

# REGISTRATION REPORT

## **Part B**

### **Section 9**

#### **Ecotoxicology**

Detailed summary of the risk assessment

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

(old code AG-CDF1-480 EC)

Product name(s): Tricera

Chemical active substances:

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

Clopyralid, 30 g/L

Fluroxypyr, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

## CORE ASSESSMENT

(authorization)

Sponsor: ADAMA Agan Ltd.

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

Submission date: September 2021

MS Finalisation date: May 2022 (initial Core Assessment)

November 2022, updated February 2023 (final Core Assessment)

## Version history

When	What
September 2019	dRR Part B – Section 9, Version 1 submitted by applicant
September 2021	dRR Part B – Section 9, Version 2 submitted by applicant, update to address the new endpoints found in the EFSA conclusion LoEP (EFSA, 2018) and to include information already submitted for the composition change in 02/2021
May 2021	Initial zRMS assessment  The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and <b>highlighted in grey</b> . Not agreed or not relevant information are <del>struck through and shaded for transparency</del> .
November 2022	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 <b>highlighted in yellow</b> . Information no longer relevant <del>is struck through and shaded</del> .
February 2023	Final report (Core Assessment updated following the Applicant's comments).  Additional information/assessments included by the zRMS in the report in response to comments received from the Applicant are <b>highlighted in green</b> . Information no longer relevant <del>is struck through and shaded</del> .

## **DATA PROTECTION CLAIM**

Under Article 59, Regulation No. 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**

The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either –

- from the owner of the data, or
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- following expiry of any period of exclusive use, by offering – in certain jurisdictions – mandatory compensation, unless the period of protection of the proprietary data concerned has expired.

## Introduction

### **General remark:**

The product ADM.3304.H.1.A (old code AG-CDF1-480 EC) is an 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 ecotoxicological studies for the product ADM.3304.H.1.A (old code AG-CDF1-480 EC) 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.

**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) 2021/566 of 30 March 2021 that fixes the new expiry date of approval to 30/04/2022. The dRR for ADM.3304.H.1.A was further updated due to the clopyralid renewal by the Commission Implementing Regulation (EU) 2021/1191 with the relevant information provided in the Renewal Report (Clopyralid, SANTE/10206/2021 Rev. 1 of 20<sup>th</sup> May 2021). The current expiry date is 30<sup>th</sup> of September 2036.

**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) 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 data have only been 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) that was updated in 2018 (EFSA Journal 2018;16(8):5389) 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 report (2018) prepared by the RMS for the a.i. (Greece).

For the implementation of the uniform principles of Regulation (EC) No. 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 renewal Regulation for the active substance **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.

In order to facilitate Member States, in granting or reviewing authorisations, to apply adequately the provisions of Article 29(1) of Regulation (EC) No 1107/2009 and the uniform principles laid down in Regulation (EU) No 546/2011, the most important endpoints were identified during the re-evaluation process. These endpoints are listed in the conclusion of the EFSA.

- 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.

**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 condition.
- The risk to aquatic organisms.

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

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 information provided by the applicant for ADM.3304.H.1.A (old code AG-CDF1-480 EC).

Appendix 3, 4 and 5 of this document contains information not previously evaluated for the active substance(s)/metabolites.

Appendix 6 of this document contains non-target terrestrial plants – SSD curves and goodness of fit toxicity data.

This dossier is being prepared to update this section on clopyralid endpoints and risk assessments to reflect the new endpoints due to the renewal process of clopyralid.

Changes are highlighted for the sake of an easy comparison. To reflect the composition change which was submitted earlier in 2021 the new code ADM.3304.H.1.A is used. Information on the detailed composition of ADM.3304.H.1.A (old code AG-CDF1-480 EC) can be found in the confidential dossier of this submission (Registration Report – Part C).

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## 9 Ecotoxicology (KCP 10)

### 9.1 Critical GAP and overall conclusions

**Table 9.1-1: Table of critical GAPs**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No.	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I	Pests or Group of pests controlled (additionally: development al stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ syner- gist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg product/ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha Min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Established grassland (NNNFW)	F	broadleaved weeds (TTTDD)	foliar spraying, overall	Mar-Aug/ BBCH 21-39	a) 1 (-) b) 1 (-)	-	a) 2 L/ha b) 2 L/ha	a) 750 <sup>1</sup> / 60 / 150 <sup>2</sup> b) 750 <sup>1</sup> / 60 / 150 <sup>2</sup>  (2,4-D / clopyralid / fluroxypyr)	200- 400	n.a.	The BBCH stages were removed since for the established grass the time of the season will be more indicative for application timing than the growth stage  Winter cereals considered as surrogate for scenarios R1 and R3	A	N	R (D3, D4, D5, R1)	A	A	A	R
2	PL	Spring cereals (umbrella GAP)	F	broadleaved weeds (TTTDD)	foliar spraying, overall	Mar-Jun/ BBCH 21-39	a) 1 (-) b) 1 (-)	-	a) 2 L/ha b) 2 L/ha	a) 750 <sup>1</sup> / 60 / 150 <sup>2</sup> b) 750 <sup>1</sup> / 60 / 150 <sup>2</sup>	200- 400	n.a.		A	A	R (D3, D4, D5, R1)	A	A	A	R

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
										(2,4-D / clopyralid / fluroxypyr)				A	A	N (R3)	A	A	A	
3	PL	Winter cereals (umbrella GAP)	F	broadleaved weeds (TTTDD)	foliar spraying, overall	Mar-May/ BBCH 21-39	a) 1 (-) b) 1 (-)	-	a) 2 L/ha b) 2 L/ha	a) 750 <sup>1</sup> / 60 / 150 <sup>2</sup> b) 750 <sup>1</sup> / 60 / 150 <sup>2</sup>  (2,4-D / clopyralid / fluroxypyr)	200-400	n.a.	Range of application rates: 1.5-2.0 L product/ha, but note that 1.5 L/ha is not supported by efficacy data  Winter cereals considered as surrogate for scenarios R1 and R3	A	A	R (D3, D4, D5, R1)  N (R3)	A	A	A	R

<sup>1</sup> equal to 1125 g of 2,4-D EHE

<sup>2</sup> equal to 216 g/L Fluroxypyr-meptyl

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

<b>Remarks table:</b>	<ol style="list-style-type: none"> <li>(1) Numeration necessary to allow references</li> <li>(2) Use official codes/nomenclatures of EU</li> <li>(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (<i>e.g.</i> fumigation of a structure)</li> <li>(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application</li> <li>(5) Scientific names <u>and</u> EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (<i>e.g.</i> biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named</li> <li>(6) Method, <i>e.g.</i> high volume spraying, low volume spraying, spreading, dusting, drench                  Kind, <i>e.g.</i> overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated</li> <li>(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application</li> <li>(8) The maximum number of application possible under practical conditions of use must be provided</li> <li>(9) Minimum interval (in days) between applications of the same product.</li> <li>(10) For specific uses other specifications might be possible, <i>e.g.</i>: g/m<sup>3</sup> in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products</li> <li>(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).</li> <li>(12) If water volume range depends on application equipments (<i>e.g.</i> ULVA or LVA) it should be mentioned under “application: method/kind”.</li> <li>(13) PHI - minimum pre-harvest interval</li> <li>(14) Remarks may include: Extent of use/economic importance/restrictions</li> </ol>
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**zRMS comments:**

It is noted that initially in the GAP table above the additional 2 uses intended in Poland were indicated. One included application to winter cereals at BBCH 21-39 at application rate 1.5 L product/ha (indicated as “fallback scenario 1”) and at BBCH 30-39 at 2.0 L product/ha (indicated as “fallback scenario 2”), which both fall into the use pattern No 3, covering all intended BBCH stages. Displaying the different stages on the label could be confusing for the end-user, since single application is possible over the entire period of BBCH 21-39, regardless of the rate. Taking this into account, the fallback scenarios were removed from the Table 9.1-1 and range of application rates in winter cereals has been indicated in Use No 3, especially no separate calculations for the lower rate were provided by the Applicant in area of Section 9 and the risk from the lower rate has been covered by evaluation performed for higher rate of the product. It should be, however, noted that lower rate (1.5 L/ha) is not supported by efficacy data and in line with the outcome of the evaluation performed in area of Section 3, the minimum effective application rate is 2.0 L/ha. Therefore, the lower rate has been included here only as option proposed by the Applicant. For the overall conclusions regarding the GAP agreed by the zRMS based on the outcome of evaluation performed in area of all sections, please refer to Section 0.

The first version of the GAP included also intended uses in UK. However, the Applicant informed that uses of ADM.3304.H.1.A in UK are no longer supported and PL is the only cMS listed in the GAP. United Kingdom has been removed as cMS from the Table 9.1-1 above for transparency.

Detailed GAP for ADM.3304.H.1.A may be found in the Core Assessment, Part B, Section 0.

## 9.1.1 Overall conclusions

### zRMS comments:

Conclusions presented in points 9.1.1.1 to 9.1.1.7 below were checked by the zRMS and amended where necessary.

### 9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

#### Birds

##### Acute risk assessment to birds

An acute LD<sub>50</sub> value is available for the formulation AG-CDF1-480 EC. There is no indication of increased toxicity of the formulation.

Acute screening/first-tier risk assessments for each active substance, variants and the formulation were conducted. All the TER<sub>a</sub> values for each active substance, variants and the formulation exceed the trigger value of 10, indicating an acceptable acute risk to birds following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use patterns proposed.

##### Reproductive risk assessment to birds

The TER<sub>lt</sub> values for each active substance were ~~above~~ below the Annex VI trigger value of 5 what indicates that the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) applied according to the intended uses do not pose a potential reproductive risk to birds. Acceptable long-term risk for the mixture was also demonstrated.

##### Secondary poisoning to birds and risk from drinking water

No risk to birds is expected via the consumption of water contaminated with the active substances and their pertinent soil metabolites from puddles on soil. A risk of secondary poisoning in terrestrial environments can be also excluded.

#### Mammals

##### Acute risk assessment

An acute LD<sub>50</sub> value is available for the formulation AG-CDF1-480 EC. Comparison of the measured and predicted toxicity of the mixture demonstrated that the formulated product is more toxic than predicted on the basis of the active substance data and the risk assessment based on the measured formulation endpoint has been performed. ~~There is no indication of increased toxicity of the formulation.~~

Since 2,4-D (regardless if an ester or acid form) was identified to drive the acute risk to birds, the TER values were calculated only for 2,4-D (acid and ester) as being protective also for clopyralid and fluroxypyr. As indicated above, the acute risk assessment was also performed for the formulated product based on the measured toxicity data. Performed calculations demonstrated acceptable acute risk to mammals ~~birds~~ from the intended uses of ADM.3304.H.1.A in cereals.

For the intended uses in grassland an acceptable risk could be concluded for large herbivorous species from 2,4-D (acid and ester) and formulation, but unacceptable risk was concluded for small herbivorous mammals based on Tier 1 calculations. The field population study provided by the Applicant to address the acute risk to small herbivores (common vole) from 2,4-D formulations was not agreed by the zRMS for the following reasons:

- The study was performed with two formulations, each containing 2,4-D in a form of DMA salt, while ADM.3304.H.1.A contains 2,4-D EHE. Behaviour of DMA salt and an ester is different and extrapolation between these two forms is not possible, so results of studies performed with 2,4-D DMA salt are not relevant to address the acute and long-term risk from formulations

containing 2,4-D EHE, especially in case of ADM.3304.H.1.A the acute risk to small herbivorous mammals is unacceptable also for 2,4-D EHE and the formulated product itself.

- The study was already evaluated in the course of the 2,4-D EU renewal and rejected during the peer-review as unreliable due to numerous uncertainties described in EFSA Journal 2014;12(9):3812. The zRMS was not in the position to challenge the decision of the MS experts and EFSA on the study reliability, as this obviously was intensively discussed and all potential arguments in favour of keeping the study for refinement of the risk were already taken into account in the course of the peer-review and rejected.

Overall, the acute dietary risk from 2,4-D EHE, 2,4-D acid and formulation to small herbivorous mammals in grassland remains unresolved and further data must be submitted by the Applicant to support authorisation in this crop.

Acceptable acute dietary risk from all active compounds and the formulation could be concluded for the intended Central Zone uses in cereals.

~~Acute screening/first tier risk assessments for 2,4-D (the toxicity “driver”) were conducted. All the  $TER_a$  values for the active substance exceed the trigger value of 10, indicating an acceptable acute risk to mammals following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use patterns proposed.~~

#### Reproductive risk assessment to mammals

Initially the Applicant performed the long-term risk assessment for 2,4-D only, explaining that this substance is a toxicity driver. However, 2,4-D was identified as a toxicity driver of the acute risk and not the long-term risk, which should have been performed for the active compounds and the mixture.

Performed evaluation demonstrated acceptable reproductive risk to mammals from clopyralid and fluroxypyr from all intended uses of ADM.3304.H.1.A.

For 2,4-D acceptable risk could be demonstrated for uses in cereals, however unacceptable risk was demonstrated for uses in grassland for both indicator species (large and small herbivore). The risk was refined using the EU agreed residue decline data and acceptable risk could be demonstrated for large herbivore, but the risk to small herbivorous species remained unacceptable. There were no other refinement options provided by the Applicant with exception of the field population study, which was, however, rejected due to reasons highlighted above in paragraph referring to the acute risk assessment.

The combined long-term mixture risk assessment was performed using simplified approach via calculation of  $TER_{mix}$  values. Based on performed calculations, an acceptable risk from the mixture could be concluded for the intended uses of ADM.3304.H.1.A in cereals, but unacceptable Tier 1 risk was concluded for both generic focal species following uses in grassland. ~~but acceptable~~ Acceptable risk could be demonstrated to large herbivorous mammal when the refined  $TER_{LT}$  value for 2,4-D was considered. However, the long-term risk to small herbivore remained unresolved.

Overall, the long-term dietary risk from 2,4-D acid and the mixture to small herbivorous mammals in grassland remains unresolved and further data must be submitted by the Applicant to support authorisation in this crop.

Acceptable long-term dietary risk from all active compounds and the mixture could be concluded for the intended Central Zone uses in cereals.

~~The  $TER_{Rt}$  values for 2,4-D to mammals in cereals exceed the Annex VI trigger value of 5, indicating that the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) applied according to the intended use do not pose a potential reproductive risk to birds.~~

~~Based on the refined  $TER_{Rt}$  values for 2,4-D, a chronic risk to mammals in grassland can be excluded.~~

### Secondary poisoning to mammals and risk from drinking water

No risk to mammals is expected via the consumption of water contaminated with the active substances and their pertinent soil metabolites from puddles on soil. A risk of secondary poisoning in terrestrial environments can be also excluded.

#### **9.1.1.2 Effects on aquatic organisms (KCP 10.2)**

The risk to aquatic organisms was evaluated for the active substances 2,4-D, Clopyralid, Fluroxypyr, their variants and its degradation products in consideration of the GAP uses envisaged for ADM.3304.H.1.A (old code AG-CDF1-480 EC). The risk of the formulated product itself was also evaluated.

Performed evaluation demonstrated acceptable risk to aquatic organisms from 2,4-D acid, clopyralid and fluroxypyr (meptyl and acid) and their metabolites with no need for risk mitigation measures.

For 2,4-D EHE acceptable risk could be demonstrated provided that the unsprayed buffer zone of 10 m to surface water bodies is respected or the spray drift is reduced by 90%.

The combined toxicity assessment demonstrated that the formulated product is more toxic to *Myriophyllum spicatum* than expected based on the active substance data. Measured toxicity to other species (fish, aquatic invertebrates, algae and *Lemna*) was either comparable or lower than the predicted mixture toxicity. The risk assessment performed for the formulation ADM.3304.H.1.A using the lowest relevant endpoint from the study performed with the most sensitive species (*Myriophyllum spicatum*) demonstrated acceptable risk from all intended Central Zone uses provided that

- 10 m unsprayed buffer zone to surface water bodies is respected in scenarios D3, D4 and D5.
- 10 m vegetated filter strip to surface water bodies is respected in scenario R1.

No acceptable risk could be concluded in scenario R3 and further assessment will be necessary at the national level in the course of the mutual recognition process.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation AG-E1-500 SC1, which was performed in line with the EU agreed methodology.

*“The endpoint  $E_rC_{50}$  is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”*

~~Following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use pattern proposed only an acute risk to fish and aquatic invertebrates is identified for 2,4-D EHE (variant 2-ethylhexyl ester of 2,4-D). Thus, risk mitigation measures are necessary in to prevent the risk to aquatic non-target organisms:~~

~~“To protect aquatic organisms respect an unsprayed buffer zone of 5 m or to use 90 % drift reducing nozzles”.~~

### 9.1.1.3 Effects on bees (KCP 10.3.1)

For the intended Central Zone uses in grassland and cereal, an acceptable acute risk for honeybees is determined for the active substances, their variants and the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC).

### 9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

For the intended Central Zone uses in grassland and cereals, an acceptable in- and off-field risk for terrestrial non-target arthropods other than bees is determined following the application of the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) with no need for risk mitigation measures. according to the use pattern proposed.

### 9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

The risk for soil macro- and mesofauna from ADM.3304.H.1.A (old code AG-CDF1-480 EC) as well as the single active substances, their variants and metabolites is acceptable for the intended Central Zone uses in grassland and cereals.

An acceptable risk for soil microbial function (N-transformation) is determined for intended Central Zone uses in grassland and cereals following the application of the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC).

### 9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

The risk assessment for non-target terrestrial plants from the intended Central Zone uses of ADM.3304.H.1.A has been performed using both, deterministic and probabilistic approach with consideration of the endpoints derived for the new variant of the formulation which will be placed in the market. The exposure was calculated using standard spray drift values and implementing potential deposition of clopyralid due to volatilisation.

On the basis of the deterministic risk assessment acceptable risk for seedling emergence could be concluded with no need for risk mitigation measures.

The deterministic risk assessment for vegetative vigour demonstrated that risk mitigation measures are necessary to demonstrate acceptable risk and for this reason probabilistic risk assessment has been performed, which, however, have not improved results of the deterministic risk assessment.

Overall, on the basis of the performed evaluation acceptable risk to non-target terrestrial plants may be concluded following the intended Central Zone uses of ADM.3304.H.1.A, provided that following risk mitigation measures are respected:

- 15 m 20 m unsprayed buffer zone to non-agricultural land, or
- 10 m unsprayed buffer zone to non-agricultural land is combined with 50% drift reduction, or
- 3 m 5 m unsprayed buffer zone to non-agricultural land is combined with 75% drift reduction.
- 3 m unsprayed buffer zone to non-agricultural land is combined with 90% drift reduction.

ADM.3304.H.1.A (old code AG-CDF1-480 EC) does not pose a risk to non-target terrestrial plants in off-crop areas following the proposed uses in grassland and/or cereals with a 5 m buffer zone or a standard buffer zone of 1 m in combination with 75 % spray drift reduction nozzles.

### 9.1.1.7 Effects on other terrestrial organism (flora and fauna) (KCP 10.7)

No other relevant data were identified in the EU review of the active substances.



