

# REGISTRATION REPORT

## **Part B**

### **Section 5**

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: GF-4021

Product names: LaDiva

Chemical active substances:

Halauxifen-methyl 10 g a.s./L (9.594 g a.e./L)

Picloram 48 g a.s./L

Aminopyralid 32 g a.s./L

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

(new submission of the product)

Applicant: Corteva AgriScience

Submission date: November 2020

Finalisation date: December 2021 (initial Core Assessment)

January 2023 (final Core Assessment)

## Version history

When	What
November 2020	New submission of GF-4021 to the Central Zone.
December 2021	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are <del>struck through and shaded for transparency</del> .
January 2023	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant <del>is struck through and shaded</del> .

## Table of Contents

<b>5</b>	<b>Analytical methods .....</b>	<b>5</b>
5.1	Conclusion and summary of assessment .....	8
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	9
5.2.1	Analysis of the plant protection product (KCP 5.1.1) .....	9
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	9
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	10
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1).....	11
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1) .....	11
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	11
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2) .....	13
5.3.1	Analysis of the plant protection product (KCP 5.2) .....	13
5.3.2	Description of analytical methods for the determination of residues XDE-729 Methyl (KCP 5.2) .....	13
5.3.2.1	Overview of residue definitions and levels for which compliance is required.....	13
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2) .....	14
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2) .....	15
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	16
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	17
5.3.2.6	Description of methods for the analysis of air (KCP 5.2) .....	17
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2) .....	18
5.3.2.8	Other studies/ information .....	18
5.3.3	Description of analytical methods for the determination of residues of aminopyralid (KCP 5.2) .....	18
5.3.3.1	Overview of residue definitions and levels for which compliance is required.....	18
5.3.3.2	Overview of residue definitions and levels for which compliance is required (Aminopyralid).....	18
5.3.3.3	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2) .....	19
5.3.3.4	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2) .....	19
5.3.3.5	Description of methods for the analysis of soil (KCP 5.2).....	20
5.3.3.6	Description of methods for the analysis of water (KCP 5.2).....	20
5.3.3.7	Description of methods for the analysis of air (KCP 5.2) .....	20
5.3.3.8	Description of methods for the analysis of body fluids and tissues (KCP 5.2) .....	20
5.3.3.9	Other studies/ information .....	21
5.3.4	Description of analytical methods for the determination of residues of picloram (KCP 5.2).....	22
5.3.4.1	Overview of residue definitions and levels for which compliance is required (Picloram).....	22
5.3.4.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2) .....	22
5.3.4.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2) .....	23
5.3.4.4	Description of methods for the analysis of soil (KCP 5.2).....	24
5.3.4.5	Description of methods for the analysis of water (KCP 5.2).....	24
5.3.4.6	Description of methods for the analysis of air (KCP 5.2) .....	25
5.3.4.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2) .....	25

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5.3.4.8	Other studies/ information .....	26
<b>Appendix 1</b>	<b>Lists of data considered in support of the evaluation.....</b>	<b>27</b>
<b>Appendix 2</b>	<b>Detailed evaluation of submitted analytical methods.....</b>	<b>36</b>
A 2.1	Analytical methods for Halauxifen-methyl, picloram and aminopyralid .....	36
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	36
A 2.2	Analytical methods for Picloram.....	50
A 2.2.1	Methods for post-authorization control and monitoring purposes (KCP 5.2) .....	50

## 5 Analytical methods

### zRMS conclusions:

#### Halauxifen-methyl

In EFSA Journal 2014;12(12):3913 – “Peer review of the pesticide risk assessment of the active substance halauxifen-methyl” EFSA concluded that *the proposed residue definition monitoring in plants, restricted to cereals, is the sum of halauxifen-methyl and metabolite X11393729 (halauxifen), expressed as halauxifen-methyl. QuEChERS (quick, easy, cheap, effective and safe) method multi-residue method and also single LC-MS/MS (liquid chromatography with tandem mass spectrometry) method exist for monitoring the compounds of the residue definition in food and feed of plant origin with LOQs (limits of quantification) of 0.01 mg/kg in all commodity groups. Residues of halauxifen-methyl and X11393729 (halauxifen), in food of animal origin can be monitored with single LC-MS/MS methods and also with the QuEChERS multi-residue method with LOQs of 0.01 mg/kg in muscle, kidney, liver, fat, milk and eggs. It should be noted, however, that no residue definition has been set for food of animal origin.*

*Residues of halauxifen-methyl, metabolite X11393729 (halauxifen) and metabolite X11449757 in soil can be monitored by LC-MS/MS with LOQs of 0.05 µg/kg for each compound. Appropriate LC-MS/MS method with LOQs of 0.05 µg/L exists for monitoring halauxifen-methyl, metabolite X11393729 (halauxifen), and metabolites X11449757 and X11406790 in surface water and drinking water. Residues of halauxifen-methyl and X11393729 (halauxifen) in air can be monitored by LC-MS/MS with LOQs of 0.82 µg/m<sup>3</sup>. The active substance is not classified as a Health Hazard under CLP and, therefore, a method of analysis is not required for body fluids and tissues.*

Considering the results of metabolism study of halauxifen-methyl in the new proposed crop group (oilseed) which are presented in section B7, the same residue definition for halauxifen-methyl for a group of pulses and oilseeds crops can be proposed and adopted as the residue definition for halauxifen-methyl for a group of cereals. Thus, the proposed residue definition for both monitoring and risk assessment for new group of crops is halauxifen-methyl and compound X11393729 (halauxifen) expressed as halauxifen-methyl.

According to the EFSA Journal 2014;12(12):3913:

#### Methods of Analysis

Analytical methods for residues (Annex IIA, point 4.2)		
Residue definitions for monitoring purposes		
Food of plant origin		The sum of halauxifen-methyl and X11393729 (halauxifen), expressed as halauxifen-methyl (restricted to cereals).
Food of animal origin		Not required.
Soil		halauxifen-methyl
Water	surface	halauxifen-methyl and X11393729 (halauxifen)
	drinking/ground	halauxifen-methyl
Air		halauxifen-methyl
Monitoring/Enforcement methods		
Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)		<p>Single method: LC-MS/MS, LOQ = 0.01 mg/kg (turnip root and wheat forage (wet crops), barley (grain, hay and straw) and wheat (grain, hay and straw) (dry crops), canola seed and soybean (oily crops), apple (whole) and orange (whole) (acidic crops), aspirated grain, bran bread, flour, germ, gluten, shorts and starch).</p> <p>NB. The method relies on mixed stable isotope labelled internal standards.</p> <p>QuEChERS Multi-Residue Method: LC-MS/MS, LOQ = 0.01 mg/kg (kale leaves (wet crops), barley grain (dry crops), oilseed rape seed (oily crops) and lemon (acidic crops)).</p> <p>N.B. although mean recoveries in acidic matrices (lemon) and wet matrices (cabbage) were acceptable for the QuEChERS method, it is noted that the individual recoveries were occasionally low.</p>
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)		<p>Single method: LC-MS/MS, LOQ = 0.01 mg/kg (bovine muscle, liver, kidney, fat, whole milk and cream and poultry muscle, liver, fat and eggs).</p> <p>NB. The method relies on mixed stable isotope labelled internal standards.</p> <p>QuEChERS Multi-Residue Method: LC-MS/MS, LOQ = 0.01 mg/kg (bovine muscle, kidney, liver, fat and whole milk and poultry muscle and eggs).</p> <p>N.B. extraction efficiencies have not been addressed as part of the</p>

	<i>method validation discussed above; however, residues are not expected to be found in products of animal origin for the proposed use. This will need to be addressed in future however if new uses give rise to positive residues.</i>
Soil (analytical technique and LOQ)	LC-MS/MS, LOQ = 0.05 µg/kg (for halauxifen-methyl, X11393729 (halauxifen) and X11449757). NB. The method relies on mixed stable isotope labelled internal standards.
Water (analytical technique and LOQ)	LC-MS/MS, LOQ = 0.05 µg/L (for halauxifen-methyl, X11393729 (halauxifen), X11449757 and X11406790). NB. The method relies on mixed stable isotope labelled internal standards.
Air (analytical technique and LOQ)	LC-MS/MS, LOQ = 0.82 µg/m <sup>3</sup> (for halauxifen-methyl and X11393729 (halauxifen)).
Body fluids and tissues (analytical technique and LOQ)	Halauxifen-methyl is not classified as toxic or highly toxic; therefore monitoring methods for human tissues and body fluids are not required.

Monitoring methods for the determination of residues in crop commodities and environmental matrices have been evaluated during the EU review of halauxifen-methyl, where they were considered adequate and acceptable.

Furthermore the Applicant submitted two methods for analysis of residues of **halauxifen-methyl, picloram and aminopyralid** for the generation of pre-authorization data. The studies are acceptable. The details of the evaluation of new and additional studies are referred in Appendix 2.

#### Picloram

In EFSA Journal 2009; 7(12):1390 – “Peer review of the pesticide risk assessment of the active substance picloram” EFSA concluded that *Only single methods for the determination of residues are available. Residues of picloram in food of plant origin can be monitored by GC-MS with a LOQ of 0.01 mg/kg in oilseed rape. It should be noted however that the experts at the PRAPeR 66 meeting (April 2009) concluded that in the method GRM 00.19 only one fragment ion has been validated and an additional one for identification, and could not agree on the acceptability of the method. It should also be noted, that following the finalization of the residue definition for monitoring, a data gap will have to be set: either to demonstrate that the methods analyse only for picloram or to demonstrate that the extraction procedures cover the picloram conjugates, too.*

*Residues in foodstuff of animal origin can be determined by GC-MS with a LOQ of 0.01 mg/kg in all relevant animal products.*

*Residues of picloram in soil can be monitored by GC-MS with a LOQ of 0.0005 mg/kg.*

*GC-MS method is available to monitor residues of picloram in surface water and drinking water with LOQs of 0.05 µg/L. It should be noted however, that the experts at the PRAPeR 66 meeting (April 2009) concluded that in the methods GRM 00.18 for soil and GRM 00.17 for water only one fragment ion has been validated and an additional one for identification, and could not agree on the acceptability of the methods. It was however considered not necessary to set a data gap for these methods at EU level.*

*Residues of picloram in air can be monitored by GC-MS method with a LOQ of 6 µg/m<sup>3</sup>.*

*Analytical methods for the determination of residues in body fluids and tissues are not required as picloram is not classified as toxic or highly toxic.*

According to the EFSA Journal 2009; 7(12):1390:

#### Methods of Analysis

Analytical methods for residues (Annex IIA, point 4.2)		
Residue definitions for monitoring purposes		
Food of plant origin		open
Food of animal origin		Picloram
Soil		Picloram
Water	surface	Picloram
	drinking/ground	Picloram
Air		Picloram
Monitoring/Enforcement methods		
Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)		GC-MS LOQ 1.0 mg/kg picloram, grass LOQ 0.01 mg/kg picloram, oilseed rape open
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)		GC-MS LOQ 0.01 mg/kg for muscle, fat, liver, kidney, milk and eggs
Soil (analytical technique and LOQ)		GC-MS (picloram) – LOQ 0.0005 mg/kg

	LC-MS/MS (XDE-750) – LOQ 0.0015 mg/kg
Water (analytical technique and LOQ)	GC-MS(picloram) –: LOQ 0.05 µg/L LC-MS/MS(XDE-750) –: LOQ 0.05 µg/L
Air (analytical technique and LOQ)	GC-MS: LOQ 6 µg/m <sup>3</sup>
Body fluids and tissues (analytical technique and LOQ)	Not required as picloram is neither toxic nor very toxic

In the EFSA Journal 2013; 11(10):3439 it is stated that *Analytical methods for the determination of picloram residues in plant commodities were assessed in the DAR and during the peer review under Directive 91/414/EEC (United Kingdom, 2007, 2009; EFSA, 2010). The available monitoring method for oilseeds is based on GC-MS with a LOQ of 0.01 mg/kg. The peer review experts could not agree on the acceptability of this method as it was unclear if the method covers conjugated picloram. Therefore a data gap concerning analytical methods for enforcement purpose was defined. Confirmatory data have not been peer reviewed yet but were submitted for the current application and were evaluated by the EMS (United Kingdom, 2013). According to the EMS, the results indicate that the method GRM 00.19 is able to quantify picloram, free and conjugated expressed as picloram in high oil content and dry commodities with an LOQ validated at 0.01 mg/kg.*

*The current enforcement residue definition set in Regulation (EC) No 396/2005 is parent picloram. The applicant did not provide analytical enforcement methods that can be used to monitor parent picloram only. Taking into account that the residue definition should be amended to the sum of picloram and its conjugates, expressed as picloram as proposed in the peer review under Directive 91/414/EEC (see 3.1.1.1) and that the residue trials on which the MRL proposal is based on were also analysed with a method that included the conjugates, the lack of an enforcement method for parent picloram is considered of minor importance.*

*EFSA concludes that a sufficiently validated analytical method for crops belonging to the group of high oil content is available to control residues of picloram and its conjugates.*

Additionally EFSA confirmed in EFSA Supporting publication 2017:EN-1258 – “Outcome of the consultation on confirmatory data used in risk assessment for picloram” that *the analytical method GRM 00.19 is able to quantify picloram residues (free and conjugated) as picloram in oilseed rape seed, forage and straw and that the monitoring analytical method applied in residue trials correctly quantifies the residues of picloram and its conjugates. It should be mentioned that the submitted study can also be considered as an assessment of the extraction efficiency.*

Taking into account the EFSA conclusions that some analytical methods provided by the notifier and validated in the picloram monograph (2007) are not considers highly specific according to SANCO/825/00 rev. 8 and a confirmatory method for the determination of picloram are required, Applicant submitted the new, highly specific analytical methods (LC-MS/MS) and its ILV for post-authorization control and monitoring purposes:

- methods for food and feed of plant origin (Vogl, E., 2012) and its ILV (Austin, R., 2012),
- methods for food and feed of animal origin (Vincent T., 2013) and its ILV (Austin, R., 2013),
- methods for soil (Vincent T., 2013),
- methods for water (Shaffer, S. R., 2012) and its ILV (Austin, R., Turner, R., 2013),
- methods for air (Bacher, R., 2012),
- methods for body fluids and tissues (Sencuic, M., Schmiedt, S., 2016).

The analytical methods are acceptable. The details of the evaluation of new and additional studies are referred in Appendix 2. No other data is required.

### Aminopyralid

According to the EFSA Journal 2013;11(9):3352: “A LC-MS/MS method involving hydrolysis and derivatization was validated to monitor aminopyralid and its conjugates determined as aminopyralid in food and feed of plant origin at LOQ of 0.01 mg/kg for all four groups of matrices (high water, high acid and high oil content and dry). Another LC-MS/MS method was validated for the analysis of aminopyralid in food of animal origin at LOQ of 0.01 mg/kg for all matrices (fat, kidney, liver, muscle, milk and eggs).

Appropriate HPLC-MS/MS methods exist for monitoring of the residues of aminopyralid in soil, water and in air with LOQs of 0.001 mg/kg, 0.05 µg/L and 7.7 µg/m<sup>3</sup> respectively. The active substance is not classified as toxic or very toxic and analytical methods for residues in body fluids and tissues are not required, however a LC-MS/MS method for analysis of aminopyralid in blood (LOQ 0.025 µg/ml) and urine (LOQ 0.01 µg/ml) was provided but without confirmatory method/data.”

