 – 99	5	5	5
Honey	Honey	10	101	97 – 103	3	3	5
Pollen	Pollen	0.001*	74	71 – 76	2	3	5
Pollen	Pollen	0.1	97	86 – 107	8	9	5
Pollen	Pollen	10	81	76 – 86	4	5	5

*Limit of quantitation, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 20% of the LOQ.

Table 3: Summary of confirmatory recovery of Clopyralid (m/z 235)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Honey	Honey	0.001*	78	74 – 86	6	8	5
Honey	Honey	0.1	96	93 – 99	2	2	5
Honey	Honey	10	101	97 – 104	3	3	5
Pollen	Pollen	0.001*	74	70 – 78	3	4	5
Pollen	Pollen	0.1	96	85 – 107	9	9	5
Pollen	Pollen	10	81	76 – 85	5	4	5

*Limit of quantitation, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 20% of the LOQ.

Table 4: Summary of confirmatory recovery of 3Clopyralid (TCP) (m/z 175)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Honey	Honey	0.001*	76	71 – 85	6	7	5
Honey	Honey	0.1	90	83 – 96	5	5	5
Honey	Honey	10	100	95 – 104	3	3	5
Pollen	Pollen	0.001*	73	71 – 76	2	3	5
Pollen	Pollen	0.1	94	84 – 101	7	7	5
Pollen	Pollen	10	79	74 – 83	4	5	5

*Limit of quantitation, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 20% of the LOQ

Repeatability

Repeatability was not assessed as a part of this study.

Working Solution Stability

The stability of clopyralid in standard solutions was investigated within the original method validation of Dow AgroSciences (DAS) ID No 171332.

Results showed that the working solutions are stable:

clopyralid propyl ester prepared in hexane for at least 10 days,

clopyralid butyl ester (internal standard) prepared in hexane for at least 10 days,

clopyralid solution prepared in methanol for at least 62 days,

final extracts at least 7 days,

when stored refrigerated (approximately 2 to 8 ^{o}C) when not in use.

Final extracts of clopyralid in honey were analyzed within the periods for which stability was demonstrated in the original method validation (stability was demonstrated after 8 days of refrigerated storage).

Extracts of clopyralid in pollen were analyzed within the periods for which stability was demonstrated in the original method validation (stability was demonstrated after 7 days of refrigerated storage). The actual storage in this ILV was up to 2 days.

Matrix Effects

No significant matrix effects were observed in pollinator matrices used for this study, except for pollen at LOQ level. The final determination for Clopyralid were quantified using internal calibration solutions in solvent (for honey and high level fortifications in pollen matrix) or in matrix (for evaluation of pollen samples fortified with clopyralid at 0.001 mg/kg level).

Extraction Efficiency

In the current study, extraction efficiency was not evaluated experimentally because the radio-labeled samples with incurred residues were not available.

Changes to Method

No major modifications to the analytical method DAS Study ID: 171332, dated 17-Apr-2018 were necessary during ILV.

The procedures were adapted to the laboratory equipment available. One minor change was that instead of a TurboVap a heating block equipped with nitrogen for blowdown were used.

Conditions of the GC/MS system were adapted to the local laboratory equipment. As indicated in the original method validation, the instrumental conditions were modified to obtain optimal chro-matographic separation and sensitivity. These minor modifications are not expected to have an impact on the outcome of the study.

The changes to the GC/MS method were as follows:

Parameter	Original Method Validation	This Study (ILV)
Injection Volume	3 µL	5 µL
Oven Temperature Program:	100 °C - 1 min 15 °C/min - 200 °C - 1 min 15 °C /min - 260 °C - 0 min 40 °C /min - 310 °C - 3 min	100 °C - 1 min 20 °C/min - 280 °C - 6 min
Transfer Line Temperature	300 °C	250 °C
Methane Flow (CI Gas)	40 %	1.5 mL/min
Quadrupole Temperature	106 °C	Not to be set
Source Temperature	150 °C	180 °C
Solvent Delay	10 min	11 min
Dwell time (Sim Ion [m/z] – time [msec]	m/z 233 – 50 msec m/z 235 – 50 msec m/z 175 – 80 msec m/z 247 – 100 msec	m/z 233 – 50 msec m/z 235 – 50 msec m/z 175 – 50 msec m/z 247 – 50 msec

CONCLUSIONS

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340 and the requirements of SANCO/825/00 rev.8.1.

The analytical method for the determination of clopyralid in pollinator matrices (honey and pollen) has been demonstrated to be satisfactory in terms of accuracy, precision, linearity, and specificity. The method was independently validated over the concentration range of 0.0010 – 10 mg/kg with a limit of quantitation of 0.0010 mg/kg. For the ILV per matrix one ILV set was necessary.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted