FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: **CHR/H/CPD 300SL**

Product name(s):

Major 300SL, Cloe 300SL, ProSto 300SL

Chemical active substance:

Clopyralid 300g/l

Central zone

Zonal Rapporteur Member State: Poland

Core assessment

(renewal of authorization)

Applicant: Innvigo sp. z o.o.

Submission date: 12.2021; 11.2022; 03.2023, 02.2024 03.2024

Version history

When	What
December 2021	New data for CHR/H/CPD based on the renewal of active substance - clopyralid. New data is highlighted in yellow.
November 2022	ZRMs evaluated submitted by Applicant dRR
March 2023	The final Registration Report
February 2024	Applicant's update
March 2024	Assessment of updated Part B5

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5 Analytical methods Conclusion and summary of assessment

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.

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

- none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Minor data gap: extraction efficiency (for plant and animal matrices). Not provided during the EU review. Will be required at re-evaluation of the product.

Commodity/crop	Supported/ Not supported
Winter wheat	Supported
Winter rape	Supported
Sugar beet	Supported

NOTE: the new alternative studies have not been assessed in this application.

March 2024 Assessment of the new data (marked in green).

The applicant provided the new study. The dark blue background of the new data provided by the applicant has been changed by zRMS to a lighter one to increase the readability of the text. The study is acceptable and is fit to purposes (pre-authorization data).

The provided studies do not change the conclusions of the evaluation.

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

5.1.1 Analysis of the plant protection product (KCP 5.1.1)

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

The study has already been evaluated during the registration process of CHR/H/CPD 300 SL.

Report:	KIIIA1 5.2.1/01, Łącka E. 2014
Title:	CLOPYRALID 300SL (CHR/H/CPD) Validation of a method for determination of the active substance content.
Document No:	BA-11/14 Institute of Industrial Organic Chemistry, Analytical Department, 6 Annopol Str., 03-236 Warsaw, Poland
Guidelines:	SANCO/3030/99 rev. 4 (11/07/00)
GLP	Yes

Method

The following analytical method for the determination of the active substance in the plant protection product performed on CHR/H/CPD has not previously been reviewed and is provided in support of the current assessment.

A method for determination of clopyralid in CHR/H/CPD was developed. The method was based on reversed phase HPLC with UV-DAD detection. It was confirmed, that the method was specific. No interference was observed between additives and the active substance clopyralid.

The content of active ingredient determined in CHR/H/CPD 26.45 ± 0.53 % (m/m).

Validation

The following validation of the analytical method for the determination of the active substance in the plant protection product, performed on standard of clopyralid (CLOPYRALID IPO 096, batch No 3C/09, purity 99.6%±0.1%). Examined sample was preparation CHR/H/CPD, has not previously been reviewed and is provided in support of the current assessment.

Summary

Linearity	The linearity of the analytical method was assessed using seven clopyralid standard solutions in the concentration range from 0.3990 mg/ml to 0.9974 mg/ml; Correlation coefficient: $R^2 = 0.9989$ Regression equation: y = 23 137 243 x + 1 506 457 Chromatograms are given; the functions are linear in the operating range.
Precision	Repeatability The repeatability of the method was assessed on the basis of five independent determinations of active substance content in CHR/H/CPD preparation.; Relative standard deviation (RSD): 1.61 % %RSDr (modified Horwitz): 1.64 % Horrat value, Hr = 0.98, acceptable
Accuracy	12 (2x6) samples -2 levels of fortification: Recovery 99.4 %, RSD 0.5 % The method shows no constant or proportional systematic error. The mean recovery is within limits (97-103%) which is acceptable.
Specificity	The UV-spectra of active ingredient and reference substance showed no peaks interfering with the examined peak of active ingredient.
Interference by other substances	Chromatograms of standards, blank formulations and samples were checked; no interferences were found.

Conclusion:

The analytical method meets the specificity, linearity, precision/repeatability and accuracy criteria specified in SANCO/3030/99 rev. 4 (11/07/00). It fulfils also the requirements of SANCO/3030/99 rev. 5.

5.1.1.2 Description of analytical methods for the determination of relevant

impurities (KCP 5.1.1)

There are no relevant impurities in clopyralid therefore no analytical methods are required.

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

With respect to toxicological, eco-toxicological or environmental aspects the product CHR/H/CPD does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.1.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

CIPAC method Number 455 is available for clopyralid, but not used for the determination of clopyralid in CHR/H/CPD (SL formulation).

5.1.2 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.

New information of analytical methods for the determination of residues in sugar beet, wheat and oilseed rape in CH/H/CPD was added.