### Methods of Analysis

Analytical methods for residues (Annex IIA, point 4.2)	
Residue definitions for monitoring purposes	
Food of plant origin	The sum of aminopyralid and its conjugates expressed as aminopyralid.
Food of animal origin	Aminopyralid

Soil		Aminopyralid
Water	surface	Aminopyralid
	drinking/ground	Aminopyralid
Air		Aminopyralid
<b>Monitoring/Enforcement methods</b>		
Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)		LC/MS/MS, analyte: aminopyralid and its conjugates measured as aminopyralid LOQ = 0.01 mg/kg (water, dry, acid and oil crop groups)
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)		LC/MS/MS, analyte: aminopyralid LOQ= 0.01 mg/kg (milk, eggs, muscle, fat , kidney, liver)
Soil (analytical technique and LOQ)		LC/MS/MS, analyte: aminopyralid and its conjugates measured as aminopyralid LOQ = 0.001 mg/kg
Water (analytical technique and LOQ)		LC/MS/MS, analyte: aminopyralid LOQ = 0.05 µg/L
Air (analytical technique and LOQ)		LC/MS/MS, analyte: aminopyralid LOQ = 7.7 µg/m <sup>3</sup>
Body fluids and tissues (analytical technique and LOQ)		Aminopyralid is not classified as toxic or very toxic.

Additionally in EFSA Journal 2020;18(8):6229 - Review of the existing MRLs for aminopyralid it is stated that *During the peer review, a hyphenated analytical method based on high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) detection was validated for the determination of aminopyralid free and conjugated (measured as aminopyralid) in all four crop matrices (high water, high acid, high oil content and dry commodities), with a limit of quantification (LOQ) of 0.01 mg/kg. The method includes hydrolytic conditions that release free aminopyralid from its conjugates. It is supported by an independent laboratory validation (ILV).*

*During the completeness check, the EURLs provided a QuEChERS multi-residue analytical method using HPLC–MS/MS with an LOQ of 0.05 mg/kg for the routine analysis of free aminopyralid in high water content, high acid content and dry commodities. During the Member State consultation, the EURLs provided an updated evaluation report and additional validation data for high oil content commodities with the same LOQ of 0.05 mg/kg. However, this method does not cover the default LOQ of 0.01 mg/kg, neither the proposed residue definition for enforcement since aminopyralid conjugates are not analysed. According to the EURLs, aminopyralid is stable under alkaline hydrolysis and as the conjugates residues of aminopyralid are mostly glucosides (easy to breakup), it is confirmed that a modified QuEChERS method including an alkaline hydrolysis step would be suitable for the determination of aminopyralid (free and conjugated) (EURLs, 2019). However, validation data for this method were not provided by the EURLs.*

No other data is required.

## 5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities 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.

Noticed data gaps are: None

Commodity/crop	Supported/ Not supported
Oilseed rape	Supported



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

### 5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of aminopyralid, picloram and halauxifen-methyl in plant protection product is provided as follows:

Comments of zRMS:	The analytical method AM-191129 was successfully validated for the determination of Aminopyralid, Picloram and Halauxifen-methyl in GF-4021 formulation according to the requirements laid down by SANCO3030/99 rev.5.
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Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of Aminopyralid, Picloram and Halauxifen-methyl in GF-4021 Formulation, Cordero Henriquez, L., 2020, AM-191129
Guideline(s):	Yes, EEC Guideline SANCO/3030/99/rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Validation - Results and discussions

**Table 5.2-1: Methods suitable for the determination of active substances aminopyralid, picloram and halauxifen-methyl in plant protection product GF-4021**

	Aminopyralid	Picloram	Halauxifen-methyl	Diphenylether
<b>Author(s), year</b>	Cordero Henriquez, L., 2020			
<b>Principle of method</b>	An aliquot of the sample is dissolved in acetonitrile containing the internal standard diphenylether and is analyzed by using an Ascentis Express C18 HPLC column with an ultra-violet detector set at 260 nm. Quantification is by internal standard calibration using peak areas.			
<b>Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)</b>	361 mg/L – 1440 mg/L, equivalent to 1.75 wt% to 6.98 wt% aminopyralid in GF-4021 r = 0.9999	593 mg/L – 2290 mg/L, equivalent to 2.87 wt% to 11.07 wt% picloram in GF-4021 r = 0.9999	140 mg/L – 517 mg/L, equivalent to 0.68 wt% to 2.52 wt% halauxifen-methyl in GF-4021 r = 0.9997	529 mg/L – 2120 mg/L r = 0.9998
<b>Precision – Repeatability Mean n = 10 (%RSD) Horrat (Hr = %RSD/%RSDr)</b>	0.60% RSD at average concentration of 3.13 wt% aminopyralid Hr= 0.27	0.8% RSD at average concentration 5.11 wt% picloram Hr= 0.67	1.8% RSD at average concentration 1.07 wt% halauxifen-methyl Hr= 0.38	Not applicable
<b>Accuracy n = 7 (% Recovery)</b>	Average recovery of 99% over a concentration range of 1.73% to 6.93% w/w aminopyralid	Average recovery of 101% over a concentration range of 2.92% to 11.17% w/w picloram	Average recovery of 100% over a concentration range of 0.69% to 2.52% w/w halauxifen-methyl	Not applicable
<b>Interference/ Specificity</b>	The solvent blank, formulation blank, internal standard, aminopyralid technical, picloram technical and halauxifen-methyl technical were assessed. No significant interferences were detected.			
<b>Comment</b>	The method is linear, precise, accurate and specific when used for the assay of GF-4021.			

## 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)

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

Comments of zRMS:	The analytical method AM-192060 was successfully validated for the determination of HCB in the GF-4021 formulation according to the requirements of SANCO/3030/99 rev. 5. and is considered fit for purpose.
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Reference:	KCP 5.1.1
Report	Analytical Method and Validation for the Determination of HCB in GF-4021, McNew, B., 2020, AM-192060
Guideline(s):	Yes, EEC Guideline SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Validation - Results and discussions

**Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) GF-4021**

	<b>HCB</b> <b>2.55ppm max. content in GF-4021</b>
<b>Author(s), year</b>	McNew, B., 2020
<b>Principle of method</b>	An aliquot of the sample is dissolved in toluene and analyzed by using a RTX-5, 30 m x 0.25 mm x 0.25 µm film column with a temperature program ranging from 50°C to 300°C over a period of 36 minutes with the use of a mass spectral detector. Quantification of HCB is by external standard calibration using peak areas.
<b>Linearity</b> (linear between mg/L) (correlation coefficient, expressed as r)	0.03 mg/L – 0.14 mg/L, equivalent to 0.0001 wt% to 0.0006 wt% HCB in GF-4021
<b>Precision – Repeatability Mean</b> <b>n = 10</b> (%RSD) <b>Horrat (Hr = %RSD/%RSDr)</b>	9.2% RSD at average concentration of 0.0003 wt% HCB in GF-4021 <b>Hr= 0.996</b>
<b>Accuracy</b> <b>n = 7</b> (% Recovery)	Average recovery of 96% over a concentration range of 0.0001 wt% - 0.0006 wt% HCB in GF-4021
<b>Interference/ Specificity</b>	The formulation blank, formulation, HCB, toluene solvent, Picloram technical, halauxifen-methyl technical and aminopyralid technical were assessed. No significant interferences were detected.
<b>LOQ</b>	9.5% RSD at average concentration of 0.0001 wt% HCB in GF-4021
<b>Comment</b>	The method is linear, precise, accurate and specific when used for the assay of GF-4021

## Conclusion

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

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

No additional 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 halauxifen-methyl, aminopyralid and picloram for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

**Table 5.2-3: Validated methods for the generation of pre-authorization data (Halauxifen-Methyl)**

Component of residue definition: Halauxifen-Methyl				
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 XDE-729 Methyl	0.01 mg/kg	LC/MS/MS	Olberding, E.L., 2011. 'Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry'. Dow AgroSciences Study Number 110005/ EU Agreed
Animal products, food of animal origin (muscle, fat, kidney, liver, milk, eggs)	Primary XDE-729 Methyl	0.01 mg/kg	LC/MS/MS	Ma, M. ; Li, Q, 2012, Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection/ EFSA Journal 2014; 12(12): 3913
Water (Ecotoxicology)	Primary	0.027 µg/L	LC/MS/MS	Goudie, O. 2020. GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga ( <i>Raphidocelis subcapitata</i> ). DAS Study ID: 190111.
		Test medium: 0.000212 µg/L  Sediment: 0.007 mg/kg	LC/MS/MS	Eser, S. 2020. GF-4021: Growth Inhibition of <i>Myrophylum spicatum</i> in a Water/Sediment System. Dow AgroSciences Study ID 190151

**Table 5.2-4: Validated methods for the generation of pre-authorization data (Aminopyralid)**

Component of residue definition: Aminopyralid				
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 Aminopyralid	0.01 mg/kg	LC/MS/MS	Method GRM 07.07, DAS 071121 / EFSA Journal 2013; 11(9): 3352
Animal products, food of animal origin (muscle, fat, , kidney, liver, milk, eggs)	Primary Aminopyralid	0.01 mg/kg	LC/MS/MS	Method GRM 07.07, DAS 071121 / EFSA Journal 2013; 11(9): 3352
Water (Ecotoxicology)	Primary	0.083 µg/L	LC/MS/MS	Goudie, O. 2020. GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga ( <i>Raphidocelis subcapitata</i> ). DAS Study ID: 190111.
		Test medium: 0.000656 µg/L Sediment: 0.007 mg/kg	LC/MS/MS	Eser, S. 2020. GF-4021: Growth Inhibition of <i>Myrophyllyum spicatum</i> in a Water/Sediment System. Dow AgroSciences Study ID 190151

**Table 5.2-5: Validated methods for the generation of pre-authorization data (Picloram)**

Component of residue definition: Picloram				
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, oily crops)	Primary Picloram	0.01 mg/kg	GC/NCI-MS	Hastings, M. J. (2003) Method GRM 00.19, DAS Study ID 021211 / EFSA Journal 2009; 7(12):1390
Plants, plant products (wet crops)	Primary Picloram	1.0 mg/kg	GC/NCI-MS	Balderrama Pinto, O., Pinheiro, A. C., Kalvan, H. C. (2001) Method GRM 01.21 / EFSA Journal 2009; 7(12):1390
Plants, plant products (wet crops, dry crops, oily crops, acidic crops)	Primary Picloram	0.01 mg/kg	LC-MS/MS	Vogl, E. (2012), Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS, DAS Study ID 120610
	ILV Picloram	0.01 mg/kg	LC-MS/MS	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”, DAS Study ID 120614
Animal products, food of animal origin (muscle, fat, , kidney, liver, milk, eggs)	Primary Picloram	0.01 mg/kg	GC/NCI-MS	Hastings, M. J., Lindsey, A. E. (2003) Method GRM 03.06, DAS Study ID 031045 / EFSA Journal 2009; 7(12):1390
	ILV Picloram	0.01 mg/kg	GC/NCI-MS	Reed, D. (2003) Method DOW-1462, DAS Study ID 030041 / EFSA Journal 2009; 7(12):1390

Component of residue definition: Picloram				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Primary Picloram	0.01 mg/kg	LC-MS/MS	Vincent T. (2013), Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection, DAS Study ID 120622
	ILV Picloram	0.01 mg/kg	LC-MS/MS	Austin, R. (2013), Independent Laboratory Validation of Dow AgroSciences Method 120622, “Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection”, DAS Study ID 120607
Water (Ecotoxicology)	Primary	0.13 µg/L	LC/MS/MS	Goudie, O. 2020. GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga ( <i>Raphidocelis subcapitata</i> ). DAS Study ID: 190111.
		Test medium: 0.00102 µg/L  Sediment: 0.007 mg/kg	LC/MS/MS	Eser, S. 2020. GF-4021: Growth Inhibition of <i>Myrophylum spicatum</i> in a Water/Sediment System. Dow AgroSciences Study ID 190151

### 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 XDE-729 Methyl (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 EFSA Scientific Report (2014); 12(12):3913 the current legal residue definition is identical.

**Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required (Halauxifen-methyl)**

Matrix	Residue definition		MRL / limit	Reference for MRL/level Remarks
High water content; Dry Agricultural Commodities; Acidic Agricultural Commodities; Oily Agricultural Commodities	XDE-729 methyl and XDE-729 acid expressed as XDE-729 methyl equivalents		0.02 mg/kg	EFSA Journal 2014;12(12):3913

Matrix	Residue definition		MRL / limit	Reference for MRL/level Remarks
Muscle Milk Eggs Fat Liver, kidney	XDE-729 methyl and XDE-729 acid		0.01 mg/kg	Note: No livestock feeding studies are required since residues in barley, rye, spelt, triticale, wheat grain and oilseed rape seeds are low. XDE-729-methyl residues in livestock diets do not reach a level where feeding studies are required. EFSA Journal 2014; 12(12): 3913
Soil (Ecotoxicology)	XDE-729 methyl		NOEC = 0.0535 mg/kg soil <sup>1</sup>	EFSA Journal 2014; 12(12): 3913
Drinking water (Human toxicology)	XDE-729 Methyl (and metabolites), XDE-729 Acid, X11406790 and X11449757		0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	XDE-729 methyl		EC50=0.149 µg as/L <sup>3</sup>	EFSA Journal 2014; 12(12): 3913
Air	XDE-729 methyl and XDE-729 acid		0.82 µg/m <sup>3</sup>	AOEL sys/AOEL inhal: NA EFSA Journal 2014; 12(12): 3913
Tissue (meat or liver)	XDE-729 methyl and XDE-729 acid		Not required	Not classified as T / T+
Body fluids			Not required	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of halauxifen-methyl in plant matrices is given in the following tables.

**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): Halauxifen-methyl**

Component of residue definition: Halauxifen-methyl ester and Halauxifen acid				
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 Dry Agricultural Commodities Acidic Agricultural Commodities Oily Agricultural Commodities Wheat Processed Products	Primary <del>and</del> confirmatory	0.01 mg/kg XDE-729 Methyl XDE-729 Acid	LC-MS/MS	Ma, M. , 2012, Method Validation Study for Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid--Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection/ EFSA Journal 2014; 12(12): 3913
High Water Content	ILV	0.01 mg/kg XDE-729 Methyl	LC-MS/MS	Robaugh, D. A., 2012, XDE-729: Independent Laboratory Validation of

Component of residue definition: Halauxifen-methyl ester and Halauxifen acid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Oily Agricultural Commodities  Wheat Processed Products		XDE-729 Acid		Method for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry/ EFSA Journal 2014; 12(12): 3913

**Table 5.3-3: Statement on extraction efficiency**

	Method for products of plant origin
Required, available from:	The data for the extraction efficiency assessment can be found in Olberding, E. L. “Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry”, Dow AgroSciences LLC Study 110005, 2011”. During the method validation study, extraction efficiency data were generated using radio labeled samples from the wheat nature of residue study, Ma, M; Smith, K. P.; Jackson, A. U. “A Nature of Residue Study with [14C]-XR-729 Methyl Applied to Wheat with and without the Safener Cloquintocet Mexyl”, Dow AgroSciences LLC Study 101080, 2011 . Samples of wheat containing ingrown residues from a nature of residue study were analyzed with the sample analysis procedure. The results obtained using this analytical method were similar to those from the nature of residue study, demonstrating the suitability of this analytical method for the determination of XDE-729 methyl ester and XDE-729 acid in agricultural commodities.
Not required, because:	Not Applicable

**zRMS comments:**

Additional information in response to comment received from the cMS-DE:

cMS-DE: *The method by Ma, M, (2012, study no. 110004) is not acceptable as confirmatory method. The method has been considered as fit for purpose under the peer review by UK, with reservations due to the fact that full validation data are only obtained for the primary MS/MS transition. Revised version including confirmatory data with calibration, recovery and precision should be provided by Applicant.*

*Alternatively the method by Daneva, E. & Täufner, A. (2011, S 11-02423, DOW-1102V, 110293; ASB2013-2724), which fulfills the requirements could be used.*

*Applicant: Report for 110004 is in the process of being amended to include the confirmatory data that is available within the study file. Amended report will be available Q1/2023.*

*The RR will be updated.*

### 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 halauxifen-methyl in animal matrices is given in the following tables.

