

# REGISTRATION REPORT

## Part B

### Section 5

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: ADM.3304.H.1.A

Product name: Tricera

Chemical active substance(s):

2,4-D, 375 g/L (562.5 g/L as 2,4-D EHE)

Clopyralid, 30 g/L

Fluroxypyr, 75 g/L (108 g/L as Fluroxypyr-meptyl)

Central Zone

Zonal Rapporteur Member State: Poland

#### CORE ASSESSMENT

(composition change)

Sponsor: ADAMA Agan Ltd.

Applicant: Country organisation / representative of ADAMA,  
as given in Part A

Submission date: February 2021

MS Finalisation date: May 2022 (initial Core Assessment)

November 2022, updated December 2022 (final Core Assessment)

### Version history

When	What
February 2021	dRR Part B – Section 5; version 1 submitted by applicant
May 2022	Initial zRMS assessment (with regard to the proposed composition change). The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency.
November 2022	Final report (Core Assessment updated following the commenting period). No additional information or assessments after the commenting period.
December 2022	Final report (Core Assessment updated following the Applicant's comments). No additional information or assessments after the commenting period.

## **DATA PROTECTION CLAIM**

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A

## **STATEMENT FOR OWNERSHIP**

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## Introduction

### **General remark:**

The product Tricera (ADM.3044.H.1.A) is a herbicide containing the active substance 2,4-D (as the ester variant 2,4-D EHE).

In the dossier below information is presented for the acid form –that will be referred as “2,4-D”- as well as for the ester form (that will be referred as “2,4-D EHE”).

This document reviews the analytical methods for the plant protection product ADM.3304.H.1.A containing the active substances 2,4-D, Clopyralid and Fluroxypyr.

**2,4-D** was reviewed as part of the renewal of approval procedure by the Member States and the Commission and the Commission review report finalised on 13.11.2015 approved 2,4-D in accordance with Regulation (EC) No. 1107/2009 (Regulation 2015/2033).

**Clopyralid** was included into Annex I of Directive 91/414/EEC according to Commission Regulation (EC) No 451/2000 (renewal of inclusion of the second and third group of active substances in Annex I, see Commission Directive 2006/64/EC of 18 July 2006, Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 that replaced the Directive 2006/64/EC after the application of Regulation 1107/2009, and Commission Implementing Regulation (EU) No 2019/168 of 31 January 2019 that fixes the new expiry date of approval to 30/04/2020.

Commission Implementing Regulation (EU) 2021/566 extended the approval period of clopyralid to 30 April 2022 in order to allow the renewal process to be completed before the expiry of the approval period of that active substance.

Clopyralid was evaluated in accordance with Regulation (EC) No 1107/2009 and Commission Implementing Regulation (EU) No 844/2012 following the submission of an application to renew the approval of this active substance expiring in April 2022. The approval of the active substance clopyralid is renewed as set out in Annex I of Commission Implementing Regulation (EU) 2021/1191 of 19 July 2021.

Date of approval - 1 October 2021

Expiration of approval - 30 September 2036

**Applicant provided LoA from Dow AgroSciences for clopyralid: access to Dow AgroSciences data on active substance clopyralid for registration of pesticide AG-CDF1-480 EC and ADM.3044.H.1.A.**

**Fluroxypyr** was included into Annex I of Directive 91/414/EEC according to Commission Regulation (EC) No 736/2011 (renewal of inclusion of the first group of active substances in Annex I).

However, all the relevant information about this last approval are indicated in Review report for active substance Fluroxypyr (SANCO/111019/201, 17 June 2011), as was evaluated within the assessment of active substance Fluroxypyr.

Where appropriate this document refers to the conclusions of the EU review or the Draft Assessment Report (DAR) of the active substances. This will be where:

- the active substance data is relied upon in the risk assessment of the formulation; *or when*
- the EU review or DAR concluded that additional data/information should be considered at national re-registration.

Note: this Part B document only reviews data (Annex II or Annex III) (Chemical Active or Chemical Product) and additional information that has not previously been considered within the EU review process, as part of the Annex I inclusion decision. New annex II (Chemical active) data have only be included if they were considered essential for the evaluation and in this case a full study summary was be provided. In the case where the formulation has been previously evaluated, at European level, detailed summaries have not been provided.

This product was not the representative formulation. The product has not been previously evaluated according to Uniform Principles.

The EFSA Report of 2,4-D (EFSA Journal 2014;12(9):3812) that was updated on 21<sup>st</sup> March 2017, the EFSA report of Clopyralid (EFSA Scientific Report (2005) 50, 1–65, ) and the EFSA Report of Fluroxypyr (EFSA Journal 2011;9(3):2091) are considered to provide the relevant review information or a reference to where such information can be found.

For the information on 2,4-D EHE, please refer to the Bridging dossier (2018) prepared by the RMS for the a.i. (Greece).

The following table provided the EU endpoint to be used in the evaluation.

#### Agreed EU Endpoints

Endpoint	2,4-D		Clopyralid		Fluroxypyr*	
	EU agreed endpoint	Endpoint used*	EU agreed endpoint (EFSA Scientific Report (2005) 50, 1–65, )	Endpoint used	EU agreed endpoint (EFSA Journal 2011;9(3):2091)	Endpoint used*
Purity of active substance	≥ 920 g/kg* (2,4-D EHE)  ≥ 970 g/kg (2,4-D Acid)	≥ 940 g/kg	≥ 950 g/kg	≥ 950g/kg	≥ 950 g/kg	≥ 985 g/kg

\*ADAMA Agan Ltd. have their own sources of 2,4-D EHE and Fluroxypyr-meptyl which have been judged as being equivalent to the respective notified reference sources.

\*\* Based on FAO specification of 2,4-D EHE

The Annex I Inclusion Directives for the active substances **2,4-D** (Commission Directive 2001/103/EC) gives specific provisions under Part B which need to be considered by the applicant in the preparation of their submission prior to granting an authorisation.

For the implementation of the uniform principles of Regulation (EC) 546/2011, the conclusions of the review report on **2,4-D**, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 28. May 2015 shall be taken into account. In this overall assessment:

Member States must pay particular attention to the:

- *Risk to aquatic organisms, terrestrial organisms and consumers in cases of uses above 750 g/ha.*

The Annex I Inclusion Directives for the active substances **Clopyralid** (Commission Directive 2006/64/CE) gives specific provisions under Part B which need to be considered by the applicant in the preparation of their submission prior to granting an authorisation.

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on the active substance Clopyralid, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 04. April 2006 shall be taken into account. In this overall assessment member states should pay particular attention to:

- The protection of non target plants and groundwater under vulnerable conditions. Conditions of authorisation should include risk mitigation measures and monitoring programmes should be initiated to verify potential groundwater contamination in vulnerable zones, where appropriate.

The Annex I Inclusion Directives for the active substances **Clopyralid** (COMMISSION IMPLEMENTING REGULATION (EU) 2021/1191 of 19 July 2021) gives specific provisions under Part B which need to be considered by the applicant in the preparation of their submission prior to granting an authorisation.

For the implementation of the uniform principles, as referred to in Article 29(6) of Regulation (EC) No 1107/2009, the conclusions of the renewal report on clopyralid, and in particular Appendices I and II thereto, shall be taken into account. In this overall assessment Member States shall pay particular attention to:

- the specification of the technical material as commercially manufactured;
- the protection of operators, ensuring that conditions of use for operators include the application of adequate personal protective equipment;
- possible presence of clopyralid residues in rotational crops;
- the possible transfer of clopyralid residues via compost or manure of animals whose feed originates from treated areas, to avoid damage to susceptible crops;
- the protection of groundwater under vulnerable conditions. Conditions of use shall include risk mitigation measures, where appropriate. The applicant shall submit to the Commission, the Member States and the Authority confirmatory information as regards the effect of water treatment processes on the nature of residues present in drinking water. The applicant shall submit this information within two years after adoption of a guidance document on evaluation of the effect of water treatment processes on the nature of residues present in surface and groundwater.

**Fluroxypyr** (Commission Implementing Regulation (EU) No 736/2011) gives specific provisions under Part B which need to be considered by the applicant in the preparation of their submission prior to granting an authorisation.

For the implementation of the uniform principles, as referred to in Article 29(6) of Regulation (EC) No 1107/2009, the conclusions of the review report on **Fluroxypyr**, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 17 June 2011 shall be taken into account.

- Only uses as herbicides may be authorised.

In this overall assessment Member States shall pay particular attention to:

- The potential contamination of groundwater by metabolite Fluroxypyr Pyridinol, when the active substance is applied in regions with alkaline or vulnerable soil and/or with vulnerable climatic conditions.
- The risk to aquatic organisms.

These concerns have, where relevant, been addressed within the current submission in the respective sections.

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 of this document contains the new data of the active substances present in AG-CDF1-480 EC in this section.

Information on the detailed composition of AG-CDF1-480 EC can be found in the confidential dossier of this submission (Registration Report - Part C).

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## 5 Analytical methods

This document summarises the analytical methods on the plant protection product ADM.3304.H.1.A (EC formulation containing 375 g/L 2,4-D (562.5 g/L as 2,4-D EHE), 30 g/L Clopyralid, 75 g/L Fluroxypyr). The dossier follows the data requirements of

- Regulation (EC) No. 544/2011 for the active substance Clopyralid,
- Regulation (EC) No. 283/2013 for the active substances 2,4-D and Fluroxypyr
- Regulation (EC) No. 284/2013 for the plant protection product ADM.3304.H.1.A.

Deviations from this is justified where relevant.

Please note that only new information and data are summarized hereafter. The studies, which were submitted with the previous dossier are not resubmitted.

### 5.1 Conclusion and summary of assessment

#### **zRMS comments:**

Please refer to the assessment prepared by zRMS-PL for AG-CDF1-480 EC (Tricera, May 2022).

Sufficiently sensitive and selective analytical methods are available for the active substances 2,4-D (and its ester variant), Clopyralid and Fluroxypyr and the 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:

According to the assessment provided by zRMS-PL for AG-CDF1-480 EC (Tricera, May 2022) the following data gaps were included in the dRR:

#### **2,4-D**

According to the EFSA Journal 2014;12(9):3812:

- Further data on the hydrolysis step and extraction efficiency for the animal and plant analytical methods

According to the SANTE/2020/12830, Rev.1, 24. February 2021:

- Analytical methods for monitoring residues in body fluids and tissues with the LOQ of 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues.

#### **Clopyralid**

According to the EFSA Journal 2018;16(8):5389:

- Verification of the efficiency of the extraction procedures used in monitoring methods for animal products
- Analytical method used in the developmental toxicity study in rats

According to the SANTE/2020/12830, Rev.1, 24. February 2021:

- Analytical methods for monitoring residues in body fluids and tissues with the LOQ of 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues.