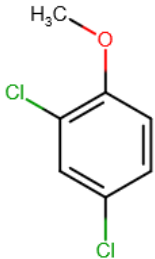
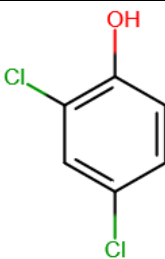
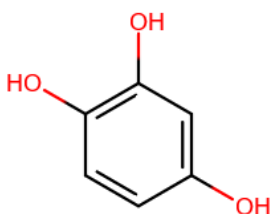
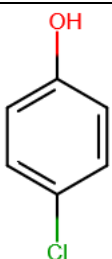
## 9.1.2 Grouping of intended uses for risk assessment

The risk envelop approach is not relevant as use pattern for grassland and cereals is the same.

## 9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of formulation is indicated in the table.

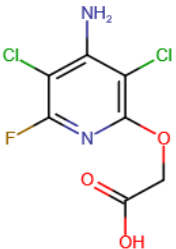
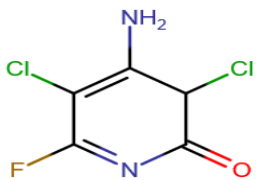
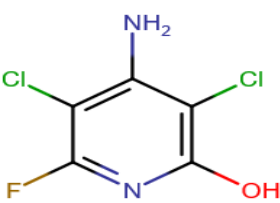
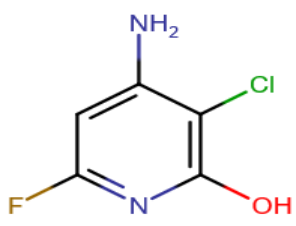
**Table 9.1-4: Metabolites of 2,4-D**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments [% of AR]		Risk assessment required?
2,4-DCA		177.03	Soil	15	Y
			Water / sediment	5.3	
2,4-DCP		163.00	Soil	aerobic: 8.7 anaerobic: 38	Y
			Water / sediment	32.1	
1,2,4-Benzenetriol (photolysis metabolite)		126.11	Water	31.7	Y
4-Chlorophenol (4-CP)		128.56	Soil (anaerobic conditions)	33	Y

**Table 9.1-2: Metabolites of Clopyralid**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
None	-	-	-	-

**Table 9.1-5: Metabolites of Fluroxypyr meptyl**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments [% of AR]		Risk assessment required?
Fluroxypyr acid		255.03	Soil	100 (calculations performed as for parent)	Y
			Water / sediment		
Methoxypyridine (DMP)		196.99	Soil	38.2	Y
Pyridinol (DCP)		197.99	Soil	23.9	Y
			Water / sediment	44 / 11.5	
3-CP		162.55	Water / sediment	17.9 / 6.5	Y

**zRMS comments:**

Information regarding metabolites of 2,4-D, clopyralid and fluroxypyr provided in Tables 9.1-3 to 9.1-5 above is in line with EU agreed data reported in:

- EFSA Journal 2014;12(9):3812 for 2,4-D,
- EFSA Journal 2018;16(7):5389 for clopyralid,
- EFSA Journal 2011;9(3):2091 for fluroxypyr.

Additional information has been added by the zRMS for completeness.

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substances Clopyralid, Fluroxypyr acid and its variant Fluroxypyr meptyl, 2,4-D acid and its variant ester 2,4-D EHE (**Tables 9.2-1** to **Table 9.2-3**). Ecotoxicology studies with 2,4-D EHE were evaluated as part of the Annex I renewal of 2,4-D acid in 2001. The subsequent Annex I Renewal (AIR) focused on 2,4-D acid alone, since the ester is considered to be a variant of 2,4-D acid. 2,4-D EHE is rapidly converted to 2,4-D acid in the environment; as a result, environmental exposure to 2,4-D EHE is transient and limited to a few hours immediately after application. Furthermore, in plants and animals, 2,4-D EHE is rapidly converted to 2,4-D acid through de-esterification, such that any uptake of 2,4-D EHE from the environment results in systemic exposure to 2,4-D acid. Thus, 2,4-D acid is the environmentally relevant chemical to be considered, particularly in long-term risk assessments. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on birds for ADM.3304.H.1.A (old code AG-CDF1-480 EC) were not evaluated as part of the EU review of Clopyralid, Fluroxypyr acid and its variant Fluroxypyr meptyl, 2,4-D acid and its variant ester 2,4-D EHE. An acute oral toxicity test with the formulation AG-CDF1-480 EC on birds is available. The results are presented in **Table 9.2-4** below. There is no indication of increased toxicity of the formulation. Thus, further data on ADM.3304.H.1.A (old code AG-CDF1-480 EC) are not considered essential.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. Justifications are provided below.

**Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds**

Species	Substance	Exposure System	Results	Reference
Canary	2,4-D	Oral, 1 d Acute	LD <sub>50</sub> = 633 mg/kg bw	EFSA Journal 2014;12(9):3812
Japanese quail	2,4-D	Oral, 1 d Acute	LD <sub>50</sub> = 617.3 mg/kg bw	EFSA Journal 2014;12(9):3812
Bobwhite quail	2,4-D	Oral, 1 d Acute	LD <sub>50</sub> = 500 mg/kg bw	EFSA Journal 2014;12(9):3812
Bobwhite quail	2,4-D	Dietary Reproductive toxicity	NOEL = 100 mg/kg bw/day <sup>1</sup>	EFSA Journal 2014;12(9):3812
Bobwhite quail	2,4-D	Dietary Reproductive toxicity	NOEL > 101 mg/kg bw/day <sup>2</sup>	EFSA Journal 2014;12(9):3812
Japanese quail	2,4-D	Dietary Reproductive toxicity	NOEL = 100 mg/kg bw/day <sup>2</sup>	EFSA Journal 2014;12(9):3812
Mallard duck	2,4-D EHE	Oral, 1 d Acute	LD <sub>50</sub> = 663 mg/kg bw (438 ae/kg bw)	<a href="#">Bridging report 2018 SANCO 7599/V1/97-final (1-October-2001)</a>
Bobwhite quail	2,4-D EHE	Dietary Reproductive toxicity	NOEL ≥ > 230 mg/kg bw/day (152 ae/kg bw)	Test provided with EU Bridging report 2018, Temple et al., 2011

ae: acid equivalents

**Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds**

Species	Substance	Exposure System	Results	Reference
Mallard duck	Clopyralid	Oral, 1 d Acute	LD <sub>50</sub> = 1465 mg/kg bw	EFSA Journal 2018;16(8):5389
Mallard duck	Clopyralid	Dietary Reproductive toxicity	NOEC = 1000 mg/kg diet equivalent to 118 mg/kg bw/day	EFSA Journal 2018;16(8):5389

**Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds**

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Fluroxypyr-meptyl	Oral, 1 d Acute	LD <sub>50</sub> > 2000 mg/kg bw	EFSA Journal 2011;9(3):2091
Bobwhite quail	Fluroxypyr acid	Oral, 1 d Acute	LD <sub>50</sub> > 2000 mg/kg bw	EFSA Journal 2011;9(3):2091
Mallard duck	Fluroxypyr-meptyl	Dietary Reproductive toxicity	NOEL = 57.8 mg/kg bw/day	EFSA Journal 2011;9(3):2091
Mallard duck	Fluroxypyr-meptyl	Dietary Reproductive toxicity	NOEL = 40.1 mg ae/kg bw/day <sup>1</sup>	EFSA Journal 2011;9(3):2091

<sup>1</sup> Equivalent of acid obtained by recalculation of the long-term Fluroxypyr-meptyl endpoint

<sup>1</sup> Estimated based on NOEC (ppm diet) × 0.1 in accordance with EFSA/2009/1438.

<sup>2</sup> Estimated based on study results.

ae: acid equivalents

**Table 9.2-4: Endpoints and effect values relevant for the risk assessment for birds**

Species	Substance	Exposure System	Results	Reference
Japanese quail	AG-CDF1-480 EC	Oral, 1 d Acute	LD <sub>50</sub> > 2000 mg/kg bw	KHIA-10.1.1/01 ...

### Metabolites of the active substances

No metabolites of 2,4-D acid, Clopyralid and Fluroxypyr acid are expected to be present in food items (plant material). No environmental risk assessment is therefore deemed necessary.

### zRMS comments:

Avian toxicity data for 2,4-D (acid form), clopyralid, and fluroxypyr are in line with EU agreed endpoints reported in EFSA Journal 2014;12(9):3812, EFSA Journal 2018;16(8):5389, and EFSA Journal 2011;9(3):2091, respectively.

The toxicity data for 2,4-D EHE are in line with endpoints presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Since the SANCO 7599/VI/97 final is not applicable anymore, reference to this document has been struck through in Table 9.2-1 and the Bridging Report has been referenced as being the relevant document where the currently agreed EU data for 2,4-D 2-EHE may be found.

The study on acute toxicity of AG-CDF1-480 EC to birds was evaluated and agreed by the zRMS. Details of the evaluation together with the study summary may be found in Appendix 2. Endpoint reported in Table 9.2-3 is confirmed to be correct.

It is noted that the study with the formulation was performed with the old version of the formulation (AG-CDF1-480 EC) while the authorisation is sought for formulation ADM.3304.H.1.A (AG-CDF1-480 EC1). The change of the composition included removal of <3% of one solvent and addition of the same amount of the other solvent. The removed solvent was toxic to aquatic organisms with EC<sub>50</sub> for algae <1.0 mg/L, while the added solvent is not toxic to aquatic species with L(E)C<sub>50</sub> values for fish, *Daphnia magna* and algae being all >100 mg/L. Based on that, no change of the ecotoxicological profile of the new version of the formulation is expected. Please note that only aquatic toxicity data are available for co-formulants, but in line with the current legislation, no further studies with other non-target species are required.

No 2,4-D, clopyralid and fluroxypyr plant metabolites were included in the EU residue definitions and no risk assessment for any of the metabolites was deemed necessary at the EU level. Taking this into account, no evaluation was required for this zonal evaluation of ADM.3304.H.1.A.

Additional information have been added in tables above is considered necessary from the informative point of view.

### 9.2.1.1 Justification for new endpoints

Screening/Tier I risk assessments for Fluroxypyr acid and its variant are based on the EU agreed toxicity endpoints for birds. The endpoints for 2,4-D acid and Clopyralid have been determined by using the approaches included in Points 2.3 and 2.4 of EFSA/2009/1438:

- For 2,4-D acid, the geometric mean derived from acute LD<sub>50</sub> values presented in **Table 9.2-3** is used in the acute risk assessment.
- For reproductive risk assessments, the NOEL from chronic bird studies is compared to the acute oral LD<sub>50</sub> value used in the acute avian assessment (either the LD<sub>50</sub> for a single species, or the geometric mean for multiple species) and divided it by 10 to obtain LD<sub>50</sub>/10. For 2,4-D acid, the ratio LD<sub>50</sub>/10 is lower than the EU agreed NOEL from chronic bird testing and provides a more conservative endpoint. Therefore, the ratio LD<sub>50</sub>/10 is used for the risk assessment.

**Table 9.2-5: Proposed Endpoints for 2,4-D and Clopyralid**

Study	Test species	EU agreed endpoints	Endpoints used in risk assessment
2,4-D acid			
Acute toxicity	Canary	LD <sub>50</sub> = 633 mg/kg bw	Geometric mean: LD <sub>50</sub> = 580.3 mg/kg bw
	Japanese quail	LD <sub>50</sub> = 617.3 mg/kg bw	
	Bobwhite quail	LD <sub>50</sub> = 500 mg/kg bw	
Reproductive toxicity (long-term)	Bobwhite quail	NOEC = 100 mg/kg bw/day <sup>1</sup>	NOEC = 100 mg/kg bw/day The risk assessment has been conducted with the lowest endpoint ( <b>58.03 mg/kg bw</b> based on LD <sub>50</sub> /10) in accordance with the current EFSA/2009/1438
	Bobwhite quail	NOEC > 101 mg/kg bw/day <sup>2</sup>	
	Japanese quail	NOEC = 100 mg/kg bw/day <sup>2</sup>	
Clopyralid			
Acute toxicity	Mallard duck	LD <sub>50</sub> = 1465 mg/kg bw	LD <sub>50</sub> = 1465 mg/kg bw/day
Reproductive toxicity (long-term)	Mallard duck	NOEC = 1000 mg/kg diet equivalent to 118 mg/kg bw/day	NOEC = 118 mg/kg bw/day. Since LD <sub>50</sub> /10 (146.5 mg/kg bw) is higher than the NOEC available (118 mg/kg bw/day), the risk assessment has been conducted with the lowest endpoint ( <b>118 mg/kg bw/day</b> based on NOEC) in accordance with the current EFSA/2009/1438.

Due to its rapid degradation in the environment, 2,4-D EHE is available to wild vertebrates only immediately after spraying. The two molecules produce similar ecotoxicological effects in wild vertebrate, since the acute, short-term and long-term endpoints for 2,4-D EHE, following exposure to birds are comparable to the same endpoints for 2,4-D acid when the ester endpoints are expressed as acid equivalents (a.e.)<sup>1</sup>. Thus, the endpoints for 2,4-D EHE and 2,4-D acid can be considered ecotoxicologically equivalent.

<sup>1</sup> Endpoints for 2,4-D 2-EHE are converted to acid equivalents (a.e.) using a molecular weight conversion factor of 0.66 (2,4-D 2-EHE molecular weight = 333.26; 2,4-D molecular weight = 221.0).

**Table 9.2-6: Comparison of 2,4-D acid and its variant 2,4-D EHE endpoints derived from avian studies**

Test species/system	Type	Lowest Endpoint		Comment
		Acid <sup>1</sup>	Ester <sup>2</sup>	
Acute toxicity to birds	LD <sub>50</sub> (mg/kg bw)	500 – 633	663 (a.e.: 438)	Endpoints comparable (within 2×) when expressed as acid equivalents.
Dietary toxicity to birds	LC <sub>50</sub> (mg/kg feed)	> 5620	> 5620 (a.e.: > 3709)	Endpoints non-definitive, but maximum concentration tested is comparable (within 2×) when expressed as acid equivalents.
Reproductive toxicity to birds	NOEL (mg/kg bw/d)	≥ 100	> 230 (a.e.: > 152)	Endpoints non-definitive, but maximum concentration tested is comparable (within 2×) when expressed as acid equivalents.

<sup>1</sup> EFSA Journal 2014;12(9):3812.

<sup>2</sup> 2,4-D 2-EFE Bridging Report (2018) SANCO-7599/VL/97-final (1-October-2001).

a.e. = acid equivalent.

#### zRMS comments:

Endpoints proposed for the risk assessment performed for 2,4-D acid and clopyralid (Table 9.2-5) are in line with values used during renewal of both active substances and thus they do not deviate from the EU agreed endpoints. Hence, specific justification for their use is not necessary.

With regard to endpoints for 2,4-D 2-EHE, they are in line with data reported in 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Although they are comparable with 2,4-D acid, respective evaluation should be performed due to higher exposure to an ester form.

An avian acute oral toxicity data is available for the formulated product AG-CDF1-480 EC. **Its LD<sub>50</sub> was estimated to be > 2000 mg/kg, indicating a low toxicity of AG-CDF1-480 EC to birds.**

#### Toxicity of mixture

According to the EFSA/2009/1438, the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD<sub>50</sub> for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

$$LD_{50}(\text{mix}) = \left( \sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

X(a.s.<sub>i</sub>) = fraction of each a.s. in the mixture

LD<sub>50</sub>(a.s.<sub>i</sub>) = acute toxicity value for each a.s.

**Table 9.2-7: LD<sub>50</sub> for ADM.3304.H.1.A (old code AG-CDF1-480 EC) mixture**

Test substance	Concentration of active substance in formulation [g a.s./L]	Fraction of active substance in the formulation mixture (X(a.s.) in the mixture) <sup>a</sup>	Endpoint LD <sub>50</sub> [mg/kg bw]	LD <sub>50</sub> (mix) <sup>b</sup> [mg/kg bw]
2,4-D acid	375	0.73	580.3	> 712
Clopyralid	30	0.06	1465	
Fluroxypyr-meptyl	108	0.21	> 2000	

<sup>a</sup> X(a.s.) = Conc. of a.s./Σ conc. a.s.<sub>i</sub>

<sup>b</sup> LD<sub>50</sub> (mix) = 1/Σ (X(a.s.<sub>i</sub>)/LD<sub>50</sub>(a.s.<sub>i</sub>))

According to the EFSA/2009/1438, measured endpoints should only be replaced by modelled endpoints if a significant change of the predicted toxicity is expected. This may be the case if one toxicant may contribute to more than 90 % of the toxicity of the mixture. The toxicity may be compared on basis of artificial “toxicity per fraction” quotients. These quotations have no biological relevance, but are

calculated for comparison only according to the equations below. In case the quotient for one single toxicant deviates from the quotient for the mixture by less than 10 % it is assumed to contribute to more than 90 % of the toxicity of the mixture (see **Table 9.2-8**).

$$\text{Tox. per fraction (a.s.)} = \frac{\text{LD}_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{Tox. per fraction (mix)} = \frac{\text{LD}_{50}(\text{mix})}{\sum X(\text{a.s.}_i)}$$

**Table 9.2-8: Comparison of measured and modelled toxicity data**

Active substance	LD <sub>50</sub> [mg a.s./kg bw]	X (a.s.) in the mixture	Tox. per fraction (a.s.)	Deviation [%] <sup>a</sup>	Endpoint used for risk assessment
LD <sub>50</sub> (mix)	712	1	712	-	-
2,4-D acid	580.3	0.73	794	10.3	
Clopyralid	1465	0.06	25052	97.2	
Fluroxypyr-meptyl	> 2000	0.21	9500	92.5	

<sup>a</sup> Deviation (%) = ((tox. per fraction (a.s.) - LD<sub>50</sub> (mix))/tox. per fraction (a.s.)) × 100.

The deviation between the tox per fraction of a.s.<sub>i</sub> and mixture is greater than 10 % for three substances. Indicating none of them contribute to ≥ 90 % the toxicity of the formulated product ADM.3304.H.1.A (old code AG-CDF1-480 EC), Consequently, the risk assessment should be performed with the data of the formulation itself.

The mixture toxicity derived from data 2,4-D EHE is also presented below.

**Table 9.2-9: LD<sub>50</sub> for ADM.3304.H.1.A (old code AG-CDF1-480 EC) mixture**

Test substance	Concentration of active substance in formulation [g a.s./L]	Fraction of active substance in the formulation mixture (X (a.s.) in the mixture) <sup>a</sup>	Endpoint LD <sub>50</sub> [mg/kg bw]	LD <sub>50</sub> (mix) <sup>b</sup> [mg/kg bw]
2,4-D EHE	562.5	0.80	663	> 759
Clopyralid	30	0.04	1465	
Fluroxypyr-meptyl	108	0.15	> 2000	

<sup>a</sup> X (a.s.) = Conc. of a.s./Σ conc. a.s.<sub>i</sub>

<sup>b</sup> LD<sub>50</sub> (mix) = 1/Σ (X(a.s.)<sub>i</sub>/LD<sub>50</sub>(a.s.)<sub>i</sub>)

**Table 9.2-10: Comparison of measured and modelled toxicity data**

Active substance	LD <sub>50</sub> [mg a.s./kg bw]	X (a.s.) in the mixture	Tox. per fraction (a.s.)	Deviation [%] <sup>a</sup>	Endpoint used for risk assessment
LD <sub>50</sub> (mix)	759	1	759	-	-
2,4-D EHE	663	0.80	825	8.1	
Clopyralid	1465	0.04	34330	97.8	
Fluroxypyr-meptyl	> 2000	0.15	13019	94.1	

<sup>a</sup> Deviation (%) = ((tox. per fraction (a.s.) - LD<sub>50</sub> (mix))/tox. per fraction (a.s.)) × 100.