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

Component of residue definition: Halauxifen-Methyl (and metabolite Halauxifen Acid)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Bovine Muscle Liver Kidney Fat Milk Cream  Poultry Muscle Liver Fat Egg	Primary	0.01 mg/kg XDE-729 Methyl XDE-729 Acid	LC/MS/MS	Ma, M. ; Li, Q, 2012, Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection/ EFSA Journal 2014; 12(12): 3913
Bovine Liver Fat  Poultry Egg	ILV	0.01mg/kg XDE-729 Methyl XDE-729 Acid	LC/MS/MS	Langridge, G , 2012, Independent Laboratory Validation of an Analytical Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices/ EFSA Journal 2014; 12(12): 3913

**Table 5.3-5: Statement on extraction efficiency**

	Method for products of animal origin
Required, available from:	The data for the extraction efficiency assessment can be found in Olberding, Ma, M. ; Li, Q, 2012, Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection”, Dow AgroSciences LLC Study 110505. During the method validation study, extraction efficiency data were generated using radio labeled samples from the ruminant and hen nature of residue studies.; Rotondaro, S. L.; Adelfinskaya, Y. A. “A Nature of the Residue Study in the Laying Hen with [14C]-XDE-729 Methyl Ester” Dow AgroSciences LLC Study 101390, 2011, unpublished report of Dow AgroSciences LLC, October 27, 2011 and Rotondaro, S. L.; Adelfinskaya, Y. A. “A Nature of the Residue Study in the Ruminant with [14C]-XDE-729 Methyl Ester” Dow AgroSciences LLC Study 101390, 2011, unpublished report of Dow AgroSciences LLC, October 27, 2011. The results obtained using this analytical method were similar to those from the nature of residue study, demonstrating the suitability of this analytical method for the determination of XDE-729 methyl ester and XDE-729 acid in animal tissues.
Not required, because:	Not applicable

**zRMS comments:**

Additional information in response to comment received from the cMS-DE:

cMS-DE: *The method by Ma, M, (2012, study no. 110505) is not acceptable as confirmatory method. The method has been considered as fit for purpose under the peer review by UK, with reservations due to the fact that full validation data are only obtained for the primary (quantitation) MS/MS transition. Revised version including confirmatory data with calibration, recovery and precision should be provided by Applicant.*

*Alternatively the method by Lindner, M. (2011, SII-02424, DOW-1103V, 110574; ASB2013-2735), which fulfills the requirements could be used.*

*Applicant: Report for 110505 is in the process of being amended to include the confirmatory data that is available within the study file. Amended report will be available Q1/2023.*

**The RR will be updated.**



### 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 halauxifen-methyl in soil is given in the following tables.

**Table 5.3-6: Validated methods for soil (Halauxifen-methyl)**

Component of residue definition: Halauxifen-Methyl (and/or metabolites Halauxifen Acid and X11449757)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 ng/g XDE-729 Methyl XDE-729 Acid X11449757	LC/MS/MS	Blakeslee, B. A ., 2012, Method Validation Study for the Determination of Residues of X11393728 (XDE-729 Methyl), X11393729 (XDE-729 Acid) and X11449757 (des-Methyl XDE-729 Acid) in Soil using High Performance Liquid Chromatography with Positive-Ion Electrospray Ionization Mass Spectrometry/ EFSA Journal 2014; 12(12): 3913

### 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 halauxifen-methyl in surface and drinking water is given in the following tables.

**Table 5.3-7: Validated methods for water (Halauxifen-methyl)**

Component of residue definition: Halauxifen-methyl (and metabolites, Halauxifen Acid, X11406790 and X11449757)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water Ground Water Surface Water	Primary	0.05 µg/L XDE-729 Methyl XDE-729 Acid X11406790 X11449757	LC/MS/MS	Rodrigues Junior, A. ; Li, Q., 2011, Method Validation Study for the Determination of Residues of XDE-729 and its Metabolites in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry (Revision) / EFSA Journal 2014; 12(12): 3913
	ILV	0.05 µg/L XDE-729 Methyl XDE-729 Acid X11406790 X11449757	LC/MS/MS	Gemrot, F., 2012, XDE-729 Methyl Ester – Independent Laboratory Validation of Analytical Method 110718 for the Determination of XDE-729 Methyl Ester and its Metabolites Residues in Water/ EFSA Journal 2014; 12(12): 3913

### 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 halauxifen-methyl in air is given in the following tables.

**Table 5.3-8: Validated methods for air (Halauxifen-methyl)**

Component of residue definition: Halauxifen-methyl (and metabolite Halauxifen Acid)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.82 µg/m <sup>3</sup> XDE-729 Methyl	LC/MS/MS	Class, T., 2011, The Development and Validation

Component of residue definition: Halauxifen-methyl (and metabolite Halauxifen Acid)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	XDE-729 Acid		of a Method for the Analysis of XDE-729 Methyl Ester and XDE-729 Acid in Air / EFSA Journal 2014; 12(12): 3913

### 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 halauxifen-methyl in body fluids and tissues is given in the following table. Although the method is presented below, it has been noted that Methods of Analysis for body fluids are not required because halauxifen-methyl is not classified as toxic or very toxic.

**Table 5.3-9: Methods for body fluids and tissues (Halauxifen-methyl)**

Component of residue definition: Halauxifen-Methyl (and metabolite Halauxifen Acid)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 mg/L XDE-729 Methyl XDE-729 Acid	LC/MS/MS	Senciuc, M., 2011, XDE-729: Development and Validation of an Analytical Method for the Determination of XDE-729 Methyl Ester and Acid in Body Fluid(s)/ EFSA Journal 2014; 12(12): 3913

### 5.3.2.8 Other studies/ information

No additional studies required.

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

### 5.3.3.1 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.

### 5.3.3.2 Overview of residue definitions and levels for which compliance is required (Aminopyralid)

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content Plant, high acid content Plant, high protein/high starch content (dry commodities) Plant, high oil content	Aminopyralid	0.01 mg/kg	Method GRM 07.07, DAS 071121 / EFSA Journal 2013; 11(9): 3352
Muscle Milk Eggs Fat Liver, kidney	Aminopyralid	0.01 mg/kg	Method GRM 07.07, DAS 071121 / EFSA Journal 2013; 11(9): 3352
Soil (Ecotoxicology)	Aminopyralid	NOEC = 1.07 mg/kg	Earthworm reproduction – EFSA Journal 2013; 11(4):3182
Surface water (Ecotoxicology)	Aminopyralid	ErC50 = 0.00257 mg/L	<i>Lemna gibba</i> – EFSA Journal 2013; 11(4):3182

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Air	Aminopyralid	1.5µg/m <sup>3</sup>	AOEL inhal: 0.05 mg/kg bw/d
Body fluids (Urine and whole blood)	Aminopyralid	Not required	not classified as T / T+, EFSA Journal 2013; 11(9): 3352

### 5.3.3.3 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 aminopyralid in plant matrices is given in the following tables.

**Table 5.3-10: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP): Aminopyralid**

Component of residue definition: Aminopyralid				
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 High acid content High oil content High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Method GRM 07.07, DAS 071121 / EFSA Journal 2013; 11(9): 3352
	ILV	0.01 mg/kg	LC-MS/MS	Method P/B 1466 G, DAS 080117 / EFSA Journal 2013; 11(9): 3352

**Table 5.3-11: Statement on extraction efficiency**

	Method for products of plant origin
Required, available from:	The analytical method GRM 07.07.R1 (Study 071121) implements an extraction procedure, including base hydrolysis, which mirrors the optimized extraction procedure within historical metabolism studies (Study 010071 and Study 020022).
Not required, because:	Not applicable

### 5.3.3.4 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 aminopyralid in animal matrices is given in the following tables.

**Table 5.3-12: Validated methods for food and feed of animal origin (Aminopyralid)**

Component of residue definition: Aminopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk Eggs Muscle Fat Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Method GRM 07.08, DAS 071121 / EFSA Journal 2013; 11(9): 3352
	ILV	0.01 mg/kg	LC-MS/MS	Method P/B 1467 G, DAS 08118 / EFSA Journal 2013; 11(9): 3352

**Table 5.3-13: Statement on extraction efficiency**

	Method for products of animal origin
Required, available from:	The analytical method GRM 07.08.R1 (Study 071121) implements a methanol extraction procedure which mirrors the optimized extraction procedure within historical metabolism studies (Study 010079).

	Method for products of animal origin
Not required, because:	Not applicable

### 5.3.3.5 Description of methods for the analysis of soil (KCP 5.2)

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

**Table 5.3-14: Validated methods for soil (Aminopyralid)**

Component of residue definition: Aminopyralid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.001 mg/kg	LC-MS/MS	Method GRM 07.09, DAS 071121 / EFSA Journal 2013; 11(9): 3352

### 5.3.3.6 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of aminopyralid in surface and drinking water is given in the following tables.

**Table 5.3-15: Validated methods for water (Aminopyralid)**

Component of residue definition: Aminopyralid				
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	Method GRM 07.10, DAS 071121 / EFSA Journal 2013; 11(9): 3352
	ILV	0.05 µg/L	LC-MS/MS	Method P/B 1464 G, DAS 080116 / EFSA Journal 2013; 11(9): 3352

### 5.3.3.7 Description of methods for the analysis of air (KCP 5.2)

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

**Table 5.3-16: Validated methods for air (Aminopyralid)**

Component of residue definition: Aminopyralid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	7.7 µg/m <sup>3</sup>	LC-MS/MS	Method P/B 1645 G, DAS 091020 / EFSA Journal 2013; 11(9): 3352

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

### 5.3.3.8 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 aminopyralid in body fluids and tissues is given in the following table.

**Table 5.3-17: Methods for body fluids and tissues (Aminopyralid)**

Component of residue definition: Aminopyralid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.025 mg/L (blood) 0.010 mg/L (urine)	LC-MS/MS	Method DOW-1419, DAS 031005 / EFSA Journal 2013; 11(9): 3352

#### **5.3.3.9 Other studies/ information**

No additional studies required.

### 5.3.4 Description of analytical methods for the determination of residues of picloram (KCP 5.2)

Compared to the residue definition proposed in the EFSA Scientific Report (2009); 7(12):1390 the current legal residue definition is identical.

#### 5.3.4.1 Overview of residue definitions and levels for which compliance is required (Picloram)

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content Plant, high acid content Plant, high protein/high starch content (dry commodities) Plant, high oil content	Picloram	0.01 mg/kg	Reg (EU) 2016/1
Muscle Milk Eggs Fat Liver, kidney	Picloram	Muscle: 0.2 mg/kg Milk: 0.05 mg/kg Eggs: 0.01 mg/kg Fat: 0.01 mg/kg Kidney: 0.01 mg/kg	Reg (EU) 2016/1
Soil (Ecotoxicology)	Picloram	NOEC = 0.167 mg ae/kg d.w.soil	Earthworm reproduction – EFSA Journal 2009; 7(12):1390
Surface water (Ecotoxicology)	Picloram	NOEC= 0.55 mg/L	Rainbow trout ELS Study – EFSA Journal 2009; 7(12):1390
Air	Picloram	AOEL sys: 0.3 mg/kg bw/d	EFSA Journal 2009; 7(12):1390
Body fluids (Urine and whole blood)	Picloram	Not required	not classified as T / T+, EFSA Journal 2009; 7(12):1390

#### 5.3.4.2 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 picloram in plant matrices is given in the following tables.

**Table 5.3-18: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP): Picloram**

Component of residue definition: Picloram				
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 High acid content High oil content High protein/high starch content (dry)	Primary	0.01 mg/kg	GC/NCI-MS	Hastings, M. J. (2003) Method GRM 00.19, DAS Study ID 021211 / EFSA Journal 2009; 7(12):1390
	Primary	1.0 mg/kg	GC/NCI-MS	Balderama Pinto, O. B, Pinheiro, A. C., Kalvan, H. C. (2001) Method GRM 01.21 / EFSA Journal 2009; 7(12):1390
	Primary	0.01 mg/kg	LC-MS/MS	Vogl, E. (2012), Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS, DAS Study ID 120610
	ILV	0.01 mg/kg	LC-MS/MS	Austin, R. (2012), Independent Laboratory Validation of Dow AgroSciences Method 120610, “Method Validation Study for the

Component of residue definition: Picloram				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS” DAS Study ID 120614
	Confirmatory	Same as the primary method	Same as the primary method	Same as the primary method

**Table 5.3-19: Statement on extraction efficiency**

	Method for products of plant origin
Required, available from:	Previous metabolism studies conducted for picloram in wheat and oilseed indicated that the majority of the extractable radioactive residues were characterised as conjugates of picloram which released picloram upon acidic or basic hydrolysis. A stand-alone analytical method (DAS Study 110573) was conducted to confirm that the conditions implemented by analytical method GRM 00.19 efficiently measured free and conjugated residue of picloram in oilseed rape seed, forage and straw.
Not required, because:	Not applicable

#### 5.3.4.3 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 picloram in animal matrices is given in the following tables.

**Table 5.3-20: Validated methods for food and feed of animal origin (Picloram)**

Component of residue definition: Picloram				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk Eggs Muscle Fat Kidney, liver	Primary	0.01 mg/kg	GC/NCI-MS	Hastings, M. J., Lindsey, A. E. (2003) Method GRM 03.06, DAS Study ID 031045 / EFSA Journal 2009; 7(12):1390
	ILV	0.01 mg/kg	GC/NCI-MS	Reed, D. (2003) Method DOW-1462, DAS Study ID 030041 / EFSA Journal 2009; 7(12):1390
	Primary	0.01 mg/kg	LC-MS/MS	Vincent T. (2013), Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection, DAS Study ID 120622
	ILV	0.01 mg/kg	LC-MS/MS	Austin, R. (2013), Independent Laboratory Validation of Dow AgroSciences Method 120622, “Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection”, DAS Study ID 120607

**Table 5.3-21: Statement on extraction efficiency**

	Method for products of animal origin
Required, available from:	The analytical method GRM 03.06 (DAS Study 031045) implements an extraction procedure which mirrors the optimized extraction procedure within historical metabolism studies (GH-C 2886) and was further demonstrated in a stand-alone verification method which determined residues using C-14 samples from the goat metabolism study (GH-C 2934).
Not required, because:	Not applicable

#### 5.3.4.4 Description of methods for the analysis of soil (KCP 5.2)

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

**Table 5.3-22: Validated methods for soil (Picloram)**

Component of residue definition: Picloram			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.0005 mg/kg	GC/NCI-MS	Hastings, M. J., Schauerma, M. (2003) Method GRM 00.18, DAS Study ID 001029 / EFSA Journal 2009; 7(12):1390
Primary	0.0005 mg/kg	LC-MS/MS	Vincent, T. P. (2013) Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS, DAS Study ID 120612

#### 5.3.4.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of picloram in surface and drinking water is given in the following tables.