Reference:	KCP 5.2.1
Report	*Validation of the Analytical Method for the Analysis of Clopyralid (Sum of Clopyralid, its salts and conjugates) in High water content, in High Oil content and Dry Commodities*, P. Schlewitz, 2023, Report No: C2135
Guideline(s):	Regulation (EC) No. 1107/2009 SANTE/2020/12830, Rev.1
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The objective of the study was to validate the analytical method for the analysis of clopyralid (sum of clopyralid, its salts and conjugates) in high water content (wheat whole plant, oilseed rape whole plant, sugar beets whole plant, leaves and roots), in high oil content (oilseed rape seeds) and dry commodities (wheat grain and straw).
A full validation was performed on wheat whole plant (high water content), on oilseed rape seeds (high oil content) and on wheat grain (dry commodities).
The applicability of the method on oilseed rape whole plant, sugar beets whole plant, leaves and roots (high water content) and on wheat straw (dry commodities) was demonstrated by concurrent recoveries (3 recoveries at LOQ and 3 recoveries at a higher level).

ANALYTICAL METHOD
Samples were analysed using a method developed by ANADIAG.

Outline of ANADIAG method:
Residues of clopyralid and its conjugates are extracted and hydrolysed from samples by heating at 60 °C for 3 hours with 2.5M KOH. After acidification with H2SO4, addition of acetonitrile, magnesium sulfate and sodium chloride, the raw extract is purified with a liquid-liquid partition. An aliquot of the upper layer is evaporated to dryness and the sample is reconstituted in 50:50, methanol/H2O + 0.1% formic acid. Extracts are analysed by LC-MS/MS.
ANADIAG References (French version) of the method are
for the preparation and extraction of the samples: SOP MP 718
for the analysis of extracts and for the calibration: SOP MA 1809

SUMMARY
The method under discussion describes the determination of clopyralid and clopyralid glycine in high water content, in high oil content and in dry commodities.
The method was validated at 0.01 mg/kg for each commodities and each analyte.

The analytical method was validated according to SANTE/2020/12830, Rev.1.
The following points were examined during the study:

Matrix effects
Matrix effects, expressed in % enhancement or suppression, were assessed for each commodities and analyte, for both primary and confirmatory methods. They were considered significant if they exceeded ±20%.
Matrix effects (enhancement or suppression) on the instrument response were considered significant for most of commodities/analyte combination. Consequently, matrix-matched calibration solutions were used for calibration.

Calibration
The analytical calibration consisted of matrix-matched calibration solutions of clopyralid, at least at 5 concentration levels, ranged from 0.4 ng/mL to 24.5 ng/mL (corresponding to 0.002 to 0.12 mg/kg for clopyralid and to 0.003 to 0.16 mg/kg for clopyralid glycine).
The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods.

Limit of detection
The limit of detection (LOD) was expressed as lowest calibration standard.
The LOD was 0.4 ng/mL for clopyralid in high water content, high oil content and in dry commodities (corresponding to 0.002 mg/kg for clopyralid and to 0.003 mg/kg for clopyralid glycine).

Limit of quantification
The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision.
The LOQ was 0.01 mg/kg for clopyralid and clopyralid glycine in high water content, in high oil content and in dry commodities.

Recovery and repeatability
For samples fortified at 0.01 mg/kg, mean recoveries were within the acceptable range 60-120% with RSD less than 30% for both primary and confirmatory methods.
For samples fortified at 0.10 mg/kg, mean recoveries were within the acceptable range 70-120% with RSD less than 20% for primary method.

Selectivity and specificity
Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte/matrix combination and for both primary and confirmatory methods were provided to prove selectivity of the method.
Mass spectra were provided to justify the selection of ions used for determination.
Untreated samples (non-fortified samples) were determined from the matrices used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods.

Confirmation
The confirmatory method was required to confirm that the primary method detected the correct analyte (analyte identity) and that the analyte signal of the primary method was quantitatively correct and not affected by any other compound.
Confirmation simultaneously to primary detection:
The confirmatory method was achieved by monitoring 1 additional transition for high water content and for dry commodities.

	Primary transition	Confirmatory transition
Clopyralid	m/z 192.0 > 110.0	m/z 192.0 > 84.0

For high oil content, the confirmatory method was achieved by analysing 2 untreated samples and 5 fortified samples at the LOQ level with an alternative analytical column.

Stability results for extracts
The stability of extracts during refrigerated storage was investigated.
Clopyralid residues were stable in extracts for at least 15 days of refrigerated storage for high water content and dry commodities and 19 days of refrigerated storage for high oil content.

Stability results for matrix-matched standard solutions
The stability of matrix-matched standard solutions during refrigerated storage was investigated.
Clopyralid residues were stable in matrix-matched calibration solutions for at least 14 days of refrigerated storage for high water content and dry commodities and 19 days of refrigerated storage for high oil content.

For details please see Appendix 2 – A 2.1.1.