#### **Fluroxypyr**

According to the SANTE/2020/12830, Rev.1, 24. February 2021:

- An Independent laboratory validation (ILV) for drinking water or ground water.

This application was submitted in October 2019 in Poland. Applicant updated the dRR in May 2022. In our opinion, these data gaps should be filled as part of the product re-authorization procedure or as part of post-registration requirement.



Commodity/crop	Supported/ Not supported
Spring wheat	Supported
Spring barley	Supported
Oats	Supported
Winter wheat	Supported
Winter barley	Supported
Winter triticale	Supported
Winter rye	Supported
Grassland	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)

Comments of zRMS:	The analytical method was successfully validated for the determination of 2,4-D (and 2,4-D EHE), Clopyralid and Fluroxypyr in Tricera formulation according to the requirements laid down by SANCO/3030/99 rev.5.
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An overview on the acceptable methods and possible data gaps for analysis of active substances, 2,4-D (and 2,4-D EHE), Clopyralid and Fluroxypyr in plant protection product is provided as follows:

Reference:	KCP 5.1.1/01, Tsesin, N. (2020)
Report	Determination of Storage Stability and Physical-Chemical Properties of Tricera (ADM.03304.H.1.A) Stored at 54 °C for 14 Days and 0 °C for 7 Days
Report No.	000106540.072FL
Document No.	000106540
Guideline(s):	Commission Regulation (EU) No 284/2013 SANCO/3030/99 rev.5, 22 March 2019
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Executive summary

The analysis was done high performance liquid chromatograph (HPLC) with UV detection using external standard technique. The HPLC method, used to quantify the active ingredients in ADM.3304.H.1.A was fully validated. For the confirmation of the identity of the active substances was performed by LC-MS.

### Materials and methods

**Material:** One representative sample of the plant protection product ADM.3304.H.1.A manufactured (Batch no.: **N6002P2/1**) was used for the study.  
External standards:  
- **Clopyralid** (CAS: 1702-17-6; batch No.: 264-3598, purity: 97.2 %, supplier: ADAMA Agan Ltd.).  
- **2,4-D EHE** (CAS: 1928-43-4; batch No.: 289-3843, purity: 97.8 %, supplier: ADAMA Agan Ltd.).  
- **Fluroxypyr-meptyl** (CAS: 81406-37-3; batch No.: 138-2312, purity: 99.4 %, supplier: ADAMA Agan Ltd.).

#### Analytical methods: 2,4-D + Clopyralid + Fluroxypyr

The content of the active ingredients (2,4-D, Clopyralid and Fluroxypyr) was determined by HPLC-UV diode array method.

HPLC system Shimadzu is equipped with UV detector, automatic injector and with Class-VP Chromatographic data system software.

About 200 mg of the formulation were weighted into a 50 mL volumetric flask. About 30 mL acetonitrile with 0.1 % TFA were added as a solvent and solutions were mixed well. Acetonitrile with 0.1 % TFA was added up to the mark and solutions were mixed well again. The solutions were analysed by injection of a 5 µL aliquots of these solution into the HPLC/DAD to quantification of active ingredients. The calibration standard solutions were injected in the same sequence.

#### HPLC-MS/MS conditions:

HPLC system: Agilent 1260 equipped with an autosampler, column oven and degasser  
Column: YMC-triart PFP, 600 bar, 150 mm × 4.6 mm, 3 µm  
Column temp.: 30 °C  
Detector: Diode Array Detector  
Mobile phase: A: 0.1 M TFA in acetonitrile  
B: 0.1 M TFA in water

Time [min]	% A	% B	Flow [mL/min]
0.0	15	85	1.0
3.0	15	85	1.0
30.0	70	30	1.0

32.0	95	5	1.0
35.0	95	5	1.0

Injection volume: 5 µL  
Wavelength: Clopyralid: 230 nm  
Fluroxypyr-meptyl: 230 nm  
2,4-D 2-EHE: 285 nm  
Retention time: Clopyralid: ~ 8.8 min  
Fluroxypyr-meptyl: ~ 30 min  
2,4-D 2-EHE: ~ 31.8 min

**Results:** The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table 5.2.1.1-04** and the following text.

**Conclusions:** The method was validated according to guideline SANCO/3030/99 rev.5 with regard to specificity, linearity of detector response, accuracy and precision for 2,4-D, Clopyralid and Fluroxypyr in ADM.3304.H.1.A and is considered acceptable.

## Validation - Results and discussions

### *Specificity*

The specificity of the method was checked by comparing the chromatograms obtained from the analysis of Tricera formulation batch before and after accelerated storage with the one of the matrix (formulation) blank samples and with the chromatograms of the solutions of Clopyralid, Fluroxypyr-meptyl and 2,4-D 2-EHE under method analysis conditions.

The chromatograms of the matrix blank, solvent blank and active ingredients standard samples are presented in the report. The figures show that the blanks chromatograms and each active ingredient chromatograms do not contain any interfering peak at the retention times corresponding to other active ingredients. It can be concluded that the analytical method is specific for the each of active ingredients determination in Tricera formulation product.

### *Linearity*

The linearity for active ingredients was teste in linear range covering at least  $\pm 20$  % of analyte nominal concentration (4.0 mg/mL for formulation) studied.

Six different solutions containing various concentrations of each Clopyralid, Fluroxypyr-meptyl and 2,4-D 2-EHE standards were prepared separately. About 30 mL acetonitrile with 0.1 % TFA were added as a solvent and solutions were mixed well (sonicated). After solutions reached room temperature, acetonitrile with 0.1 % TFA was added up to the mark and solutions were mixed well again and injected into the HPLC.

Clopyralid:

$$y = 11386.5493 x + 16.9651, R^2 = 0.9999$$

Fluroxypyr-meptyl:

$$y = 7335.1755 x + 62.1724, R^2 = 0.9998$$

2,4-D 2-EHE:

$$y = 1526.5967 x + 11.6478, R^2 = 1.0000$$

The resulting linearity curves have correlation coefficients  $R^2 > 0.99$  (as required by SANCO/3030/99 rev. 5, 22 March 2019) indicating that each of the active ingredient are linear in the range of interest.

### *Repeatability (precision)*

In the order to determine the repeatability of the analytical method for active ingredients determination of five weightings, about 200 mg each, of Tricera formulation product were made into separate 50 mL volumetric flasks. Acetonitrile with 0.1 % TFA was added as a solvent and solutions were mixed well (sonicated). These solutions were injected into the HPLC, under conditions described previously. The relative standard deviation of the area per weight response (RF) and Horrat ratio obtained for active ingredients from these injections, where taken as the indication of analytical method repeatability.

Two repeatability assays were performed on two different days. The relative standard deviation of the RF

and Horrat ratio obtained for active ingredients from 10 injections from two assays was taken as the indication of analytical method intermediate precision.

The obtained repeatability RSD values are less than the threshold values, 2.31 % for Clopyralid, 1.90 % for Fluroxypyr-meptyl and 1.48 % for 2,4-D 2-EHE concentrations according to SANCO guidelines.

Horrat ratio,  $H_r$ , (where  $H_r = \% \text{RSD} / \% \text{RSD}_r$ ) are less than 1 for all active ingredients and for both repeatability analyses. Therefore, it can be concluded that the analytical method has a good repeatability (precision).

#### **Recovery (accuracy)**

To three sets of two matrix blank samples containing appropriate amount of each material, Clopyralid, Fluroxypyr-meptyl and 2,4-D 2-EHE standards were added at maximal, medium and minimal concentration levels in final solutions. A blank, containing about 70 mg matrix blank without standard addition, was prepared and used to obtain an indication of the contribution of the active ingredients content in the sample to the overall peak area. Acetonitrile with 0.1 % TFA was used as the solvent in sample preparation. Prepared samples were assayed for active ingredients content, under analytical conditions described above using external standard solutions.

**Table 5.2.1.1-01: Accuracy (recovery) of 2,4-D EHE in formulated product ADM.3304.H.1.A**

Analyte	Fortification level [mg/mL]	Spiked level	Recoveries			
			Single values [%]	n	Mean [%]	RSD* [%]
ADM.3304.H.1.A	2.1852	120 %	100.61, 100.43, 100.40, 100.33	4	100	0.12
	1.9950	100 %	100.43, 100.43, 100.71, 100.29	4	100	0.17
	1.6471	80 %	100.48, 100.33, 100.42, 100.25	4	100	0.10

\* RSD: Relative Standard Deviation

**Table 5.2.1.1-02: Accuracy (recovery) of Clopyralid in formulated product ADM.3304.H.1.A**

Analyte	Fortification level [mg/mL]	Spiked level	Recoveries			
			Single values [%]	n	Mean [%]	RSD* [%]
ADM.3304.H.1.A	0.1415	120 %	99.49, 99.38, 99.41, 99.23	4	99	0.11
	0.1182	100 %	99.43, 99.61, 99.43, 99.28	4	99	0.14
	0.0797	80 %	99.55, 99.46, 99.64, 99.42	4	100	0.10

\* RSD: Relative Standard Deviation

**Table 5.2.1.1-03: Accuracy (recovery) of Fluroxypyr-meptyl in formulated product ADM.3304.H.1.A**

Analyte	Fortification level [mg/mL]	Spiked level	Recoveries			
			Single values [%]	n	Mean [%]	RSD* [%]
ADM.3304.H.1.A	0.5694	120 %	99.96, 99.84, 99.83, 99.76	4	100	0.08
	0.3960	100 %	101.23, 101.22, 101.54, 101.15	4	101	0.17
	0.3157	80 %	101.10, 100.90, 101.03, 100.84	4	101	0.12

\* RSD: Relative Standard Deviation

**Table 5.2.1.1-04: Methods suitable for the determination of active substances 2,4-D, Clopyralid and Fluroxypyr-meptyl in plant protection product ADM.3304.H.1.A**

	2,4-D EHE	Clopyralid	Fluroxypyr-meptyl
Author(s), year	Tsesin, N. (2020)	Tsesin, N. (2020)	Tsesin, N. (2020)

		<b>2,4-D EHE</b>	<b>Clopyralid</b>	<b>Fluroxypyr-meptyl</b>
<b>Principle of method</b>		HPLC- UV/DAD	HPLC- UV/DAD	HPLC- UV/DAD
<b>Linearity</b> (linear between mg/L / % range of the declared con- tent) (correlation coefficient)		$y = 1526.5967 x + 11.6478$ $R^2 = 1.0000$ (range: 1.2 mg/mL to 3.2 mg/mL)	$y = 11386.549 x + 16.9651$ $R^2 = 0.9999$ (range: 0.06 mg/mL to 0.16 mg/mL)	$y = 7335.1755 x + 62.1724$ $R^2 = 0.9999$ (range: 0.25 mg/mL to 0.70 mg/mL)
<b>Precision – System Re- peatability Mean</b> [% RSD] $H_r$		RSD = 0.06 % $H_r = 0.04$	RSD = 0.31 % $H_r = 0.13$	RSD = 0.04 % $H_r = 0.02$
<b>Accuracy</b>	<b>120 %</b>	100 ± 0.12	99 ± 0.11	100 ± 0.08
	<b>100 %</b>	100 ± 0.17	99 ± 0.14	101 ± 0.11
	<b>80 %</b>	100 ± 0.10	100 ± 0.10	101 ± 0.12
<b>Interference/ Specificity</b>		Specific method, no interference	Specific method, no interference	Specific method, no interference

## Conclusion

The HPLC method, used to quantify the active ingredients in ADM.3304.H.1.A was fully validated.