**Table 5.3-23: Validated methods for water (picloram)**

Component of residue definition: Picloram				
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	GC/NCI-MS	Hastings, M. J. (2001) Method GRM 00.17, DAS Study ID 001030 / EFSA Journal 2009; 7(12):1390
	Primary	0.05 µg/L	LC-MS/MS	Shaffer, S. R. (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, DAS Study ID 120611



Component of residue definition: Picloram				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	ILV	0.05 µg/L	LC-MS/MS	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”, DAS Study ID 120613

#### 5.3.4.6 Description of methods for the analysis of air (KCP 5.2)

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

**Table 5.3-24: Validated methods for air (picloram).**

Component of residue definition: Picloram			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	6.0 µg/m <sup>3</sup>	GC/MSD	Atkinson, S. (2003) Method GRM 02.29, DAS Study ID GHE-P-10114 / EFSA Journal 2009; 7(12):1390
Primary	9.0 µg/m <sup>3</sup>	LC-MS/MS	Bacher, R. (2012) The Development and Validation of a Method for the Analysis of Picloram in Air, DAS Study ID 120603

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

#### 5.3.4.7 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 Picloram in body fluids and tissues is given in the following table.

**Table 5.3-25: Methods for body fluids and tissues (picloram).**

Component of residue definition: Picloram			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/L (blood) 0.02 mg/L (urine)	GC/MSD	Freshour, N. L., Hermann, E. A. (1983) DAS Study ID HET-K-038323-036 / EFSA Journal 2009; 7(12):1390
Primary	0.05 mg/L	LC-MS/MS	Sencuic, M., Schmiedt, S. (2016) Development and Validation of a Method for the Analysis of Picloram, Aminopyralid and Triclopyr (All Free Acids) in Body Fluids, DAS Study ID 160866

#### **5.3.4.8            Other studies/ information**

No additional studies required.

## 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	Cordero Henriquez, L.	2020	Analytical Method and Validation for the Determination of Aminopyralid, Picloram and Halauxifen-Methyl in GF-4021 Formulation. DAS Report No.: AM-191129. Product and Process Technology R&D, Dow AgroSciences LLC. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.1.1	McNew, B.	2020	Analytical Method and Validation for the Determination of HCB in GF-4021. DAS Report No.: AM-192060. Product and Process Technology R&D, Dow AgroSciences LLC. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.1.2 (for the full summary please see KCP 10.2.1)	Goudie, O., et al.	2020	GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga ( <i>Raphidocelis subcapita</i> ) DAS Report No.: 190111 Eurofins EAG Agorscience, LLC GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.1.2 (for the full summary please see KCP 10.2.1)	Eser, S.	2020	GF-4021: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System DAS Report No.: 190151 Eurofins Agrosience Services EcoChem GmbH GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
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 DAS Report No.: 120610 ABC Laboratories, Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
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” DAS Report No.: 120614 Battelle UK Ltd. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Vincent, T.	2013	Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection DAS Report No.: 120622 ABC Laboratories, Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Austin, R.	2013	Independent Laboratory Validation of Dow AgroSciences Method 120622, “Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection” DAS Report No.: 120607 Battelle UK Ltd. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
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. DAS Report No.: 120612. ABC Laboratories, Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Shaffer, S. R.	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 DAS Report No.: 120611 ABC Laboratories, Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

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., Turner, R.	2012	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” DAS Report No.: 120613 Battelle UK Ltd. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Bacher, R.	2012	The Development and Validation of a Method for the Analysis of Picloram in Air DAS Report No.: 120603 PTRL Europe GMBH. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Sencuic, M., Schmiedt, S.	2016	Development and Validation of a Method for the Analysis of Picloram, Aminopyralid and Triclopyr (All Free Acids) in Body Fluids. DAS Report No.: 160866 EAG Laboratories, PTRL Europe. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2 (for the full summary please see KCP 8/ KCA 6.6.2)	White, T.	2019	Determination of Residues of Picloram in Rotational Crops (Wheat, Turnip and Kale) After One Application of GF-224 to Bare Soil at Two Sites in Northern Europe and Two Sites Southern Europe 2014 – 2017 DAS Report No.: 140651 Eurofins Agrosience Services Ltd. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2 (for the full summary please see KCP 8/ KCA 6.6.2)	White, T.	<del>2019</del> 2018	Determination of Residues of Picloram in Winter and Spring Wheat Grown as Rotational Crops After One Application of GF-224 to Bare Soil at Eight Sites in Northern Europe and Eight Sites in Southern Europe 2014-2016 DAS Report No.: <del>140652</del> 140642 Eurofins Agrosience Services Ltd. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.2 (for the full summary please see KCP 8/ KCA 6.6.2)	Delmotte, R.	2016	Magnitude of the Residues of Halauxifen methyl and Picloram in Oilseed rape (RAC Whole plant, Seed, and Straw), following One Application of GF-3447, Northern and Southern Europe – 2015 DAS Report No.: 150006 Staphyt GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

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

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.1.2	Olberding, E. L	2011	Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry DAS Report No.: 110005 Dow AgroSciences LLC, Indianapolis, Indiana, United States GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Robaugh, D. A.	2012	Independent Laboratory Validation of Method for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry DAS Report No.: 110825 Pyxant Labs Inc, Colorado Springs, Colorado, United States GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Ma, M.; Li, Q	2012	Method Validation Study for the Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Bovine and Poultry Tissues using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection DAS Report No.: 110505 Dow AgroSciences LLC, Indianapolis, Indiana, United States GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.2	Langridge, G	2012	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-729 Methyl Ester and XDE-729 Acid in Animal Matrices DAS Report No.: 110828 CEM Analytical Services Ltd Glendale Park (CEMAS), North Ascot, Berkshire, United Kingdom GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Blakeslee, B. A.	2012	Method Validation Study for the Determination of Residues of X11393728 (XDE-729 Methyl), X11393729 (XDE-729 Acid) and X11449757 (des-Methyl XDE-729 Acid) in Soil using High Performance Liquid Chromatography with Positive-Ion Electrospray Ionization Mass S DAS Report No.: 110716 Dow AgroSciences LLC, Indianapolis, Indiana, United States GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Rodrigues Jr, A.; Li, Q.	2011	Method Validation Study for the Determination of Residues of XDE-729 and its Metabolites in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry (Revision) DAS Report No.: 110718S2 Dow AgroSciences Industrial Ltd., Mogi-Mirim, Sao Paulo, Brazil GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Class, T.	2011	The Development and Validation of a Method for the Analysis of XDE-729 Methyl Ester and XDE-729 Acid in Air DAS Report No.: 110028 PTRL Europe, Ulm, Germany GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Senciuc, M.	2011	XDE-729: Development and Validation of an Analytical Method for the Determination of XDE-729 Methyl Ester and Acid in Body Fluid(s) DAS Report No.: 110029 PTRL Europe, Ulm, Germany GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.2	Wendelburg, B. M., Olberding, E. L.	2008	Validation Report for Methods GRM 07.07.R1 – Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometric Detection, GRM 07.08.R1 - Determination of Residues of Aminopyralid in Bovine and Poultry Tissues, Milk, and Eggs by Liquid Chromatography with Tandem Mass Spectrometric Detection, GRM 07.09.R1 - Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometric Detection, and GRM 07.10.R1 - Determination of Residues of Aminopyralid in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection DAS Report No.: 071121 Dow AgroSciences LLC GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Beck, I., Class, T.	2008	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 07.07 – Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometric Detection DAS Report No.: 080117 PTRL Europe GmbH. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Beck, I., Class, T.	2008	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 07.08 – Determination of Residues of Aminopyralid in Bovine and Poultry Tissues, Milk and Eggs by Liquid Chromatography with Tandem Mass Spectrometric Detection DAS Report No.: 080118 PTRL Europe GmbH. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Beck, I., Class, T.	2008	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 07.10 – Determination of Residues of Aminopyralid in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection DAS Report No.: 080116 PTRL Europe GmbH. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Bacher, R.	2009	The Development and Validation of a Method for the Analysis of Aminopyralid in Air. DAS Report No.: 091020. PTRL Europe GmbH. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)



<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.2	Mollica, J., West, S.	2003	Method Validation for the Analysis of XDE-750 in Human Blood and Urine. DAS Report No.: 031005. Pyxant Labs Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Hastings, M. J.	2003	Determination of Residues of Clopyralid and Picloram in Canola by Gas Chromatography with Negative-Ion Chemical Ionization Spectrometry Method Number: GRM 00.19 DAS Report No.: 021211. Dow AgroSciences LLC GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Balderrama Pinto, O., Pinheiro, A. C., Kalvan, H. C.	2001	Determination of Picloram and 2,4-D in Grass DAS Report No.: 030026. Morse Laboratories, Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Lindsey, A. E., Hastings, M. J.	2003	Method Validation for the Determination of Residues of Picloram in Animal Tissues by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry Detection Using Dow AgroSciences Method GRM 03.06 DAS Report No.: 031045 Dow AgroSciences LLC. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Reed, D.	2003	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 03.06 – Determination of Residues of Picloram in Animal Tissues by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry. DAS Report No.: 030041. Pyxant Labs Inc. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Shackelford, D. D., et al.	2003	Conjugate Analyses with [14C]-Picloram Applied to Oilseed Rape DAS Report No.: 110573. Ricerca Biosciences LLC. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 5.2	Hastings, M. J., Schaeuerman, M.	2001	Determination of Clopyralid and Picloram Residues in Soil by Gas Chromatography with Mass Selective Detection. Method Number: GRM 00.18. DAS Report No.: 001029. Dow AgroSciences Letcombe Laboratory. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Hastings, M. J., Schaeuerman, M.	2001	Determination of Residues of Clopyralid and Picloram in Waters (Drinking Water, Surface Water, and Ground Water) by Gas Chromatography with Mass Selective Detection Method Number: GRM 00.17. DAS Report No.: 001030. Dow AgroSciences Letcombe Laboratory. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Atkinson, S.	2003	Determination of Picloram in Air by Capillary Gas Chromatography with Mass Spectrometric Detection Method Number: GRM 02.29 DAS Report No.: GHE-P-10114. CEMAS. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)
KCP 5.2	Freshour, N.L., Hermann, E.A.	1983	Picloram: Quantitative Determination in Human Blood and Urine DAS Report No.: 833368; K-038323-036. Dow Chemical Company LLC. GLP (Y/N): Y. Published (Y/N): N.	N	Corteva Agriscience (Dow AgroSciences)

**List of data submitted by the applicant and not relied on**

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

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

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

## Appendix 2 Detailed evaluation of submitted analytical methods

### A 2.1 Analytical methods for Halauxifen-methyl, picloram and aminopyralid

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

#### A 2.1.1.1 Description of analytical methods for the determination of residues in water (KCP 5.1.2)

##### A 2.1.1.1.1 Analytical Method 1

Comments of zRMS:	<p>The analytical method has been validated for the determination of GF-4021 (the concentrations of <b>aminopyralid, picloram and halauxifen-methyl</b>) in freshwater AAP medium.</p> <p>The analyses were performed using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).</p> <p>The method limit of quantitation (LOQ) for these analyses is set at 0.00250 mg GF-4021/L (0.083 µg a.i./L aminopyralid, 0.13 µg a.i./L picloram, 0.027 µg a.i./L halauxifen-methyl) defined as the lowest nominal concentration of a matrix fortification sample for which a mean recovery of 70-110% and relative standard deviation of <math>\leq 20\%</math> has been obtained.</p> <p>The analytical method is satisfactorily validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentrations of aminopyralid, picloram, and halauxifen-methyl in Freshwater AAP algal media.</p> <p>The study is acceptable.</p>
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Method Identifier No.:	190111
Performing Laboratory:	Eurofins EAG Agrosience, LLC Easton, Maryland, U.S.A.
Reference:	KCP 5.1.2
Report:	Goudie, O., Sneckenberger, G.W., Arnie, J.R., Zhang, L.; 2020; GF-4021: A 72-Hour Toxicity Test with the Freshwater Alga ( <i>Raphidocelis subcapitata</i> ); Eurofins EAG Agrosience, LLC, 8598 Commerce Drive, Easton, MD 21601, USA; Lab Study No. 379P-159; DAS Study No. 190111; 02 October 2020; Unpublished
Guideline(s):	OECD Guideline 201, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

## MATERIALS AND METHODS

### Method Principle

Residues of GF-4021 (analysed for active ingredients aminopyralid, picloram, and halauxifen-methyl) are determined via extraction (aminopyralid and picloram) or dilution (halauxifen-methyl) from samples of freshwater AAP algal medium. For analysis of aminopyralid and picloram, samples were extracted twice using ethyl acetate following pH adjustment with 10% HCl in HPLC-grade water. The combined extracts were evaporated and reconstituted with 20 : 80 : 0.1 (v/v/v) methanol : HPLC-grade water : formic acid. Additional dilutions were performed, as necessary to bring all samples into the range of the calibration curve, using 20 : 80 : 0.1 (v/v/v) methanol : HPLC-grade water : formic acid. The samples for halauxifen-methyl were diluted initially with 0.5% formic acid in methanol to achieve a solvent composition of 20 : 80 : 0.1 (v/v/v) methanol : freshwater AAP medium : formic acid. Additional dilutions were performed,

as necessary to bring all samples into the range of the calibration curve, using 20 : 80 : 0.1 (v/v/v) methanol : freshwater AAP medium : formic acid. The final samples are analysed for aminopyralid, picloram, and/or halauxifen-methyl 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 1:** Recovery results from method validation of aminopyralid (*m/z* 207.1/161.0) using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP algal media	aminopyralid	0.083	99	4.1	5	
Freshwater AAP algal media	aminopyralid	83	108	1.1	5	

**Table A 2:** Recovery results from method validation of picloram (*m/z* 241.1/194.9) using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP algal media	picloram	0.13	105	4.0	5	
Freshwater AAP algal media	picloram	130	110	1.2	5	

**Table A 3:** Recovery results from method validation of halauxifen-methyl (*m/z* 345.0/285.0) using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP algal media	halauxifen-methyl	0.027	97	3.8	5	
Freshwater AAP algal media	halauxifen-methyl	27	101	1.9	5	

**Table A 4:** Procedural recovery results for aminopyralid (*m/z* 207.1/161.0) using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP algal media	aminopyralid	0.083	108	5.7	5	
Freshwater AAP algal media	aminopyralid	83	107	5.4	5	

**Table A 5: Procedural recovery results for picloram (m/z 241.1/194.9) using the analytical method**

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP algal media	picloram	0.13	102	9.2	5	
Freshwater AAP algal media	picloram	130	108	7.1	5	

**Table A 6: Procedural recovery results for halauxifen-methyl (m/z 345.0/285.0) using the analytical method**

Matrix	Analyte	Fortification level (µg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater AAP algal media	halauxifen-methyl	0.027	102	3.0	5	
Freshwater AAP algal media	halauxifen-methyl	27	100	5.2	5	

**Table A 7: Characteristics for the analytical method used for matrix fortification of GF-4021 (analysed for active ingredients aminopyralid, picloram, and halauxifen-methyl) residues in freshwater AAP algal media**

	aminopyralid	picloram	halauxifen-methyl
Specificity	m/z 207.1/161.0 blank value <30% LOQ	m/z 241.1/194.9 blank value <30% LOQ	m/z 345.0/285.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ 5 data points	linear regression analysis with 1/x weighting $r \geq 0.999$ 5 data points	linear regression analysis with 1/x weighting $r \geq 0.998$ 5 data points
Calibration range	Concentration range of 0.500-10.0 µg a.s./L (equivalent to 0.0098 – 0.20 mg GF-4021/L)	Concentration range of 0.500-10.0 µg a.s./L (equivalent to 0.015 – 0.30 mg GF-4021/L)	Concentration range of 0.00750-0.150 µg a.s./L (equivalent to 0.00069 – 0.014 mg GF-4021/L)
Limit of determination/quantification	LOQ= 0.083 µg a.s./L (0.0025 mg GF-4021/L) LOD= 0.025 µg a.s./L (0.0075 mg GF-4021/L)	LOQ=0.13 µg a.s./L (0.0025 mg GF-4021/L) LOD=0.038 µg a.s./L (0.0075 mg GF-4021/L)	LOQ=0.027 µg a.s./L (0.0025 mg GF-4021/L) LOD= 0.0081 µg a.s./L (0.0075 mg GF-4021/L)

## CONCLUSION

The method was considered acceptable for the determination of GF-4021 (analysed for active ingredients aminopyralid, picloram and halauxifen-methyl) in freshwater AAP algal media because the precision and mean recoveries of matrix fortification samples met acceptance criteria.