Table 5.1-1: Validated methods for the generation of pre-authorization data

Component of residue definition: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar 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	Primary	0.01 mg/kg	LC–MS/MS	EFSA Journal 2018;16(7):5389
	Primary	0.01 mg/kg	LC–MS/MS	P. Schlewitz, 2023, C2135
	Confirmatory (if required)	N/A	N/A	N/A
Component of residue definition: Clopyralid and its salts				
Animal products, food of animal origin	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Component of residue definition: Clopyralid				
Surface water Drinking water	Primary	0.05 µg/L	LC -MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Soil	Primary	0.5 µg/kg	LC -MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Air	Primary	4.5 µg/m³	LC-MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Body fluids and tissues	Primary	0.05 mg/L (urine, blood)	LC-MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A

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

5.2.1 Analysis of the plant protection product (KCP 5.2)

For active substance Clopyralid all presented methods are sufficient and no new methods are necessary. Please refer to KCP 5.1.2

5.2.2 Description of analytical methods for the determination of residues

For active substance Clopyralid all presented methods are sufficient and no new methods are necessary.

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

Table 5.2-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Component of residue definition: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat forage (wet crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Lettuce (wet crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Wheat grain (dry crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012; RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Rye grain (dry crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*

	Confirmatory (if required)	N/A		
Orange (acidic crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012; RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Lemon (acidic crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013),RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Canola seed (oily crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012; RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Oilseed Rape Seed((oily crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013), RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Component of residue definition: Clopyralid and its salts				
Milk	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*EFSA Journal 2018;16(7):5389 120483(2012), 120484(2012),; 130729(2013)Volume3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		
Eggs	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 (2012)120483; 120484; 130729(2013)RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		

Muscle	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 120483(2012); 120484(2013); 130729(2013)RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		
Liver	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 (2012)120483; 130729(2013) RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A	N/A	N/A
Fat	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*EFSA Journal 2018;16(7):5389 (2012)120483; 130729(2013) RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		
Component of residue definition: Clopyralid and its salts				
Surface water Drinking water	Primary	0.05 µg/L	LC -MS/MS	*EFSA Journal 2018;16(7):5389 Shaffer, S. (2012); RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2019, S19-00449*
	Confirmatory (if required)	N/A		
Soil	Primary	0.5 µg/kg	LC -MS/MS	*EFSA Journal 2018;16(7):5389 Vincent, T.P. (2013); RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2019, S19-00448*
	Confirmatory (if required)	N/A		
Air	Primary	4.5 µg/m³	LC-MS/MS	*EFSA Journal 2018;16(7):5389 Bacher, R. (2012);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Kirchherr, 2019, S19-00451*
	Confirmatory (if required)	N/A		

Body fluids and tissues	Primary	0.05mg/L (urine, blood)	LC-MS/MS	*EFSA Journal 2018;16(7):5389 130727(2014);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00450*
	Confirmatory (if required)	N/A		

5.2.2.2 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 and its metabolites in plant matrices is given in the following tables.

Table 5.2-2: 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 common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar 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 wet crop	Primary	Wheat forage 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,2020, S19-00446(tomato)*
		Lettuce 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR, Volume 3, Annex B-Clopyralid which is equivalent to Knop M.,(2020), S19-00446(tomato)*
	ILV	Lettuce 0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014) RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richer S., (2020), S19-00438(tomato)*
		Wheat Whole Plant	LC-MS/MS	*Austin, R. (2012), RAR, Volume 3, Annex B-Clopyralid Clopyralid to which is equivalent to Richer S., (2020), S19-00438(tomato)*
	Confirmatory (if required)	N/A		
High protein/high starch content (dry)	Primary	Wheat grain 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(rice)*
		Rye grain 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR, Volume 3, Annex B-Clopyralid which is equivalent to Knop M.,(2020), S19-00446 (rice)*

Component of residue definition: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	ILV	0.01 mg/kg	LC-MS/MS	*Austin, R. (2012), RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richer S.,(2020), S19-00438 (rice)*
	Confirmatory (if required)	N/A		
(High oil content) Oily crop	Primary	Canola seed 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(olive)*
	ILV	Oilseed Rape Seed 0.01 mg/kg	LC-MS/MS	*Austin, R. (2012), RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richer S., (2020), S19-00438(olive)*
	Confirmatory (if required)	N/A		
(High acid content) acid crop	Primary	Orange 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(grape)*
		Lemon 0.01 mg/kg	LC-MS/MS	*130729(2013),RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(grape)*
	ILV	Lemon 0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014) RAR, Volume 3, Annex B-Clopyralid*
	Confirmatory (if required)	N/A		

Table 5.2-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	RAR clopyralid Vol. 3 Section B.5
Not required, because:	Extraction efficiency requirement was described in the guideline SANTE 2017/10632 rev. 3, which was noted in November 2017, thus it is applicable only to submissions after 11.2019. EFSA for clopyralid was published in 2018, thus before the guideline enter into force.

5.2.2.3 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.