Tsesin, N. (2020)

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

No new information compared to the previously submitted dossier is presented. The tests were performed with a comparable composition.

For more details please refer to Part C – business confidential information.

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

None of the formulants is of toxicological, environmental or ecotoxicological relevance within the formulation ADM.3304.H.1.A. Therefore, no analytical method is required.

### 5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

For technical 2,4-D a CIPAC method is available:

Acid content (CIPAC method 1/TC/M3/5.2); Esters (CIPAC 1.3/EC/m2/-).

For Clopyralid and Fluroxypyr no CIPAC methods are available.

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

An overview on the new acceptable methods and possible data gaps for analysis of residues of 2,4-D, Clopyralid and Fluroxypyr and for the generation of pre-authorization data is given in **Table 5.2.2-01** to **Table 5.2.2-03**. For the detailed evaluation of new studies it is referred to Appendix 2.

**Table 5.2.2-01: Validated methods for the generation of pre-authorization data of 2,4-D**

<b>Component of residue definition:</b> <b>Please refer to the respective matrix below for the residue definition</b>				
<b>Matrix type</b>	<b>Method type</b>	<b>Method LOQ</b>	<b>Principle of method (i.e. GC-MS or HPLC-UV)</b>	<b>Author(s), year / missing / EU agreed</b>
Soil, water,... (Ecotoxicology)	Growth Inhibition of <i>Myriophyllum</i>	0.158 µg/L (test medium)	HPLC-MS/MS	Eser, S. (2019) Report No: S19-03357 (Sponsor ID: 000102708)  KCP 5.1.2/01
	(2,4-D)	0.005 mg/kg (sediment)		
	Growth Inhibition of <i>Myriophyllum</i>	0.158 µg/L (test medium)	HPLC-GC/MS	
	(2,4-D-EHE)	0.05 mg/kg (sediment)		

**Table 5.2.2-02: Validated methods for the generation of pre-authorization data of Clopyralid**

<b>Component of residue definition:</b> <b>Please refer to the respective matrix below for the residue definition</b>				
<b>Matrix type</b>	<b>Method type</b>	<b>Method LOQ</b>	<b>Principle of method (i.e. GC-MS or HPLC-UV)</b>	<b>Author(s), year / missing / EU agreed</b>
Soil, water,... (Ecotoxicology)	Growth Inhibition of <i>Myriophyllum</i>	0.0422 µg/L (test medium)	HPLC-MS/MS	Eser, S. (2019) Report No: S19-03357 (Sponsor ID: 000102708)  KCP 5.1.2/01
	(Clopyralid)	0.005 mg/kg (sediment)		
	Sedling Emergence and Seedling Growth	2.83 mg/L	HPLC-MS/MS	Duffner, A.. (2019a) Report No.: S19-03358 (Sponsor ID: 000102902)  KCP 5.1.2/02
	(Clopyralid)			
	Vegetative vigour	2.83 mg/L	HPLC-MS/MS	Duffner, A.. (2019b) Report No.: S19-03359 (Sponsor ID: 000102903)  KCP 5.1.2/03
	(Clopyralid)			

**Table 5.2.2-03: Validated methods for the generation of pre-authorization data of Fluroxypyr**

Component of residue definition: Fluroxypyr (Fluroxypyr, its esters, salts and its conjugates expressed as Fluroxypyr)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Soil, water,... (Ecotoxicology)	Growth Inhibition of <i>Myriophyllum spicatum</i> (Fluroxypyr)	0.0302 µg/L (test medium) 0.005 mg/kg (sediment)	HPLC-MS/MS	Eser, S. (2019) Report No: S19-03357 (Sponsor ID: 000102708)  KCP 5.1.2/01
	Growth Inhibition of <i>Myriophyllum spicatum</i> (Fluroxypyr-meptyl)	0.0316 µg/L (test medium) 0.005 mg/kg (sediment)	HPLC-MS/MS	
	Sedling Emergence and Seedling Growth (Fluroxypyr-meptyl)	10.1 mg/L	HPLC-MS/MS	Duffner, A.. (2019a) Report No.: S19-03358 (Sponsor ID: 000102902)  KCP 5.1.2/02
	Vegetative vigour (Fluroxypyr-meptyl)	10.1 mg/L	HPLC-MS/MS	Duffner, A.. (2019b) Report No.: S19-03359 (Sponsor ID: 000102903)  KCP 5.1.2/03

For the old studies, please refer to original dRR, previously submitted to the zRMS Poland.

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

No new information compared to the previously submitted dossier is presented. All relevant information was already submitted to the zRMS Poland previously.

#### **zRMS comments:**

Please refer to the assessment prepared by zRMS-PL for AG-CDF1-480 EC (Tricera, May 2022).

## Appendix 1 Lists of data considered in support of the evaluation

### List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner*
KCP 5.1.1/01	Tsesin, N.	2020	Determination of Storage Stability and Physical-Chemical Properties of Tricera (ADM.03304.H.1.A) Stored at 54 °C for 14 Days and at 0 °C for 7 Days Registration Laboratory & Research and Development Division ADAMA Agan Ltd., Israel Report No.: 000106540.072FL Sponsor No.: 000106540 GLP: yes Published: no	N	ADM
KCP 5.1.2/01	Eser, S.	2019	ADM.3304.H.1.A: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agrosience Services EcoChem GmbH, Niefern, Germany Report No.: S19-03357 Sponsor No.: 000102708 GLP: yes Published: no <b>Submitted in KCP 10.2.1/06</b>	N	ADM
KCP 5.1.2/02	Duffner, A.	2019a	ADM.3304.H.1.A: Effects on the Seedling Emergence and Seedling Growth of Non.Target Terrestrial Plant Species under Greenhouse Conditions Report No.: S19-03358 Sponsor No.: 000102902 GLP: yes Published: no <b>Submitted in KCP 10.6.2/04</b>	N	ADM
KCP 5.1.2/03	Duffner, A.	2019b	ADM.3304.H.1.A: Effects on the Vegetative Vigour of Non-Target Terrestrial Plant Species under Greenhouse Conditions Report No.: S19-03359 Sponsor No.: 000102903 GLP: yes Published: no <b>Submitted in KCP 10.6.2/03</b>	N	ADM

\* ADM: ADAMA Agan Ltd.



**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>
-	-	-	-	-	-

**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 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>
-	-	-	-	-	-

## **Appendix 2 Detailed evaluation of submitted analytical methods**

### **A 2.1 Analytical methods for 2,4-D**

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

The studies have been evaluated in the renewal of the active substance (AIR), for more detail please refer to Final addendum RAR Volume 3 CA, Section B-5 of 2,4-D (Greece, 2014).

##### **A 2.1.1.1 Determination of active substance and/or variant in the plant protection product**

The study summary is given above (chapter 5.2.1.1, Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)).

##### **A 2.1.1.2 Description of analytical methods for the determination of relevant impurities**

2,4-D EHE as well as 2,4-D acid and Fluroxypyr have relevant impurities. The study summaries are given above (chapter 5.2.1.2, Description of analytical methods for the determination of relevant impurities (KCP 5.1.1))

For more details please refer to dRR Part C - Confidential Information under point 5.1.1/03 (evaluation report on the equivalence of technical material for the active 2,4-D 2-EHE) and further information on Fluroxypyr impurities.

##### **A 2.1.1.3 Methods for the determination of residues in soil, water and non-target organisms**

The recent studies to support the residues, e-fate and ecotoxicological sections have been evaluated in the renewal of the active substance (AIR), for more detail refer to Final addendum RAR Volume 3 CA, Section B9 of 2,4-D (Greece, 2014).

New studies to support residues section are described below, not previously evaluated in a peer reviewed process at EU level:

Comments of zRMS:	Analytical methods for the determination of 2,4-D ester, 2,4-D acid, fluroxypyr-MHE, fluroxypyr acid and clopyralid in test medium and sediment were validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The following limits of detection (LOQ) of the methods were confirmed in test medium and sediment:
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Analyte	LOQ in Test Medium Samples	LOQ in Sediment Samples
2,4-D Ester	0.158 µg/L (0.298 µg/L of test item)	0.0500 mg/kg
2,4-D Acid	0.158 µg/L	0.00500 mg/kg
Fluroxypyr-MHE	0.0316 µg/L	0.00500 mg/kg
Fluroxypyr Acid	0.0302 µg/L	0.00500 mg/kg
Clopyralid	0.0422 µg/L (1.49 µg/L of test item)	0.00500 mg/kg

The limit of detection LOD was set at 30 % of the LOQ:

Analyte	LOD in Test Medium Samples	LOD in Sediment Samples
2,4-D Ester	0.0474 µg/L	0.0150 mg/kg
2,4-D Acid	0.0474 µg/L	0.00150 mg/kg
Fluroxypyr-MHE	0.00948 µg/L	0.00150 mg/kg
Fluroxypyr Acid	0.00906 µg/L	0.00150 mg/kg
Clopyralid	0.0127 µg/L	0.00150 mg/kg

The analytical methods fulfil the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.

The study is acceptable.

Reference:	KCP 5.1.2/01, Eser, S. (2019) (submitted in KCP 10.2.1/06)
Report	ADM.3304.H.1.A: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System
Report No.	S19-03357 (Sponsor No: 000102708)
Guideline(s):	OECD 239 and SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Summary

The objective of this study was to quantify the effect of the test item on the growth of the rooted aquatic macrophyte, *Myriophyllum spicatum*. Plants were maintained for 14 days in a static water-sediment system under controlled environment conditions. Measurements of shoot length, shoot fresh weight, shoot dry weight and number and length of side shoots were made at specified intervals. Data was used to determine EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values, and NOEC/LOEC values where possible.

## Materials and methods

### Determination:

**2,4-D 2-EHE:** test medium samples analysis was performed by liquid-liquid extraction with toluene and quantification by GC/MS (two methods were used, M1 and M3).