### A 2.1.1.1.2 Analytical Method 2

Comments of zRMS:	<p>The analytical method has been validated for the determination of the concentrations of <b>aminopyralid, picloram and halauxifen-methyl</b> in test medium and sediment.</p> <p>The analyses were performed using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).</p> <p>In test medium, the limit of quantification (LOQ) of the analytical method was 0.0200 µg/L of the test item (0.000212 µg/L of halauxifen-methyl, 0.000656 µg/L of aminopyralid and 0.00102 µg/L of picloram).</p> <p>In sediment samples the limit of quantification (LOQ) of the analytical method was 0.000700 mg/kg for halauxifen-methyl and 0.00700 mg/kg for aminopyralid and picloram.</p>
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	<p>The mean recoveries at each fortification level were in the range between 70 % and 110 % with relative standard deviations below 20 %.</p> <p>The analytical method is satisfactorily validated with regard to recovery, limit of quantification, precision and detector linearity in accordance with SANCO/3029/99 rev. 4. for the determination of the concentrations of aminopyralid, picloram, and halauxifen-methyl in test medium and sediment.</p> <p>The study is acceptable.</p>
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Method Identifier No.:	190151 Appendix H
Performing Laboratory:	Eurofins Agrosience Services EcoChem GmbH, 75223 Niefern - Öschelbronn, Germany
Reference:	KCP 5.1.2
Report:	Eser, S.; 2020; GF-4021: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System; Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24 D-75223 Niefern-Öschelbronn Germany; Lab Study No. S19-00162; DAS Study No. 190151; September 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 halauxifen-methyl are determined from samples of test medium by direct injection. The final samples are analysed for halauxifen-methyl by liquid chromatography coupled with positive ion electrospray tandem mass spectrometry (LC-MS/MS).

Residues of aminopyralid and picloram are determined from samples of test medium by liquid-liquid extraction with ethyl acetate. The final samples are analysed for aminopyralid and picloram by liquid chromatography coupled with positive ion electrospray tandem mass spectrometry (LC-MS/MS).

Residues of halauxifen-methyl are determined from samples of sediment by extraction with acetonitrile/water (1:1, v/v) + 2% formic acid. The final samples are analysed for halauxifen-methyl by liquid chromatography coupled with positive ion electrospray tandem mass spectrometry (LC-MS/MS).

Residues of aminopyralid and picloram are determined from samples of sediment by extraction with acetonitrile/water (80:20, v/v) + 2% formic acid. The final samples are analysed for aminopyralid and picloram 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 31:** Recovery results from method validation of halauxifen-methyl (*m/z* 345/250 Q) using the analytical method

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Test medium (Smart and Barko)	Halauxifen-methyl	0.000212 µg/L	70	17	5	-
Test medium (Smart and Barko)	Halauxifen-methyl	0.276 µg/L	90	3	5	-

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Sediment (Artificial soil)	Halauxifen-methyl	0.000700 mg/kg	98	1	5	-
Sediment (Artificial soil)	Halauxifen-methyl	0.300 mg/kg	76	4	5	-

**Table A 2: Procedural recovery results of halauxifen-methyl (m/z 345/250 Q) using the analytical method**

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Test medium (Smart and Barko)	Halauxifen-methyl	0.000212 µg/L	95	13	3	-
Test medium (Smart and Barko)	Halauxifen-methyl	0.276 µg/L	95	3	6	-
Sediment (Artificial soil)	Halauxifen-methyl	0.000700 mg/kg	75	3	3	-

**Table A 3: Recovery results from method validation of aminopyralid (m/z 207/134Q) using the analytical method**

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Test medium (Smart and Barko)	Aminopyralid	0.000656 µg/L	98	15	5	-
Test medium (Smart and Barko)	Aminopyralid	0.853 µg/L	101	3	5	-
Sediment (Artificial soil)	Aminopyralid	0.00700 mg/kg	91	7	5	-
Sediment (Artificial soil)	Aminopyralid	0.300 mg/kg	94	15	5	-

**Table A 4: Procedural recovery results of aminopyralid (m/z 207/134Q) using the analytical method**

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Test medium (Smart and Barko)	Aminopyralid	0.853 µg/L	92	9	6	-
Sediment (Artificial soil)	Aminopyralid	0.00700 mg/kg	94	5	3	-
Sediment (Artificial soil)	Aminopyralid	0.300 mg/kg	97	5	3	-

**Table A 5: Recovery results from method validation of picloram (m/z 243/170Q) using the analytical method**

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Test medium (Smart and Barko)	Picloram	0.00102 µg/L	107	9	5	-
Test medium	Picloram	1.32 µg/L	107	4	5	-



Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
(Smart and Barko)						
Sediment (Artificial soil)	Picloram	0.00700 mg/kg	86	9	5	-
Sediment (Artificial soil)	Picloram	0.300 mg/kg	99	6	5	-

**Table A 6: Procedural recovery results of picloram (m/z 243/170Q) using the analytical method**

Matrix	Analyte	Fortification level	Mean Recovery (%)	RSD (%)	n	Comments
Test medium (Smart and Barko)	Picloram	0.00102 µg/L	75	14	3	-
Test medium (Smart and Barko)	Picloram	1.32 µg/L	91	10	6	-
Sediment (Artificial soil)	Picloram	0.00700 mg/kg	105	3	3	-
Sediment (Artificial soil)	Picloram	0.300 mg/kg	106	5	3	-

**Table A 7: Characteristics for the analytical method used for validation of halauxifen-methyl residues in test medium**

	Halauxifen-methyl
Specificity	m/z 345/250Q m/z 345/235C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ≥0.995 minimum of five data points
Calibration range	Concentration range of 0.00006 ng/mL to 0.0015 ng/mL, corresponding to 0.0000600 µg/L to 0.00150 µg/L
Limit of determination/quantification	LOQ=0.000212 µg/L

**Table A 8: Characteristics for the analytical method used for validation of halauxifen-methyl residues in sediment**

	Halauxifen-methyl
Specificity	m/z 345/250Q m/z 345/235C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r ≥0.995 minimum of five data points
Calibration range	Concentration range of 0.05 ng/mL to 1 ng/mL, corresponding to 0.000210 mg/kg to 0.00420 mg/kg
Limit of determination/quantification	LOQ=0.000700 mg/kg

**Table A 9: Characteristics for the analytical method used for validation of aminopyralid residues in test medium**

	Aminopyralid
Specificity	<i>m/z</i> 207/134Q <i>m/z</i> 207/161C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ minimum of five data points
Calibration range	Concentration range of 0.01 ng/mL to 1 ng/mL, corresponding to 0.0002 µg/L to 0.0200 µg/L
Limit of determination/quantification	LOQ=0.000656 µg/L

**Table A 10: Characteristics for the analytical method used for validation of aminopyralid residues in sediment**

	Aminopyralid
Specificity	<i>m/z</i> 207/134Q <i>m/z</i> 207/161C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ minimum of five data points
Calibration range	Concentration range of 0.25 ng/mL to 2.5 ng/mL, corresponding to 0.00205 mg/kg to 0.0205 mg/kg
Limit of determination/quantification	LOQ=0.00700 mg/kg

**Table A 7: Characteristics for the analytical method used for validation of picloram residues in test medium**

	Picloram
Specificity	<i>m/z</i> 243/170Q <i>m/z</i> 243/143C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ minimum of five data points
Calibration range	Concentration range of 0.015 ng/mL to 1.5 ng/mL, corresponding to 0.000300 µg/L to 0.0300 µg/L
Limit of determination/quantification	LOQ=0.00102 µg/L

**Table A 8: Characteristics for the analytical method used for validation of picloram residues in sediment**

	Picloram
Specificity	<i>m/z</i> 243/170Q <i>m/z</i> 243/143C blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.995$ minimum of five data points

Calibration range	Concentration range of 0.25 ng/mL to 2.5 ng/mL, corresponding to 0.00205 mg/kg to 0.0205 mg/kg
Limit of determination/quantification	LOQ=0.00700 mg/kg

## CONCLUSION

The methods were successfully validated for the determination of halauxifen-methyl, aminopyralid and picloram in test medium and sediment.

### A 2.1.1.2 Description of analytical methods for the determination of residues in plant matrices (CA 6.6.2)

#### A 2.1.1.2.1 Analytical Method 1

Comments of zRMS:	<p>The method validation study was conducted to determine the recovery levels and the precision of the method (when using positive electrospray ionisation LC-MS/MS) for the determination of residues of <b>picloram</b> in wheat (whole plant, grain and straw), turnip (roots and tops, including leaves) and kale (leaves). The efficiency of the analytical method was determined at the time of validation for each set of samples by fortifying aliquots of the appropriate control crop matrix with picloram and analysing the samples for recovery. Unfortified control matrix and a reagent blank were included in each sample set. Fortified recovery samples were analysed over a sample concentration range of 0.01-0.1 mg/kg for all matrices. The validated limit of quantification of the method was 0.01mg/kg. In all cases the mean recovery at each fortification level for each of the sample sets was between 70% and 110% and the relative standard deviation was less than 20%. The results from this evaluation support validation of the Dow AgroSciences method 120610 as in ABC report 68930, for the determination of picloram in wheat (whole plant, grain and straw), turnip (roots and tops, including leaves) and kale (leaves) according to SANCO/3029/99 rev. 4. Therefore, it is concluded that this method is suitable for use in analysis of picloram in wheat (whole plant, grain and straw), turnip (roots and tops, including leaves) and kale (leaves) generated in this study.</p> <p>Additionally the analytical method 120612 was validated for the determination of residues of picloram in soil matrix. Fortifications were performed at the level of 0.001 mg/kg, 0.01 mg/kg and 0.02 mg/kg. In the soil analytical phase S14-01962-L2 of this study specimens of soil were analysed for residues of picloram with a LOQ of 0.001 mg/kg. Single recoveries were in the range of 60-120% each, while the mean recoveries at each fortification level were in the range of 70-110%. The relative standard deviation was <math>\leq 20\%</math> for each level for all combinations of matrix types and analytes.</p> <p>With regard to selectivity, accuracy and precision, the analytical methods were validated in accordance with SANCO/3029/99 rev. 4.</p> <p>The study is acceptable.</p>
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Method Identifier No.:	120610, 120612
Performing Laboratory:	Eurofins Agroscience Services Ltd., Slade Lane, Wilson, Melbourne, Derbyshire DE73 8AG, UK
Reference:	CA 6.6.2
Report:	White, T.; 2019; Determination of Residues of Picloram in Rotational Crops (Wheat, Turnip and Kale) After One Application of GF-224 to Bare Soil at Two Sites in Northern Europe and Two Sites Southern Europe 2014 – 2017; Eurofins Agroscience Services Ltd, Slade Lane, Wilson, Melbourne, Derbyshire DE73 8AG, UK; Lab Study No. S14-01962; DAS Study No. 140651; 15 March 2019; Unpublished
Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	No

GLP: Yes  
Acceptability: Yes  
Method Alterations: No

## MATERIALS AND METHODS

### Method Principle

Residues of picloram were extracted from wheat, kale, and turnip samples by homogenizing and shaking with an organic solution and were allowed to settle overnight. An aqueous solution was added to an aliquot of the sample. The organic portion of the sample was then removed under a gently stream of nitrogen. An organic solvent was then added to perform a liquid-liquid extraction. After centrifugation, the organic phase from the sample was transferred to a reversed phase polymeric solid-phase extraction. Following elution with an organic solvent from the SPE cartridge, the sample was dried down with nitrogen and reconstituted with an aqueous solution. The sample was then filtered and analyzed with liquid chromatography with negative-ion electrospray ionization tandem mass spectrometry (LC-MS/MS).

Residues of picloram were extracted from soil with an organic solvent. An aliquot of the extract was evaporated and reconstituted with an aqueous solution. Samples were purified using reversed phase polymeric solid phase extraction. Following elution, the samples were evaporated, reconstituted, and filtered prior to injection and analysis by liquid chromatography 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 1: Procedural recovery results for picloram ( $m/z$  241.0/196.8) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat whole plant	picloram	0.01	86	5.9	5	
Wheat whole plant	picloram	0.1	87	8.6	5	
Wheat grain	picloram	0.01	95	2.8	5	
Wheat grain	picloram	0.1	91	5.4	5	
Wheat straw	picloram	0.01	74	13.4	5	
Wheat straw	picloram	0.1	80	12.7	5	
Turnip roots	picloram	0.01	89	8.2	7	
Turnip roots	picloram	0.1	88	13.1	7	
Turnip tops, including leaves	picloram	0.01	98	7.3	9	
Turnip tops, including leaves	picloram	0.1	95	8.8	9	
Kale leaves	picloram	0.01	101	7.9	5	
Kale leaves	picloram	0.1	99	8.5	5	

**Table A 42: Recovery results from procedural recoveries of Picloram ( $m/z$  239/195) using the soil analytical method**

Matrix	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Soil	0.001	90	18	9	
Soil	0.01	78	8	14	

**Table A 3: Characteristics for the analytical method used for the determination of picloram residues in wheat, turnip, and kales samples.**

	Picloram
Specificity	<i>m/z</i> 241.0/196.8 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ min. 5 data points
Calibration range	Concentration range of 0.6 - 60 ng/mL (equivalent to 0.003 – 3.0 mg/kg)
Limit of determination/quantification	LOQ= 0.01 mg/kg

**Table A 4 Characteristics for the analytical method used for the determination of picloram residues in soil samples.**

	Picloram
Specificity	<i>m/z</i> 239/195 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ min. 5 data points
Calibration range	Concentration range of 0.4-100 ng/mL, (equivalent to 0.00011-0.0267 mg/kg)
Limit of determination/quantification	LOQ= 0.001 mg/kg

## CONCLUSION

The methods were considered acceptable for the determination of picloram in soil, wheat, turnip, and kale samples.