Table 5.2-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Clopyralid and its salts				
Milk	Primary	Bovine milk 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013),RAR,Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	Bovine milk 0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014); RAR,Volume3,Annex B-Clopyralid*
	Primary	Bovine milk 0.01 mg/kg	LC-MS/MS	*120483(2012),RAR,Volume3, Annex B-Clopyralid to which is equivalent to abe Abe,Ch.(2019) S19-00447*
	ILV	Bovine milk 0.01 mg/kg	LC-MS/MS	*120484(2012) RAR,Volume3, Annex B-Clopyralid*
	Confirmatory (if required)	N/A		
Eggs	Primary	Poultry eggs 0.01 mg/kg	LC-MS/MS	*120483(2012), RAR Volume3, Annex B-Clopyralid Clopyralid to which is equivalent to Abe,Ch.(2019)S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012);), RAR Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019)P 5210 G*
	Primary	Poultry eggs 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013),RAR,Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	Confirmatory (if required)	N/A		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	*120483 (2012); RAR Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019)P 5210 G*

	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019)P 5210 G*
	Confirmatory (if required)	N/A	N/A	*N/A*
Liver, kidney	Primary	0.01 mg/kg	LC-MS/MS	*120483(2012); RAR Volume3, Annex B-Clopyralid o which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012); RAR,Volume3, Annex B-Clopyralid*
	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	N/A		
	Confirmatory (if required)	N/A		
Fat	Primary	0.01 mg/kg	LC-MS/MS	*120483(2012); RAR Volume3, Annex B-Clopyralid o which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019);P 5210 G*
	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013); RAR Volume3, Annex B-Clopyralid o which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019);P 5210 G*
	Confirmatory (if required)	N/A		

Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	RAR clopyralid Vol. 3 Section B.5
Not required, because:	Extraction efficiency requirement was described in the guideline SANTE 2017/10632 rev. 3, which was noted in November 2017, thus it is applicable only to submissions after 11.2019. EFSA for clopyralid was published in 2018, thus before the guideline enters into force.

5.2.2.4 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 and its metabolites in soil is given in the following tables.

Table 5.2-5: Validated methods for soil (if appropriate)

Component of residue definition: Clopyralid				
Soil	Primary	0.5 µg/kg	LC -MS/MS	*Vincent, T.P. (2013); RAR, Volume 3, Annex B-Clopyralid to which is equivalent to* Knop, M.,(2019) *S19-00448*
	ILV	Not required.		
	Confirmatory (if required)	N/A		

5.2.2.5 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.

Table 5.2-6: 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 Surface water Ground water	Primary	0.05 µg/L	LC-MS/MS A validation for drinking water was not necessary because the limit of quantitation for surface water is below the drinking water limit of 0.1 µg/L.	*Shaffer, S. (2012); RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop, M.,(2019); S19-00449*
	ILV	0.05µg/L	LC-MS/MS	*Austin, R., Turner, R. (2013) RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richter, S. (2019); P 5211 G*
	Confirmatory	N/A		

5.2.2.6 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 5.2-7: 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);RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Kirchher, M.(2019); S19-00451*
ILV	N/A		
Confirmatory	N/A		

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

Table 5.2-5: Validated 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	human blood, human urine 0.05 mg/L	LC-MS/MS	*130727 (2014), RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Abe Ch.(2019); S19-00450*
ILV	N/A		
Confirmatory	N/A		

5.2.2.8 Other studies/ information

Not relevant.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Łącka, E.	2014	*CLOPYRALID 300SL (CHR/H/CPD) Validation of a method for determination of the ac-tive substance content.* BA-11/14 Institute of Industrial Organic Chemistry, Analytical Department, 6 Annopol Str., 03-236 Warsaw, Poland GLP-yes unpublished	N	Chemirol
KCP 5.1.2	P. Schlewitz	2023	*Validation of the Analytical Method for the Analysis of Clopyralid (Sum of Clopyralid, its salts and conjugates) in High water content, in High Oil content and Dry Commodities* C2135 ANADIAG, 16, rue Ampère, 67500 HAGUENAU, France GLP-yes unpublished	N	Chemirol

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2		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* 130729 GLP-yes unpublished	N	DAS
KCP 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS

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KCP 5.2		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* 130729 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* 120483 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* 120484 GLP-yes unpublished	N	DAS
KCP 5.2		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* 130729 GLP-yes unpublished	N	DAS

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	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 120611 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2	Vincent, T.P.	2013	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS* 120612 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2	Bacher, R.	2012	*The Development and Validation of a Method for the Analysis of Clopyralid in Air* 120601 PTRL Europe GmbH, D-89081 Ulm, Germany GLP-yes unpublished	N	DAS
KCP 5.2		2014	*Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)* 130727 GLP-yes unpublished	N	DAS
KCP 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	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"* 120614 Battelle UK Ltd, Ongar, Essex, United Kingdom GLP-yes unpublished	N	DAS
KCP 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
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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" 120613 Battelle UK Ltd, Ongar, Essex, United Kingdom GLP-yes unpublished	N	DAS
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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2		2014	*Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)* 130727 GLP-yes unpublished	N	DAS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for clopyralid

A 2.1.1 Description of analytical methods for the determination of residues in plant matrices

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.

zRMS: Method is accepted.