**Fluroxypyr (free acid) and Fluroxypyr meptyl:** test medium sample analysis was performed by extraction with ethyl acetate and quantification by HPLC-MS/MS.

**2,4-D acid and Clopyralid:** test medium samples analysis was performed by direct injection of test medium samples and quantification by HPLC-MS/MS.

Sediment sample analysis for **2,4-D acid, Fluroxypyr-meptyl, Fluroxypyr acid** and **Clopyralid** was performed by extraction of sediment samples with acetonitrile/water (80:20, v/v) + 2 % formic acid and quantification by HPLC-MS/MS detection.

For **2,4-D ester** the analysis of sediment samples was performed by extraction of sediment samples with acetonitrile/water (80:20, v/v) + 2 % formic acid, further liquid-liquid extraction with toluene and quantification by GC-MS detection (two methods were used, M1 and M3).

**Material:** Test item: ADM.3304.H.1.A (batch No.: N6903-A; Supplier: ADAMA Agan Ltd.)

External standards:

- **Fluroxypyr (free acid)** (CAS No.: 69377-81-7; batch No.: SZBF100XV; purity: 98.9 %; Supplier: Sigma-Aldrich).
- **Fluroxypyr meptyl** (CAS No.: 81406-37-3; batch No.: BCBT2272; purity: 98.5 %; Supplier: Sigma-Aldrich).
- **2,4-D PESTANAL®** (CAS No.: 94-75-7; batch No.: SDHG-014; purity: > 98 %; Supplier: Cambridge Isotope Laboratories, Inc.).
- **2,4-D 2-EHE (2-ethylhexyl ester)** (CAS No.: 1928-43-4; batch No.: G976505; purity: 99.29 %; Supplier: Dr. Ehrenstorfer).
- **Clopyralid PESTANAL®** (CAS No.: 1702-17-6; batch No.: BCBZ5263; purity: 99.3 %; Supplier: Sigma-Aldrich).

### Sample preparation

Test item: Stock solutions (1020 mg/L, 1032 mg/L, 1070 mg/L, 1018 mg/L, purity not considered) and dilutions (100 mg/L, 10 mg/L, 1 mg/L and 0.1 mg/L) were prepared in demineralised water. Further stock solutions (1010 mg/L, 1032 mg/L, 1036 mg/L, purity not considered) and dilutions (100 mg/L, 10 mg/L and 1 mg/L) were prepared in acetonitrile/water (1:1, v/v).

2,4-D ester: stock solutions (1082 mg/L and 1056 mg/L, purity considered) and dilutions (100 mg/L and 10 mg/L) were prepared in acetonitrile. The dilutions were used for fortification of 2,4-D ester sediment recovery samples.

For calibration purpose further stock solutions (1025 mg/L and 1112 mg/L, purity considered) and dilutions (1 mg/L) were prepared in acetonitrile. From the 1 mg/L dilution further calibration solution in test medium blank extract and sediment blank extract were prepared. Further dilutions as appropriate was perform to cover the calibration range.

2,4-D acid: stock solutions (98.3 mg/L in acetonitrile, purity considered) was used for fortification of high recoveries in test medium. For LOQ recoveries in test medium the 98.3 mg/L stock solution was diluted with acetonitrile to a final concentration of 0.1 mg/L. The stock solution and the dilution (1 ng/mL) were used for fortification of 2,4-D acid sediment recovery samples. For calibration purpose a further 1 mg/L dilution was prepared in acetonitrile. Further dilutions as appropriate was perform to cover the calibration range.

Fluroxypyr acid and Fluroxypyr-meptyl: for Fluroxypyr-meptyl stock solutions (1034 mg/L, 1044 mg/L and 1015 mg/L, purity considered) and dilutions (10 mg/L, 1 mg/L and 0.01 mg/L) were prepared in acetonitrile. The 0.01 mg/L dilution was used for fortification of LOQ test medium recovery samples of Fluroxypyr-meptyl.

For Fluroxypyr acid stock solutions (1009 mg/L, 1027 mg/L and 999 mg/L, purity considered) and dilutions ( 100 mg/L, 10 mg/L and 0.1 mg/L) were prepared in acetonitrile + 0.5 % formic acid. The 100 mg/L and 0.1 mg/L dilutions were used for fortification of test medium recovery samples.

For fortification of sediment recovery samples mix standard solutions of the stock solutions Fluroxypyr-meptyl (1034 mg/L), Fluroxypyr acid (1009 mg/L) and Clopyralid (1102 mg/L) were prepared in acetonitrile with the final concentrations 100 mg/L and 1 mg/L each.

For calibration purpose mixed standard solutions with the concentration 1 mg/L (each) were prepared from stock solutions of Fluroxypyr-meptyl (1034 mg/L, 1044 mg/L) and from a stock solution Fluroxypyr acid (999 mg/L) in acetonitrile/water (1:1, v/v). From the 1 mg/L mixed dilution further calibration solutions in test medium blank extract and sediment blank extract were prepared.

Clopyralid: stock solutions (1102 mg/L and 1043 mg/L, purity considered) were prepared in acetonitrile/water (80:20, v/v). A further stock solutions (1003 mg/L) was prepared in acetonitrile. For fortification of sediment recovery samples mix standard solutions with concentrations 100 mg/L and 1 mg/L (each) were prepared in acetonitrile from stock solution of Fluroxypyr-meptyl (1034 mg/L), Fluroxypyr acid (1009 mg/L) and Clopyralid (1102 mg/L).

For calibration purpose dilutions (1 mg/L) were prepared from the stock solutions in acetonitrile/water (80:20, v/v). From the 1 mg/L dilutions further calibration solutions in test medium + 0.5 % acetic acid and sediment blank extract were prepared. For calibration of Clopyralid in test medium the following

dilutions were prepared in test medium + 0.5 % acetic acid.

## Sample preparation

### Analysis of Test medium Samples

- Extraction of 2,4-D 2EHE from test medium samples for analysis with GC-MS (Method M1)  
After sampling, 100 mL of test medium samples were stirred with 2 mL toluene for 30 min on a magnetic stirrer. After phase separation the organic phase was transferred into a 4 mL glass vial and stored deep-frozen ( $\leq -18\text{ }^{\circ}\text{C}$ ) until analysis. On the day of analysis, samples were brought to ambient temperature and further diluted with test medium blank extract (M1), if necessary.  
Non-spiked test medium blank extract for M1 was prepared as described in the report and further used for dilution of recovery samples and preparation of calibration standards.  
Recovery samples were prepared by fortification of untreated test medium (100 mL) with the test item and analysed as described below.
- Extraction of 2,4-D 2EHE from test medium samples for analysis with GC-MS (Method M3)  
After sampling, 50 mL of test medium samples stabilised with 250  $\mu\text{L}$  acetic acid were stored deep-frozen ( $\leq -18\text{ }^{\circ}\text{C}$ ) until analysis. At the analytical laboratory, test medium samples (50 mL + 250  $\mu\text{L}$  acetic acid) were thawed to ambient temperature. The samples were extracted with 25 mL toluene in 100 mL Schott bottles for 30 min on a horizontal flatbed shaker. After phase separation the organic phase is transferred into a 4 mL glass vial and stored deep-frozen ( $\leq -18\text{ }^{\circ}\text{C}$ ) until analysis. On the day of analysis, samples were brought to ambient temperature and further diluted with test medium blank extract (M3), if necessary.  
Non-spiked test medium blank extract for M3 was prepared as described in the report and further used for dilution of recovery samples and preparation of calibration standards.  
For recovery samples, 50 mL of test medium were fortified with test item. 250  $\mu\text{L}$  acetic acid were added and analysed as described below.
- Extraction of Fluroxypyr-meptyl and Fluroxypyr acid in test medium samples for LC-MS/MS analysis  
After sampling, 250  $\mu\text{L}$  acetic acid were added to the medium samples (50 mL) and samples were stored deep-frozen ( $\leq -18\text{ }^{\circ}\text{C}$ ) until analysis. At the analytical laboratory, the samples were thawed to ambient temperature and shaken well using a Vorex-Mixer. Then 0.5 mL phosphoric acid, 18 g sodium chloride (saturation) and 15 mL ethyl acetate were added. Samples were shaken on a horizontal flatbed shaker for 10 min. Samples were extracted twice with 15 mL ethyl acetate. The organic extracts were combined and dried over about 2 g anhydrous sodium sulfate. The organic layer was decanted into a 50 mL round bottom flask and evaporated to dryness using a rotary vacuum evaporator at  $40\text{ }^{\circ}\text{C}$ .  
The residue was reconstituted in 2 mL of methanol/water (1:1, v/v). Aliquots of about 1 mL were transferred into auto sampler glass vials. If necessary, the samples were further diluted with test medium blank extract prior to analysis by HPLC-MS/MS.  
Non-spiked test medium blank extract was prepared as described above and further used for dilution of recovery samples and preparation of calibration solutions.  
For LOQ recovery samples of Fluroxypyr-meptyl, 50 mL of test medium were fortified with the analytical standard of Fluroxypyr-meptyl and stabilised with 250  $\mu\text{L}$  acetic acid and analysed as described below. For recovery samples of Fluroxypyr acid, 50 mL of test medium were fortified with the analytical standard of Fluroxypyr acid, stabilised with 250  $\mu\text{L}$  acetic acid and analysed as described below. If necessary, the samples were further diluted with test medium blank extract prior to analysis by HPLC-MS/MS.
- Preparation of test medium samples for 2,4-D acid LC-MS/MS analysis  
After sampling, 50  $\mu\text{L}$  of acetic acid were added to the test medium samples (10 mL) and stored deep-frozen ( $\leq -18\text{ }^{\circ}\text{C}$ ) until analysis. If the analytical laboratory, test medium samples (10 mL + 50  $\mu\text{L}$  acetic acid) were thawed to ambient temperature and shaken well using a Vortex-Mixer. Aliquots of about 1 mL were transferred into auto sampler glass vials. If necessary, the samples were further diluted with test medium + 0.5 % acetic acid prior to analysis by HPLC-MS/MS.

For recovery samples, 10 mL of test medium were fortified with the analytical standard of 2,4-D acid. 50 µL acetic acid were added and analysed as described below. If necessary, the samples were further diluted with test medium + 0.5 % acetic acid prior to analysis by HPLC-MS/MS.

- Preparation of test medium samples for Clopyralid LC-MS/MS analysis  
After sampling, 50 µL of acetic acid were added to the test medium samples (10 mL) and stored deep-frozen ( $\leq -18^{\circ}\text{C}$ ) until analysis. If the analytical laboratory, test medium samples (10 mL + 50 µL acetic acid) were thawed to ambient temperature and shaken well using a Vortex-Mixer. Aliquots of about 1 mL were transferred into auto sampler glass vials. If necessary, the samples were further diluted with test medium + 0.5 % acetic acid prior to analysis by HPLC-MS/MS.  
For recovery samples, 10 mL, respectively 5 mL of test medium were fortified with test item. 50 µL, respectively 25 µL acetic acid were added and analysed as described above.