### A 2.1.1.2.2 Analytical Method 2

Comments of zRMS:	<p>The analytical method using liquid chromatography with negative-ion or positive-ion electrospray ionisation (ESI) with tandem mass spectrometry (LC-MS/MS) has been validated and reported in Dow AgroSciences study no. 120610 / ABC study no. 68930. The limit of detection (LOD) and limit of quantitation (LOQ) for <b>picloram</b> in all matrices (wheat grain and straw) were 0.003 mg/kg and 0.01 mg/kg, respectively.</p> <p>To verify method performance in terms of recovery efficiency during analysis of each set, subsamples of untreated field samples were fortified at the method LOQ and at a higher rate 0.1 mg/kg, as well as at the LOD for qualitative assessment of detectability. Concurrent fortification recovery results for picloram showed excellent accuracy and consistency. Individual recovery values over all matrices were within the range of 71 to 112% with RSD values ranging from 5.4 to 8.8% within each analyte-matrix-level combination.</p> <p>Recoveries in wheat grain averaged 95% for picloram. Recoveries in wheat straw averaged 85% for picloram.</p> <p>The analytical method was validated in accordance with SANCO/3029/99 rev. 4. The study is acceptable.</p>
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Method Identifier No.: 120610  
Performing Laboratory: Eurofins Agroscience Services Ltd., Derbyshire, UK  
Reference: CA 6.6.2  
Report: White, T.; 2018; Determination of Residues of Picloram in Winter and Spring Wheat Grown as Rotational Crops After One Application of GF-224 to Bare Soil at Eight Sites in Northern Europe and Eight Sites in Southern Europe 2014-2016; Eurofins Agroscience Services Ltd.,

Derbyshire, UK; Lab Study No. S14-01961; DAS Study No. 140642; 08  
November 2018; 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 picloram were extracted from wheat samples by homogenizing and shaking with an organic solution and were allowed to settle overnight. An aqueous solution was added to an aliquot of the sample. The organic portion of the sample was then removed under a gently stream of nitrogen. An organic solvent was then added to perform a liquid-liquid extraction. After centrifugation, the organic phase from the sample was transferred to a reversed phase polymeric solid-phase extraction. Following elution with an organic solvent from the SPE cartridge, the sample was dried down with nitrogen and reconstituted with an aqueous solution. The sample was then filtered and analyzed with liquid chromatography with negative-ion electrospray ionization 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 5: Recovery results from method validation of Picloram (m/z 241/141) using the analytical method**

Matrix	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat grain	0.01	95	6.9	10	
Wheat grain	0.1	95	8.8	10	
Wheat straw	0.01	88	5.4	10	
Wheat straw	0.1	82	8.4	10	

**Table A 6: Characteristics for the analytical method used for validation of Picloram**

	Picloram
Specificity	m/z 241/141 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression r≥0.998 7 data points
Calibration range	Concentration range of 0.6-60 ng/mL, corresponding to 0.003-0.3 mg/kg
Limit of determination/quantification	LOQ=0.01 mg/kg

## CONCLUSION

This method was successfully validated for the determination of picloram in wheat grain and straw.

### A 2.1.1.2.3 Analytical Method 3

Comments of zRMS:	<b>Halauxifen (XDE-729)</b> All samples were analysed for XDE-729 methyl (X11393728) and XDE-729 acid (X11393729) using the analytical method described in Dow AgroSciences Study Number
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	<p>110005, “Determination of Residues of XDE-729 Methyl Ester and XDE-729 Acid in Agricultural Commodities and Wheat Processed Products using Online Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry”. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively.</p> <p>Recoveries in whole plants averaged 87%, in straw 98% and 90% in seeds for halauxifen methyl, and averaged 86% in whole plants, 99% in straw and 100% in seeds, for halauxifen-acid.</p> <p><b>Picloram</b></p> <p>All samples were analysed for picloram using Dow Agrosciences study number 120610, “Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS”. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively.</p> <p>Recoveries in whole plants averaged 91%, in straw 101% and 78% in seeds.</p> <p>The analytical methods were validated in accordance with SANCO/3029/99 rev. 4. The study is acceptable.</p>
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Method Identifier No.:	110005, 120610
Performing Laboratory:	Staphyt, Inchy En Artois, France.
Reference:	CA 6.6.2
Report:	Delmotte, R.; 2016; Magnitude of the Residues of Halauxifen-methyl and Picloram in Oilseed rape (RAC Whole Plant, Seed and Straw), following One Application of GF-3447, Northern and Southern Europe - 2015; Staphyt, 23 Route de Moeuvres, 62860 Inchy En Artois, France; Lab Study No. RDE-15-20345; DAS Study No. 150006; 18 March 2016; Unpublished
Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

## MATERIALS AND METHODS

### Method Principle

All oilseed rape samples (RAC whole plant, seed and straw) were analyzed for halauxifen methyl and halauxifen acid using the analytical method described in Dow AgroSciences Study Number 110005. Residues of halauxifen methyl and halauxifen acid were extracted from samples by homogenizing and shaking with an organic solution. After centrifugation, an aliquot of the sample was transferred to a 96-well plate followed by the addition of an internal standard solution. The sample was concentrated to remove the organic solvent and then reconstituted in an aqueous solution. The sample was then purified using an online reversed phase polymeric solid-phase extraction cartridge coupled with liquid chromatography with positive-ion electrospray ionization tandem mass spectrometry (LC-MS/MS).

All oilseed rape samples (RAC whole plant, seed and straw) were analyzed for picloram using the analytical method described in Dow AgroSciences Study Number 120610. Residues of picloram were extracted from samples by homogenizing and shaking with an organic solution and were allowed to settle overnight. An aqueous solution was added to an aliquot of the sample. The organic portion of the sample was then removed under a gently stream of nitrogen. An organic solvent was then added to perform a liquid-liquid extraction. After centrifugation, the organic phase from the sample was transferred to a reversed phase polymeric solid-phase extraction. Following elution with an organic solvent from the SPE cartridge, the sample was dried down with nitrogen and reconstituted with an aqueous solution. The

sample was then filtered and analyzed with liquid chromatography with negative-ion electrospray ionization 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 1: Procedural recovery results for picloram (*m/z* 190/146) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Seeds	picloram	0.01	80	5.2	9	
Seeds	picloram	0.10	75	4.3	6	
Straw	picloram	0.01	103	12.3	9	
Straw	picloram	0.10	104	3.0	4	
Straw	picloram	0.5	87	9.0	2	
Whole Plants	picloram	0.01	90	10.5	9	
Whole Plants	picloram	0.10	93	11.5	2	
Whole Plants	picloram	1.0	75	2.8	2	
Whole Plants	picloram	2.0	108	2.0	2	

**Table A 2: Procedural recovery results for halauxifen-methyl (*m/z* 344.9/250.1) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Seeds	Halauxifen-methyl	0.01	90	4.9	9	
Seeds	Halauxifen-methyl	0.10	91	11.0	6	
Straw	Halauxifen-methyl	0.01	96	4.3	6	
Straw	Halauxifen-methyl	0.10	100	4.2	4	
Whole Plants	Halauxifen-methyl	0.01	86	4.5	6	
Whole Plants	Halauxifen-methyl	0.10	91	0.8	2	
Whole Plants	Halauxifen-methyl	1.0	87	7.4	2	

**Table A 3: Procedural recovery results for halauxifen acid (*m/z* 330.9/250.0) using the analytical method**

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Seeds	Halauxifen acid	0.01	103	10.2	9	
Seeds	Halauxifen acid	0.10	94	12.6	6	
Straw	Halauxifen acid	0.01	98	7.4	6	
Straw	Halauxifen acid	0.10	101	3.4	4	
Whole Plants	Halauxifen acid	0.01	83	14.6	6	
Whole Plants	Halauxifen acid	0.10	93	4.6	2	
Whole Plants	Halauxifen acid	1.0	87	4.1	2	



**Table A 4:**                      **Characteristics for the analytical method used for the determination of picloram, halauxifen-methyl, and halauxifen acid residues in oilseed rape samples (RAC whole plant, seed, and straw).**

	<b>Picloram</b>	<b>Halauxifen-methyl</b>	<b>Halauxifen acid</b>
Specificity	<i>m/z</i> 190/146 blank value <30% LOQ	<i>m/z</i> 344.9/250.1 blank value <30% LOQ	<i>m/z</i> 330.9/250.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.9965$ min. 5 data points	linear regression analysis with 1/x weighting $r \geq 0.9985$ min. 5 data points	linear regression analysis with 1/x weighting $r \geq 0.9995$ min. 5 data points
Calibration range	Concentration range of 0.5 – 50 ng/mL (equivalent to 0.0025 – 0.25 mg/kg)	Concentration range of 0.075 – 25 ng/mL (equivalent to 0.003 – 5.0 mg/kg)	Concentration range of 0.075 – 25 ng/mL (equivalent to 0.003 – 5.0 mg/kg)
Limit of determination/quantification	LOQ= 0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

## CONCLUSION

The methods were considered acceptable for the determination of picloram, halauxifen-methyl, and halauxifen acid in oilseed rape (RAC whole plant, seed and straw).

## A 2.2 Analytical methods for Picloram

### A 2.2.1 Methods for post-authorization control and monitoring purposes (KCP 5.2)

#### A 2.2.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

##### A 2.2.1.1.1 Analytical method 1

Comments of zRMS:	<p>The method was validated for the determination of residues of clopyralid and <b>picloram</b> in agricultural commodities representative of the four European crop groupings (wheat forage, canola seed, orange fruit, and wheat grain) over the concentration range of 0.010 mg/kg to 1.0 mg/kg with a verification of the limit of detection at 0.003 mg/kg.</p> <p>The average recoveries at each fortification level in each crop matrix group ranged from 70 to 110%. Relative standard deviations at each fortification level were all less than 20%. This study was conducted to fulfill data requirements outlined in the SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.</p> <p>The study is acceptable.</p>
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Reference: 120610

Report Vogl, E.; 2012; Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS; ABC Laboratories, Inc., Columbia, Missouri, USA; Lab Study ID 68930; DAS Study ID 120610; 21 Sep 2012; Unpublished.

Guideline(s): SANCO/3029/99 rev. 4; SANCO 825/00 rev 8.1; OPPTS 860.1340; Dir98-02

Deviations: No

GLP: Yes

Acceptability: Yes

### Method Scope

This method is applicable for the quantitative determination of residues of picloram and clopyralid in agricultural commodities. The method was validated over the concentration range of 0.01 – 1.0 mg/kg with a validated limit of quantitation of 0.01 mg/kg.

### Method Principle

Residues of clopyralid and picloram are 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 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 cleanup for crops is affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the 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).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in agricultural commodities by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram  $m/z$  Q1/Q3 241/197 (quantitation)

## Picloram $m/z$ Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the coefficients of determination ( $r$ ) were greater than or equal to 0.990 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.

### Limits of Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all analytes in all tested matrices.

### Standard Stability

As part of this method validation study, the stability of the fortification solutions and the calibration standards was evaluated over a period of 13 days. The results indicate that clopyralid and picloram fortification solutions prepared in methanol and clopyralid and picloram calibration standard solutions prepared in a 0.1% formic acid:methanol (90:10) solution are stable for at least 13 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram and clopyralid were tested after 12 days of storage under refrigerated conditions and were found to be stable.

### Matrix Effect

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 demonstrate that matrix effects are within  $\pm 20\%$ .

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study as the work was conducted in a separate study.

### Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The results obtained for picloram are summarised in the following table.

Validation Data	Matrix	Fortification	Recovery Rate (%)		RSD	n
		Level (mg/kg)	mean	range	(%)	
Picloram $m/z$ 241/197	Acidic Crop (Oranges)	0.01	80	83-98	7.9	5
		1.0	87	74-92	8.6	5
Picloram $m/z$ 241/197	Dry Crop (Wheat Grain)	0.01	85	81-93	5.5	5
		1.0	89	75-99	11.2	5
Picloram $m/z$ 241/197	Oily Crop (Canola Seed)	0.01	80	71-94	11.7	5
		1.0	83	79-86	3.5	5
Picloram $m/z$ 241/197	Wet Crop (Wheat Forage)	0.01	83	81-87	3.2	5
		1.0	84	80-87	3.4	5

Picloram <i>m/z</i> 239/195	Acidic Crop (Oranges)	0.01	88	81-94	7.1	5
		1.0	84	67-90	11.4	5
Picloram <i>m/z</i> 239/195	Dry Crop (Wheat Grain)	0.01	89	84-94	4.6	5
		1.0	88	77-100	10.5	5
Picloram <i>m/z</i> 239/195	Oily Crop (Canola Seed)	0.01	78	65-90	11.7	5
		1.0	82	77-87	5.3	5
Picloram <i>m/z</i> 239/195	Wet Crop (Wheat Forage)	0.01	89	86-94	3.4	5
		1.0	88	79-94	7.3	5

## Conclusion

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

### A 2.2.1.1.2 Analytical method 2

Comments of zRMS:	<p>This report contains independent laboratory validation data for Dow AgroSciences method 120610, “Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS”.</p> <p>The method was successfully independently validated in oilseed rape seed (a commodity with high oil content) and wheat whole plant (a commodity with high-water content) over the concentration range of 0.01 - 0.1 mg/kg with a verification of the limit of quantification of 0.010 mg/kg.</p> <p>Average recoveries at each fortification level were all within the acceptance range of 70-120%. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the analytes.</p> <p>The study is acceptable.</p>
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Reference: 120614

Report 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”; Battelle UK Ltd, Ongar, Essex, CM5 0GZ, UK; Lab Study ID YR/12/017; DAS Study ID 120614; 12 Oct 2012; Unpublished.

Guideline(s): EC Regulation No. 1107/2009 (21-Oct-09); SANCO 825/00 rev 8.1; OPPTS 860.1340; PR Notice 96-1 and PR Notice 2011-3

Deviations: No

GLP: Yes

Acceptability: Yes

## Method Scope

This method is applicable for the quantitative determination of residues of picloram and clopyralid in agricultural commodities. The method was validated over the concentration range of 0.01 – 0.1 mg/kg with a validated limit of quantitation of 0.01 mg/kg.

## Method Principle

Residues of clopyralid and picloram are extracted from crop samples with methanol/10 N sodium hydroxide (100:1) by blending for approximately 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to stand at room temperature 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 cleanup for crops is performed by partitioning the basic extract with dichloromethane. An aliquot of the extract is acidified with hydrochloric acid and submitted to a solid phase extraction column (Waters HLB) cleanup and elution with dichloromethane. After removal of the dichloromethane using nitrogen blow down, the sample is reconstituted in methanol/0.1% formic acid (10:90). 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).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in agricultural commodities by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram  $m/z$  Q1/Q3 241/197 (quantitation)  
Picloram  $m/z$  Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

Linear regression with 1/x weighting was used to describe the detector response as a function of the standard calibration curve concentrations, and the correlation coefficients (r) were always greater than or equal to 0.995 for all of the calibration curve determinations during the method validation study.

### Limits of Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all analytes in all tested matrices.

### Matrix Effect

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 demonstrate that matrix effects are within  $\pm 20\%$ .

### Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The results obtained for picloram are summarised in the following table.

Validation Data	Matrix	Fortification	Recovery Rate (%)		RSD	n
		Level (mg/kg)	mean	range	(%)	
Picloram $m/z$ 241/197	Wet Crop (Wheat Forage)	0.01	83	77-92	6.7	5
		0.10	85	78-92	7.4	5
Picloram $m/z$ 241/197	Oily Crop (Canola Seed)	0.01	73	72-75	1.8	5
		0.10	76	73-80	3.6	5
Picloram $m/z$ 239/195	Wet Crop (Wheat Forage)	0.01	83	79-87	4.5	5
		0.10	83	77-89	6.0	5
Picloram $m/z$ 239/195	Oily Crop (Canola Seed)	0.01	84	81-90	4.4	5
		0.10	76	73-80	3.9	5

### Conclusion

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

#### A 2.2.1.1.3 Analytical method 3

Comments of zRMS:	<p>The method was validated for the determination of residues of <b>picloram</b> in bovine and poultry matrices (bovine muscle, bovine fat, bovine liver, bovine kidney, bovine milk, poultry muscle, poultry fat, poultry liver, and poultry egg) over the concentration range from the limit of quantitation (0.010 mg/kg) to 100 x the limit of quantitation (1.0 mg/kg), with a limit of detection verification of 0.003 mg/kg.</p> <p>The individual recoveries for all samples ranged from 70 to 110% and the average recoveries at each fortification level in each animal tissue matrix group also fell within the range of 70 to 110%. Relative standard deviations at each fortification level were all less</p>
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	<p>than 20%.</p> <p>This study was conducted to fulfill data requirements outlined in SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.</p> <p>The study is acceptable.</p>
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Reference: 120622

Report Vincent, T.; 2013; Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry by Liquid Chromatography with Tandem Mass Spectrometry Detection; ABC Laboratories Inc., Columbia, Missouri, USA; Lab Study ID 68615; DAS Study ID 120622; 11 Feb 2013; Unpublished.