The deviation between the tox per fraction of a.s.<sub>i</sub> and mixture is greater than 10 % in case of Clopyralid and Fluroxypyr-meptyl, but < 10 % (i.e. 8.1 %) for 2,4-D EHE. This indicates that 2,4-D EHE contributes to ≥ 90 % the toxicity of the formulated product ADM.3304.H.1.A (old code AG-CDF1-480 EC) so the other components of the mixture will only have a marginal impact on the predicted risk. Consequently, the risk assessment can be performed for the most toxic active substance alone, i.e. 2,4-D EHE (the active substance identified as “driver” of the toxicity of the mixture).

Regardless of the outcome of analysis of mixture toxicity, it is important to note the acute risk assessment for birds has been conducted for all active substances and/or variants: 2,4-D, 2,4-D EHE, Clopyralid, Fluroxypyr meptyl and Fluroxypyr acid. The risk assessment for the formulated product AG-CDF1-480 EC was conducted using the measured LD<sub>50</sub> value as EFSA/2009/1438

**recommends if there is no significant change between the modelled data and experimental one.**

**zRMS comments:**

The presented above acute mixture toxicity is agreed by the zRMS. The combined acute risk assessment should be performed using the measured toxicity for the formulation and due to different outcome of the calculations depending whether the ester or acid form of 2,4-D is considered, the risk assessment for each form of the active compounds is relevant.

**Mixture toxicity effects relevant for long-term exposure**

For mixtures of compounds acting the same way, i.e. with the same molecular targets causing similar effects via similar mechanisms driving the risk assessments, assessments for combined effects on a case-by-case basis are recommended.

A simplified approach recommended in EFSA/2009/1438 is to express all active substances belonging to the same group on a molar basis to account for differences in molar weight in terms of their most toxic representative. Risk assessments are then performed based on the NOEC for the most toxic compound. As Fluroxypyr-meptyl clearly drives the risk assessment, considerations of combined effects are not deemed necessary for reproductive risk assessments.

**zRMS comments:**

The Applicants' statement that fluroxypyr-meptyl is driving the long-term risk from ADM.3304.H.1.A is not substantiated by any relevant data of calculations.

It has to be noted that in case of the risk assessment, it cannot be simply assumed that substance with the lowest endpoint is driving the risk since also the exposure should be taken into account and in case of uses of ADM.3304.H.1.A the greatest exposure will be from 2,4-D (750 g a.s./ha), being 5 times higher comparing to fluroxypyr (on the acid equivalents basis). Furthermore, the long-term risk assessment for 2,4-D is based on LD<sub>50</sub>/10 (58.03 mg ae/kg bw/d), being comparable with fluroxypyr endpoint (57.8 mg ae/kg bw/d). Taking into account both, toxicity and exposure, it seems that 2,4-D is more likely to drive the long term risk from ADM.3304.H.1.A.

Nevertheless, in case of the long-term combined risk assessment it is not relevant to estimate the endpoint for the mixture, since the NOEL values for particular active compound may be based on different parameters. Taking this into account, the simplified approach with calculation of the TER<sub>mix</sub> should be taken, which will be done by the zRMS in point 9.2.2.1 below.

## **9.2.2 Risk assessment for spray applications**

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

### **9.2.2.1 First-tier assessment (screening/generic focal species)**

The results of the acute and reproductive screening/first-tier risk assessments for each active substance, variants and the formulation are summarised in the following tables.



### **Risk assessment for 2,4-D EHE and 2,4-D acid**

The variant 2,4-D EHE is rapidly degraded to 2,4-D acid in the environment. As a result, environmental exposure to 2,4-D EHE is transient and limited to a few hours immediately after application. In plants and animals, 2,4-D EHE is also rapidly converted to 2,4-D acid through de-esterification, such that any uptake of 2,4-D EHE from the environment results in systemic exposure to 2,4-D acid. Thereby, it can be concluded that repeated and long-term exposure 2,4-D acid is the only environmentally relevant substance to be considered in the long-term risk assessment. This means that no chronic risk assessment for the variant 2,4-D EHE is deemed necessary.

**Table 9.2-11: Screening assessment of the acute risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use		Grassland				
Active substance/product		2,4-D EHE				
Application rate (g/ha)		1 × 1125				
Acute toxicity (mg/kg bw)		663				
TER criterion		40				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
Grassland	Large herbivorous bird	30.5	1.0	34.31	19.3	

SV: shortcut value; MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-12: First-tier assessment of the acute risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use		Cereals (BBCH 21–39)				
Active substance/product		2,4-D EHE				
Application rate (g/ha)		1 × 1125				
Acute toxicity (mg/kg bw)		663				
TER criterion		40				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
Cereals, early (shoots) autumn–winter BBCH 10–29	Large herbivorous bird “goose”	30.5	1.0	34.31	19.3	
Cereals, early (shoots) autumn–winter BBCH 10–29	Small omnivorous bird “lark”	24.0	1.0	27.00	24.6	

SV: shortcut value; MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-13: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use		Grassland				
Active substance/product		2,4-D acid				
Application rate (g/ha)		1 × 750				
Acute toxicity (mg/kg bw)		580.3				
TER criterion		40				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
Grassland	Large herbivorous bird	30.5	1.0	22.88	25.4	
Reprod. toxicity (mg/kg bw/d)		58.03				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>	
Growth stage						
Grassland	Large herbivorous bird	16.2	0.53	6.44	9.0	

SV: shortcut value; MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-14: First tier assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use		Cereals (BBCH 21—39)				
Active substance/product		2,4-D acid				
Application rate (g/ha)		1 × 750				
Acute toxicity (mg/kg bw)		580.3				
TER criterion		40				
Crop scenario Growth stage	Indicator/generic focal species		SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>ca</sub>
Cereals, BBCH 10—29	Small omnivorous bird “lark”		24.0	1.0	18.00	32.2
Cereals, BBCH 30—39	Small omnivorous bird “lark”		12.0	1.0	9.0	64.5
Cereals, early (shoots) autumn-winter BBCH 10—29	Large herbivorous bird “goose”		30.5	1.0	22.88	25.4
Reprod. toxicity (mg/kg bw/d)		58.03				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species		SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals, BBCH 10—29	Small omnivorous bird “lark”		10.9	0.53	4.33	13.4
Cereals, BBCH 30—39	Small omnivorous bird “lark”		5.4	0.53	2.15	27.0
Cereals, early (shoots) autumn-winter BBCH 10—29	Large herbivorous bird “goose”		16.2	0.53	6.44	9.0

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

#### **zRMS comments:**

Although the dietary risk assessment for birds from ester and acid form of 2,4-D could be partially agreed, it was performed for only part of the generic indicator species listed in EFSA (2009) for the intended BBCH stages of grassland and cereals, while all respective species should be included for completeness, even if some of them will have lower SV values. Furthermore, for cereals also large herbivorous bird (goose) was considered, as being in theory relevant for BBCH stages 10-29, however in Annex I of EFSA (2009) it is clearly indicated that this species is relevant for autumn-winter, while ADM.3304.H.1.A is intended to be used during spring only. Taking this into account, large herbivore is not relevant for the intended uses of ADM.3304.H.1.A in cereals.

As to variants of 2,4-D considered by the Applicant, in point 9.2.1.1 it was indicated that endpoints for an acid and ester form are equivalent and that the risk assessment will be focused on acid form. Nevertheless, the Applicant decided to perform the acute risk assessment for 2,4-D EHE, but waived the long-term risk assessment for this form. This is not agreed by the zRMS, as in case it is decided to include this form in evaluation, both, acute and long-term risk, should be covered, regardless of the rate of dissipation of the substance in the environment. It has to be noted that fluroxypyr-meptyl is also rapidly transformed into the acid form, but the full risk assessment has been performed for both forms of this compound. Consistent approach should be taken for all active substances.

Since correction of tables above would require inclusion of additional calculations and struck through of some calculations, the presentation of the evaluation would become less transparent. Taking this into account it was decided by the zRMS to struck through whole risk assessment for 2,4-D above and to provide correct calculations below. For the long-term risk assessment for 2,4-D EHE LD<sub>50</sub>/10 has been used as being lower than NOEL derived from the reproductive toxicity study. All calculations were performed with unrounded values.

Risk assessment for 2,4-D EHE from uses in grassland

Intended use	ADM.3304.H.1.A, grassland, BBCH 21-39				
Active substance/product	2,4-D EHE				
Application rate (g/ha)	1 x 1125				
Acute toxicity (mg/kg bw)	663				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Growth stage					
Grassland	Large herbivorous bird “goose”	30.5	1	34.31	19.3
Growing shoots	Small insectivorous bird “wagtail”	26.8		30.15	22.0
Reprod. toxicity (mg/kg bw/d)	66.3 (LD <sub>50</sub> /10)				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> ×	DDD <sub>m</sub>	TER <sub>lt</sub>
Growth stage			TWA	(mg/kg bw/d)	
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	9.66	6.9
Growing shoots	Small insectivorous bird “wagtail”	11.3		6.74	9.8

Based on above calculations acceptable acute and long-term dietary risk to birds from 2,4-D EHE following intended uses of ADM.3304.H.1.A in grassland may be concluded.

Risk assessment for 2,4-D EHE from uses in cereals

Intended use	ADM.3304.H.1.A, cereals, BBCH 21-39				
Active substance/product	2,4-D EHE				
Application rate (g/ha)	1 x 1125				
Acute toxicity (mg/kg bw)	663				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Growth stage					
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24.0	1	27.0	24.6
Cereals, BBCH 30-39	Small omnivorous bird “lark”	12.0		13.5	49.1
Reprod. toxicity (mg/kg bw/d)	66.3 (LD <sub>50</sub> /10)				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> ×	DDD <sub>m</sub>	TER <sub>lt</sub>
Growth stage			TWA	(mg/kg bw/d)	
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	1 x 0.53	6.50	10.2
Cereals, BBCH 30-39	Small omnivorous bird “lark”	5.4		3.22	20.6

Based on above calculations acceptable acute and long-term dietary risk to birds from 2,4-D EHE following intended uses of ADM.3304.H.1.A in cereals may be concluded.

Risk assessment for 2,4-D acid from uses in grassland

Intended use	ADM.3304.H.1.A, grassland, BBCH 21-39				
Active substance/product	2,4-D acid				
Application rate (g/ha)	1 x 750				
Acute toxicity (mg/kg bw)	580.3				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Growth stage					
Grassland	Large herbivorous bird “goose”	30.5	1	22.88	25.4
Growing shoots	Small insectivorous bird “wagtail”	26.8		20.10	28.9
Reprod. toxicity (mg/kg bw/d)	58.03 (LD <sub>50</sub> /10)				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> ×	DDD <sub>m</sub>	TER <sub>lt</sub>
Growth stage			TWA	(mg/kg bw/d)	
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	6.44	9.01
Growing shoots	Small insectivorous bird “wagtail”	11.3		4.49	12.9

Based on above calculations acceptable acute and long-term dietary risk to birds from 2,4-D acid following intended uses of ADM.3304.H.1.A in grassland may be concluded.

Risk assessment for 2,4-D acid from uses in cereals

<b>Intended use</b>	<b>ADM.3304.H.1.A, cereals, BBCH 21-39</b>				
<b>Active substance/product</b>	2,4-D acid				
<b>Application rate (g/ha)</b>	1 x 750				
<b>Acute toxicity (mg/kg bw)</b>	580.3				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>
<b>Growth stage</b>					
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24.0	1	18.0	32.2
Cereals, BBCH 30-39	Small omnivorous bird “lark”	12.0		9.0	64.5
<b>Reprod. toxicity (mg/kg bw/d)</b>	58.03 (LD <sub>50</sub> /10)				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
<b>Growth stage</b>					
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	1 x 0.53	4.33	13.4
Cereals, BBCH 30-39	Small omnivorous bird “lark”	5.4		2.15	27.0

Based on above calculations acceptable acute and long-term dietary risk to birds from 2,4-D acid following intended uses of ADM.3304.H.1.A in cereals may be concluded.

No 2,4-D plant metabolites are included in the residue definition and no risk assessment for any of the metabolites was deemed necessary at the EU level. Taking this into account, no evaluation was required for this zonal evaluation of ADM.3304.H.1.A.

**Risk assessment for Clopyralid**

**Table 9.2-15: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

<b>Intended use</b>	<b>ADM.3304.H.1.A, grassland, BBCH 21-39</b>				
<b>Active substance/product</b>	Clopyralid				
<b>Application rate (g/ha)</b>	1 x 60				
<b>Acute toxicity (mg/kg bw)</b>	1465				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>
<b>Growth stage</b>					
Grassland	Large herbivorous bird “goose”	30.5	1	1.83	800.5
<b>Reprod. toxicity (mg/kg bw/d)</b>	118				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
<b>Growth stage</b>					
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	0.52	229.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-16: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use	ADM.3304.H.1.A, cereals (BBCH 21 – 39)				
Active substance/product	Clopyralid				
Application rate (g/ha)	1 x 60				
Acute toxicity (mg/kg bw)	1465				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals	Small omnivorous bird	158.8	1	9.53	153.8
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals	Small omnivorous bird	64.8	1 x 0.53	2.06	57.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**zRMS comments:**

The screening risk assessment for birds exposed to clopyralid used as ADM.3304.H.1.A in grassland and cereals is agreed by the zRMS. Acceptable acute and long-term dietary risk to birds may be concluded from both intended uses of the product.

No clopyralid plant metabolites are included in the residue definition and no risk assessment for any of the metabolites was deemed necessary at the EU level. Taking this into account, no evaluation was required for this zonal evaluation of ADM.3304.H.1.A.

Although the Tier 1 risk assessment was not necessary, long-term Tier 1 TER values were calculated by the zRMS in tables below as being necessary for purposes of the long-term combined risk assessment. All calculations were performed with unrounded values

Uses in grassland

Intended use	ADM.3304.H.1.A, grassland, BBCH 21-39				
Active substance/product	Clopyralid				
Application rate (g/ha)	1 x 60				
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	0.52	229.1
Growing shoots	Small insectivorous bird “wagtail”	11.3		0.36	328.4

Uses in cereals

Intended use	ADM.3304.H.1.A, cereals (BBCH 21 – 39)				
Active substance/product	Clopyralid				
Application rate (g/ha)	1 x 60				
Reprod. toxicity (mg/kg bw/d)	118				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	1 x 0.53	0.35	340.4
Cereals, BBCH 30-39	Small omnivorous bird “lark”	5.4		0.17	687.2

### **Risk assessment for Fluroxypyr-meptyl and Fluroxypyr acid**

**Table 9.2-17: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use	<b>ADM.3304.H.1.A, grassland, BBCH 21-39</b>				
Active substance/product	Fluroxypyr-meptyl				
Application rate (g/ha)	1 x 216				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Grassland	Large herbivorous bird “goose”	30.5	1	6.59	> 303.6
Reprod. toxicity (mg/kg bw/d)	57.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	1.85	31.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-18: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use	<b>ADM.3304.H.1.A, cereals (BBCH 21 – 39)</b>				
Active substance/product	Fluroxypyr-meptyl				
Application rate (g/ha)	1 x 216				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals	Small omnivorous bird	158.8	1	34.30	> 58.3
Reprod. toxicity (mg/kg bw/d)	57.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals	Small omnivorous bird	64.8	1 x 0.53	7.42	7.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-19: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use	<b>ADM.3304.H.1.A, grassland, BBCH 21-39</b>				
Active substance/product	Fluroxypyr acid				
Application rate (g/ha)	1 x 150				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Grassland	Large herbivorous bird “goose”	30.5	1	4.58	> 437.2
Reprod. toxicity (mg/kg bw/d)	40.1				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	1.29	31.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-20: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use	ADM.3304.H.1.A, cereals (BBCH 21 – 39)				
Active substance/product	Fluroxypyr acid				
Application rate (g/ha)	1 x 150				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals	Small omnivorous bird	158.8	1	23.82	> 84.0
Reprod. toxicity (mg/kg bw/d)	40.1				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals	Small omnivorous bird	64.8	1 x 0.53	5.15	7.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**zRMS comments:**

The screening risk assessment for birds exposed to fluroxypyr in a form of ester and acid used as ADM.3304.H.1.A in grassland and cereals is agreed by the zRMS. Acceptable acute and long-term dietary risk to birds may be concluded from both intended uses of the product.

No fluroxypyr plant metabolites are included in the residue definition and no risk assessment for any of the metabolites was deemed necessary at the EU level. Taking this into account, no evaluation was required for this zonal evaluation of ADM.3304.H.1.A.

Although the Tier 1 risk assessment was not necessary, long-term Tier 1 TER values were calculated by the zRMS in tables below as being necessary for purposes of the long-term combined risk assessment. All calculations were performed with unrounded values

Uses of fluroxypyr-meptyl in grassland

Intended use	ADM.3304.H.1.A, grassland, BBCH 21-39				
Active substance/product	Fluroxypyr-meptyl				
Application rate (g/ha)	1 x 216				
Reprod. toxicity (mg/kg bw/d)	57.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	1.85	31.2
Growing shoots	Small insectivorous bird “wagtail”	11.3		1.29	44.7

Uses of fluroxypyr-meptyl in cereals

Intended use	ADM.3304.H.1.A, cereals (BBCH 21 – 39)				
Active substance/product	Fluroxypyr-meptyl				
Application rate (g/ha)	1 x 216				
Reprod. toxicity (mg/kg bw/d)	57.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	1 x 0.53	1.25	46.3
Cereals, BBCH 30-39	Small omnivorous bird “lark”	5.4		0.62	93.5

Uses of fluroxypyr acid in grassland					
Intended use	ADM.3304.H.1.A, grassland, BBCH 21-39				
Active substance/product	Fluroxypyr acid				
Application rate (g/ha)	1 x 150				
Reprod. toxicity (mg/kg bw/d)	40.1				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Grassland	Large herbivorous bird “goose”	16.2	1 x 0.53	1.29	31.1
Growing shoots	Small insectivorous bird “wagtail”	11.3		0.90	44.6

Uses of fluroxypyr acid in cereals					
Intended use	ADM.3304.H.1.A, cereals (BBCH 21 – 39)				
Active substance/product	Fluroxypyr acid				
Application rate (g/ha)	1 x 150				
Reprod. toxicity (mg/kg bw/d)	40.1				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Cereals, BBCH 10-29	Small omnivorous bird “lark”	10.9	1 x 0.53	0.87	46.3
Cereals, BBCH 30-39	Small omnivorous bird “lark”	5.4		0.43	93.4

### **Risk assessment for the formulated product AG-CDF1-480 EC**

**Table 9.2-21: Screening assessment of the acute risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use	Grassland				
Active substance/product	AG-CDF1-480 EC				
Application rate (g/ha)	1 x 1.401 (1.125 kg 2,4 D-EHE + 0.060 kg Clopyralid + 0.216 kg Fluroxypyr meptyl)				
Acute toxicity (mg/kg bw)	≥ 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Grassland	Large herbivorous bird	30.5	1.0	42.73	≥ 46.8

SV: shortcut value; MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.2-22: Screening assessment of the acute risk for birds due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use	Cereals (BBCH 21 – 39)				
Active substance/product	AG-CDF1-480 EC				
Application rate (g/ha)	1 x 1.401 (1.125 kg 2,4 D-EHE + 0.060 kg Clopyralid + 0.216 kg Fluroxypyr meptyl)				
Acute toxicity (mg/kg bw)	≥ 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals, BBCH 10 – 29	Small omnivorous bird “lark”	24.0	1.0	33.62	≥ 59.5
Cereals, BBCH 30 – 39	Small omnivorous bird “lark”	12.0	1.0	16.81	≥ 119.0
Cereals, early (shoots) autumn-winter BBCH 10 – 29	Large herbivorous bird “goose”	30.5	1.0	42.73	≥ 46.8

SV: shortcut value; MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.