#### Analysis of Sediment Samples

- Extraction of 2,4-D 2EHE in Sediment Samples for GC-MS  
Sediment samples were stored deep-frozen ( $\leq -18^{\circ}\text{C}$ ) until analysis. At the day of analysis the samples were thawed to ambient temperature and mixed manually. A 10 g aliquot was transferred into a 250 mL glass bottle and extracted with 100 mL acetonitrile/water (80:20, v/v) + 2 % formic acid on a horizontal flatbed shaker for 2 hours. After sedimentation for about 5 min, a 40 mL aliquot was transferred into a 50 mL PE centrifuge tube and centrifuged for 5 min at 4000 rpm. After centrifugation, 10 mL of the extract were transferred into a 100 mL volumetric flask, diluted with water to 100 mL and stirred with 2 mL of toluene for 20 minutes. After phase separation, an aliquot of the toluene extract was transferred into an autosampler glass vial for GC-MS analysis. If necessary, the samples were further diluted with sediment blank extract prior to analysis by GC-MS.  
Sediment blank extract was prepared of untreated sediment, prepared as describe above. If necessary, the samples were further diluted with sediment blank extract prior to analysis by GC-MS.
- Extraction of 2,4-D 2EHE in Sediment Samples for GC-MS  
Sediment samples were stored deep-frozen ( $\leq -18^{\circ}\text{C}$ ) until analysis. At the day of analysis the samples were thawed to ambient temperature and mixed manually. A 10 g aliquot was transferred into a 250 mL glass bottle and extracted with 100 mL acetonitrile/water (80:20, v/v) + 2 % formic acid on a horizontal flatbed shaker for 2 hours. After sedimentation for about 5 min, a 40 mL aliquot was transferred into a 50 mL PE centrifuge tube and centrifuged for 5 min at 4000 rpm. After centrifugation, 0.5 mL of the clear extract was transferred into an autosampler glass vial.  
Sediment blank extract was prepared of untreated sediment, prepared as described above and used for dilution of recovery samples and preparation of calibration solutions.  
Recovery samples were prepared by fortification of untreated sediment with the analytical standards and analysed as described below. If necessary, the sampels were further diluted with sediment blank extract prior to analysis by HPLC-MS/MS.

#### **Chromatographic conditions**

<b>HPLC-GC/MS conditions for 2,4-D 2-EHE:</b>	HPLC system:	Thermo ISQ-LT GC/MS system with TriPlus RSH
	Column:	Restek Rxi-5Sil MS, 20 m × 0.18 mm, 0.18 µm film thickness with guard column Restek Rxi®, 1 m × 0.25 mm
	Carrier gas:	Helium, constant flow 1.0 mL/min
	Injection volume:	1 µL
	Injection port:	SSL
	Temperature:	150 °C (hold 0.6 min), 35 °C/min to 270 °C, 60 °C/min to 340 °C (hold 1 min)
	Detector:	MS in SIM Mode
	Ionizer:	250 °C
	SIM masses (m/z):	2,4-D 2-EHE: 222 (quantifier); 162 (qualifier 1), 332 (qualifier 2)
	Retention time:	~ 4.3 min
<b>HPLC-GC/MS conditions for</b>	HPLC system:	Thermo ISQ GC/MS system with AS 3000
	Column:	Restek Rxi-5Sil MS, 20 m × 0.18 mm, 0.18 µm film thickness with

## 2,4-D 2-EHE:

guard column Restek Rxi®, 1 m × 0.25 mm  
Carrier gas: Helium, constant flow 1.0 mL/min  
Injection volume: 1 µL  
Injection port: PTV  
Temperature: 150 °C (hold 0.6 min), 35 °C/min to 270 °C, 60 °C/min to 340 °C (hold 1 min)  
Detector: MS in SIM Mode  
Ionizer: 250 °C  
SIM masses (m/z): 2,4-D 2-EHE: 222 (quantifier); 162 (qualifier 1), 332 (qualifier 2)  
Retention time: ~ 4.0 min

## HPLC-MS/MS conditions for 2,4-D:

HPLC system: Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP  
Column: Supelco Ascentis Express C18, 50 mm × 2.1 mm, 2.7 µm with 2.1 mm UHPLC guard column  
Column temp.: 40 °C  
Detector: SCIEX API5500  
Interface: Electrospray ionization (ESI)  
Source polarity: Negative ion mode  
Mobile phase: A: Water + 0.5 % (v/v) formic acid  
B: Methanol

Time [min]	% A	% B	Flow [µL/min]
0.10	80	20	600
2.50	5	95	600
3.00	5	95	600
3.01	80	20	600
4.00	80	20	600
4.01	80	20	600

Injection volume: 30 µL  
Retention time: ~ 1.9 min  
Mass transitions: m/z 219 → 161, CE -10 eV, quantification  
m/z 221 → 163, CE -10 eV, confirmation

## HPLC-MS/MS conditions for Fluroxypyr and Fluroxypyr meptyl:

HPLC system: Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP  
Column: Phenomenex Synergi 4 µ Polar RP 80A, 150 mm × 3 mm, 4 µm with 4 mm fusion RP guard column  
Column temp.: 40 °C  
Detector: SCIEX API 5500  
Interface: Electrospray ionization (ESI)  
Source polarity: Negative/Positive ion mode (4500 V / -4500 V)  
Mobile phase: A: Water + 0.5 % (v/v) formic acid  
B: Methanol + 0.5 % (v/v) formic acid

Time [min]	% A	% B	Flow [µL/min]
0.10	90	10	800
4.50	5	95	800
6.40	5	95	800
6.50	90	10	800
8.50	90	10	800
8.51	90	10	800

Injection volume: 50 µL  
Retention time: Fluroxypyr: ~ 4.3 min  
Fluroxypyr meptyl: ~ 5.6 min  
Mass transitions: Fluroxypyr:  
m/z 255 → 197, CE -19 eV, quantification  
m/z 253 → 195, CE -20 eV, confirmation  
Fluroxypyr-meptyl:  
m/z 367 → 255, CE 16 eV, quantification  
m/z 369 → 257, CE 16 eV, confirmation

## HPLC-MS/MS

HPLC system: Shimadzu LC-30 AD HPLC pump with autosampler SIL-30ACMP

**conditions for Clopyralid:**

Column: Phenomenex Luna 3  $\mu\text{m}$ , 150 mm  $\times$  2 mm, 3  $\mu\text{m}$  with 4 mm guard column  
Column temp.: 40  $^{\circ}\text{C}$   
Detector: SCIEX API 5500  
Interface: Electrospray ionization (ESI)  
Source polarity: Positive (5000 V)  
Mobile phase: A: Water + 0.5% (v/v) formic acid  
B: Methanol + 0.5% (v/v) formic acid

Time [min]	% A	% B	Flow [ $\mu\text{L}/\text{min}$ ]
0.01	90	10	500
1.50	10	90	500
2.50	10	90	500
2.51	90	10	500
4.00	90	10	500
4.01	90	10	500

Injection volume: 50  $\mu\text{L}$   
Retention time:  $\sim$  1.4 min  
Mass transitions: m/z 192  $\rightarrow$  110, CE 13 eV, quantification  
m/z 192  $\rightarrow$  146, CE 13 eV, confirmation

**Results:** The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table A 2.1.1.3-01** to **Table A 2.1.1.3-06**.

**Results and discussions**

**Table A 2.1.1.3-01: Recovery results from method of Fluroxypyr into test medium and sediment**

Matrix	Fortification level	Recovery [%]	Replicates	Mean Recovery [%]	RSD [%]
<b>Mass transition m/z 255 <math>\rightarrow</math> 197 (quantification)</b>					
<b>Test medium</b>	0.0302 $\mu\text{g}/\text{L}$	79, 79, 75, 74, 62	5	74	9
	131 $\mu\text{g}/\text{L}$	82, 86, 81, 89, 83	5	86	6
<b>Sediment</b>	0.005 mg/kg	102, 117, 109, 113, 105	5	109	6
	1.00 mg/kg	115, 113, 108, 107, 109	5	110	3
<b>Mass transition m/z 253 <math>\rightarrow</math> 195 (confirmation)</b>					
<b>Test medium</b>	0.0302 $\mu\text{g}/\text{L}$	109, 109, 117, 93, 92	5	104	11
	131 $\mu\text{g}/\text{L}$	82, 84, 84, 102, 97	5	90	10
<b>Sediment</b>	0.005 mg/kg	101, 110, 105, 118, 108	5	108	6
	1.00 mg/kg	107, 114, 112, 109, 109	5	110	3

RSD: Relative Standard Deviation

**Table A 2.1.1.3-02: Recovery results from method of Fluroxypyr-meptyl into test medium and sediment**

Matrix	Fortification level	Recovery [%]	Replicates	Mean Recovery [%]	RSD [%]
<b>Mass transition m/z 367 <math>\rightarrow</math> 255 (quantification)</b>					
<b>Test medium</b>	0.0316 $\mu\text{g}/\text{L}$	103, 99, 84, 91, 108	5	97	10



Matrix	Fortification level	Recovery [%]	Replicates	Mean Re-covery [%]	RSD [%]
Sediment	1.49 µg/L	95, 85, 104, 97, 87	5	94	5
	1300 µg/L	76, 78, 71, 81, 83	5	78	6
	0.005 mg/kg	107, 111, 110, 112, 111	5	110	2
	1.00 mg/kg	111, 111, 108, 104, 105	5	108	3
Mass transition m/z 369 → 257 (confirmation)					
Test medium	0.0316 µg/L	106, 98, 88, 90, 108	5	98	9
	1.49 µg/L	93, 84, 102, 93, 87	5	92	8
	1300 µg/L	81, 82, 74, 84, 86	5	81	6
Sediment	0.005 mg/kg	105, 111, 104, 111, 110	5	110	3
	1.00 mg/kg	112, 112, 108, 104, 107	5	109	3

RSD: Relative Standard Deviation

**Table A 2.1.1.3-03: Recovery results from method of 2,4-D into test medium and sediment**

Matrix	Fortification level	Recovery [%]	Replicates	Mean Re-covery [%]	RSD [%]
Mass transition m/z 219 → 161 (quantification)					
Test medium	0.158 µg/L	98, 98, 97, 97, 99	5	98	1
	691 µg/L	101, 101, 101, 101, 101	5	101	0
Sediment	0.005 mg/kg	92, 103, 92, 103, 99	5	98	6
	1.00 mg/kg	106, 121, 109, 98, 103	5	107	8
Mass transition m/z 221 → 163 (confirmation)					
Test medium	0.158 µg/L	101, 103, 103, 103, 103	5	103	1
	691 µg/L	100, 99, 100, 102, 100	5	100	1
Sediment	0.005 mg/kg	109, 105, 99, 108, 95	5	103	6
	1.00 mg/kg	114, 121, 109, 101, 105	5	110	7