Guideline(s): SANCO/3029/99 rev. 4; SANCO 825/00 rev 8.1; OPPTS 860.1340; PMRA Dir98-02

Deviations: No

GLP: Yes

Acceptability: Yes

### Method Scope

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

### Method Principle

Residues of picloram are extracted from a 1 gram animal tissue sample by homogenizing and shaking with 20.0 mL of a methanol solution saturated with sodium bicarbonate. After extraction, 1.0 mL of 1 N sodium hydroxide is added to 5.0 mL of the extract and the methanol is evaporated under a gentle stream of nitrogen. The remaining solution is adjusted to 5.0 mL with 1 N sodium hydroxide and heated at approximately 100 °C for approximately 1 hour to hydrolyze the tissue. After hydrolysis, the sample is partitioned with dichloromethane, and 4.0 mL of the aqueous layer is subsequently acidified with 5.0 mL of 1 N hydrochloric acid. The sample is then purified using a polymeric reversed-phase solid-phase extraction (SPE) column. The analyte is eluted with 14 mL of dichloromethane which is then evaporated to dryness. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution, filtered through a 0.2 µm PTFE filter, and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (LC-MS/MS).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in bovine and poultry matrices by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram  $m/z$  Q1/Q3 241/197 (quantitation)  
Picloram  $m/z$  Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of five standards over the concentration range of 0.50-50 ng/mL (or the sample equivalent range of 0.0025-0.250 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999.

### Limits of Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram stock and spiking solutions prepared in methanol and picloram calibration standards prepared in a methanol/0.1% formic acid (10:90) solution are stable for at least 75 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram were tested after 7 days of storage under refrigerated conditions and were found to be stable.

### Matrix Effect

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 demonstrate that matrix effects are within  $\pm 20\%$ .

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study as the work was conducted in a separate study.

## Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The results obtained for picloram are summarised in the following table.

### Summary of quantitative recovery of Picloram (m/z Q1/Q3 241/197)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Bovine Muscle	0.010	83	75-93	6.6	8.0	5
Animal	Bovine Muscle	1.00	89	85-92	2.9	3.3	5
Animal	Bovine Fat	0.010	89	87-93	2.5	2.8	5
Animal	Bovine Fat	1.00	94	90-97	3.0	3.2	5
Animal	Bovine Liver	0.010	84	75-90	5.6	6.7	5
Animal	Bovine Liver	1.00	84	79-91	4.7	5.6	5
Animal	Bovine Kidney	0.010	84	76-93	6.4	7.6	5
Animal	Bovine Kidney	1.00	93	88-98	4.6	4.9	5
Animal	Bovine Milk	0.010	87	79-92	5.4	6.2	5
Animal	Bovine Milk	1.00	87	83-90	2.7	3.1	5
Animal	Poultry Muscle	0.010	85	79-92	5.7	6.7	5
Animal	Poultry Muscle	1.00	90	88-92	1.7	1.8	5
Animal	Poultry Fat	0.010	91	84-95	5.0	5.5	5
Animal	Poultry Fat	1.00	96	94-101	3.0	3.1	5
Animal	Poultry Liver	0.010	77	72-90	7.5	9.7	5
Animal	Poultry Liver	1.00	89	84-93	3.4	3.8	5
Animal	Poultry Eggs	0.010	85	80-91	4.1	4.8	5
Animal	Poultry Eggs	1.00	88	83-94	4.1	4.6	5

### Summary of confirmatory recovery of Picloram (m/z Q1/Q3 239/195)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Bovine Muscle	0.010	83	76-93	6.3	7.5	5
Animal	Bovine Muscle	1.00	89	84-94	3.7	4.1	5

Animal	Bovine Fat	0.010	87	86-89	1.3	1.5	5
Animal	Bovine Fat	1.00	94	91-97	2.8	3.0	5
Animal	Bovine Liver	0.010	78	66-92	11	14	5
Animal	Bovine Liver	1.00	84	79-91	4.4	5.3	5
Animal	Bovine Kidney	0.010	94	66-92	7.0	7.5	5
Animal	Bovine Kidney	1.00	93	88-104	4.1	4.4	5
Animal	Bovine Milk	0.010	78	74-86	4.4	5.6	5
Animal	Bovine Milk	1.00	85	81-89	3.2	3.8	5
Animal	Poultry Muscle	0.010	81	75-86	4.7	5.8	5
Animal	Poultry Muscle	1.00	90	88-92	1.6	1.8	5
Animal	Poultry Fat	0.010	93	87-101	5.3	5.7	5
Animal	Poultry Fat	1.00	96	93-101	3.1	3.3	5
Animal	Poultry Liver	0.010	82	71-90	7.1	8.7	5
Animal	Poultry Liver	1.00	89	85-93	2.8	3.1	5
Animal	Poultry Eggs	0.010	87	82-91	3.5	4.0	5
Animal	Poultry Eggs	1.00	88	84-95	4.1	4.7	5

## Conclusion

Method is acceptable based on current guidelines.

### A 2.2.1.1.4 Analytical method 4

Comments of zRMS:	<p>This report contains independent laboratory validation data for Dow AgroSciences method 120622, “Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection”</p> <p>The method was successfully independently validated in bovine milk, bovine meat and chicken liver over the concentration range of 0.01 - 0.1 mg/kg with a verification of the limit of quantification of 0.010 mg/kg.</p> <p>Average recoveries at each fortification level were all within the acceptance range of 70-120%. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the analytes.</p> <p>The study is acceptable.</p>
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Reference: 120607

Report Austin, R.; 2013; Independent Laboratory Validation of Dow AgroSciences Method 120622, “Method Validation Study for the Determination of Residues of Picloram in Bovine and Poultry by Liquid Chromatography with Tandem Mass Spectrometry Detection”; Battelle UK Ltd., Ongar, Essex, CM5 0GZ, UK; Lab Study ID YR/12/022; DAS Study ID 120607; 28 Feb 2013; Unpublished.

Guideline(s): EC Regulation No. 1107/2009 (21-Oct-09); SANCO 825/00 rev 8.1; OPPTS 860.1340; PR Notice 96-1 and PR Notice 2011-3

Deviations: No

GLP: Yes

Acceptability: Yes

## Method Scope

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

## Method Principle

Residues of picloram are extracted from a 1 gram animal tissue sample by homogenizing and shaking with 20.0 mL of a methanol solution saturated with sodium bicarbonate. After extraction, 1.0 mL of 1 N



sodium hydroxide is added to 5.0 mL of the extract and the methanol is evaporated under a gentle stream of nitrogen. The remaining solution is adjusted to 5.0 mL with 1 N sodium hydroxide and heated at approximately 100 °C for approximately 1 hour to hydrolyze the tissue. After hydrolysis, the sample is partitioned with dichloromethane, and 4.0 mL of the aqueous layer is subsequently acidified with 5.0 mL of 1 N hydrochloric acid. The sample is then purified using a polymeric reversed-phase solid-phase extraction (SPE) column. The analyte is eluted with 14 mL of dichloromethane which is then evaporated to dryness. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution, filtered through a 0.2 µm PTFE filter, and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (LC-MS/MS).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in bovine and poultry matrices by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram  $m/z$  Q1/Q3 241/197 (quantitation)

Picloram  $m/z$  Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of five standards over the concentration range of 0.50-50 ng/mL (or the sample equivalent range of 0.0025-0.250 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999.

### Limits of Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram stock and spiking solutions prepared in methanol and picloram calibration standards prepared in a methanol/0.1% formic acid (10:90) solution are stable for at least 29 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram in bovine milk were tested after 12 days of storage under refrigerated conditions and were found to be stable. Sample extracts of picloram in bovine meat and chicken liver were tested after 12 days of storage under refrigerated conditions and were found to be stable.

### Matrix Effect

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 demonstrate that matrix effects are within ±20%.

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study as the work was conducted in a separate study.

## Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The results obtained for picloram are summarised in the following table.

### Summary of quantitative recovery of Picloram ((m/z Q1/Q3 241/197))

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Bovine Meat	0.010	91	87-98	4.10	4.5	5
Animal	Bovine Meat	1.00	99	94-102	3.17	3.2	5
Animal	Bovine Milk	0.010	97	93-101	3.20	3.3	5
Animal	Bovine Milk	1.00	94	91-96	1.97	2.1	5
Animal	Poultry Liver	0.010	80	76-84	3.04	3.8	5
Animal	Poultry Liver	1.00	91	90-92	0.82	0.9	5

### Summary of confirmatory recovery of Picloram (m/z Q1/Q3 239/195)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Bovine Meat	0.010	89	83-98	5.70	6.4	5
Animal	Bovine Meat	1.00	100	96-102	2.40	2.4	5
Animal	Bovine Milk	0.010	97	91-101	4.46	4.6	5
Animal	Bovine Milk	1.00	92	91-94	1.47	1.6	5
Animal	Poultry Liver	0.010	84	80-86	2.35	2.8	5
Animal	Poultry Liver	1.00	91	90-92	0.73	0.8	5

## Conclusion

Independent laboratory validation is acceptable based on current guidelines.

### A 2.2.1.1.5 Analytical method 5

Comments of zRMS:	<p>The method was validated for the determination of residues of <b>picloram</b> in loamy sand, sandy clay loam, loam, and silt loam soil, per USDA Soil Class (equivalent to loamy sand, sandy clay, clay loam, and clay loam, respectively, per International Soil Class) over the concentration range from the limit of quantitation (0.50 µg/kg) to 2000x the limit of quantitation (1000 µg/kg) with a limit of detection verification of 0.15 µg/kg.</p> <p>The average picloram recoveries at each fortification level in each soil matrix group ranged 70 to 120%, with the exception of the loamy sand at 0.50 µg/kg (69%). The average picloram recoveries for all fortification levels in each soil matrix group fell within the range of 70 to 120%. Relative standard deviations at each fortification level were all less than 20%.</p> <p>This study was conducted to fulfill data requirements outlined in SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.</p> <p>The study is acceptable.</p>
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Reference: 120612

Report Vincent, T.; 2013; Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS; ABC Laboratories Inc., Columbia, Missouri, USA; Lab Study ID 68931; DAS Study ID 120612; 20 Feb 2013; Unpublished.

Guideline(s): SANCO/3029/99 rev. 4; SANCO 825/00 rev 8.1; OPPTS 850-6100; PMRA Dir98-02

Deviations: No

GLP: Yes

Acceptability: Yes

## Method Scope

This method is applicable for the quantitative determination of residues of picloram and clopyralid in soil. The method was validated over the concentration range of 0.5 - 1000 µg/kg with a validated limit of quantitation of 0.5 µg/kg.

### Method Principle

Residues of clopyralid and picloram are extracted from soil samples by adding 25 mL of acetone:1N hydrochloric acid (90:10) then shaking and centrifuging, followed by 10 mL of additional acetone:1N hydrochloric acid (90:10) and further shaking and centrifuging. The acetone is then evaporated using nitrogen and brought to 8 mL final volume with 1N sodium hydroxide before vortexing and sonication. Approximately 8 mL of dichloromethane is added, with sonication, vortexing, and centrifuging to mix well, and the upper 6 mL extract layer is transferred to a clean glass tube and 6 mL of 1N hydrochloric acid is added. The sample is then passed through a pre-conditioned Waters HLB solid phase extraction (SPE) column. The sample bottle is then rinsed with 1N hydrochloric acid which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle steam of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution 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).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in soil by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram	<i>m/z</i> Q1/Q3 241/197 (quantitation)
Picloram	<i>m/z</i> Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of ten standards over the concentration range of 0.40-50 ng/mL (or the sample equivalent range of 0.11-13 µg/kg) demonstrated linearity with correlation coefficients (*r*) of at least 0.9969 for picloram.

### Limits of 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 0.5 µg/kg for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram stock and spiking solutions prepared in methanol are stable for at least 75 days and picloram calibration standards prepared in a methanol/0.1% formic acid (10:90) solution are stable for at least 24 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram were tested after 7 days of storage under refrigerated conditions and were found to be stable.

## Matrix Effect

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 demonstrate that matrix effects are within  $\pm 20\%$ .

## Extraction Efficiency

Extraction efficiency was not assessed as a part of this study as the work was conducted in a separate study.

## Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ), with the exception of the 0.5  $\mu\text{g/kg}$  level in soil (484) for picloram in both the quantitative and confirmatory transitions. However, the 0.5  $\mu\text{g/kg}$  picloram average values in soil (484) were slightly below the 70% level and had a low RSD; therefore, the results were deemed acceptable. The results obtained for picloram are summarised in the following table.

**Summary of quantitative recovery of picloram (m/z 241/197)**

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		( $\mu\text{g/kg}$ )	mean	range	(%)	(%)	
Soil	Soil (484)	0.50	69	64-75	4.6	6.7	5
Soil	Soil (484)	1000	75	73-77	1.7	2.2	5
Soil	Soil (485)	0.50	87	77-93	6.5	7.4	5
Soil	Soil (485)	1000	85	79-89	3.7	4.3	5
Soil	Soil (498)	0.50	91	73-118	16.7	18.3	5
Soil	Soil (498)	1000	94	84-104	7.9	8.4	5
Soil	Soil (508)	0.50	85	75-103	11.3	13.3	5
Soil	Soil (508)	1000	80	65-97	11.9	14.8	5

**Summary of confirmatory recovery of picloram (m/z 239/195)**

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		( $\mu\text{g/kg}$ )	mean	range	(%)	(%)	
Soil	Soil (484)	0.50	65	61-69	3.1	4.8	5
Soil	Soil (484)	1000	75	73-78	2.2	3.0	5
Soil	Soil (485)	0.50	80	74-86	4.6	5.7	5
Soil	Soil (485)	1000	85	80-89	3.5	4.2	5
Soil	Soil (498)	0.50	85	67-116	20.2	23.9	5
Soil	Soil (498)	1000	94	85-104	7.4	7.9	5
Soil	Soil (508)	0.50	85	66-99	15.2	17.8	5
Soil	Soil (508)	1000	81	66-98	12.0	14.8	5

## Conclusion

Method is acceptable based on current guidelines.

### A 2.2.1.1.6 Analytical method 6

Comments of zRMS:	<p>The method was validated for the determination of residues of <b>picloram</b> in drinking water, ground water, and surface water over the concentration range of 0.050 <math>\mu\text{g/L}</math> to 10.0 <math>\mu\text{g/L}</math> with a verification of the limit of detection of 0.015 <math>\mu\text{g/L}</math>.</p> <p>The limit of quantitation was 0.05 <math>\mu\text{g/L}</math>.</p> <p>Average recoveries at each fortification level were all within the acceptance range of 70-120%. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the matrix.</p> <p>The study is acceptable.</p>
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Reference: 120611

Report 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; ABC Laboratories Inc., Columbia, Missouri,

	USA; Lab Study ID 68631; DAS Study ID 120611; 04 Dec 2012; Unpublished.
Guideline(s):	SANCO/3029/99 rev. 4; SANCO 825/00 rev 8.1; OPPTS 850.6100; PMRA Dir98-02
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Method Scope

This method is applicable for the quantitative determination of residues of picloram and clopyralid in drinking water, ground water, and surface water. The method was validated over the concentration range of 0.05 - 10 µg/L with a validated limit of quantitation of 0.05 µg/L.