#### zRMS comments:

The acute combined dietary risk assessment performed by the Applicant in tables above is not agreed by the zRMS since for calculation of the exposure the sum of active substances in the product was used while the toxicity endpoint is expressed in terms of the formulation. In addition to that, in case of the risk assessment for cereals also large herbivorous bird (goose) was considered, as being in theory relevant for BBCH stages 10-29. However, in Annex I of EFSA (2009) it is clearly indicated that this species is relevant for autumn-winter period, while ADM.3304.H.1.A is intended to be used during spring only. Taking this into account, large herbivore is not relevant for the intended uses of ADM.3304.H.1.A in cereals.

For transparency reasons the acute combined risk assessment was re-calculated by the zRMS in tables below, while calculations provided by the Applicant were struck through. All calculations were performed with unrounded values.

#### Screening combined acute risk assessment for ADM.3304.H.1.A from uses in grassland

Intended use	Grassland				
Active substance/product	ADM.3304.H.1.A				
Application rate (g/ha)	1 x 2.0 L/ha (corresponding to 2180 g/ha, based on density of 1.09 g/mL)				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Grassland	Large herbivorous bird	30.5	1.0	66.49	> 30.1

#### Screening combined acute risk assessment for ADM.3304.H.1.A from uses in cereals

Intended use	Cereals, BBCH 21-39				
Active substance/product	ADM.3304.H.1.A				
Application rate (g/ha)	1 x 2.0 L/ha (corresponding to 2180 g/ha, based on density of 1.09 g/mL)				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals, BBCH 10-29	Small omnivorous bird "lark"	24.0	1.0	52.32	>38.2
Cereals, BBCH 30-39	Small omnivorous bird "lark"	12.0		26.16	>76.5

Based on above calculations acceptable acute combined dietary risk to birds from the intended uses of ADM.3304.H.1.A in grassland cereals may be concluded.

No chronic combined risk assessment was performed by the Applicant. Justification provided in point 9.2.1.1 above was, however, not agreed by the zRMS since the toxicity driver for the long-term (and also acute) risk cannot be determined based solely on toxicity data, which was done by the Applicant. As already indicated in the zRMS comment in point 9.2.1.1, in case of chronic combined risk assessment the simplified approach with calculation of TER<sub>mix</sub> is most suitable. Respective calculations are provided by the zRMS below. Evaluation was performed with consideration of Tier 1 TER values derived for particular active compounds. For sake of simplicity, only the lowest TER values were considered, covering also risk to generic focal species producing lower TER values. For completeness, calculations were performed with two mixtures assumed:

- 2,4-D EHE + clopyralid + fluroxypyr-meptyl,
- 2,4-D acid + clopyralid + fluroxypyr acid.

Compound						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
2,4-D EHE		Clopyralid		Fluroxypyr-meptyl				
TER <sup>1)</sup>	1/TER	TER <sup>1)</sup>	1/TER	TER <sup>1)</sup>	1/TER			
Grassland								
6.9	0.145	229.1	0.004	31.2	0.032	0.181	5.5	5
Cereals								
10.2	0.098	340.4	0.003	46.3	0.022	0.123	8.2	5

<sup>1)</sup> Lowest Tier 1 TER

Compound						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
2,4-D acid		Clopyralid		Fluroxypyr acid				
TER <sup>1)</sup>	1/TER	TER <sup>1)</sup>	1/TER	TER <sup>1)</sup>	1/TER			
Grassland								
9.01	0.111	229.1	0.004	31.1	0.032	0.148	6.8	5
Cereals								
13.4	0.075	340.4	0.003	46.3	0.022	0.099	10.1	5

<sup>1)</sup> Lowest Tier 1 TER

The calculated Tier 1 TER<sub>mix</sub> is above the trigger of 5 for both uses and mixtures demonstrating acceptable long-term combined risk to birds exposed to the mixture of 2,4-D, clopyralid and fluroxypyr applied as ADM.3304.H.1.A.

### Overall conclusion:

All the TERa values for each active substance, variants and the formulated product exceed the trigger value of 10, indicating an acceptable acute risk to birds following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use patterns proposed.

An acceptable chronic risk to birds is expected in grassland and also cereals following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) at the proposed label rates.

### 9.2.2.2 Higher-tier risk assessment

Based on the outcome of the acute and/or chronic risk assessments, no refinement of the risk is necessary.

### 9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

#### Leaf scenario

Since the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

#### Puddle scenario

The ‘puddle scenario’ is considered relevant for the proposed uses of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in grassland and cereals.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ( $K_{OC} < 500$  L/kg) or 3000 in the case of more sorptive substances ( $K_{OC} \geq 500$  L/kg).

With arithmetic mean  $K_{OC}$  values of 58.6 (median of 42 soils), 1.41 and 68 L/kg for 2,4-D acid, Clopyralid and Fluroxypyr acid, these compounds belong to the group of less sorptive substances. With  $K_{fOC}$  of 19550 L/kg, fluroxypyr-meptyl belongs to less sorptive substances for which the trigger of 3000 is applicable.

The formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) contains in its composition the variant 2-ethylhexyl ester of 2,4-D (i.e. 2,4-D EHE). This compound is rapidly converted to 2,4-D acid in the environment. Due to its quick degradation in water, no experimental adsorption/desorption coefficients

could be ~~not~~ derived. It is important to emphasize non-target organisms exposed via drainflow or run-off will be only exposed to 2,4-D acid. To obtain an estimate for PPP concentrations in puddles formed on a field after rainfall (predicted environmental concentration  $PEC_{\text{puddle}}$ ), it is assumed that this concentration is the same as the concentration in runoff water as calculated for the assessment of surface water exposure. A simplified model can be used to calculate  $PEC_{\text{puddle}}$  in mg/L as a function of application rate to bare soil without degradation and the organic carbon adsorption coefficient ( $K_{\text{oc}}$ ) of a substance.

~~In light of all above mentioned, the puddle scenario (runoff water) is not deemed as relevant for 2,4-D EHE.~~

The effective application rate is calculated by multiplying the proposed application rates by MAF values based on the  $DT_{50}$  in soil for the active substances (EFSA/2009/1438). Since the formulated product ADM.3304.H.1.A (old code AG-CDF1-480 EC) is applied once per season,  $MAF = 1$ . The ratios of the effective application rates to the relevant endpoints for each active substance are presented in the following tables.

<b>2,4-D acid</b>				
Effective application rate (g/ha)	=	750		
Acute toxicity (mg/kg bw)	=	580.3	quotient =	1.3
Reprod. toxicity (mg/kg bw/d)	=	58.03	quotient =	12.8
<b>2,4-D EHE</b>				
Effective application rate (g/ha)	=	1125		
Acute toxicity (mg/kg bw)	=	663	quotient =	1.7
Reprod. toxicity (mg/kg bw/d)	=	66.3	quotient =	16.9

<b>Clopyralid</b>				
Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	1465	quotient =	0.04
Reprod. toxicity (mg/kg bw/d)	=	118	quotient =	0.51

<b>Fluroxypyr acid</b>				
Effective application rate (g/ha)	=	150		
Acute toxicity (mg/kg bw)	=	> 2000	quotient =	< 0.1
Reprod. toxicity (mg/kg bw/d)	=	40.1	quotient =	3.6
<b>Fluroxypyr-meptyl</b>				
Effective application rate (g/ha)	=	216		
Acute toxicity (mg/kg bw)	=	> 2000	quotient =	<0.1
Reprod. toxicity (mg/kg bw/d)	=	57.8	quotient =	3.7

Since the ratios of effective application rates (in g/ha) to relevant endpoints (in mg/kg bw/d) are below the critical value of 50, no quantitative risk assessment (calculation of TER values) for each active substance is required.

The above assessments demonstrate that no unacceptable risk to birds emerges from exposure to drinking water from puddles. Thus, no further refinement is necessary.

#### **zRMS comments:**

The drinking water risk assessment for 2,4-acid, clopyralid and fluroxypyr acid presented in tables above is agreed by the zRMS. Additional calculations for fluroxypyr-meptyl were included for completeness.

Although 2,4-D EHE degradation is extremely fast so no soil sorption/desorption studies could be performed, the drinking water risk assessment was included by the zRMS above for precautionary reasons. Due to nature of the compound it may be expected that soil sorption would be >500 L/kg (similarly as in case of other ester forms of phenoxyacids). Nevertheless, both ratios between the effective rates and the respective toxicity data are low (1.7 and

16.9 for acute and long-term risk), so the drinking water risk would be covered even in case the  $K_{foc}$  was <500 L/kg.

Acceptable risk from all forms of active compounds may be concluded for birds exposed via drinking water.

It should be, however, noted that the drinking water risk assessment should be also performed for the pertinent soil metabolites of active substances, which was not done by the Applicant. Respective calculations were thus performed by the zRMS and are presented below. In absence of the toxicity data, 10 times toxicity of the parent was assumed representing worst case. In opinion of the zRMS in case of the risk assessment for metabolites for which no toxicity data are available, it is more appropriate to consider experimentally derived parent endpoints and for this reason for 2,4-D metabolites the lowest NOEL of 100 mg a.s./kg bw/d was taken into account resulting with surrogate endpoint of 10 mg/kg bw/d. The pseudo-application rates of metabolites were calculated with consideration of the parent rate, maximum occurrence in soil and molar ratio. Crop interception was not considered representing worst case.

2,4-D metabolites				
2,4-DCA effective application rate (g/ha)	90.1 (molar ratio 0.801, max occur. 15%)			
Acute toxicity (mg/kg bw)	58.03	quotient =	1.6	Trigger: 50
Reprod. toxicity (mg/kg bw/d)	10.0	quotient =	9.01	
2,4-DCP effective application rate (g/ha)	210.2 (molar ratio 0.738, max occur. 38% (soil anaerobic study))			
Acute toxicity (mg/kg bw)	58.03	quotient =	3.6	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	10.0	quotient =	21.0	
4-CP effective application rate (g/ha)	144 (molar ratio 0.582, max occur. 33%)			
Acute toxicity (mg/kg bw)	58.03	quotient =	2.5	Trigger: 50
Reprod. toxicity (mg/kg bw/d)	10.0	quotient =	14.4	
Fluroxypyr metabolites				
Methoxy pyridine effective application rate (g/ha)	44.3 (molar ratio 0.773, max occur. 38.2%)			
Acute toxicity (mg/kg bw)	>200	quotient =	0.2	Trigger: 50
Reprod. toxicity (mg/kg bw/d)	4.01	quotient =	11.0	
Pyridinol effective application rate (g/ha)	27.8 (molar ratio 0.776, max occur. 23.9%)			
Acute toxicity (mg/kg bw)	>200	quotient =	0.1	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	4.01	quotient =	6.9	

Ratios between the effective rates and toxicity endpoints are below the respective triggers for all pertinent soil metabolites of 2,4-D and fluroxypyr demonstrating acceptable acute and long-term risk to birds exposed via drinking water.

Since no metabolites are formed from clopyralid, no additional calculations were deemed necessary for this compound.

#### 9.2.2.4 Effects of secondary poisoning

According to EFSA/2009/1438<sup>2</sup>, substances with a log  $K_{ow}$  greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

**Table 9.2-23: Log  $K_{ow}$  and BCF values for the variant 2,4-D EHE**

Substance	2,4-D EHE
Log $K_{ow}$	5.78
Bio-concentration factor (BCF)	Not required due to rapid degradation in water (DT <sub>50</sub> of 0.26 d)

<sup>2</sup> European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. [139 pp.]. doi:10.2903/j.efsa.2009.1438. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu)

**Table 9.2-24: Log K<sub>OW</sub> and BCF values for the active substance 2,4-D acid and its metabolites found in fish**

Substance	2,4-D acid	2,4-DCA	2,4-DCP
Log K <sub>OW</sub>	-0.82	3.36	3.06
Bioconcentration factor (BCF)	Not required	31	340

**Table 9.2-25: Log K<sub>OW</sub> and BCF values for the active substance Clopyralid**

Substance	Clopyralid
Log K <sub>OW</sub>	-2.63
Bioconcentration factor (BCF)	Not required

**Table 9.2-26: Log K<sub>OW</sub> and BCF values for the active substance Fluroxypyr acid**

Substance	Fluroxypyr acid
Log K <sub>OW</sub>	0.0393
Bioconcentration factor (BCF)	Not required

**Table 9.2-27: Log K<sub>OW</sub> and BCF values for the variant Fluroxypyr meptyl and its metabolites found in fish**

Substance	Fluroxypyr meptyl	Pyridinol	3-CP	Methoxypyridine
Log K <sub>OW</sub>	4.53	0.039	0.65	3.09
Bioconcentration factor (BCF)	167 (total <sup>14</sup> C) 26 (Fluroxypyr-meptyl)	Not required	Not required	1.41 (estimated with QSAR model)

The log K<sub>OW</sub> values of 2,4-D (i.e. -0.82 at pH 7) and Clopyralid (i.e. -2.63) are below 3, and it is therefore not necessary to consider the risk from secondary poisoning further.

Although for Fluroxypyr-meptyl the log K<sub>OW</sub> is > 3 (i.e. 4.53), the risk from secondary poisoning was not conducted because Fluroxypyr-meptyl is rapidly degraded to Fluroxypyr (acid) and does not accumulate in fish and earthworm.

Although the octanol/water coefficient expressed as log K<sub>OW</sub> is greater than 3 (5.78) the potential for bioaccumulation of 2,4-D EHE in fish is low due to rapid degradation in water (half-life of 6.2 hours in natural water). It is important to note that 2,4-D EHE is also rapidly metabolised to 2,4-D in vertebrates, so bioaccumulation of the ester in fish will not occur.

A risk assessment for secondary poisoning is required for the 2,4-D metabolites 2,4-DCA and 2,4-DCP and the Fluroxypyr meptyl metabolite Methoxypyridine.

### **Risk assessment for earthworm-eating birds via secondary poisoning**

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

**Table 9.2-28: Assessment of the risk for earthworm-eating birds due to exposure to 2,4-DCA via bioaccumulation in earthworms (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCA	Comments
PEC <sub>soil</sub> (twa = 21 d) (mg/kg soil)	0.0870	Minimum interception of 20 % for the application rate of 0.75 kg 2,4-D acid
Log K <sub>ow</sub> / K <sub>ow</sub>	3.36 / 2291	
K <sub>oc</sub>	1028	EU agreed value for groundwater modelling
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	1.38	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.12	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.13	DDD = PEC <sub>worm</sub> × 1.05
NOEL (mg/kg bw/d) <sup>1</sup>	10.0 <del>5.80</del>	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	<b>76.9</b> <del>44.6</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to birds was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid.

**Table 9.2-29: Assessment of the risk for earthworm-eating birds due to exposure to 2,4-DCP via bioaccumulation in earthworms (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCP	Comments
PEC <sub>soil</sub> (twa = 21 d) (mg/kg soil)	0.0832	Minimum interception of 20 % for the application rate of 0.75 kg 2,4-D acid
Log K <sub>ow</sub> / K <sub>ow</sub>	3.06 / 1148	
K <sub>oc</sub>	512	EU agreed value for groundwater modelling
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	1.43	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.12	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.13	DDD = PEC <sub>worm</sub> × 1.05
NOEL (mg/kg bw/d) <sup>1</sup>	10.0 <del>5.80</del>	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	<b>76.9</b> <del>44.6</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to birds was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid.

**Table 9.2-30: Assessment of the risk for earthworm-eating birds due to exposure to Fluroxypyr methoxy pyridine via bioaccumulation in earthworms (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	Fluroxypyr methoxy pyridine	Comments
PEC <sub>soil</sub> (plateau) (mg/kg soil)	0.09	Minimum interception of 20 % for the application rate of 0.15 kg Fluroxypyr
Log K <sub>ow</sub> / K <sub>ow</sub>	3.09 / 1230	
K <sub>oc</sub>	321	
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	2.43	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.22	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.23	DDD = PEC <sub>worm</sub> × 1.05
NOEL (mg/kg bw/d) <sup>1</sup>	4.01 <del>5.78</del>	10 times toxicity of the parent (fluroxypyr acid)
TER <sub>it</sub>	<b>17.4</b> <del>25.2</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to birds was available, it was assumed that it is 10 times more toxic than the parent Fluroxypyr meptyl.

The TER values estimated for the metabolites are above the trigger value of 5, indicating there is no risk to earthworm-eating birds via secondary poisoning following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the GAP Table.

### Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

**Table 9.2-31: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCA via bioaccumulation in fish (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCA	Comments
PEC <sub>sw</sub> max. (Focus Step 2) (mg/L)	0.0062	
TWA factor	0.53	
BCF <sub>fish</sub>	31	
BMF	1.0	Biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.102 <del>0.099</del>	PEC <sub>fish</sub> = PEC <sub>water</sub> × TWA × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.016	DDD = PEC <sub>fish</sub> × 0.159
NOEL (mg/kg bw/d) <sup>1</sup>	<b>10.0</b> <del>5.80</del>	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	<b>638</b> <del>362.5</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to birds was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid.

**Table 9.2-32: Assessment of the risk for fish-eating birds due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCP	Comments
PEC <sub>sw</sub> max. (Focus Step 2) (mg/L)	0.013	
TWA factor	0.53	
BCF <sub>fish</sub>	340	
BMF	1.0	Biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	2.343	PEC <sub>fish</sub> = PEC <sub>water</sub> × TWA × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.371	DDD = PEC <sub>fish</sub> × 0.159
NOEL (mg/kg bw/d) <sup>1</sup>	<b>10.0</b> <del>5.80</del>	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	<b>27.0</b> <del>15.5</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to birds was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid.

**Table 9.2-33: Assessment of the risk for fish-eating birds due to exposure to Fluroxypyr methoxy pyridine via bioaccumulation in fish (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	Fluroxypyr methoxy pyridine	Comments
PEC <sub>sw</sub> max. (Focus Step 2) (mg/L)	0.0055	
TWA factor	0.53	
BCF <sub>fish</sub>	1.41	
BMF	1.0	Biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.004	PEC <sub>fish</sub> = PEC <sub>water</sub> × TWA × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.0006	DDD = PEC <sub>fish</sub> × 0.159
NOEL (mg/kg bw/d) <sup>1</sup>	<b>4.01</b> <del>5.78</del>	10 times toxicity of the parent (fluroxypyr acid)
TER <sub>it</sub>	<b>6750</b> <del>9633.3</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to birds was available, it was assumed that it is 10 times more toxic than the parent Fluroxypyr meptyl.