New data on Validation of the Analytical Method for the Analysis of Clopyralid (Sum of Clopyralid, its salts and conjugates) in High water content, in High Oil content and Dry Commodities were described below.

Analytical method 1

Reference:	KCP 5.2.1
Report	*Validation of the Analytical Method for the Analysis of Clopyralid (Sum of Clopyralid, its salts and conjugates) in High water content, in High Oil content and Dry Commodities*, P. Schlewitz, 2023, Report No: C2135
Guideline(s):	Regulation (EC) No. 1107/2009 SANTE/2020/12830, Rev.1
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The objective of the study was to validate the analytical method for the analysis of clopyralid (sum of clopyralid, its salts and conjugates) in high water content (wheat whole plant, oilseed rape whole plant, sugar beets whole plant, leaves and roots), in high oil content (oilseed rape seeds) and dry commodities (wheat grain and straw).
A full validation was performed on wheat whole plant (high water content), on oilseed rape seeds (high oil content) and on wheat grain (dry commodities).
The applicability of the method on oilseed rape whole plant, sugar beets whole plant, leaves and roots (high water content) and on wheat straw (dry commodities) was demonstrated by concurrent recoveries (3 recoveries at LOQ and 3 recoveries at a higher level).

Equipment
- Usual laboratory glassware
- Centrifuge with centrifuge tube of 50 mL or other equivalent material
- Vortex
- Ultrasonic bath

- Evaporation under nitrogen system (sand bath at 40 °C)
- Water bath at 60 °C
- pH paper

Solvents and reagents:

Name	CAS No.	Formula	Quality
Methanol	67-56-1	CH3OH	HPLC
Acetonitrile	75-05-8	C2H3N	HPLC
Water	7732-18-5	H2O	Type 1
Sulfuric acid conc.	7664-93-9	H2SO4	for analysis
Potassium hydroxide	1310-58-3	KOH	for analysis
Formic acid (conc.)	64-18-6	HCOOH	for analysis
Supel QuE Non-Buffered Tube	-	4 g $MgSO_4$ + 1 g NaCl	Sigma-Aldrich ref. 55294-U
Ammonium formate	540-69-2	HCO_2NH_4	for analysis
PTFE 0.45 µm filter	-	-	for analysis

Extraction and Clean-up
Dry commodities (straw and grain):
Weigh exactly 2 g of homogenized sample material into a 50 mL centrifuge tube
Fortify if necessary.
Add 14 ml of 2.5 M KOH, mix for 10 sec. and heat the mixture (60°C) in a water bath for 3 hours.
Place the centrifuge tube in an ice bath.
Add 3 mL of 9 N H2SO4 and mix for 10 sec. Check the pH (<1).
Add 10 mL of acetonitrile and shake vigorously manually for 1 min.
Add the content of two Supel QuE Non-Buffered Tubes.
Shake vigorously manually for 1 min. and centrifuge for 5 min at 4500 rpm.
Transfer 2 mL of the upper layer into a 5 mL volumetric cylinder.
Evaporate the extract to dryness under a gentle stream of nitrogen.
Dissolve the final residues with 1 mL of methanol + 0.1% formic acid (vortex and ultrasonic bath).
Adjust to 2 mL with water + 0.1% of formic acid.
Filter through filter PTFE 0.45 µm.
Transfer into a vial.
Store samples refrigerated.

High water content (wheat whole plant, sugar beet (whole plant, leaves and roots)) and high oil content (oilseed seeds):
Weigh exactly 2 g of homogenized sample material into a 50 mL centrifuge tube
Fortify if necessary.
Add 8 ml of 2.5 M KOH, mix for 10 sec. and heat the mixture (60°C) in a water bath for 3 hours.
Place the centrifuge tube in an ice bath.
Add 2 mL of 9 N H2SO4 and mix for 10 sec. Check the pH (<1).
Add 10 mL of acetonitrile and shake vigorously manually for 1 min.
Add the content of one Supel QuE Non-Buffered Tube.
Shake vigorously manually for 1 min. and centrifuge for 5 min at 4500 rpm.
Transfer 2 mL of the upper layer into a 5 mL volumetric cylinder.
Evaporate the extract to dryness under a gentle stream of nitrogen.
Dissolve the final residues with 1 mL of methanol + 0.1% formic acid (vortex and ultrasonic bath).
Adjust to 2 mL with water + 0.1% of formic acid.
Filter through filter PTFE 0.45 µm.
Transfer into a vial.

Store samples refrigerated.