### Method Principle

Residues of clopyralid and picloram are extracted from water samples by passing 100 mL of water through a pre-conditioned Waters HLB solid phase extraction (SPE) column after adjusting the pH to below 2 with 1N HCl. The sample bottle is then rinsed with 1N HCl which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle stream of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution 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).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in water by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram	<i>m/z</i> Q1/Q3 241/197 (quantitation)
Picloram	<i>m/z</i> Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of five standards over the concentration range of 1.0-50.0 ng/mL (or the sample equivalent range of 0.010-0.50 µg/L) demonstrated linearity with correlation coefficients (r) of at least 0.9995.

### Limits of 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 0.05 µg/L for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram stock and spiking solutions prepared in methanol are stable for at least 23 days and picloram calibration standards prepared in a methanol/0.1% formic acid (10:90) solution are stable for at least 24 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram were tested after 15 days of storage under refrigerated conditions and were found to be stable.

### Matrix Effect

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 demonstrate that matrix effects are within  $\pm 20\%$ .

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

### Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The results obtained for picloram are summarised in the following table.

#### Summary of quantitative recovery of picloram (m/z 241/197)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		( $\mu\text{g/L}$ )	mean	range	(%)	(%)	
Water	Ground water	0.050	100	94-105	3.9	3.9	5
Water	Ground water	10	99	96-101	2.0	2.0	5
Water	Drinking water	0.050	101	96-105	3.6	3.6	5
Water	Drinking water	10	98	96-101	2.2	2.2	5
Water	Surface water	0.050	93	85-102	6.5	7.0	5
Water	Surface water	10	92	81-100	7.3	7.9	5

#### Summary of confirmatory recovery of picloram (m/z 239/195)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		( $\mu\text{g/L}$ )	mean	range	(%)	(%)	
Water	Ground water	0.050	98	95-100	2.1	2.1	5
Water	Ground water	10	98	94-102	3.3	3.3	5
Water	Drinking water	0.050	99	96-100	1.7	1.7	5
Water	Drinking water	10	98	95-101	2.5	2.5	5
Water	Surface water	0.050	91	86-100	5.7	6.2	5
Water	Surface water	10	91	80-97	6.6	7.2	5

### Conclusion

Method is acceptable based on current guidelines.

#### A 2.2.1.1.7 Analytical method 7

Comments of zRMS:	<p>This report contains independent laboratory validation data for 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".</p> <p>The method was successfully independently validated in drinking water, ground water and surface water over the concentration range of 0.05 - 0.5 <math>\mu\text{g/L}</math> with a verification of the limit of quantification of 0.050 <math>\mu\text{g/L}</math>.</p> <p>Average recoveries at each fortification level were all within the acceptance range of 70-120%. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the matrix.</p> <p>This study was conducted to fulfill data requirements outlined in SANCO/825/00 rev.8.1.</p> <p>The study is acceptable.</p>
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Reference: 120613

Report	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”; Battelle UK Ltd., Ongar, Essex, CM5 0GZ, UK; Lab Study ID YR/12/023; DAS Study ID 120613; 05 Apr 2013; Unpublished.
Guideline(s):	SANCO/825/00 rev. 8.1, (16-Nov-10), EPA Guideline; OCSPP 850.6100, PR Notice 96-1 and PR Notice 2011-3
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Method Scope

This method is applicable for the quantitative determination of residues of picloram and clopyralid in drinking water, ground water, and surface water. The method was validated over the concentration range of 0.05 – 0.5 µg/L with a validated limit of quantitation of 0.05 µg/L.

### Method Principle

Residues of clopyralid and picloram are extracted from water samples by passing 100 mL of water through a pre-conditioned Waters HLB solid phase extraction (SPE) column after adjusting the pH to below 2 with 1N HCl. The sample bottle is then rinsed with 1N HCl which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle stream of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution 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).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in water by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram	<i>m/z</i> Q1/Q3 241/197 (quantitation)
Picloram	<i>m/z</i> Q1/Q3 239/195 (confirmation)

### Confirmation

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

### Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of five standards over the concentration range of 1.0-50 ng/mL (or the sample equivalent range of 0.01-0.5 µg/L) demonstrated linearity with correlation coefficients (*r*) of at least 0.996.

### Limits of 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 0.05 µg/L for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram stock and spiking solutions prepared in methanol and calibration standards prepared in a methanol/0.1% formic acid (10:90) solution are stable for at least 29 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram in surface water, drinking water, and ground water were tested after 8, 12, and 14 days of storage, respectively, under refrigerated conditions and were found to be stable.

### Matrix Effect

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 demonstrate that matrix effects are within ±20%.

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

## Results and discussions

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

### Summary of quantitative recovery of Picloram (m/z 241/197)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(µg/L)	mean	range	(%)	(%)	
Water	Drinking Water	0.05	100	99-101	0.8	0.8	5
Water	Drinking Water	0.5	99	96-102	2.2	2.2	5
Water	Ground Water	0.05	89	84-95	4.7	5.3	5
Water	Ground Water	0.5	96	93-100	3.0	3.2	5
Water	Surface Water	0.05	97	92-100	3.8	4.0	5
Water	Surface Water	0.5	97	95-100	1.8	1.8	5

### Summary of confirmatory recovery of Picloram (m/z 239/195)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(µg/L)	mean	range	(%)	(%)	
Water	Drinking Water	0.05	97	93-101	4.1	4.2	5
Water	Drinking Water	0.5	99	98-99	0.5	0.6	5
Water	Ground Water	0.05	94	90-100	3.9	4.2	5
Water	Ground Water	0.5	97	92-99	2.8	2.9	5
Water	Surface Water	0.05	95	93-99	2.3	2.4	5
Water	Surface Water	0.5	92	88-93	2.1	2.3	5

## Conclusion

Method is acceptable based on current guidelines.

### A 2.2.1.1.8 Analytical method 8

Comments of zRMS:	<p>The method was successfully developed and validated for the determination of residues of <b>picloram</b> in ambient as well as warm and humid air with a limit of quantitation (LOQ) of approximately 9 µg/m<sup>3</sup>. Final determination of picloram was performed by LC-MS/MS, using the transition 239 m/z =&gt; 195 m/z as the primary transition of the analyte for quantification and the transition 241 m/z =&gt; 197 m/z as the secondary transition for confirmation of the presence of the analyte.</p> <p>Average recoveries at each fortification level ranged between 70% and 120%, with relative standard deviations of ≤20% for both LC-MS/MS transitions. The method was demonstrated to be applicable for use in the determination of picloram in air.</p>
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	This study was conducted to fulfill data requirements outlined in SANCO/3029/99 rev. 4 and SANCO/825/00 rev.8.1. The study is acceptable.
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Reference: 120603

Report Bacher, R.; 2012; The Development and Validation of a Method for the Analysis of Picloram in Air; PTRL Europe GmbH, Ulm, Germany; Lab Study ID P 2581 G; DAS Study ID 120603; 12 Nov 2012; Unpublished.

Guideline(s): EC Regulation No. 1107/2009 (21-Oct-09) repealing Directive 91/414/EEC; SANCO/825/00 rev. 8.1 (16/11/10); SANCO/3029/99 rev. 4 (11/07/2000).

Deviations: No

GLP: Yes

Acceptability: Yes

### Method Scope

This method is applicable for the quantitative determination of residues of picloram in air at ambient temperature and normal humidity conditions, as well as under warm, high humid air conditions. The method was validated over the approximate concentration range of 9 – 900 µg/m<sup>3</sup> with an approximate validated limit of quantitation of 9 µg/m<sup>3</sup>.

### Method Principle

After sampling of air (6 hours), the front and the back adsorbent portions of the adsorption material were separated and both sections were extracted separately 1 three times, each time with 3 mL of acetonitrile. The three extracts from the front portion were combined, and the volumes were adjusted to 10 mL with acetonitrile. Extracts obtained from recoveries fortified at 100xLOQ (front portion) were further diluted by a factor of 100 using acetonitrile/water (1/9). Combined extracts of the blank control, LOQ, and 100xLOQ from the back portion of the tubes used to check for breakthrough were diluted by a factor of 10 using acetonitrile/water (1/9). The sample were analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS-MS).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in air by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram *m/z* Q1/Q3 241/197 (confirmation)  
Picloram *m/z* Q1/Q3 239/195 (quantitative)

### Confirmation

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

### Linearity

For analysis of picloram by LC-MS/MS, calibration functions were established by injecting calibration solutions in neat solvent at ≥ 6 different concentration levels in a range from 5.0 to 500 ng/mL. Calibration functions were calculated by linear regression calculation, applying "1/x" weighting. Correlation coefficients (r) for all calibration curves were always > 0.99.

### Limits of 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 approximately 9 µg/m<sup>3</sup> for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram calibration standards prepared in an acetonitrile/water (10:90) solution are stable for at least 10 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram were tested after 4 days of storage under refrigerated conditions and were found to be stable.

### Extraction Efficiency

Extraction efficiency as well as the storage stability of picloram when adsorbed onto the XAD material and in extracts were both examined by additional experiments and observed to be acceptable.

### Results and discussions

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

#### Summary of quantitative recovery of Picloram (m/z 239/195)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(µg/m <sup>3</sup> )	mean	range	(%)	(%)	
Air	Warm, humid	3.0	84	77-96	7	9	5
Air	Warm, humid	300	104	92-112	8	8	5
Air	Ambient	3.0	75	73-75	2	2	5
Air	Ambient	300	74	70-77	2	3	5

#### Summary of confirmatory recovery of Picloram (m/z 241/197)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(µg/m <sup>3</sup> )	mean	range	(%)	(%)	
Water	Warm, humid	3.0	83	75-94	7	8	5
Water	Warm, humid	300	103	91-110	8	8	5
Water	Ambient	3.0	73	71-75	3	3	5
Water	Ambient	300	72	68-75	3	4	5

### Conclusion

Method is acceptable based on current guidelines.

#### A 2.2.1.1.9 Analytical method 9

Comments of zRMS:	<p>The method was successfully developed and validated for the determination of residues of <b>picloram and aminopyralid</b> in body fluids (human blood plasma and urine) with a limit of quantitation (LOQ) of 0.05 mg/L.</p> <p>The average recoveries for the two parent-daughter ion transitions monitored were within the acceptable ranges of 70% to 110% with relative standard deviations (RSD) of ≤ 20% for all analytes.</p> <p>A summary of the recovery results for aminopyralid is given below:</p>
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Validation Data	Matrix	Fortification (mg/L)	Recovery		SD	RSD	n
			mean	range			
Aminopyralid 207 m/z -> 161 m/z	Urine	0.05	86%	80%-90%	4%	4%	5
		5.0	91%	88%-95%	2%	3%	5
		0.05 and 5.0	89%	80%-95%	4%	4%	10
	Blood Plasma	0.05	87%	78%-96%	7%	8%	5
		5.0	89%	79%-94%	6%	7%	5
		0.05 and 5.0	88%	78%-96%	6%	7%	10
Aminopyralid 207 m/z -> 189 m/z	Urine	0.05	83%	77%-95%	7%	8%	5
		5.0	91%	86%-95%	3%	4%	5
		0.05 and 5.0	87%	77%-95%	7%	8%	10
	Blood Plasma	0.05	87%	78%-99%	8%	10%	5
		5.0	89%	79%-95%	6%	7%	5
		0.05 and 5.0	88%	78%-99%	7%	8%	10
SD: standard deviation; RSD: relative standard deviation; n: number of replicates.							

Average recoveries at each fortification level ranged between 70% and 120%, with relative standard deviations of  $\leq 20\%$  for both LC-MS/MS transitions. The method was demonstrated to be applicable for use in the determination of picloram and aminopyralid in body fluids (human blood plasma and urine)

It is concluded that the applied residue method fulfils all guideline criteria of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4.

The study is acceptable.

Reference: 160866

Report Schmiedt, S., Senciuc, M.; 2016; Development and Validation of a Method for the Analysis of Picloram, Aminopyralid, and Triclopyr (All Free Acids) in Body Fluids; EAG Laboratories, PTRL Europe, Ulm, Germany; Lab Study ID P 4065 G; DAS Study ID 160866; 17 Oct 2016; Unpublished.

Guideline(s): SANCO/825/00 rev. 8.1, (16-Nov-10), EPA Guideline; OPPTS 860.1340, Dir 98-02

Deviations: No

GLP: Yes

Acceptability: Yes

### Method Scope

This method is applicable for the quantitative determination of residues of picloram, aminopyralid, and triclopyr in body fluids. The method was validated over the concentration range of 0.05 - 5 mg/L with a validated limit of quantitation of 0.05 mg/L.

### Method Principle

Residues of picloram, aminopyralid and triclopyr are extracted from human blood plasma or urine by using a QuEChERS-like extraction using acidified acetonitrile and modified salt mixture. After centrifugation no further clean-up procedure was necessary and an aliquot was diluted by adding internal standards (isotopically labelled) in acetonitrile and water. The final sample is analysed for picloram, aminopyralid and triclopyr by liquid chromatography coupled with polarity switching electrospray ionization tandem mass spectrometry (LC-MS/MS).

### Specificity/Selectivity

The method is highly selective for the determination of picloram in body fluids by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, additional confirmatory ion transitions can be monitored as follows:

Picloram	m/z Q1/Q3 243/197 (quantitative)
Picloram	m/z Q1/Q3 241/195 (confirmatory)

Picloram IS (m+3)

m/z Q1/Q3 248/202 (internal standard)

### Confirmation

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

### Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of at least five standards over the concentration range of 0.10-20 ng/mL (or the sample equivalent range of 0.01-2.0 mg/L) containing 5.0 ng/mL (0.50 mg/L) of each internal standard demonstrated linearity with correlation coefficients (r) of at least 0.999.

### Limits of 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 0.05 mg/L for all analytes in all tested matrices.

### Standard Stability

The stability of picloram was tested and the results indicate that picloram stock prepared in methanol are stable for at least 15 days, fortification solutions prepared in acetonitrile are stable for at least 12 days, and calibration standards prepared in an acetonitrile/water (2/8) containing 0.1% formic acid solution are stable for at least 12 days when stored under refrigerated conditions.

### Extract Stability

Sample extracts of picloram in were tested after 8 days (urine) and 12 days (blood plasma) of storage under refrigerated conditions and were found to be stable.

### Matrix Effect

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 demonstrate that matrix effects are within  $\pm 20\%$ .

### Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

### Results and discussions

Results obtained were within guideline requirements (mean recovery 70-110%;  $RSD \leq 20\%$ ). The results obtained for picloram are summarised in the following table.

#### Summary of quantitative recovery of Picloram (m/z 243/197)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/L)	mean	range	(%)	(%)	
Body fluids	Urine	0.05	97%	95%-99%	2%	2%	5
Body fluids	Urine	5.0	100%	97%-102%	2%	2%	5
Body fluids	Blood plasma	0.05	87%	78%-95%	7%	8%	5
Body fluids	Blood plasma	5.0	96%	89%-101%	5%	5%	5

#### Summary of confirmatory recovery of Picloram(m/z 241/195)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/L)	mean	range	(%)	(%)	
Body fluids	Urine	0.05	91%	89%-93%	2%	2%	5
Body fluids	Urine	5.0	100%	97%-104%	3%	3%	5
Body fluids	Blood plasma	0.05	89%	78%-96%	8%	8%	5
Body fluids	Blood plasma	5.0	95%	89%-100%	4%	4%	5

## Conclusion

Method is acceptable based on current guidelines.