RSD: Relative Standard Deviation

**Table A 2.1.1.3-04: Recovery results from method of 2,4-D 2EHE into test medium and sediment**

Matrix	Fortification level	Recovery [%]	Replicates	Mean Re-covery [%]	RSD [%]
Fragment ion m/z 213 (qualifier)					
Test medium (M1)	0.298 µg/L	99, 91, 98, 96, 163*	4	96	4
	4.00 µg/L	89, 978, 94, 104, 99	5	96	7

Matrix	Fortification level	Recovery [%]	Replicates	Mean Re-covery [%]	RSD [%]
	20.0 µg/L	87, 103, 77, 88, 92	5	89	12
Test medium (M3)	4.00 µg/L	90, 95, 81, 88, 96	5	89	7
	20.0 µg/L	85, 85, 82, 85, 91	5	84	2
	1300 µg/L	103, 95, 96, 95, 99	5	98	4
Sediment	0.050 mg/kg	73, 74, 72, 81, 72	5	74	5
	1.00 mg/kg	68, 81, 90, 66, 70	5	75	14
Fragment ion m/z 332 (confirmatory)					
Test medium (M1)	0.298 µg/L	107, 98, 104, 100, 172*	4	102	4
	4.00 µg/L	91, 95, 105, 103, 94	5	98	7
	20.0 µg/L	88, 103 84, 89, 91	5	91	9
Test medium (M3)	4.00 µg/L	95, 90, 87, 93, 94	5	92	4
	20.0 µg/L	86, 84, 86, 83, 87	5	85	2
	1300 µg/L	104, 97, 96, 92, 99	5	98	4
Sediment	0.050 mg/kg	68, 67, 72, 78, 66	5	70	7
	1.00 mg/kg	68, 82, 92, 67, 72	5	76	14

RSD: Relative Standard Deviation, \* significant outlier by Grubb's test

**Table A 2.1.1.3-05: Recovery results from method of Clopyralid into test medium and sediment**

Matrix	Fortification level	Recovery [%]	Replicates	Mean Re-covery [%]	RSD [%]
Mass transition m/z 192 → 110 (quantification)					
Test medium	1.49 µg/L	90, 89, 91, 89, 93	5	90	2
	1300 µg/L	92, 92, 85, 97, 96	5	92	5
Sediment	0.005 mg/kg	110, 101, 108, 112, 104	5	107	4
	1.00 mg/kg	109, 104, 103, 105, 102	5	105	3
Mass transition m/z 192 → 146 (confirmation)					
Test medium	1.49 µg/L	91, 88, 88, 96, 92	5	91	4
	1300 µg/L	92, 91, 87, 97, 94	5	92	4
Sediment	0.005 mg/kg	104, 96, 102, 105, 95	5	100	5
	1.00 mg/kg	107, 105, 104, 104, 104	5	105	1

RSD: Relative Standard Deviation

Mean recoveries and relative standard deviations of all analytes per fortification level and matrix fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery,  $\leq 20$  % RSD).

**Table A 2.1.1.3-06: Characteristics for the analytical method used**

	<b>Formulated product ADM.3304.H.1.A</b>																																																																																	
Specificity and Selectivity	<p>The analytes 2,4-D acid, Fluroxypyr-meptyl, Fluroxypyr acid and Clopyralid were determined in the final sample dilutions and extracts by use of LC-MS/MS detection. One MS/MS mass transition was evaluated for 2,4-D acid, Fluroxypyr-meptyl, Fluroxypyr acid and Clopyralid. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of samples. Only for validation purposed two mass transicions were evaluated for recovery samples.</p> <p>2,4-D ester was determiend in the final sample dilutions and extracts by use of GC-MS detection. One selected MS fragment ion was evaluated for 2,4-D ester. Two additional fragments were monitored for confirmation of peak identity but were not be used for quantification. Only for validation purposes two fragments were evaluated for recovery samples.</p> <p>Untreated test medium and sediment samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention times of 2,4-D ester, 2,4-D acid, Fluroxypyr-meptyl, Fluroxypyr and Clopyralid. The samples showed no significant interference (above 30 % of LOQ) at the retention times of the analytes in test medium and sediment respectively, therefore showing that the methods are highly specific.</p>																																																																																	
Linearity	<p>The ranges cover the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detection in any sample dilution or extract.</p> <p>The calibration curves were linear with correaltion coefficients <math>r \geq 0.995</math> for each analyte in test medium and sediment samples.</p> <p>The calibration graphs for all analytes were linear within the calibrated ranges and included in the report:</p> <table> <tr> <th>Matrix</th><th>Ranges [ng/mL]</th><th>Mass transition m/z</th><th>Calibration fucntion</th><th>Correlation coefficient (r)</th></tr> <tr> <td colspan="5"><b>Fluroxypyr</b></td></tr> <tr> <td rowspan="2">Test medium samples</td><td rowspan="2">0.15 - 10</td><td>255 → 197</td><td><math>y = 1.3 \cdot 10^5 x + 3.24 \cdot 10^4</math></td><td>0.9961</td></tr> <tr> <td>253 → 195</td><td><math>y = 2.14 \cdot 10^5 x + 9.61 \cdot 10^3</math></td><td>0.9984</td></tr> <tr> <td rowspan="2">Sediment samples</td><td rowspan="2">0.1 – 7.5</td><td>255 → 197</td><td><math>y = 8.97 \cdot 10^4 x - 866</math></td><td>0.9997</td></tr> <tr> <td>253 → 195</td><td><math>y = 1.45 \cdot 10^5 x - 1.34 \cdot 10^3</math></td><td>0.9998</td></tr> </table> <table> <tr> <th>Matrix</th><th>Ranges [ng/mL]</th><th>Mass transition</th><th>Calibration fucntion</th><th>Correlation coefficient (r)</th></tr> <tr> <td colspan="5"><b>Fluroxypyr-meptyl</b></td></tr> <tr> <td rowspan="2">Test medium samples</td><td rowspan="2">0.15 - 10</td><td>367 → 255</td><td><math>y = 1.01 \cdot 10^6 x + 4.4 \cdot 10^3</math></td><td>0.9996</td></tr> <tr> <td>369 → 257</td><td><math>y = 6.0 \cdot 10^5 x + 3.29 \cdot 10^4</math></td><td>0.9997</td></tr> <tr> <td rowspan="2">Sediment samples</td><td rowspan="2">0.1 – 7.5</td><td>367 → 255</td><td><math>y = 5.12 \cdot 10^5 x - 1.04 \cdot 10^4</math></td><td>0.9998</td></tr> <tr> <td>369 → 257</td><td><math>y = 3.06 \cdot 10^5 x + 2.84 \cdot 10^3</math></td><td>1.0000</td></tr> </table> <table> <tr> <th>Matrix</th><th>Ranges [ng/mL]</th><th>Mass transition</th><th>Calibration fucntion</th><th>Correlation coefficient (r)</th></tr> <tr> <td colspan="5"><b>2,4-D</b></td></tr> <tr> <td rowspan="2">Test medium samples</td><td rowspan="2">0.03 - 10</td><td>219 → 161</td><td><math>y = 1.84 \cdot 10^5 x + 103</math></td><td>1.0000</td></tr> <tr> <td>221 → 163</td><td><math>y = 1.17 \cdot 10^5 x - 1.07 \cdot 10^3</math></td><td>1.0000</td></tr> <tr> <td rowspan="2">Sediment samples</td><td rowspan="2">0.1 – 7.5</td><td>219 → 161</td><td><math>y = 6.05 \cdot 10^4 x + 256</math></td><td>0.9996</td></tr> <tr> <td>221 → 163</td><td><math>y = 3.67 \cdot 10^4 x - 513</math></td><td>0.9990</td></tr> </table>				Matrix	Ranges [ng/mL]	Mass transition m/z	Calibration fucntion	Correlation coefficient (r)	<b>Fluroxypyr</b>					Test medium samples	0.15 - 10	255 → 197	$y = 1.3 \cdot 10^5 x + 3.24 \cdot 10^4$	0.9961	253 → 195	$y = 2.14 \cdot 10^5 x + 9.61 \cdot 10^3$	0.9984	Sediment samples	0.1 – 7.5	255 → 197	$y = 8.97 \cdot 10^4 x - 866$	0.9997	253 → 195	$y = 1.45 \cdot 10^5 x - 1.34 \cdot 10^3$	0.9998	Matrix	Ranges [ng/mL]	Mass transition	Calibration fucntion	Correlation coefficient (r)	<b>Fluroxypyr-meptyl</b>					Test medium samples	0.15 - 10	367 → 255	$y = 1.01 \cdot 10^6 x + 4.4 \cdot 10^3$	0.9996	369 → 257	$y = 6.0 \cdot 10^5 x + 3.29 \cdot 10^4$	0.9997	Sediment samples	0.1 – 7.5	367 → 255	$y = 5.12 \cdot 10^5 x - 1.04 \cdot 10^4$	0.9998	369 → 257	$y = 3.06 \cdot 10^5 x + 2.84 \cdot 10^3$	1.0000	Matrix	Ranges [ng/mL]	Mass transition	Calibration fucntion	Correlation coefficient (r)	<b>2,4-D</b>					Test medium samples	0.03 - 10	219 → 161	$y = 1.84 \cdot 10^5 x + 103$	1.0000	221 → 163	$y = 1.17 \cdot 10^5 x - 1.07 \cdot 10^3$	1.0000	Sediment samples	0.1 – 7.5	219 → 161	$y = 6.05 \cdot 10^4 x + 256$	0.9996	221 → 163	$y = 3.67 \cdot 10^4 x - 513$	0.9990
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Accuracy and Precision	<p>The method’s applicability in terms on accuracy and repeatability was assessed by fortification of untreated test medium and sediment and subsequent determination of the recoveries upon applying the test methods.</p> <p>Results are detailed in previous tables. The relative standard deviation per fortification level was within the guideline requirements (<math>\leq 20\%</math>).</p>																																																													
Limit of determination / quantification	<p>The Limit of Detection (LOD) of the method is defined as 30% of the limit of quantification</p> <p>The Limit of Quantification (LOQ) was determined as the lowest fortification level at which an acceptable mean recovery (70% to 110% of nominal) with a relative standard deviation (RSD) <math>&lt; 20\%</math> was obtained.</p>																																																													
		Fluroxypyr	Fluroxypyr meptyl	2,4-D acid	2,4-D 2-EHE	Clopyralid																																																								
	Test medium LOD [μg/L]	0.00906	0.00948	0.0474	0.0474	0.0127																																																								
	Test medium LOQ[ <del>mg/L</del> μg/L]	0.0302	0.0316	0.158	0.158	0.0422																																																								
	Sediment samples LOD [mg/kg]	0.00150	0.00150	0.00150	0.0150	0.00150																																																								
	Sediment samples LOQ [mg/kg]	0.00500	0.00500	0.00500	0.0500	0.00500																																																								

## Conclusion

The method was validated according to guideline SANCO/3029/99 rev. 4 with regard to specificity, linearity of detector response, accuracy and precision for the active substances and is considered acceptable.