The TER values estimated for the metabolites are above the trigger value of 5, indicating there is no risk to fish-eating birds via secondary poisoning following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the GAP Table.

**zRMS comments:**

The evaluation of the risk of secondary poisoning was triggered for two metabolites of 2,4-D (2,4-DCA and 2,4-DCP) and one metabolite of fluroxypyr (methoxypyridine).

In line with conclusions from the EU review of 2,4-D and fluroxypyr, the evaluation was not triggered for the ester forms of active compounds despite log Pow >3. This is explained by extremely rapid degradation in water and negligible possibility for bioaccumulation.

The approach of the Applicant as presented in tables above is agreed, however in opinion of the zRMS in case of the risk assessment for metabolites for which no toxicity data are available, it is more appropriate to consider experimentally derived parent endpoints. For this reason for 2,4-D metabolites the lowest NOEL of 100 mg a.s./kg bw/d was taken into account resulting with surrogate endpoint of 10 mg/kg bw/d.

It was also noted that for methoxypyridine the fluroxypyr-meptyl endpoint divided by 10 was considered, while methoxypyridine is formed from fluroxypyr acid and for this reason endpoint agreed for the acid form was considered resulting with the surrogate endpoint of 4.01 mg/kg bw/d. Additional corrections were made in case the exposure agreed in area of Section 8 deviated from PEC values considered by the Applicant. Information of K<sub>ow</sub> has been added in Tables 9.2-28 to 9.2-30 for completeness.

Based on the performed calculations acceptable risk of secondary poisoning could be concluded for earthworm- and fish-eating birds exposed to 2,4-DCA, 2,4-DCP and methoxypyridine.

### 9.2.2.5 Biomagnification in terrestrial food chains

No risk of biomagnification in terrestrial food chains is expected according to the data given in the Point 9.2.2.4.

### 9.2.3 Overall conclusions

#### Acute risk assessment to birds

An acute LD<sub>50</sub> value is available for the formulation AG-CDF1-480 EC. There is no indication of increased toxicity of the formulation.

Acute screening/first-tier risk assessments for each active substance, variants and the formulation were conducted. All the TER<sub>a</sub> values for each active substance, variants and the formulation exceed the trigger value of 10, indicating an acceptable acute risk to birds following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use patterns proposed.

#### Reproductive risk assessment to birds

The TER<sub>lt</sub> values for each active substance were ~~above~~ ~~below~~ the Annex VI trigger value of 5 what indicates that the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) applied according to the intended uses do not pose a potential reproductive risk to birds. Acceptable long-term risk for the mixture was also demonstrated.

#### Secondary poisoning to birds and risk from drinking water

No risk to birds is expected via the consumption of water contaminated with the active substances and their pertinent soil metabolites from puddles on soil. A risk of secondary poisoning in terrestrial environments can be also excluded.



## 9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

### 9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with the active substances Clopyralid, Fluroxypyr acid and its variant Fluroxypyr meptyl, 2,4-D acid and its variant ester 2,4-D EHE (**Tables 9.3-1 to Table 9.3-3**). Ecotoxicology studies with 2,4-D EHE were evaluated as part of the Annex I renewal of 2,4-D acid in 2001. The subsequent Annex I Renewal (AIR) focused on 2,4-D alone, since the ester is considered to be a variant of 2,4-D acid. 2,4-D EHE is rapidly converted to 2,4-D acid in the environment; as a result, environmental exposure to 2,4-D EHE is transient and limited to a few hours immediately after application. Furthermore, in plants and animals, 2,4-D EHE is rapidly converted to 2,4-D acid through de-esterification, such that any uptake of 2,4-D EHE from the environment results in systemic exposure to 2,4-D acid. Thus, 2,4-D acid is the environmentally relevant chemical to be considered, particularly in long-term risk assessments. Full details of these studies are provided in the respective EU RAR and related documents.

An acute oral toxicity study in rat conducted with AG-CDF1-480 EC is available. This test was not evaluated as part of the EU review of the active substances 2,4-D acid, Clopyralid and Fluroxypyr acid. The LD<sub>50</sub> from test was determined to be 500 mg/kg bw. An analysis of mixture toxicity is presented below. There is no increased toxicity of the formulation as indicated by the MDR (model deviation ratio) =  $EC_{Xmix-CA}/EC_{XPP} = 692/500 = 1.4$  (SANTE-2015-00080<sup>3</sup>). Further data on ADM.3304.H.1.A (old code AG-CDF1-480 EC) are therefore not considered essential.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. Justifications are provided below.

**Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals**

Species	Substance	Exposure System	Results	Reference
Rat	2,4-D	Oral, 1 d Acute	LD <sub>50</sub> = 699 mg/kg bw	EFSA Journal 2014;12(9):3812
Rat	2,4-D	Oral, 1 d Acute	LD <sub>50</sub> = 486 mg/kg bw	EFSA Journal 2014;12(9):3812
Rat	2,4-D	Oral, 1 d Acute	LD <sub>50</sub> > 500 mg/kg bw	EFSA Journal 2014;12(9):3812
		Geometric mean LD <sub>50</sub>	LD <sub>50</sub> > 554 mg/kg bw	EFSA Journal 2014;12(9):3812
Rat	2,4-D	Dietary Reproductive toxicity	NOEL = 20.6 mg/kg bw/day	EFSA Journal 2014;12(9):3812
Rat	2,4-D EHE	Oral, 1 d Acute	LD <sub>50</sub> = 896 mg/kg bw	Bridging report 2018 <del>SANCO-7599/VI/97-final</del> (1 October 2001)
Rat	2,4-D EHE	Dietary Reproductive toxicity	NOEL = 16 mg/kg bw/day	Bridging report 2018 <del>SANCO-7599/VI/97-final</del> (1 October 2001)

<sup>1</sup> Estimated based on NOEC (ppm diet) × 0.1 in accordance with EFSA/2009/1438.

<sup>2</sup> Estimated based on study results.

<sup>3</sup> Guidance Document on Tiered Risk Assessment for Plant Protection Products for Aquatic Organisms in Edge-of-Field Surface Waters in the context of Regulation (EC) No. 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015)

**Table 9.3-2: Endpoints and effect values relevant for the risk assessment for mammals**

Species	Substance	Exposure System	Results	Reference
Rat	Clopyralid	Oral, 1 d Acute	LD <sub>50</sub> > 5000 mg/kg bw	EFSA Journal 2018;16(8):5389
Rat	Clopyralid	Dietary Reproductive toxicity	NOEC = 50 mg/kg bw/day <sup>1</sup>	EFSA Journal 2018;16(8):5389

<sup>1</sup> The NOEL used in the wild mammals risk assessment is based on an endpoint derived from a two-year rat study. It is noted that this study was not considered acceptable in the Pesticide Peer Review experts' meeting 175 (mammalian toxicology) due to fundamental deviations from the study protocol. The outcome of the risk assessment does not change when the available reproductive NOEL is considered being the latter higher than the endpoint used in the risk assessment. It is noted that uncertainties still stand on whether the endpoint used in the risk assessment would cover developmental effects in rabbit noting that a developmental LOEL of 50 mg/kg bw/d (based on mean foetal weight decrease and delayed ossification) was agreed at the Pesticide Peer Review experts' meeting 175. Considering that a clear dose response was not observed in the effects on body weight and delayed ossification and that the incidence of delayed ossification was relatively low, the ecotoxicological relevance of the effects seen in the rabbit study is not confirmed as a consequence the risk assessment was not changed; the NOEL of 50 mg/kg bw/d was still considered.

**Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals**

Species	Substance	Exposure System	Results	Reference
Rat	Fluroxypyr-meptyl	Oral, 1 d Acute	LD <sub>50</sub> > 2000 mg/kg bw	EFSA Journal 2011;9(3):2091
Rabbit Rat	Fluroxypyr acid	Dietary Reproductive toxicity	NOEL = 100 mg/kg bw/day	EFSA Journal 2011;9(3):2091

**Table 9.3-4: Endpoints and effect values relevant for the risk assessment for mammals**

Species	Substance	Exposure System	Results	Reference
Rat	AG-CDF1-480 EC	Oral, 1 d Acute	LD <sub>50</sub> = 500 mg/kg bw	Submitted in Part B Section 6 as KCP 7.1.1/01 Allingham (2015) Study code: 144994

### Metabolites of the active substances

No metabolites of 2,4-D acid, Clopyralid and Fluroxypyr acid are expected to be present in food items (plant material). No environmental risk assessment is therefore deemed necessary.

### zRMS comments:

Mammalian toxicity data for 2,4-D (acid form), clopyralid, and fluroxypyr are in line with EU agreed endpoints reported in EFSA Journal 2014;12(9):3812, EFSA Journal 2018;16(8):5389, and EFSA Journal 2011;9(3):2091, respectively.

The geometric mean LD<sub>50</sub> as agreed in the course of the renewal process of 2,4-D has been added by the zRMS in Table 9.3-1 as being used in the risk assessment.

The toxicity data for 2,4-D EHE are in line with endpoints presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Since the SANCO 7599/VI/97 final is not applicable anymore, reference to this document has been struck through in Table 9.3-1 and the Bridging Report has been referenced as being the relevant document where the currently agreed EU data for 2,4-D 2-EHE may be found.

The study on acute toxicity of AG-CDF1-480 EC to mammals was evaluated by the zRMS toxicology expert in area of Section 6. Endpoint reported in Table 9.3-3 is confirmed to be correct.

It is noted that the study with the formulation was performed with the old version of the formulation (AG-CDF1-480 EC) while the authorisation is sought for formulation ADM.3304.H.1.A (AG-CDF1-480 EC1). The change of the composition included removal of <3% of one solvent and addition of the same amount of the other solvent. The removed solvent was toxic to aquatic organisms with EC<sub>50</sub> for algae <1.0 mg/L, while the added solvent is not toxic to aquatic species with L(E)C<sub>50</sub> values for fish, *Daphnia magna* and algae being all >100 mg/L. Based on that, no change of the ecotoxicological profile of the new version of the formulation is expected. Please note that only

aquatic toxicity data are available for co-formulants, but in line with the current legislation, no further studies with other non-target species are required.

No 2,4-D, clopyralid and fluroxypyr plant metabolites were included in the EU residue definitions and no risk assessment for any of the metabolites was deemed necessary at the EU level. Taking this into account, no evaluation was required for this zonal evaluation of ADM.3304.H.1.A.

## Toxicity of mixture

According to the EFSA/2009/1438, the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD<sub>50</sub> for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

$$LD_{50}(\text{mix}) = \left( \sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

X (a.s.<sub>i</sub>) = fraction of each a.s. in the mixture

LD<sub>50</sub>(a.s.<sub>i</sub>) = acute toxicity value for each a.s.

**Table 9.3-5: LD<sub>50</sub> for ADM.3304.H.1.A (old code AG-CDF1-480 EC) mixture**

Test substance	Concentration of active substance in formulation [g a.s./L]	Fraction of active substance in the formulation mixture (X (a.s.) in the mixture) <sup>a</sup>	Endpoint LD <sub>50</sub> [mg/kg bw]	LD <sub>50</sub> (mix) <sup>b</sup> [mg/kg bw]
2,4-D acid	375	0.73	> 554	692
Clopyralid	30	0.06	> 5000	
Fluroxypyr-meptyl	108	0.21	> 2000	

<sup>a</sup> X (a.s.) = Conc. of a.s./Σ conc. a.s.<sub>i</sub>

<sup>b</sup> LD<sub>50</sub> (mix) = 1/Σ (X(a.s.)/LD<sub>50</sub>(a.s.<sub>i</sub>))

According to the EFSA/2009/1438, measured endpoints should only be replaced by modelled endpoints if a significant change of the predicted toxicity is expected. This may be the case if one toxicant may contribute to more than 90 % of the toxicity of the mixture. The toxicity may be compared on basis of artificial “toxicity per fraction” quotients. These quotations have no biological relevance, but are calculated for comparison only according to the equations below. In case the quotient for one single toxicant deviates from the quotient for the mixture by less than 10 % it is assumed to contribute to more than 90 % of the toxicity of the mixture (see **Table 9.3-5**).

$$\text{Tox. per fraction (a.s.)} = \frac{LD_{50}(a.s._i)}{X(a.s._i)}$$

$$\text{Tox. per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum X(a.s._i)}$$

**Table 9.3-6: Comparison of measured and modelled toxicity data**

Active substance	LD <sub>50</sub> [mg a.s./kg bw]	X (a.s.) in the mixture	Tox. per fraction (a.s.)	Deviation [%] <sup>a</sup>	Endpoint used for risk assessment
LD <sub>50</sub> (mix)	692	1	692	-	-
2,4-D acid	> 554	0.73	758	8.1	
Clopyralid	> 5000	0.06	85500	99.2	
Fluroxypyr-meptyl	> 2000	0.21	9500	92.7	

<sup>a</sup> Deviation (%) = ((tox. per fraction (a.s.) - LD<sub>50</sub> (mix))/tox. per fraction (a.s.)) × 100.

The deviation between the tox per fraction of a.s.<sub>i</sub> and mixture is greater than 10 % in case of Clopyralid and Fluroxypyr-meptyl, but < 10 % (i.e. 8.1 %) for 2,4-D acid. This indicates that 2,4-D acid contributes

to  $\geq 90$  % the toxicity of the formulated product ADM.3304.H.1.A (old code AG-CDF1-480 EC), so the other components of the mixture will only have a marginal impact on the predicted risk. Consequently, the risk assessment can be performed for the most toxic active substance alone, i.e. 2,4-D acid (the active substance identified as “driver” of the toxicity of the mixture). The same conclusion is reached for mixture toxicity based on data of 2,4-D EHE (see tables below).

**Table 9.3-7: LD<sub>50</sub> for ADM.3304.H.1.A (old code AG-CDF1-480 EC) mixture**

Test substance	Concentration of active substance in formulation [g a.s./L]	Fraction of active substance in the formulation mixture (X (a.s.) in the mixture) <sup>a</sup>	Endpoint LD <sub>50</sub> [mg/kg bw]	LD <sub>50</sub> (mix) <sup>b</sup> [mg/kg bw]
2,4-D EHE	562.5	0.80	896	1018
Clopyralid	30	0.04	> 5000	
Fluroxypyr-meptyl	108	0.15	> 2000	

<sup>a</sup> X (a.s.) = Conc. of a.s./Σ conc. a.s.<sub>i</sub>

<sup>b</sup> LD<sub>50</sub> (mix) = 1/Σ (X(a.s.)/LD<sub>50</sub>(a.s.)<sub>i</sub>)

**Table 9.3-8: Comparison of measured and modelled toxicity data**

Active substance	LD <sub>50</sub> [mg a.s./kg bw]	X (a.s.) in the mixture	Tox. per fraction (a.s.)	Deviation [%] <sup>a</sup>	Endpoint used for risk assessment
LD <sub>50</sub> (mix)	1018	1	1018	-	-
2,4-D EHE	896	0.80	1115	8.7	
Clopyralid	> 5000	0.04	116750	99.1	
Fluroxypyr-meptyl	> 2000	0.15	12972	92.1	

<sup>a</sup> Deviation (%) = ((tox. per fraction (a.s.) - LD<sub>50</sub> (mix))/tox. per fraction (a.s.)) × 100.

#### zRMS comments:

The presented above acute mixture toxicity is agreed by the zRMS with some minor corrections made in Table 9.3-8, since Applicants' calculation of the tox per fraction could not be reproduced.

Performed calculations demonstrated that 2,4-D drives the acute toxicity of the mixture, regardless which form is considered. Clopyralid and fluroxypyr contribution to the acute mixture toxicity is negligible and may be ignored, in line with indications of EFSA (2009).

However, as there are the measured toxicity data for the formulation data, it should be checked if there are any indications of increased toxicity of the formulated product due to presence of co-formulants. According to EFSA (2009) the following equation is being used for that comparison:

$$\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} = \frac{1}{LD_{50}(mix)}$$

With:

X(a.s.<sub>i</sub>) = fraction of active substance [i] in the mixture (here: formulation)

LD<sub>50</sub>(a.s.<sub>i</sub>) = acute toxicity value for active substance [i]

LD<sub>50</sub>(mix) = measured acute toxicity value for the mixture (here: formulation)

Conclusion is based on following indications (EFSA, 2009):

*A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity).*

The formulation endpoint must be expressed in terms of the active substance in order to make comparison depicted in equation above. The LD<sub>50</sub> for the formulation was determined to be 500 mg product/kg bw, which based on active substance content in the formulation tested (information taken from the study report) corresponds to:

- 245 mg sum of a.s./kg bw (when 2,4-D acid and fluroxypyr-meptyl are considered),
- 335 mg sum of a.s./kg bw (when 2,4-D EHE and fluroxypyr-meptyl are considered),

Substance	Active substance LD <sub>50</sub> [mg a.s./kg bw]	fraction/LD <sub>50</sub>	Formulation LD <sub>50</sub> [mg sum a.s./kg bw]	1/LD <sub>50</sub> mix
2,4-D	>554	0.00132	245	0.0041
Clopyralid	>5000	0.000012		
Fluroxypyr-meptyl	>2000	0.00011		
		Σ 0.00144		

Substance	Active substance LD <sub>50</sub> [mg a.s./kg bw]	fraction/LD <sub>50</sub>	Formulation LD <sub>50</sub> [mg sum a.s./kg bw]	1/LD <sub>50</sub> mix
2,4-D EHE	896	0.0009	335	0.0030
Clopyralid	>5000	0.0000086		
Fluroxypyr-meptyl	>2000	0.000077		
		Σ 0.00098		

Regardless which variant of 2,4-D is considered, the value on the right side is greater comparing to the left side indicating that the formulated product may be more toxic than predicted based on the active substance data, potentially due to the presence of co-formulants.

Taking into account performed acute mixture toxicity assessment it may be concluded that the acute risk assessment performed for 2,4-D will be protective for the remaining active substances (clopyralid and fluroxypyr) since 2,4-D is the toxicity driver. However, the acute toxicity study performed with the formulated product indicates that the formulation may be more acutely toxic than predicted based on the active substance data and for this reason separate acute risk assessment should be performed using measured formulation toxicity data.

### Mixture toxicity effects relevant for long-term exposure

For mixtures of compounds acting the same way, i.e. with the same molecular targets causing similar effects via similar mechanisms driving the risk assessments, assessments for combined effects on a case-by-case basis are recommended.

A simplified approach recommended in EFSA/2009/1438 is to express all active substances belonging to the same group on a molar basis to account for differences in molar weight in terms of their most toxic representative. Risk assessments are then performed based on the NOEC for the most toxic compound. As 2,4-D acid clearly drives the risk assessment, considerations of combined effects are not deemed necessary for reproductive risk assessments.

#### zRMS comments:

In decision on not performing the long-term risk assessment for clopyralid and fluroxypyr the Applicant refers to the outcome of the acute mixture toxicity assessment, which demonstrated that 2,4-D is an acute toxicity driver of the mixture. However, conclusion of the acute mixture toxicity is not applicable to the long-term mixture toxicity.