Standard solutions
Clopyralid
Prepare independent stock solution at approximately 1 mg/mL by accurately weighing 10 mg of clopyralid analytical standard into a 10 mL volumetric flask and bringing to volume with acetonitrile.
Clopyralid glycine
Weigh approximately 10 mg of clopyralid glycine analytical standard into a 10 mL volumetric flask. Add 5 mL HPLC methanol and mix well. Fill the flask to the mark with methanol and mix well.

Spiking solutions
Prepare individual spiking solutions of clopyralid or clopyralid glycine at 0.2 µg/mL (for spiked samples at LOQ) and 2.0 µg/mL (for spiked samples at 10xLOQ) by dilution of the stock solution with acetonitrile.
Fortifications were performed by adding known amounts of these spiking solutions to control samples just prior to the extraction step (meaning that working solution of standard is added to the homogenized subsample, before mixing with the extraction solvent).

Calibration solutions
Intermediate calibration solutions
From clopyralid spiking solution, prepare an intermediate solution at 200 and 20 ng/mL by dilution with control extract.
Matrix-matched calibration solutions
Prepare matrix-matched calibration solutions at 0.4, 1, 2, 5, 10, 20 and 24 ng/mL by dilution of intermediate calibration solutions with control extract.
Standard solution to test matrix effects
Prepare a standard solution at 20 ng/mL by dilution of the spiking solution at 200 ng/mL with methanol / water (50/50) + 0.1% formic acid.

Summary

	Solvent	Concentration	Storage conditions	Expiry date (Preparation + …)
Stock solution Clopyralid	Acetonitrile	≈ 1 mg/mL	Frozen	6 months
Stock solution Clopyralid glycine	Methanol	≈ 1 mg/mL	Frozen	6 months
Spiking solutions All analyte separately	Acetonitrile	≈ 0.2 and 2 µg/mL	Frozen	1 month
Intermediate calibration solutions Clopyralid	Control extract	≈ 200 and 20 ng/mL	Refrigerated	14 days
Matrix-matched Calibration solutions Clopyralid	Control extract	≈ 0.4 to 24 ng/mL	Refrigerated	14 days
Matrix-effect testing Calibration solution Clopyralid	Methanol / water (50/50) + 0.1% formic acid	≈ 20 ng/mL	Refrigerated	14 days

Calibration curves
Aliquots of the calibration solutions were injected into the analytical system using the same conditions as the specimens.

Peak areas of clopyralid obtained from chromatograms were plotted versus concentration and the calibration functions were determined by least square fit.
Number and concentrations of standards used, as well as acceptability criteria are described in SOP No. PG 0118. According to this SOP, the correlation coefficient for a curve (r) should be ≥ 0.990 for the calibration to be acceptable with regression residuals randomly distributed.

Analytical conditions
Primary and confirmatory method for high water content and dry commodities
Primary method for high oil content
Analytical conditions LC-MS/MS – 6500, n° MA_1809-03

Column: TRIART, C18, 3 µm
Mobile phase:

A =	Ultra-pure water + 5 mM ammonium formate + 0.1% formic acid
B =	HPLC methanol + 5 mM ammonium formate + 0.1% formic acid

Sample temperature: 15°C
Column temperature: 40°C

Elution:

Elution	Time	Flow	Composition (%)		Curve*	Elution	Time	Flow	Composition (%)		Curve*
	min	mL/min	A	B	(type)		min	mL/min	A	B	(type)
Pg1	0.00	0.8	100	0	-	Pg4	9.50	0.8	0	100	0
Pg2	6.00	0.8	60	40	0	Pg5	10.00	0.8	100	0	0
Pg3	6.50	0.8	0	100	0	Pg6	12.00	0.8	100	0	0

*0=linear

Detector: IONISATION mode ES, Polarity Pos, Capillary voltage 5500 V
Retention time: Clopyralid: ≈ 5.8 min.
Injected volume: 5 or 10 µL

Analytical Conditions:
Confirmatory method for high oil content
Analytical conditions LC-MS/MS – 6500, n° MA_1809-04

Column: BEH Phenyl, 1.7 µm
Mobile phase:
A = Ultra-pure water + 5 mM ammonium formate + 0.1% formic acid
B= HPLC methanol + 5 mM ammonium formate + 0.1% formic acid

Sample temperature: 15°C
Column temperature: 40°C
Elution:

Elution	Time	Flow	Composition (%)		Curve*	Elution	Time	Flow	Composition (%)		Curve*
	min	mL/min	A	B	(type)		min	mL/min	A	B	(type)
Pg1	0.00	0.4	90	10	-	Pg4	6.00	0.4	0	100	0
Pg2	4.00	0.4	60	40	0	Pg5	6.50	0.4	90	10	0
Pg3	4.50	0.4	0	100	0	Pg6	8.00	0.4	90	10	0

*0=linear

Detector: IONISATION mode ES, Polarity Pos, Capillary voltage (IS) 5500 V
Retention time: Clopyralid: ≈ 2 min.
Injected volume: 10 µL