Eser, S. (2019)

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

Please refer to original dRR, previously submitted to zRMS Poland.

## **A 2.2 Analytical methods for Clopyralid**

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

Please refer to original dRR, previously submitted to zRMS Poland.

#### **A 2.2.1.1 Determination of active substance and/or variant in the plant protection product**

A new study to determine active substance Clopyralid in formulated product have been submitted in the point A 2.1.1.1.

#### **A 2.2.1.2 Description of analytical methods for the determination of relevant impurities**

Please refer to original dRR, previously submitted to zRMS Poland.

#### **A 2.2.1.3 Methods for the determination of residues in soil, water and non-target organisms**

Two new studies are submitted to support this dossier concerning the active substance Clopyralid and Fluroxypyr and are detailed below.

Comments of zRMS:	An analytical method for the determination of clopyralid and fluroxypyr-meptyl in tap water was successfully validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The limit of quantification (LOQ) of the analytical method was 100 mg/L of test item (2.83 mg/L of clopyralid and 10.1 mg/L of fluroxypyr-meptyl). The analytes were not detectable in the tap water used for recovery samples. The limit of detection (LOD) was defined as 30% of the limit of quantification (0.849 mg/L of clopyralid and 3.03 mg/L of fluroxypyr-meptyl). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The analytical methods fulfil the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The study is acceptable.
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Reference:	KCP 5.1.2/02, Duffner, A. (2019a) (submitted in KCP 10.6.2/04)
Report	ADM.3304.H.1.A: Effects on the Seedling Emergence and Seedling Growth of Non-Target Terrestrial Plant Species under Greenhouse Conditions
Report No.	S19-03358 (Sponsor ID: 000102902)
Guideline(s):	OECD 208 and SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## **Summary**

An analytical method for the determination of Clopyralid and Fluroxypyr-meptyl in tap water was validated with regard to recovery, linearity of detector response, repeatability, and specificity, limit of quantification and limit of detection in order to support the study of effects on seedling emergence and growth

of non-target terrestrial plant species under greenhouse conditions. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev.4.

Sample analysis was performed by dilution with acetonitrile + 1 % formic acid, further dilution with acetonitrile/water (1:1) + 0.25 % formic acid of tap water samples and quantification by HPLC-MS/MS detection.

## Materials and methods

A stock solution (2436mg/L, purity not considered) was prepared in tap water and used for fortification of 100 mg/L of test item recovery samples. For fortification of 55000 mg/L of test item recovery samples  $55 \pm 0.55$  mg were dissolved in tap water

For Clopyralid a stock solution (1000 mg/L, purity considered) was prepared in acetonitrile/water (8:2, v/v). For Fluroxypyr-meptyl a stock solution (877 mg/L, purity considered) was prepared in acetonitrile + 1 % formic acid.

From the stock solutions a mix standard with the concentrations 1 mg/L for both analytes was prepared in acetonitrile/water (1:1, v/v) + 0.25 % formic acid. Dilutions for calibration of HPLC-MS/MS analysis were prepared in acetonitrile/water (1:1, v/v) + 0.25 % formic acid from the mix standard.

**Material:** Test item: ADM.3304.H.1.A (Clopyralid 30 g/L + 2,4-D 380 g/L –present as 2,4-D 2-Ethylhexyl ester 574 g/L– + Fluroxypyr 75 g/L –present as Fluroxypyr-meptyl 109 g/L-); (batch No.: **N6903-A**) was used for the study.

External standards:

- **Clopyralid**: (CAS No.: 1702-17-6; batch No.: BCBZ5263; purity: 99.3 %; Supplier: Sigma-Aldrich).

- **Fluroxypyr-meptyl**: (CAS No.: 81406-37-3; batch No.: BCBT2272; purity: 98.5 %; Supplier: Sigma-Aldrich).

## HPLC-MS/MS conditions:

HPLC system: Agilent 1290 Infinity  
Column: Agilent ZORBAX Eclipse XDB-C18, 600 bar, 50 mm × 4.6 mm, 1.8 µm mean particle size with 2.1 mm UHPLC guard column  
Column temp.: 40 °C  
Detector: SCIEX API 5500  
Interface: ESI  
Source polarity: Positive (5500 V)  
Mobile phase: A: water + 0.5 % (v/v) formic acid  
B: methanol

Time [min]	% A	% B	Flow [µL/min]
0.0	90	10	600
3.0	20	80	600
4.0	10	90	600
6.0	10	90	600
6.1	90	10	600
8.0	90	10	600

Injection volume: 20 µL for Clopyralid, 5 µL for Fluroxypyr-meptyl

Retention time: Clopyralid: ~ 2.6 min  
Fluroxypyr-meptyl: ~ 5.4 min

Mass transitions:  
Clopyralid  
m/z 192 → 110, CE 49 eV, quantification  
m/z 192 → 146, CE 31 eV, confirmation  
m/z 195 → 75, CE 77 eV, confirmation  
Fluroxypyr-meptyl  
m/z 367 → 255, CE 17 eV, quantification  
m/z 367 → 257, CE 16 eV, confirmation

**Results:** The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table A 2.2.1.3-01** and **Table A 2.2.1.3-02** and the following text.

## Results and discussions

**Table A 2.2.1.3-01: Recovery results from method in tap water for Clopyralid and Fluroxypyr-meptyl**

Analyte	Fortification level [mg/L]	Recoveries					
		Single values [%]	n	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Clopyralid	Mass transition m/z 192 → 110 (quantification)						
	100	99, 96, 104, 99, 106	5	101	4	105	5.5
	55000	112, 113, 107, 110, 106	5	110	3		
	Mass transition m/z 192 → 146 (confirmation)						
	100	101, 96, 101, 98, 106	5	100	4	105	5.9
	55000	112, 115, 109, 108, 108	5	110	3		
	Mass transition m/z 192 → 75 (confirmation)						
	100	97, 95, 100, 96, 106	5	99	4	105	6.7
	55000	110, 115, 108, 111, 108	5	110	3		
Fluroxypyr-meptyl	Mass transition m/z 367 → 255 (quantification)						
	100	107, 108, 110, 104, 110	5	108	2	105	3.7
	55000	99, 102, 100, 103, 105	5	102	3		
	Mass transition m/z 367 → 257 (confirmation)						
	100	106, 108, 110, 103, 109	5	107	3	105	3.3
	55000	100, 102, 101, 103, 106	5	103	3		

**Table A 2.2.1.3-02: Characteristics for the analytical method used**

	Clopyralid and Fluroxypyr-meptyl
Specificity	<p>The analytes were determined in the final diluted sample by use of LC-MS/MS detection.</p> <p>For Clopyralid three MS/MS mass transitions were evaluated. The second and third mass transition were monitored for confirmation of peak identity but was only used for quantification of recovery sample.</p> <p>For Fluroxypyr-meptyl two MS/MS mass transitions were evaluated. The second mass transition was monitored for confirmation of peak identity.</p> <p>Untreated tap water samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention times of Clopyralid and Fluroxypyr-meptyl. The samples showed no significant interference (above 30 % of LOQ) at the retention times of the analytes in any investigated tap water, therefore showing that the method is highly specific.</p>
Linearity	<p>The linearity of the detector response was demonstrated by single determination of solvent calibration standards at a minimum of seven concentration levels ranging from 1 ng/mL to 35 ng/mL. This range covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) sample.</p> <p>The calibration curve was linear with correlation coefficient <math>r \geq 0.995</math>.</p> <p>Clopyralid</p> <p><math>y = 7.11 \cdot 10^3 x - 2.61 \cdot 10^3</math>, <math>r = 0.9997</math> (quantification)</p> <p><math>y = 7.47 \cdot 10^3 x - 2.29 \cdot 10^3</math>, <math>r = 0.9998</math> (confirmation)</p> <p><math>y = 6.37 \cdot 10^3 x - 1.95 \cdot 10^3</math>, <math>r = 0.9998</math> (confirmation)</p> <p>Fluroxypyr-meptyl</p>

	<b>Clopyralid and Fluroxypyr-meptyl</b>
	$y = 2.62 \cdot 10^5 x - 7.0 \cdot 10^4$ , $r = 0.9986$ (quantification) $y = 1.75 \cdot 10^5 x - 4.3 \cdot 10^4$ , $r = 0.9982$ (confirmation) The calibration graphs for all analytes are included in the report.
Accuracy and precision	The method's applicability in terms of accuracy and repeatability was assessed by fortification of untreated tap water and subsequent determination of the recoveries upon applying the test method. Five recovery determination at 100 mg/L of test item (LOQ) and five recovery determination at 55000 mg/L of the test item were performed. The results are detailed in the <b>Table A 2.2.1.3-01</b> . The relative standard deviation per fortification level was within the guideline requirements ( $\leq 20\%$ ).
Limit of determination/quantification	The Limit of Detection (LOD) of the method is defined as 30% of the limit of quantification. LOD = 0.849 mg/L to Clopyralid LOD = 3.03 mg/L to Fluroxypyr-meptyl The Limit of Quantification (LOQ) was determined as the lowest fortification level at which an acceptable mean recovery (70% to 110% of nominal) with a relative standard deviation (RSD) < 20% was obtained. LOQ = 2.83 mg/L to Clopyralid LOQ = 10.1 mg/L to Fluroxypyr-meptyl

## Conclusion

The method was validated according to guideline SANCO/3029/99 rev. 4 with regard to specificity, linearity of detector response, accuracy and precision for the active substances and is considered acceptable.