It should be also pointed out that the long-term mixture assessment is mandatory regardless if the active compounds have the same or different MoA.

Taking this into account, the long-term combined risk assessment is deemed necessary and will be performed in point 9.3.1.1 based on simplified approach with calculation of the TERmix.

### 9.3.1.1 Justification for new endpoints

Based on the approach proposed in EFSA/2009/1438 for data from multiple species, the acute endpoint for 2,4-D acid (the toxicity “driver”) is defined as geometric mean from acute LD<sub>50</sub> values presented in **Table 9.3-3**. Chronic risk assessments for 2,4-D acid are based on the EU agreed toxicity endpoint for mammals (see **Table 9.3-9** below).

**Table 9.3-9: Proposed Endpoints for 2,4-D acid**

Study	Test species	EU agreed endpoints	Endpoints used in risk assessment
<b>2,4-D acid</b>			
Acute toxicity	Rat	LD <sub>50</sub> = 699 mg/kg bw	Geometric mean: LD <sub>50</sub> > 554 mg/kg bw
	Rat	LD <sub>50</sub> = 486 mg/kg bw	
	Rat	LD <sub>50</sub> > 500 mg/kg bw	
Reproductive toxicity (long-term)	Rat	NOAEL = 20.6 mg/kg bw/day	NOAEL = 20.6 mg/kg bw/day

Due to its rapid degradation in the environment, 2,4-D EHE is available to wild vertebrates only immediately after spraying. The two molecules produce similar ecotoxicological effects in wild vertebrate, since the acute, short-term and long-term endpoints for 2,4-D EHE, following exposure to mammals are comparable to the same endpoints for 2,4-D acid when the ester endpoints are expressed as acid equivalents (a.e.)<sup>4</sup>. Thus, the endpoints for 2,4-D EHE and 2,4-D acid can be considered ecotoxicologically equivalent.

**Table 9.3-10: Comparison of 2,4-D acid and its variant 2,4-D EHE endpoints derived from mammalian studies**

Test species/system	Type	Lowest Endpoint		Comment
		Acid <sup>1</sup>	Ester <sup>2</sup>	
Acute toxicity to Mammals	LD <sub>50</sub> (mg/kg bw)	> 500 – 699	896 (a.e.: 591)	Endpoints comparable (within 2×) when expressed as acid equivalents.
Reproductive toxicity to Mammals	NOEL (mg/kg bw/d)	20.6	16 (a.e.: 10.6)	Endpoints comparable (within 2×) when expressed as acid equivalents.

<sup>1</sup> EFSA Journal 2014;12(9):3812.

<sup>2</sup> SANCO 7599/VI/97-final (1 October 2001).

a.e. = acid equivalent.

**zRMS comments:**

Endpoints for the risk assessment performed for 2,4-D acid proposed in tables above are in line with values used during renewal of this active compound and thus they do not deviate from the EU agreed endpoints. Hence, specific justification for their use it not necessary.

With regard to endpoints for 2,4-D 2-EHE, the are in line with data reported in 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018).

## 9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

### 9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments for the toxicity “driver” 2,4-D and its variant are summarised in the following tables.

<sup>4</sup> Endpoints for 2,4-D 2-EHE are converted to acid equivalents (a.e.) using a molecular weight conversion factor of 0.66 (2,4-D 2-EHE molecular weight = 333.26; 2,4-D molecular weight = 221.0).

### **Risk assessment for 2,4-D EHE and 2,4-D acid**

The variant 2,4-D EHE is rapidly hydrolysed to 2,4-D acid in the environment. As a result, environmental exposure to 2,4-D EHE is transient and limited to a few hours immediately after application. In plants and animals, 2,4-D EHE is also rapidly converted to 2,4-D acid through de-esterification, such that any uptake of 2,4-D EHE from the environment results in systemic exposure to 2,4-D acid. Thereby, it can be concluded that repeated and long-term exposure 2,4-D acid is the only environmentally relevant substance to be considered in the long-term risk assessment. This means that no chronic risk assessment for the variant 2,4-D EHE is deemed necessary. Further, the refinement which is presented for 2,4-D below fully covers 2,4-D-EHE.

**Table 9.3-11: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use		Grassland				
Active substance/product		2,4-D EHE				
Application rate (g/ha)		1 × 1125				
Acute toxicity (mg/kg bw)		896				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Grassland, all season	Large herbivorous mammal “lagomorph”	32.6	1.0	36.68	24.4	
Grassland, all season	Small herbivorous mammal “vole”	136.4	1.0	153.45	<b>5.8</b>	
<del>Grassland, late</del>	<del>Small insectivorous mammal “shrew”</del>	<del>5.4</del>	<del>1.0</del>	<del>6.08</del>	<del>147.5</del>	
<del>Grassland, late season (seed heads)</del>	<del>Small omnivorous mammal “mouse”</del>	<del>14.4</del>	<del>1.0</del>	<del>16.20</del>	<del>55.3</del>	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.3-12: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use		Cereals (BBCH 21 – 39)				
Active substance/product		2,4-D EHE				
Application rate (g/ha)		1 × 1125				
Acute toxicity (mg/kg bw)		896				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Cereals, BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.0	6.08	147.5	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1.0	19.35	46.3	
Cereals, BBCH 30 – 39	Small omnivorous mammal “mouse”	8.6	1.0	9.68	92.6	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.3-13: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland**

Intended use		Grassland				
Active substance/product		2,4-D acid				
Application rate (g/ha)		1 × 750				
Acute toxicity (mg/kg bw)		> 554				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
Grassland, all season	Large herbivorous mammal “lagomorph”	32.6	1.0	24.45	> 22.7	
Grassland, all season	Small herbivorous mammal “vole”	136.4	1.0	102.30	> 5.4	
Grassland, late	Small insectivorous mammal “shrew”	5.4	1.0	4.05	≥ 136.8	
Grassland, late season (seed heads)	Small omnivorous mammal “mouse”	14.4	1.0	10.80	≥ 51.3	
Reprod. toxicity (mg/kg bw/d)		20.6				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>	
Growth stage						
Grassland, all season	Large herbivorous mammal “lagomorph”	17.3	0.53	6.88	3.0	
Grassland, all season	Small herbivorous mammal “vole”	72.3	0.53	28.74	0.7	
Grassland, late	Small insectivorous mammal “shrew”	1.9	0.53	0.76	27.3	
Grassland, late season (seed heads)	Small omnivorous mammal “mouse”	6.6	0.53	2.62	7.9	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Table 9.3-14: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Cereals**

Intended use		Cereals (BBCH 21 – 39)				
Active substance/product		2,4-D acid				
Application rate (g/ha)		1 × 750				
Acute toxicity (mg/kg bw)		> 554				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Growth stage						
Cereals, BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.0	4.05	> 136.8	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2	1.0	12.90	> 42.9	
Cereals, BBCH 30 – 39	Small omnivorous mammal “mouse”	8.6	1.0	6.45	> 85.9	
Reprod. toxicity (mg/kg bw/d)		20.6				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>	
Growth stage						
Cereals, BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	0.53	0.76	27.3	
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8	0.53	3.10	6.6	
Cereals, BBCH 30 – 39	Small omnivorous mammal “mouse”	3.9	0.53	1.55	13.3	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

### Overall conclusion:

No acute and/or chronic risk is expected to mammals from the use of the formulated product ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals according to the use pattern proposed

An unacceptable acute risk to small herbivorous mammals “vole” is expected in grassland following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC). TER<sub>lt</sub> values for large and small



herbivorous mammals in grassland are lower than the trigger value of 5, indicating an unacceptable chronic risk to mammals following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) at the proposed label rate.

The refinements required for the acute and chronic risk to mammals in grassland are presented in Point 9.3.2.2 below.

#### **zRMS comments:**

The risk assessment for 2,4-D acid and ester presented in tables above is in general agreed by the zRMS.

It is noted that ADM.3304.H.1.A is intended to be used in grassland at BBCH stages 21-39 and for this reason the risk assessment for late stages is considered not necessary and was thus struck through in tables above.

The zRMS agrees that due to rapid transformation of 2,4-D EHE to the acid form, there will be no long-term exposure to the ester form. It has to be noted that actually, the transformation of the ester to acid begins already during preparation of the application solution, so actually even the acute exposure to the ester will be reduced at the moment of application. However, there are no relevant data to estimate the reduction, so the full ester rate is used for the acute risk assessment purposes. Nevertheless, in the long-term the acid form is more relevant to be used in the risk assessment.

Based on the above calculations an acceptable acute and long-term dietary risk to mammals may be concluded from both forms of 2,4-D following the intended uses of ADM.3304.H.1.A in cereals.

For uses in grassland, acceptable acute dietary risk could be concluded for single generic focal species (large herbivore), but unacceptable acute dietary risk has been shown for small herbivore. The long-term dietary risk has been shown to be unacceptable for both generic focal species. Refinement of the risk from 2,4-D is presented in point 9.3.2.2 below.

As already indicated in point 9.3.1 above, the acute risk from the formulation should be performed with consideration of the measured toxicity data since due to the presence of co-formulants the formulation was demonstrated to be more toxic than predicted based on the active substance data.

Furthermore, the Applicant waived the long-term risk assessment for clopyralid and fluroxypyr explaining that the mixture toxicity assessment demonstrated that 2,4-D is a toxicity driver and the risk assessment performed for this substance covers the risk from other compounds. What was, however, ignored by the Applicant is that only the acute mixture risk assessment has been performed and its conclusions are not applicable for the long-term evaluation. Taking this into account, the respective calculations of the long-term TER values for clopyralid and fluroxypyr were performed by the zRMS below together with the combined long-term risk assessment, which is mandatory regardless of the MoA of the particular active compounds. Only Tier 1 calculations were performed in order to comply with approach taken in evaluation of the long-term risk for 2,4-D and be able to use derived TER values in the TERmix calculations.

All calculations were performed on unrounded values.

#### **Formulation acute risk assessment**

##### Grassland

<b>Intended use</b>	<b>ADM.3304.H.1.A, grassland, BBCH 21-39</b>				
<b>Active substance/product</b>	Product				
<b>Application rate (g/ha)</b>	1 x 2.0 L/ha (corresponding to 2180 g/ha, based on density of 1.09 g/mL)				
<b>Acute toxicity (mg/kg bw)</b>	500				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>
<b>Growth stage</b>					
Grassland, all season	Large herbivorous mammal “lagomorph”	32.6	1	71.1	7.04
Grassland, all season	Small herbivorous mammal “vole”	136.4		297.4	<b>1.7</b>

<b>Cereals</b>					
<b>Intended use</b>	<b>ADM.3304.H.1.A, cereals, BBCH 21-39</b>				
<b>Active substance/product</b>	Product				
<b>Application rate (g/ha)</b>	1 x 2.0 L/ha (corresponding to 2180 g/ha, based on density of 1.09 g/mL)				
<b>Acute toxicity (mg/kg bw)</b>	500				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>
<b>Growth stage</b>					
Cereals, BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1	11.8	42.5
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	17.2		37.5	13.3
Cereals, BBCH 30-39	Small omnivorous mammal “mouse”	8.6		18.7	26.7

Based on above calculation, acceptable acute dietary risk from the formulation may be concluded following uses in cereals. For uses in grassland, acceptable acute dietary risk may be concluded for large herbivore, but unacceptable acute risk is demonstrated for small herbivorous species.

#### **Clopyralid long-term risk assessment**

##### **Grassland**

<b>Intended use</b>	<b>ADM.3304.H.1.A, grassland, BBCH 21-39</b>				
<b>Active substance/product</b>	Clopyralid				
<b>Application rate (g/ha)</b>	1 x 60				
<b>Reprod. toxicity (mg/kg bw/d)</b>	50				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
<b>Growth stage</b>					
Grassland, all season	Large herbivorous mammal “lagomorph”	17.3	1 x 0.53	0.55	90.9
Grassland, all season	Small herbivorous mammal “vole”	72.3		2.30	21.7

##### **Cereals**

<b>Intended use</b>	<b>ADM.3304.H.1.A, cereals, BBCH 21-39</b>				
<b>Active substance/product</b>	Clopyralid				
<b>Application rate (g/ha)</b>	1 x 60				
<b>Reprod. toxicity (mg/kg bw/d)</b>	50				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
<b>Growth stage</b>					
Cereals, BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 x 0.53	0.06	827.5
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8		0.25	201.6
Cereals, BBCH 30-39	Small omnivorous mammal “mouse”	3.9		0.12	403.2

Based on above calculation acceptable long-term dietary risk may be concluded for mammals from clopyralid following the intended uses of ADM.3304.H.1.A.

#### **Fluroxypyr long-term risk assessment**

##### **Grassland**

<b>Intended use</b>	<b>ADM.3304.H.1.A, grassland, BBCH 21-39</b>				
<b>Active substance/product</b>	Fluroxypyr (acid)				
<b>Application rate (g/ha)</b>	1 x 150				
<b>Reprod. toxicity (mg/kg bw/d)</b>	100				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
<b>Growth stage</b>					
Grassland, all season	Large herbivorous mammal “lagomorph”	17.3	1 x 0.53	1.38	72.7

Grassland, all season	Small herbivorous mammal “vole”	72.3		5.75	17.4
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### Cereals

Intended use	ADM.3304.H.1.A, cereals, BBCH 21-39				
Active substance/product	Fluroxypyr (acid)				
Application rate (g/ha)	1 x 150				
Reprod. toxicity (mg/kg bw/d)	100				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Cereals, BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1 x 0.53	0.15	662.0
Cereals, BBCH 10-29	Small omnivorous mammal “mouse”	7.8		0.62	161.3
Cereals, BBCH 30-39	Small omnivorous mammal “mouse”	3.9		0.31	322.5

Based on above calculations, acceptable long-term dietary risk may be concluded for mammals from fluroxypyr following the intended uses of ADM.3304.H.1.A.

Please note that from the EU review only the long-term risk for acid form of fluroxypyr is available and no risk assessment was deemed necessary for the ester form for reasons the same as in case of 2,4-D EHE described above (rapid transformation of an ester to acid).

### Combined long-term risk assessment

As already indicated in the zRMS comment in point 9.3.1, in case of chronic combined risk assessment the simplified approach with calculation of TER<sub>mix</sub> is most suitable. Nevertheless, as an initial step it may be checked if one of the active compounds contributes to >90% toxicity of the mixture. In such a case, the combined risk assessment may be waived as being covered by evaluation performed for the toxicity driver. Calculation of the Toxic Units was not available in the initial version of the dRR submitted by the Applicant and respective calculations were presented in the Reporting Table during the commenting period. Provided calculations were in general correct, but it was noted that the endpoint considered by the Applicant for fluroxypyr meptyl was actually relevant for fluroxypyr acid, since no mammalian long-term endpoint is available from the EU review of this substance and for this reason the ester form of fluroxypyr cannot be considered in the performed calculations. The TU calculations were thus amended by the zRMS accordingly and are presented below, separately for 2,4-D in an ester and acid form.

Group	Test substance	Endpoint	Fraction in formulation	Toxicity of active substance [mg/L]	Toxic Unit (TU)	Toxic Unit [%]
Mammals, chronic	2,4-D EHE	NOEC	0.803	16	0.050187366	95.44
	Clopyralid		0.043	50	0.000856531	1.63
	Fluroxypyr acid		0.154	100	0.001541756	2.93
				SUM TU	0.052585653	--

Group	Test substance	Endpoint	Fraction in formulation	Toxicity of active substance [mg/L]	Toxic Unit (TU)	Toxic Unit [%]
Mammals, chronic	2,4-D acid	NOEC	0.781	20.6	0.037924757	93.09
	Clopyralid		0.063	50	0.00125	3.07
	Fluroxypyr acid		0.156	100	0.0015625	3.84
				SUM TU	0.040737257	--

Calculations performed above for both combinations indicate that 2,4-D clearly drives the long-term toxicity of the mixture, regardless if it is in form of an ester or acid. The long-term risk assessment resulting from exposure to the mixture is thus covered by evaluation performed for 2,4-D and the long-term TER<sub>mix</sub> values calculated by the zRMS in the first version of the Core Assessment are struck through below as being no longer necessary.

Respective calculations are provided by the zRMS below. Evaluation was performed with consideration of Tier 1-TER values derived for particular active compounds. For sake of simplicity, only the lowest TER values were considered, covering also risk to generic focal species producing lower TER values.

Compound			Σ1/TER	Σ1/TER <sup>+</sup>	Trigger
2,4-D acid	Clopyralid	Fluroxypyr acid			

TER <sup>†)</sup>	1/TER	TER <sup>†)</sup>	1/TER	TER <sup>†)</sup>	1/TER			
<b>Grassland</b>								
0.7	1.429	21.7	0.046	17.4	0.057	1.532	0.653	5
<b>Cereals</b>								
6.6	0.152	201.6	0.005	161.3	0.006	0.163	6.15	5

<sup>†)</sup> Lowest Tier 1 TER

~~Based on above calculations, acceptable long term combined risk to mammals exposed to the mixture of 2,4-D, clopyralid and fluroxypyr applied as ADM.3304.H.1.A in cereals may be concluded. The long term combined risk for the mixture is unacceptable for uses in cereals.~~

#### **Overall conclusion on Tier 1 risk assessment for mammals**

Based on the performed evaluation, acceptable acute and long-term dietary risk to mammals from 2,4-D, clopyralid, fluroxypyr and the mixture may be concluded for the intended uses of ADM.3304.H.1.A in cereals.

For clopyralid and fluroxypyr acceptable long-term risk could be concluded for uses in grassland, however unacceptable risk was concluded for 2,4-D and the mixture for the following species:

1. Acute risk:
  - small herbivorous mammal (2,4-D EHE, 2,4-D acid and formulation)
2. Long-term risk
  - large herbivorous mammal (2,4-D acid ~~and formulation~~),
  - small herbivorous mammal (2,4-D acid ~~and mixture~~).

The higher-tier risk assessment is presented in point 9.3.2.2 below.

### **9.3.2.2 Higher-tier risk assessment**

The Guidance Document on Risk Assessment for Birds and Mammals (The EFSA Journal (2009) 7(12):1438) provides several options that can be applied to refine the exposure of the animal group to be assessed. Formulas for the calculation of refined TER-values are provided in the “Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC”, SANCO/4145/2000 (2002).

**General refinement** options are e.g.:

- The evaluation of actual residue data to obtain lower RUD for the intended use.
- The evaluation of actual residue data to obtain lower DT<sub>50</sub>-values for the degradation of the substance.
- The evaluation of actual use practice on the risk assessment – e.g. by considering the likelihood of several applications at the earliest growth stages at minimum interval.
- The re-evaluation of the relevant endpoint on its ecotoxicological relevance.
- The re-evaluation of residue levels and/or residue decline in the food sources (e.g. arthropods).
- The exclusion of species which have been defined as irrelevant for national risk evaluation and / or risk management.

If these general refinements are not possible or not sufficient, there is also the possibility to choose actual focal species for the relevant diet guild and foraging strata (instead of generic focal species) based on actual information from field studies.

Specific refinement options after the choice of a focal species are e.g.:

- Evaluation of the diet composition obtained from the treated area (PD).
- Refinement of the proportion of the animal’s daily diet obtained in the habitat treated with the pesticide (PT).