The amount of clopyralid or clopyralid glycine in the specimens was determined using the following equation:

$$\text{Amount found (µg/kg)} = \frac{\text{Conc. x V x d x i}}{\text{M x \% aliquot purified}}$$

Calculations were based on real values. Intermediate values were not rounded.
Where: Conc. Concentration in extract (ng/mL)
V Final volume of the extract (mL)
d Dilution factor
M Sample weight (g)
% aliquot purified % of the crude extract used for purification
i Conversion factor
Conversion factor (i) for clopyralid glycine::
i = MM (clopyralid glycine) / MM (clopyralid) = 249.05 / 192.0 = 1.297
Conversion factor (i) for clopyralid:
i = 1.000

Results and discussions
A full validation was performed on wheat whole plant (high water content), on oilseed rape seeds (high oil content) and on wheat grain (dry commodities).

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

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Clopyralid	High water content (Wheat whole plant)	0.01	92.0%	3.8%	87.0%	96.5%	5
		0.10	97.1%	2.5%	94.8%	100.6%	5
Clopyralid glycine		0.01	75.3%	6.6%	68.7%	82.5%	5
		0.10	80.3%	1.5%	79.0%	82.3%	5
Clopyralid	High oil content (oilseed rape seeds)	0.01	97.0%	3.7%	92.9%	102.6%	5
		0.10	111.8%	2.3%	107.6%	114.1%	5
Clopyralid glycine		0.01	74.7%	6.3%	68.1%	81.2%	5
		0.10	87.5%	3.9%	83.3%	92.4%	5
Clopyralid	Dry commodities (wheat grain)	0.01	96.9%	6.0%	88.9%	104.5%	5
		0.10	94.7%	6.1%	86.4%	102.5%	5
Clopyralid glycine		0.01	95.6%	3.0%	91.6%	98.7%	5
		0.10	88.5%	3.3%	84.8%	91.4%	5

For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Clopyralid	Oilseed rape whole plant (High water content)	0.01	84.2%	3.8%	80.5%	86.3%	3
		0.10	96.8%	1.7%	95.6%	98.7%	3
Clopyralid glycine		0.01	75.5%	6.3%	70.0%	78.3%	3
		0.10	83.6%	6.7%	79.5%	89.9%	3
Clopyralid	Sugar beets whole plant (High water content)	0.01	82.6%	6.1%	79.3%	88.4%	3
		0.10	98.3%	3.1%	95.4%	101.5%	3
Clopyralid glycine		0.01	64.0%	5.0%	60.3%	66.1%	3
		0.10	78.0%	2.8%	75.9%	80.3%	3
Clopyralid	Sugar beets leaves (High water content)	0.01	88.8%	7.7%	83.3%	96.4%	3
		0.10	102.2%	6.4%	96.3%	109.3%	3
Clopyralid glycine		0.01	61.7%	5.4%	58.3%	64.9%	3
		0.10	90.1%	6.5%	85.6%	96.7%	3
Clopyralid	Sugar beets roots (High water content)	0.01	80.5%	1.3%	79.3%	81.2%	3
		0.10	88.6%	3.3%	85.8%	91.7%	3
Clopyralid glycine		0.01	63.4%	4.4%	60.5%	66.0%	3
		0.10	72.8%	1.4%	71.8%	73.9%	3
Clopyralid	Wheat straw (Dry commodities)	0.01	88.8%	3.3%	86.2%	91.9%	3
		0.10	100.9%	8.0%	95.7%	110.2%	3
Clopyralid glycine		0.01	76.9%	3.5%	74.9%	79.9%	3
		0.10	74.1%	6.6%	69.4%	79.1%	3

For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

Confirmatory method

Summary of recoveries – Confirmatory method

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Clopyralid	High water content (Wheat whole plant)	0.01	87.3%	17.5%	70.2%	106.1%	5
Clopyralid glycine		0.01	73.7%	25.5%	56.2%	105.2%	5
Clopyralid	High oil content (Oilseed rape seeds)	0.01	93.4%	4.0%	87.6%	97.7%	5
Clopyralid glycine		0.01	76.2%	4.8%	71.8%	81.4%	5
Clopyralid	Dry commodities (wheat grain)	0.01	96.7%	20.2%	72.4%	121.3%	5
Clopyralid glycine		0.01	85.3%	13.3%	78.4%	105.4%	5

For the confirmatory method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% and RSD were less than 30% for spiked samples at the LOQ level.

Primary method

Fortified samples (High water content (Wheat whole plant), High oil content (oilseed rape seeds), Dry commodities (wheat grain)).

For the primary method, recovery and repeatability (as precision, % RSD) tests were performed by untreated control samples spiked with clopyralid and clopyralid glycine before extraction at the following

fortification levels for each commodities:
- LOQ (5 samples),
- 10 x LOQ (5 samples).
For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

Confirmatory method
Fortified samples
For the confirmatory method, recovery and repeatability (as precision, % RSD) tests were performed by untreated control samples spiked with clopyralid and clopyralid glycine at the LOQ (5 samples) before extraction for each commodities.
For the confirmatory method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% and RSD were less than 30% for spiked samples at the LOQ level.