Duffner, A. (2019a)

Comments of zRMS:	An analytical method for the determination of clopyralid and fluroxypyr-meptyl in tap water was validated with regard to recovery, linearity of detector response, repeatability, and specificity, limit of quantification and limit of detection. The limit of quantification (LOQ) of the analytical method was 100 mg/L of test item (2.83 mg/L of clopyralid and 10.1 mg/L of fluroxypyr-meptyl). The analytes were not detectable in the tap water used for recovery samples. The limit of detection (LOD) was defined as 30% of the limit of quantification (0.849 mg/L of clopyralid and 3.03 mg/L of fluroxypyr-meptyl). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The analytical methods fulfil the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The study is acceptable.
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Reference:	KCP 5.1.2/03, Duffner, A. (2019b) (submitted in KCP 10.6.2/03)
Report	ADM.3304.H.1.A: Effects on the Vegetative Vigour of Non-Target Terrestrial Plant Species under Greenhouse Conditions
Report No.	S19-03359 (Sponsor ID: 000102903)
Guideline(s):	OECD 227 and SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Summary

An analytical method for the determination of Clopyralid and Fluroxypyr-meptyl in tap water was validated with regard to recovery, linearity of detector response, repeatability, and specificity, limit of quantification and limit of detection in order to support the study of effects on vegetative vigour of non-target terrestrial plant species under greenhouse conditions. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev.4.

Sample analysis was performed by dilution with acetonitrile + 1 % formic acid, further dilution with acetonitrile/water (1:1) + 0.25 % formic acid of tap water samples and quantification by HPLC-MS/MS detection.

## Materials and methods

Two stock solutions (2180 mg/L and 2102 mg/L, purity not considered) were prepared in tap water and used for fortification of 100 mg/L of test item recovery samples. For fortification of 55000 mg/L of test item recovery samples  $55 \pm 0.55$  mg were dissolved in tap water.

For Clopyralid a stock solution (1000 mg/L, purity considered) was prepared in acetonitrile/water (8:2, v/v). For Fluroxypyr-meptyl a stock solution (877 mg/L, purity considered) was prepared in acetonitrile + 1 % formic acid.

From the stock solutions a mix standard with the concentrations 1 mg/L for both analytes was prepared in acetonitrile/water (1:1, v/v) + 0.25 % formic acid. Dilutions for calibration of HPLC-MS/MS analysis were prepared in acetonitrile/water (1:1, v/v) + 0.25 % formic acid from the mix standard.

**Material:** Test item: ADM.3304.H.1.A (Clopyralid 30 g/L + 2,4-D 380 g/L –present as 2,4-D 2-Ethylhexyl ester 574 g/L– + Fluroxypyr 75 g/L –present as Fluroxypyr-meptyl 109 g/L-); (batch No.: **N6903-A**) was used for the study.

External standards:

- **Clopyralid:** (CAS No.: 1702-17-6; batch No.: BCBZ5263; purity: 99.3 %; Supplier: Sigma-Aldrich).

- **Fluroxypyr-meptyl:** (CAS No.: 81406-37-3; batch No.: BCBT2272; purity: 98.5 %; Supplier: Sigma-Aldrich).

## HPLC-MS/MS conditions:

HPLC system: Agilent 1290 Infinity  
Column: Agilent ZORBAX Eclipse XDB-C18, 600 bar, 50 mm × 4.6 mm, 1.8 µm mean particle size with 2.1 mm UHPLC guard column  
Column temp.: 40 °C  
Detector: SCIEX API 5500  
Interface: ESI  
Source polarity: Positive (5500 V)  
Mobile phase: A: water + 0.5 % (v/v) formic acid  
B: methanol

Time [min]	% A	% B	Flow [µL/min]
0.0	90	10	600
3.0	20	80	600
4.0	10	90	600
6.0	10	90	600
6.1	90	10	600
8.0	90	10	600

Injection volume: 20 µL for Clopyralid, 5 µL for Fluroxypyr-meptyl

Retention time: Clopyralid: ~ 2.6 min  
Fluroxypyr-meptyl: ~ 5.4 min

Mass transitions: Clopyralid  
m/z 192 → 110, CE 49 eV, quantification  
m/z 192 → 146, CE 31 eV, confirmation  
m/z 195 → 75, CE 77 eV, confirmation  
Fluroxypyr-meptyl  
m/z 367 → 255, CE 17 eV, quantification  
m/z 367 → 257, CE 16 eV, confirmation

**Results:** The parameters linearity, precision, accuracy and specificity were checked. Typical calibration curves and chromatograms are presented in the report. Information concerning the validation of the method please refer to **Table A 2.2.1.3-03** and **Table A 2.2.1.3-04** and the following text.

## Results and discussions

**Table A 2.2.1.3-03: Recovery results from method in tap water for Clopyralid and Fluroxypyr-meptyl**

Analyte	Fortification level [mg/L]	Recoveries						
		Single values [%]	n	Mean [%]	RSD [%]	Mean [%]	RSD [%]	
Clopyralid	Mass transition m/z 192 → 110 (quantification)							
	100	100, 106, 107, 104, 106	5	105	3	105	3.5	
	55000	107, 113, 106, 103, 101	5	106	4			
	Mass transition m/z 192 → 146 (confirmation)							
	100	100, 108, 108, 108, 108	5	106	3	106	3.5	
	55000	108, 113, 103, 103, 101	5	106	5			
	Mass transition m/z 192 → 75 (confirmation)							
	100	101, 103, 108, 106, 104	5	104	3	105	3.3	
	55000	104, 112, 104, 104, 100	5	105	4			
Fluroxypyr-meptyl	Mass transition m/z 367 → 255 (quantification)							
	100	105, 109, 113, 112, 110	5	110	3	109	2.1	
	55000	110, 109, 108, 109, 107	5	109	1			
	Mass transition m/z 367 → 257 (confirmation)							
	100	105, 110, 115, 112, 110	5	110	3	109	2.7	
	55000	109, 109, 106, 109, 106	5	108	2			

**Table A 2.2.1.3-04: Characteristics for the analytical method used**

Clopyralid and Fluroxypyr-meptyl	
Specificity	<p>The analytes were determined in the final diluted sample by use of LC-MS/MS detection. For Clopyralid three MS/MS mass transitions were evaluated. The second and third mass transition were monitored for confirmation of peak identity but was only used for quantification of recovery sample.</p> <p>For Fluroxypyr-meptyl two MS/MS mass transitions were evaluated. The second mass transitions were monitored for confirmation of peak identity.</p> <p>Untreated tap water samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention times of Clopyralid and Fluroxypyr-meptyl. The samples showed no significant interference (above 30 % of LOQ) at the retention times of the analytes in any investigated tap water, therefore showing that the method is highly specific.</p>
Linearity	<p>The linearity of the detector response was demonstrated by single determination of solvent calibration standards at a minimum of seven concentration levels ranging from 1 ng/mL to 35 ng/mL. This range</p>

	<p>covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) sample.</p> <p>The calibration curve was linear with correlation coefficient <math>r \geq 0.995</math>.</p> <p>Clopyralid  <math>y = 2.53 \cdot 10^4 x + 1.46 \cdot 10^3</math>, <math>r = 0.9998</math> (quantification)  <math>y = 2.86 \cdot 10^4 x - 2.47 \cdot 10^3</math>, <math>r = 0.9996</math> (confirmation)  <math>y = 2.57 \cdot 10^4 x + 521</math>, <math>r = 0.9998</math> (confirmation)</p> <p>Fluroxypyr-meptyl  <math>y = 2.34 \cdot 10^5 x + 7.39 \cdot 10^4</math>, <math>r = 0.9998</math> (quantification)  <math>y = 1.51 \cdot 10^5 x + 3.58 \cdot 10^4</math>, <math>r = 0.9998</math> (confirmation)</p> <p>The calibration graphs for all analytes are included in the report.</p>
Accuracy and precision	<p>The method's applicability in terms of accuracy and repeatability was assessed by fortification of untreated tap water and subsequent determination of the recoveries upon applying the test method. Five recovery determination at 100 mg/L of test item (LOQ) and five recovery determination at 55000 mg/L of the test item were performed.</p> <p>The results are detailed in the <b>Table A 2.2.1.3-03</b>.</p> <p>The relative standard deviation per fortification level was within the guideline requirements (<math>\leq 20\%</math>).</p>
Limit of determination/quantification	<p>The Limit of Detection (LOD) of the method is defined as 30% of the limit of quantification.</p> <p>LOD = 0.849 mg/L to Clopyralid  LOD = 3.03 mg/L to Fluroxypyr-meptyl</p> <p>The Limit of Quantification (LOQ) was determined as the lowest fortification level at which an acceptable mean recovery (70% to 110% of nominal) with a relative standard deviation (RSD) <math>&lt; 20\%</math> was obtained.</p> <p>LOQ = 2.83 mg/L to Clopyralid  LOQ = 10.1 mg/L to Fluroxypyr-meptyl</p>

## Conclusion

The method was validated according to guideline SANCO/3029/99 rev. 4 with regard to specificity, linearity of detector response, accuracy and precision for the active substances and is considered acceptable.

Duffner, A. (2019b)

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

Please refer to original dRR, previously submitted to zRMS Poland.

## A 2.3 Analytical methods for Fluroxypyr

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

#### A 2.3.1.1 Determination of active substance and/or variant in the plant protection product

A new study to determine active substance Fluroxypyr in formulated product have been submitted in the point A 2.1.1.1.

#### A 2.3.1.2 Description of analytical methods for the determination of relevant impurities

No studies in order to determine relevant impurities in formulated product are submitted.

**A 2.3.1.3                    Methods for the determination of residues in soil, water and non-target organisms**

Two new studies to support ecotoxicological section have been submitted and detailed in the point A 2.1.1.3 and A 2.2.1.3.

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

**A 2.3.2.1                    Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)**

**A 2.3.2.1.1                    Analytical method 1**

**A 2.3.2.1.1.1                    Method validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.1.1.2                    Independent laboratory validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.1.1.3                    Confirmatory method (if required)**

Please refer to original dRR.

**A 2.3.2.1.1.4                    Extraction efficiency**

No extraction efficiency.

**A 2.3.2.2                    Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)**

**A 2.3.2.2.1                    Analytical method 1**

**A 2.3.2.2.1.1                    Method validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.2.1.2                    Independent laboratory validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.2.1.3                    Confirmatory method (if required)**

Please refer to original dRR.

**A 2.3.2.2.1.4                    Extraction efficiency**

Please refer to original dRR.

**A 2.3.2.3                    Description of Methods for the Analysis of Soil (KCP 5.2)**

**A 2.3.2.3.1                    Analytical method 1**

**A 2.3.2.3.1.1                    Method validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.4            Description of Methods for the Analysis of Water (KCP 5.2)**

**A 2.3.2.4.1            Analytical method 1**

**A 2.3.2.4.1.1            Method validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.4.1.2            Independent laboratory validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.5            Description of Methods for the Analysis of Air (KCP 5.2)**

**A 2.3.2.5.1            Analytical method 1**

**A 2.3.2.5.1.1            Method validation**

Please refer to original dRR, previously submitted to zRMS Poland.

**A 2.3.2.6            Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)**

Please refer to original dRR, previously submitted to zRMS Poland.