- Refinement of risk by taking into account avoidance behaviour (e.g. by dehusking seeds).
- The collection of wildlife incident data (mainly for acute toxicity).
- The conduction of field studies with the product.

#### Refinement steps used for 2,4-D risk assessment:

- The actual residue data of the substance 2,4-D on cereals (i.e. 2.3 days) will be used in order to refine the risk (RAR Addendum, March 2014).
- The rapid excretion of 2,4-D by mammals (RAR Addendum, February 2014).
- A field effects study on vole populations in grassland was conducted to evaluate the potential for visible mortality and long-term impact on populations of 2,4-D (...; 2012; RAR Addendum, February 2014, B.9.3.2.1/01). Please also refer to Appendix 2, KCP 10.1.2.2 – Higher tier data on mammals.

#### 1. Refined DT<sub>50</sub> value with the use of measured substance-specific residue data for 2,4-D

In the Tier 1 assessment the default assumption for exposure to residues of 2,4-D on vegetation was used (i.e. DT<sub>50</sub> = 10 days). The long-term risk assessment for mammals exposed to 2,4-D was refined by use data available from a total of 30 trials on cereals (8 on winter cereals and 22 on spring cereals). These data confirm that residues of 2,4-D decline quickly with a mean **DT<sub>50</sub> = 2.30 days on cereals** (2.09 days on winter cereals and 2.39 days on spring cereals). Details of the residue trial and the resulting DT<sub>50</sub> value are summarised in the RAR Addendum, February 2014, B.9.3.3-12).

This value corresponds to a 21-day time-weighted-average factor (f<sub>TWA</sub>) of **0.16**, which will be considered in the refined long-term risk assessment for the herbivorous mammal.

**Table 9.3-15: Higher-tier assessment of the ~~acute~~ and long-term/reproductive risk for mammals due to the use of 2,4-D in Grassland – refined parameters (\*) are above described and justified in the text**

Intended use		Grassland					
Active substance/product		2,4-D					
Application rate (g/ha)		1 × 750					
Reprod. toxicity (mg/kg bw/d)		20.6					
TER criterion		5					
Generic focal species	Food category, % in diet	FIR/bw	RUD <sub>m</sub> × DF (mg/kg food)	MAF <sub>m</sub> × TWA*	PT	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>LT</sub>
Brown hare	Grass, 100 %	0.32	54.2	1 × 0.16	1.0	2.1	9.9
Common vole	Grass, 100 %	1.33	54.2	1 × 0.16	1.0	8.7	<b>2.4</b>

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

The TER<sub>LT</sub> value for large herbivorous mammals as represented by the hare is above the trigger value of 5.

The TER<sub>LT</sub> value for small herbivorous mammals as represented by the common vole is below the trigger value of 5, a further refinement is required. In order to further refine the risk to voles please refer to the results of the field effect monitoring study presented below.

#### zRMS comments:

The refinement of the long-term dietary risk to herbivorous mammals exposed to 2,4-D following application of ADM.3304.H.1.A to grassland is agreed by the zRMS. The title of the Table 9.3-15 has been corrected since only long-term higher-tier risk assessment has been presented. It was also indicated by the zRMS in Table 9.3-15 that the

species used in evaluation were actually generic focal species taken from Appendix A of EFSA (2009) since no specific monitoring studies were performed in order to determine the actual focal species.

The refined DT<sub>50</sub> of 2.3 days was agreed at the EU level and originates from 30 residue decline trials performed in winter and spring cereals. It is possible to extrapolate from cereals to grass since both crops belong to the same botanical group (monocots). The fTWA of 0.16 calculated with consideration of DT<sub>50</sub> of 2.3 days is confirmed to be correct. The other parameters (FIR/bw and RUD) were taken from Appendix A of EFSA (2009).

Based on the above calculation, the long-term combined risk assessment was performed by the zRMS using Tier 1 TER values for clopyralid and fluroxypyr and refined TER for 2,4-D taken from Table 9.3-15 above.

Compound						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
2,4-D acid		Clopyralid		Fluroxypyr acid				
TER <sup>-1</sup>	1/TER	TER <sup>-1</sup>	1/TER	TER <sup>-1</sup>	1/TER			
Grassland (large herbivorous mammal)								
9.9	0.101	90.9	0.011	72.7	0.014	0.126	7.95	5
Grassland (small herbivorous mammal)								
2.4	0.417	21.7	0.046	17.4	0.057	0.520	1.92	5

Based on the above calculations, acceptable risk could be concluded for large herbivorous mammal from 2,4-D and the mixture of all three compounds following application of ADM.3304.H.1.A in grassland, but the risk to small herbivores remains unresolved. Further evaluation is presented in chapter below.

As already indicated in the zRMS commenting box in point 9.3.2.1 above, the Toxic Units calculated following the commenting period demonstrated that 2,4-D (regardless of its form) clearly drives the long-term toxicity of the mixture to mammals and for this reason the long-term risk from the formulated product is considered to be covered by evaluation performed for 2,4-D. Taking this into account, the refined long-term TERmix values were struck through above as being no longer necessary. The text of the conclusion was also amended accordingly.

## 2. Field effect monitoring study on common voles (*Microtus arvalis*) for 2,4-D

The risk assessment above indicates a potential risk to small herbivorous mammals after the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) at a single application rate of 750 g a.s./ha 2,4-D on grassland. Therefore, a higher tier risk assessment is required.

In order to explore potential effects of 2,4-D on small herbivorous mammals, a field effects study on vole populations in grassland was conducted using the representative formulation Chardol 600 SL (containing 600 g a.s./L 2,4-D). The study was previously submitted to support the Annex I Renewal of 2,4-D and a summary is provided in the RAR (see .... (2012) KCP 10.1.2.2/01, for more details) to support the application of 2,4-D-containing products in maize.

Acute and long-term effects of 2,4-D on the population dynamics of the common vole *Microtus arvalis* were studied during spring and summer 2011 in Germany (Central Zone) and France (Southern Zone). 2,4-D was applied once with an application rate of 750 g a.s./ha in grassland. Studies in grassland are generally considered as highly conservative, as the individuals remain resident throughout the reproductive season (unlike in arable crops). Therefore, PT = 1 and PD = 1 can be assumed for the risk assessment.

All techniques that were applied to monitor acute (radio-tracking approach) and long-term effects (capture-mark-recapture approach) are in line with the recommendations of the current EFSA GD on birds and mammals (2009). Acute effects were monitored within 14 days after application. Long-term effects on population level were monitored over the season. Abundance, population structure and dynamics were assessed over a period of at least two months after application of 2,4-D using live trapping (capture-mark-recapture).

### Acute effects

The potential for acute effects on small herbivorous mammals was assessed using telemetry for up to 14 days following application of 2,4 D. During the first week after application, there were no treatment-related mortalities detected amongst the 39 radio-tagged and tracked individuals on treatment plots in Southern France and Germany. After seven days radio-tracking signals could not be obtained for an increasing number of tagged individuals, making it difficult to determine status of these individuals via telemetry (see study report for more details). Because the residues on the diet of voles exposed (via plants) decreased within the first week after application to just 22 % and 7 % in Southern France and Germany, respectively, potential acute effects on voles caused by 2,4 D applications after DAT 7 seem to be highly unlikely. This assumption is supported by the observed fast dissipation of 2,4 D from treated vegetation ( $DT_{50}$  between 0.5 to 3.3 day on grassland; please refer to the RAR Addendum, February 2014, B.9.3.3-12) and the acute toxicity data obtained from studies conducted by Meyer (1981), Wandrag (1993), Dange (1994) and Leonie (2011). In these studies, mortality was observed within 7 days of administration, indicating that treatment-induced mortality in the field study would have been detectable within the first week of the telemetry monitoring period (see RAR Addendum, February 2014, B.6.2.1/01-04 for details).

**In conclusion, based on the presented data, there was no evidence of any treatment related acute toxicity (mortality) to voles after application of 2,4-D under field conditions in Southern France or Germany.**

#### Long-term effects

The potential for long term effects on small herbivorous mammals was assessed using live trapping data for up to 120 days following 2,4 D application. For the common vole, the population dynamics as described by the MNA (Minimum Number Alive) followed the similar seasonal pattern on control and treated plots at each of the two study sites. The recapture rate for the common vole was even slightly higher on treatment plots at both study sites compared to control plots indicating that treated habitats exhibited a stable population as well.

With respect to the reproductive status or body weight of exposed voles no substantial differences could be found for treatment and control plots at both study sites (for details, please refer to the study report provided in Part B, Section 9, KCP 10.1.2.2.3/01). Finally, the population increase was calculated by means of the number of trapped individuals per trapping session. This comparison showed no significant differences between treatment and control plots across all trapping sessions for the common vole. The lack of long term effects is most likely linked to the fast dissipation of the substance in treated vegetations (e.g.  $DT_{50}$  = 0.5–3.3 days in grassland (please refer to the Part B, Section 9, KCP 10.1.2.2/01), which is a common behaviour of 2,4 D (see RAR Addendum, February 2014, B.9.3.3 for more details).

**In conclusion, based on the presented data, there was no evidence of any treatment related long-term toxicity to voles after application of 2,4-D under field conditions in Southern France or Germany.**

#### **Additional information**

In the course of the Annex I Renewal, EFSA concluded that the field study by ... (2012) is “*not considered suitable for the risk assessment because of a number of shortcomings*” (EFSA, 2014). For that reason, ... (2015) re-evaluated the study conducted by .... (2012) to address the concerns raised by EFSA and to provide further supportive information for the validity of the field study (refer to Appendix 2, KCP 10.1.2.2 – Higher tier data on mammals). A summary of the response to EFSA, providing clarification to the concerns raised, is provided below. The report is provided in Part B, Section 9, KCP 10.1.2.2/02.

The field study was designed to monitor the potential for acute and long-term effects of 2,4 D application on common vole (*Microtus arvalis*) populations in grassland in Germany and France (...). EFSA concluded that the field study by ... (2012) was not considered suitable for the risk assessment because of the following shortcomings:

1. ~~Mean trapping efficiency between the selected sites was significantly different~~ (19.4 captures/100 trap nights for Southern France vs. 89.1 captures/100 trap nights for Germany in the treated plots);
2. ~~Uncertainty as to whether the number of tagged individuals per plot (~7 per treated plot and 5–6 per control plot) can be considered representative of the whole population;~~
3. ~~Carcass examination was not performed;~~
4. ~~Radio-tracking signals could not be obtained for all voles after 7 days~~ maybe due to the battery life of the tags;
5. ~~In the treated plots a 50 % survival in Southern France (80 % in the control) and 79 % survival in Germany (84 % in the control) was recorded one week after the treatment and for a period of 2 weeks, and it was not clear whether the 50 % loss was treatment-related~~ because the status of the missing individuals remained unknown.

The following response is focused on the concerns highlighted by EFSA, regarding the methods employed and interpretation of the results by the study director, to provide further supportive information on the validity of the results from the field study by ... (2012).

### ***1. Mean trapping efficiency between the selected sites was significantly different***

... (2012) detected different trapping efficiencies at the investigated regions (Central versus Southern zone); such differences are not surprising in spatially distinct populations given the seasonal as well as annual natural fluctuations in population densities of common voles. What is important, for the purposes of this study, is that both local populations, i.e. vole populations in the investigated regions in southern France and in Germany, respectively, showed no differences in the population development in treated sites compared to untreated control sites. In conclusion, despite general regional differences between vole populations in southern France and in Germany, the application of 2,4-D did not affect the populations in a negative way in both regions: populations on treated sites in both regions showed similar developments as the control sites within the respective regions.

### ***2. Number of tagged individuals used for radio tracking***

For the purpose of this effect study it is reasonable to consider the total number of individuals per treatment group, rather than the number of individuals per plot, since each individual is monitored independently from each other, but conditions between plots (within one study site) were similar. Thus, pooling the data for all plots of a treatment group is appropriate, especially as the analysed data in the study by ... (2012) are descriptively presented as percentage of animals proven to be alive after a certain number of days following application without statistical evaluation.

In general, there is no advice for the number of animals assumed to be “sufficient” for an effect study. For the purpose of estimating PT values, the GD (2009) recommends that 20 individuals are radio-tracked (Appendix P in EFSA 2009). Based on this, the total of 39 individuals tracked in this study on treatment plots (20 in France and 19 in Germany) and 34 on control plots (15 in France and 19 in Germany) can be considered sufficient. Indeed, overall, this sums up to a total of 73 individuals that were monitored by radio-tracking. Thus, the total number of individuals radio-tracked in the study can be considered sufficient and representative for the local common vole populations.

### ***3. Carcass examination was not performed***

The detection of potential carcasses is influenced by several factors including search efficiency, the toxicity of the substance under field conditions, carcass removal by scavengers, and visibility of the carcass since intoxicated rodents might die in their subterranean burrows or concealed shelters (dense vegetation). Radio tracking can therefore be more efficient for monitoring acute effects (see below) as the location of individuals can be determined even if they are hidden in deep vegetation or underground burrows. In addition, radio-tags can be located even if the individual is separated from the tag (as may occur following predation).

In this study 92 % (36/39 animals) of all tagged animals on the treated plots, and 91 % (31/34 animals) of all tagged animals on the control plots, were recovered alive during the first week after treatment. In the treated plots the 3 remaining tags were recovered in surrounding shrub land, and had been removed from



the vole (presumably by a predator), and in the control plots 1 remaining tag was found in an owl pellet (vole presumed predated) while 2 tags were never recovered. Thus, within the first week, 71/73 tags (97 %) were located and an assessment of acute toxicity was possible.

Even if a carcass had been found after this period, the post mortem examination would be highly unlikely to reveal a clear cause of death as it would not be linked to acute toxicity, residues of 2,4-D within the carcass would be expected to be low or non-detectable (given the rapid degradation on vegetation, and metabolism/excretion by mammals), and so it would not be possible to link more general symptoms to the active substance.

#### ***4. Radio-tracking signals could not be obtained for all voles after 7 days***

Acute toxicity (mortality) would have been detected within the first seven days of radio-tracking of tagged individuals. Therefore, the lack of radio signals after seven days or more has no influence on the integrity of the study regarding acute effects, because the relevant (acute) period is the first (few) day(s) following exposure. In the study by ... (2012), radio-tracking was not intended to investigate long-term effects, i.e. several weeks after treatment, for which the capture-mark-recapture method was applied.

In addition, residues of 2,4-D on vegetation declined rapidly following application ( $DT_{50} \leq 3.3$  days; ... 2012), confirming that exposure of herbivorous mammals to 2,4-D is transient, i.e. potential acute effects are most likely immediately or shortly after treatment (when the residue loading on the potential food items is highest). The radio-tracking data clearly exclude any acute effects or mortalities until seven days after application (... 2012).

#### ***5. Not clear whether the 50 % loss was treatment-related***

As mentioned above, in this study 92 % of tagged animals on the treated plots, and 91 % of all tagged animals on the control plots, were recovered alive during the first week after treatment. After the first week, the battery life on the tags declined making it impossible to obtain a signal for some animals, and there was some evidence of predation. Therefore, interpretation of the decline in number of voles recovered (based on detection of a radio tag signal) should be treated with caution as a lack of signal from the tag cannot be assumed to represent mortality of the individual.

Considering each site, it should be noted that the difference between control and treated plots observed in Germany represents a single vole since 15/19 (79 %) of voles were found alive on treated plots vs 16/19 (84 %) found alive on the control plots.

In France, the difference was greater, with 10/20 (50 %) found alive on treated plots vs 12/15 (80 %) on control plots. However, in 5 cases in the treated plots the tag was recovered but no carcass found—the loss of the tag was attributed to predation, but could also be due to removal by the vole. The lack of carcass does not indicate death of the vole; indeed, if the lower numbers of voles detected in the treated plots in Southern France were indicative of treatment-related mortality, then this should be detectable in the overall population numbers using the capture-mark-recapture method which was applied to assess the potential for long-term effects on the population.

#### **Conclusion**

The field study by ... (2012) was conducted according to recommendations of the current guidance document (EFSA 2009). The methods applied are widely accepted for the monitoring of potential acute (via radio-tracking) and long-term effects (via capture-mark-recapture approach), e.g. by EFSA (2009). Local common vole populations were investigated in two different geographic regions. In both areas no indication for acute effects and no contrasting population developments were recorded on treatment compared to control plots.

#### **zRMS comments:**

First of all it has to be emphasised that formulations Chardol 600 SL and Spritz Hormin 500 SL used in the field study by ... (2012) on the common vole population dynamics in grassland in the Southern France and Germany contain 2,4-D in a form of DMA salt, while in ADM.3304.H.1.A an ester form of 2,4-D is present. Behaviour of

DMA salt and an ester is different and extrapolation between these two forms is not possible, so results of studies performed with 2,4-D DMA salt are not relevant to address the acute and long-term risk from formulations containing 2,4-D EHE, especially in case of ADM.3304.H.1.A the acute risk to small herbivorous mammals is unacceptable also for 2,4-D EHE and the formulated product itself. Taking this into account, the field effect study should be performed with ADM.3304.H.1.A in order to resolve all risks to small herbivores identified in the Tier 1 evaluation.

In addition to that, the study by ... (2012) has been evaluated in the course of the EU renewal process of 2,4-D and although it was initially agreed by the RMS, its reliability was challenged during the peer-review. Following consultations of EFSA and MS experts numerous uncertainties were noted as listed by the Applicant above and the study was eventually concluded to be not reliable and not relevant for purposes of the risk refinement. The following is stated in EFSA Journal 2014;12(9):3812:

*To further address the risk to small herbivorous mammals, a higher tier study (field study) was submitted. The purpose of this study was to monitor the potential for acute and long-term effects on small herbivorous mammal populations with the common vole *Microtus avails*. The study was not considered suitable for the risk assessment because of a number of shortcomings: the mean trapping efficiency between the selected sites is significantly different (19.4 captures/100 trap nights for Southern France vs 89.1 captures/100 trap nights for Germany in the treated plots); it is unclear whether the number of tagged individuals per plot (~7 per treated plot and 5 - 6 per control plot) can be considered representative of the whole population; carcasses examination was not performed; after 7 days radio-tracking signals could not be obtained maybe due to the battery life of the tags; in the treated plots a 50 % survival in Southern France (80 % in the control) and 79 % survival in Germany (84 % in the control) was recorded one week after the treatment and for a period of 2 weeks, and it was not clear whether the 50 % loss was treatment-related because the status of the missing individuals remained unknown.*

Since the study was already discussed and rejected as being not reliable, the zRMS is not in the position to challenge the decision of the MS experts and EFSA on the study reliability, as this obviously was intensively discussed and all potential arguments in favour of keeping the study for refinement of the risk were already taken into account in the course of the peer-review and rejected.

Taking all this into account, the field study by ... (2012) is not agreed for refinement of the acute risk from 2,4-D EHE and 2,4-D acid and long-term risk from 2,4-D acid following uses of ADM.3304.H.1.A in grassland. The study summary above and discussion on its results were thus struck through above.

Overall, the acute risk from 2,4-D EHE, 2,4-D acid and formulation as well as long-term risk from 2,4-D ~~and the mixture~~ to small herbivorous mammals in grassland remains unresolved and further data must be submitted by the Applicant to support authorisation in this crop.