The applicability of the method on oilseed rape whole plant, sugar beets whole plant, leaves and roots (high water content) and on wheat straw (dry commodities) was demonstrated by concurrent recoveries (3 recoveries at LOQ and 3 recoveries at a higher level).
Fortified samples
For the primary method, recovery and repeatability (as precision, % RSD) tests were performed by untreated control samples spiked with clopyralid and clopyralid glycine before extraction at the following fortification levels for each commodities:
- LOQ (3 samples),
- 10 x LOQ (3 samples).
For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

Table A 1: Characteristics for the analytical method used for validation of clopyralid residues in all matrices

	clopyralid
Specificity	Selectivity and specificity Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte/matrix combination and for both primary and confirmatory methods were provided to prove selectivity of the method (detailed described in Appendix IV of report C2135). Mass spectra were provided to justify the selection of ions used for determination (detailed described in Appendix III of report C2135).. Untreated samples (non-fortified samples) were determined from the matrices used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods.
Calibration (type, number of data points)	matrix-matched calibration solutions of clopyralid, at least at 5 concentration levels The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods.
Calibration range	The analytical calibration ranged from 0.4 ng/mL to 24.5 ng/mL (corresponding to 0.002 to 0.12 mg/kg for clopyralid and to 0.003 to 0.16 mg/kg for clopyralid glycine). The calibration covered two orders of

magnitude and ranged from 20% of the LOQ to 20% above the highest level for clopyralid and ranged from 30% of the LOQ to 60% above the highest level for clopyralid glycine. Standard concentrations were distributed evenly over the full calibration range.
Calibration curves were run for each analysis sequence for both primary and confirmatory methods.
An example of a typical calibration plot, the equation of the calibration line, the linear correlation coefficient and the regression residuals plot is given in Appendix V of the validation report for both primary and confirmatory methods.

Assessment of matrix effects is presented

Matrix effects, expressed in % enhancement or suppression, were assessed for each commodities and analyte, for both primary and confirmatory methods. They were considered significant if they exceeded ±20%.
Matrix effects (enhancement or suppression) on the instrument response were considered significant for most of commodities/analyte combination. Consequently, matrix-matched calibration solutions were used for calibration.

Assessment of matrix effects was performed by comparing the analyte response of at least one individual standard prepared in solvent to at least one prepared in blank matrix for both primary and confirmatory methods and for all commodities.
Matrix effects, expressed in % enhancement or suppression were evaluated according to the following equation:
Recovery for matrix effect (%) = 100 x (FRe (matrix) / FR (solvent)– 100
FRe (matrix) = average response factor (matrix)
FR (solvent) = response factor (solvent)
Matrix effects were considered significant if they exceed ±20%.

Matrix effects – Primary method

Matrix	Analyte	Concentration (ng/mL)	Matrix effect (%)
High water content (Wheat whole plant)	Clopyralid	20.0	**16.4%**
High oil content (Oilseed rape seeds)	Clopyralid	20.0	**-46.9%**
Dry commodities (Wheat grain)	Clopyralid	20.0	**21.6%**

Matrix effects – Confirmatory method

Matrix	Analyte	Concentration (ng/mL)	Matrix effect (%)
High water content (Wheat whole plant)	Clopyralid	20.0	**18.0%**
High oil content (Oilseed rape seeds)	Clopyralid	20.0	**-29.8%**
Dry commodities (Wheat grain)	Clopyralid	20.0	**17.0%**

The detailed data are given in appendix II.

Matrix effects (enhancement or suppression) on the instrument response were considered significant for most of commodities/analyte combination. Consequently, matrix-matched calibration solutions were used for calibration.

Limit of determination/quantification

The limit of detection (LOD) was expressed as lowest calibration standard.
The LOD was 0.4 ng/mL for clopyralid in high water content, high oil content and in dry commodities (corresponding to 0.002 mg/kg for clopyralid and to 0.003 mg/kg for clopyralid glycine).
The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision.
The LOQ was 0.01 mg/kg for clopyralid and clopyralid glycine in high water content, in high oil content and in dry commodities.

A 2.1.2 Description of analytical methods for the determination of residues in animal matrices

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.

A 2.1.3 Description of analytical methods for the determination of residues in soil

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.

A 2.1.4 Description of analytical methods for the determination of residues in water

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.

A 2.1.5 Description of analytical methods for the determination of residues in air

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.

A 2.1.6 Description of analytical methods for the determination of residues in body fluids

Data matching studies have been evaluated by RMS - Finland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CPD300SL/Major 300SL, Cloe 300SL, ProSto 300SL INNVIGO is allowed to refer to EU approved reports.