### 9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

#### Puddle scenario

The 'puddle scenario' is considered relevant for the proposed uses of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in grassland and cereals.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ( $K_{OC} < 500$  L/kg) or 3000 in the case of more sorptive substances ( $K_{OC} \geq 500$  L/kg).

With arithmetic mean  $K_{OC}$  values of 58.6 (median of 42 soils), 1.41 4.9 and 68 L/kg for 2,4-D acid, Clopyralid and Fluroxypyr acid, these compounds belong to the group of less sorptive substances. With  $K_{fOC}$  of 19550 L/kg, fluroxypyr-meptyl belongs to less sorptive substances for which the rigger of 3000 is applicable.

The formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) contains in its composition the variant 2-ethylhexyl ester of 2,4-D (i.e. 2,4-D EHE). This compound is rapidly converted to 2,4-D acid in the environment. Due to its quick degradation in water, no experimental adsorption/desorption coefficients could be ~~not~~ derived. It is important to emphasize non-target organisms exposed via drainflow or run-off will be only exposed to 2,4-D acid. To obtain an estimate for PPP concentrations in puddles formed on a field after rainfall (predicted environmental concentration  $PEC_{\text{puddle}}$ ), it is assumed that this concentration is the same as the concentration in runoff water as calculated for the assessment of surface water exposure. A simplified model can be used to calculate  $PEC_{\text{puddle}}$  in mg/L as a function of application rate to bare soil without degradation and the organic carbon adsorption coefficient ( $K_{OC}$ ) of a substance.

~~In light of all above mentioned, the puddle scenario (runoff water) is not deemed as relevant for 2,4-D EHE.~~

The effective application rate is calculated by multiplying the proposed application rates by MAF values based on the  $DT_{50}$  in soil for the active substances (EFSA/2009/1438). Since the formulated product ADM.3304.H.1.A (old code AG-CDF1-480 EC) is applied once per season,  $MAF = 1$ . The ratios of the effective application rates to the relevant endpoints for each active substance are presented in the following tables.

<b>2,4-D acid</b>				
Effective application rate (g/ha)	=	750		
Acute toxicity (mg/kg bw)	=	> 554	quotient =	< 1.4
Reprod. toxicity (mg/kg bw/d)	=	20.6	quotient =	36.3
<b>2,4-D EHE</b>				
Effective application rate (g/ha)	=	1125		
Acute toxicity (mg/kg bw)	=	896	quotient =	1.3
Reprod. toxicity (mg/kg bw/d)	=	<b>Not required</b> 16.0	quotient =	<del>70.3</del>

<b>Clopyralid</b>				
Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	> 5000	quotient =	< 0.01
Reprod. toxicity (mg/kg bw/d)	=	50	quotient =	1.2

<b>Fluroxypyr acid</b>				
Effective application rate (g/ha)	=	150		
Acute toxicity (mg/kg bw)	=	> 2000	quotient =	< 0.08
Reprod. toxicity (mg/kg bw/d)	=	100	quotient =	1.5
<b>Fluroxypyr-meptyl</b>				
Effective application rate (g/ha)	=	216		
Acute toxicity (mg/kg bw)	=	>2000	quotient =	<0.1
Reprod. toxicity (mg/kg bw/d)	=	No EU agreed endpoint	quotient =	-

Since the ratios of effective application rates (in g/ha) to relevant endpoints (in mg/kg bw/d) are below the critical value of 50, no quantitative risk assessment (calculation of TER values) for each active substance is required.

The above assessments demonstrate that no unacceptable risk to mammals emerges from exposure to drinking water from puddles. Thus, no further refinement is necessary.

#### zRMS comments:

The drinking water risk assessment for 2,4-acid, clopyralid and fluroxypyr acid presented in tables above is agreed by the zRMS. Additional calculations for fluroxypyr-meptyl were included for completeness.

Although initially the long-term drinking water assessment has been included by the zRMS for 2,4-D EHE, during the commenting period it was pointed out that this was not necessary as in line with conclusions presented in the Bridging Report (2018), no long-term exposure of mammals is anticipated for this form of 2,4-D. As no dietary long-term risk assessment was performed for an ester form in point 9.3.2.1 above, consequently the long-term drinking water risk assessment also should be waived. Taking this into account, calculations initially provided by the zRMS have been struck through in table above.

~~Although 2,4-D EHE degradation is extremely fast so no soil sorption/desorption studies could be performed, the drinking water risk assessment was included by the zRMS above for precautionary reasons. Due to nature of the compound it may be expected that soil sorption would be >500 L/kg (similarly as in case of other ester forms of phenoxyacids). Nevertheless, both ratios between the effective rates and the respective toxicity data are low (1.7 and 16.9 for acute and long term risk), so the drinking water risk would be covered even in case the  $K_{OC}$  was <500 L/kg.~~

Acceptable risk from all forms of active compounds may be concluded for **mammals** ~~birds~~ exposed via drinking water.

It should be, however, noted that the drinking water risk assessment should be also performed for the pertinent soil metabolites of active substances, which was not done by the zRMS. Respective calculations were thus performed by the zRMS and are presented below. In absence of the toxicity data, 10 times toxicity of the parent was assumed representing worst case. The pseudo-application rates of metabolites were calculated with consideration of the parent rate, maximum occurrence in soil and molar ratio. Crop interception was not considered representing worst case.

2,4-D metabolites				
2,4-DCA effective application rate (g/ha)	90.1 (molar ratio 0.801, max occur. 15%)			
Acute toxicity (mg/kg bw)	>55.4	quotient =	<1.6	Trigger: 50
Reprod. toxicity (mg/kg bw/d)	2.06	quotient =	43.7	
2,4-DCP effective application rate (g/ha)	210.2 (molar ratio 0.738, max occur. 38% (soil anaerobic study))			
Acute toxicity (mg/kg bw)	>55.4	quotient =	<3.8	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	2.06	quotient =	102.0	
4-CP effective application rate (g/ha)	144 (molar ratio 0.582, max occur. 33%)			
Acute toxicity (mg/kg bw)	>55.4	quotient =	<2.6	Trigger: 50
Reprod. toxicity (mg/kg bw/d)	2.06	quotient =	<b>69.9</b>	
Fluroxypyr metabolites				
Methoxypyridine effective application rate (g/ha)	44.3 (molar ratio 0.773, max occur. 38.2%)			
Acute toxicity (mg/kg bw)	>200	quotient =	<0.2	Trigger: 50
Reprod. toxicity (mg/kg bw/d)	10.0	quotient =	4.4	
Pyridinol effective application rate (g/ha)	27.8 (molar ratio 0.776, max occur. 23.9%)			
Acute toxicity (mg/kg bw)	>200	quotient =	<0.1	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	10.0	quotient =	2.8	

Ratios between the effective rates and toxicity endpoints are below the respective triggers for majority of pertinent soil metabolites of 2,4-D and fluroxypyr with exception of metabolite 4-CP, for which the ratio for reproductive risk from drinking water was above the trigger 50. The acute ratio was at acceptable level.

Since the ratio for reproductive risk for metabolite 4-CP was above the trigger, the Tier 1 risk assessment was performed with consideration of the  $PEC_{PUDDLE}$  calculated using the following equation (EFSA, 2009):

$PEC_{\text{puddle}} = \frac{AR/10}{1000(w + Koc \times s)}$ <p>With:</p> <p>AR = application rate [g/ha]; divisor of 10 to achieve rate in mg/m<sup>2</sup></p> <p>w = 0.02 (pore water term: volume)</p> <p>s = 0.0015 (soil term: volume, density, organic carbon content)</p>						
<p>The long-term drinking water risk assessment for the indicator species (small granivorous mammal) from 4-CP based on EFSA (2009) indications is presented below.</p>						
<b>Intended use</b>		Grassland, cereals (0% crop interception as a worst case)				
<b>Active substance</b>		4-CP (2,4-D metabolite)				
<b>Application rate (g/ha)</b>		144 (based on parent rate of 750 g/ha, molar ratio 0.582 and peak occurrence of 33%)				
<b>Reprod. toxicity (mg/kg bw/d)</b>		2.06 (10 times toxicity of the parent)				
<b>TER criterion</b>		5				
<b>Soil-relevant applic. rate (g/ha)</b>	<b>Kfoc (L/kg)</b>	<b>PEC<sub>puddle</sub> (mg/L)</b>	<b>DW uptake (L/kg bw/d)</b>	<b>Daily dose (mg/kg bw/d)</b>	<b>NO(A)EL (mg/kg bw/d)</b>	<b>TER<sub>it</sub></b>
144	155 (lowest)	0.057	0.24	0.0137	2.06	150.4
<p>PEC<sub>puddle</sub>: concentration in puddles; DW: drinking water; TER: toxicity to exposure ratio.</p> <p>Based on above calculations, acceptable long-term risk to mammals exposed to 4-CP via drinking water may be concluded.</p> <p>Overall, acceptable acute and long-term risk to mammals exposed to 2,4-D, clopyralid, fluroxypyr and their metabolites via drinking water may be concluded following the intended Central Zone uses of ADM.3304.H.1.A.</p> <p>Since no metabolites are formed from clopyralid, no additional calculations were deemed necessary for this compound.</p>						

### 9.3.2.4 Effects of secondary poisoning

According to EFSA/2009/1438<sup>5</sup>, substances with a log K<sub>ow</sub> greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

**Table 9.3-16: Log K<sub>ow</sub> and BCF values for the variant 2,4-D EHE**

<b>Substance</b>	<b>2,4-D EHE</b>
<b>Log K<sub>ow</sub></b>	5.78
<b>Bio-concentration factor (BCF)</b>	Not required due to rapid degradation in water (DT <sub>50</sub> of 0.26 d)

**Table 9.3-17: Log K<sub>ow</sub> and BCF values for the active substance 2,4-D acid and its metabolites found in fish**

<b>Substance</b>	<b>2,4-D acid</b>	<b>2,4-DCA</b>	<b>2,4-DCP</b>
<b>Log K<sub>ow</sub></b>	-0.82	3.36	3.06
<b>Bioconcentration factor (BCF)</b>	Not required	31	340

**Table 9.3-18: Log K<sub>ow</sub> and BCF values for the active substance Clopyralid**

<b>Substance</b>	<b>Clopyralid</b>
<b>Log K<sub>ow</sub></b>	-2.63
<b>Bioconcentration factor (BCF)</b>	Not required

<sup>5</sup> European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. [139 pp.]. doi:10.2903/j.efsa.2009.1438. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu)

**Table 9.3-19: Log K<sub>ow</sub> and BCF values for the active substance Fluroxypyr acid**

Substance	Fluroxypyr acid
Log K <sub>ow</sub>	0.0393
Bioconcentration factor (BCF)	Not required

**Table 9.3-20: Log K<sub>ow</sub> and BCF values for the variant Fluroxypyr meptyl and its metabolites found in fish**

Substance	Fluroxypyr meptyl	Pyridinol	3-CP	Methoxypyridine
Log K <sub>ow</sub>	4.53	0.039	0.65	3.09
Bioconcentration factor (BCF)	167 (total <sup>14</sup> C) 26 (Fluroxypyr-meptyl)	Not required	Not required	1.41 (estimated with QSAR model)

The log K<sub>ow</sub> values of 2,4-D (i.e. -0.82 at pH 7) and Clopyralid (i.e. -2.63) are below 3, and it is therefore not necessary to consider the risk from secondary poisoning further.

Although for Fluroxypyr-meptyl the log K<sub>ow</sub> is > 3 (i.e. 4.53), the risk from secondary poisoning was not conducted because Fluroxypyr-meptyl is rapidly degraded to Fluroxypyr (acid) and does not accumulate in fish and earthworm.

Although the octanol/water coefficient expressed as log K<sub>ow</sub> is greater than 3 (5.78) the potential for bioaccumulation of 2,4-D EHE in fish is low due to rapid degradation in water (half-life of 6.2 hours in natural water). It is important to note that 2,4-D EHE is also rapidly metabolised to 2,4-D in vertebrates, so bioaccumulation of the ester in fish will not occur.

A risk assessment for secondary poisoning is required for the 2,4-D metabolites 2,4-DCA and 2,4-DCP and the Fluroxypyr meptyl metabolite Methoxypyridine.

### Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

**Table 9.3-20: Assessment of the risk for earthworm-eating mammals due to exposure to 2,4-DCA via bioaccumulation in earthworms (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCA	Comments
PEC <sub>soil</sub> (twa = 21 d) (mg/kg soil)	0.0870	Minimum interception of 20 % for the application rate of 0.75 kg 2,4-D acid
Log K <sub>ow</sub> / K <sub>ow</sub>	3.36 / 2291	
K <sub>oc</sub>	1028	EU agreed value for groundwater modelling
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	1.38	BCF <sub>worm/soil</sub> = (PEC <sub>worm,ww</sub> /PEC <sub>soil,dw</sub> ) = (0.84 + 0.012 × K <sub>ow</sub> ) / f <sub>oc</sub> × K <sub>oc</sub>
PEC <sub>worm</sub>	0.12	PEC <sub>worm</sub> = PEC <sub>soil</sub> × BCF <sub>worm/soil</sub>
Daily dietary dose (mg/kg bw/d)	0.15	DDD = PEC <sub>worm</sub> × 1.28
NOEL (mg/kg bw/d) <sup>1</sup>	2.06	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>lt</sub>	13.7	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to mammals was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid

**Table 9.3-21: Assessment of the risk for earthworm-eating mammals due to exposure to 2,4-DCP via bioaccumulation in earthworms (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCP	Comments
PEC <sub>soil</sub> (twa = 21 d) (mg/kg soil)	0.0832	Minimum interception of 20 % for the application rate of 0.75 kg 2,4-D acid
Log K <sub>ow</sub> / K <sub>ow</sub>	3.06 / 1148	
K <sub>oc</sub>	512	EU agreed value for groundwater modelling
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	1.43	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.12	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.15	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d) <sup>1</sup>	2.06	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	13.7	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to mammals was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid

**Table 9.3-22: Assessment of the risk for earthworm-eating mammals due to exposure to Fluroxypyr methoxy pyridine via bioaccumulation in earthworms (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	Fluroxypyr methoxy pyridine	Comments
PEC <sub>soil</sub> (plateau) (mg/kg soil)	0.09	Minimum interception of 20 % for the application rate of 0.15 kg Fluroxypyr
Log K <sub>ow</sub> / K <sub>ow</sub>	3.09 / 1230	
K <sub>oc</sub>	321	
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	2.43	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.22	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.28	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d) <sup>1</sup>	10.0	10 times toxicity of the parent (fluroxypyr acid)
TER <sub>it</sub>	35.7 <del>45.7</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to mammals was available, it was assumed that it is 10 times more toxic than the parent Fluroxypyr meptyl.

The TER values estimated for the metabolites are above the trigger value of 5, indicating there is no risk to earthworm-eating mammals via secondary poisoning following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the GAP Table.

### Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

**Table 9.3-23: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCA via bioaccumulation in fish (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCA	Comments
PEC <sub>sw</sub> max. (Focus Step 2) (mg/L)	0.0062	
TWA factor	0.53	
BCF <sub>fish</sub>	31	
BMF	1.0	Biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.102 <del>0.099</del>	PEC <sub>fish</sub> = PEC <sub>water</sub> × TWA × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.014	DDD = PEC <sub>fish</sub> × 0.142
NOEL (mg/kg bw/d) <sup>1</sup>	2.06	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	147.13	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to mammals was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid.

**Table 9.3-24: Assessment of the risk for fish-eating mammals due to exposure to 2,4-DCP via bioaccumulation in fish (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	2,4-DCP	Comments
PEC <sub>sw</sub> max. (Focus Step 2) (mg/L)	0.013	
TWA factor	0.53	
BCF <sub>fish</sub>	340	
BMF	1.0	Biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	2.343	PEC <sub>fish</sub> = PEC <sub>water</sub> × TWA × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.333 <del>0.32</del>	DDD = PEC <sub>fish</sub> × 0.142
NOEL (mg/kg bw/d) <sup>1</sup>	2.06	10 times toxicity of the parent (experimental NOEL for 2,4-D acid)
TER <sub>it</sub>	6.19 <del>6.44</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to mammals was available, it was assumed that it is 10 times more toxic than the parent 2,4-D acid.

**Table 9.3-25: Assessment of the risk for fish-eating mammals due to exposure to Fluroxypyr methoxy pyridine via bioaccumulation in fish (secondary poisoning) for the intended use in Grassland/Cereals**

Parameter	Fluroxypyr methoxy pyridine	Comments
PEC <sub>sw</sub> max. (Focus Step 2) (mg/L)	0.0055	
TWA factor	0.53	
BCF <sub>fish</sub>	1.41	
BMF	1.0	Biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.004	PEC <sub>fish</sub> = PEC <sub>water</sub> × TWA × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.0006 <del>0.0005</del>	DDD = PEC <sub>fish</sub> × 0.142
NOEL (mg/kg bw/d) <sup>1</sup>	10.0 <del>0.10</del>	10 times toxicity of the parent (fluroxypyr acid)
TER <sub>it</sub>	16667 <del>188.5</del>	

TER values shown in **bold** fall below the relevant trigger.

<sup>1</sup> Since no information on toxicity to mammals was available, it was assumed that it is 10 times more toxic than the parent Fluroxypyr meptyl.

The TER values estimated for the metabolites are above the trigger value of 5, indicating there is no risk to fish-eating mammals via secondary poisoning following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the GAP Table.

**zRMS comments:**

The evaluation of the risk of secondary poisoning was triggered for two metabolites of 2,4-D (2,4-DCA and 2,4-DCP) and one metabolite of fluroxypyr (methoxy pyridine).



In line with conclusions from the EU review of 2,4-D and fluroxypyr, the evaluation was not triggered for the ester forms of active compounds despite log Pow >3. This is explained by extremely rapid degradation in water and negligible possibility for bioaccumulation.

The risk assessment presented by the Applicant in Tables 9.3-20 to 9.3-25 is in general agreed by the zRMS with some minor corrections and additional information included for clarity. It was also noted that in the risk assessment for fish-eating mammals exposed to methoxy pyridine not correct endpoint has been taken into account (0.10 mg/kg bw/d was used instead of 10 mg/kg bw/d, which results from division of the parent endpoint of 100 mg/kg bw/d by 10).

Overall, based on the performed calculations acceptable risk of secondary poisoning could be concluded for earthworm- and fish-eating mammals exposed to 2,4-DCA, 2,4-DCP and methoxy pyridine.

### 9.3.2.5 Biomagnification in terrestrial food chains

No risk of biomagnification in terrestrial food chains is expected according to the data given in the Point 9.3.2.4.

### 9.3.3 Overall conclusions

#### Acute risk assessment to mammals

An acute LD<sub>50</sub> value is available for the formulation AG-CDF1-480 EC. Comparison of the measured and predicted toxicity of the mixture demonstrated that the formulated product is more toxic than predicted on the basis of the active substance data and the risk assessment based on the measured formulation endpoint has been performed. ~~There is no indication of increased toxicity of the formulation.~~

Since 2,4-D (regardless if form of an ester or acid) was identified to drive the acute risk to birds and for this reason the TER values were calculated only for 2,4-D (acid and ester) as being protective also for clopyralid and fluroxypyr. As indicated above, the acute risk assessment was also performed for the formulated product based on the measured toxicity data. Performed calculations demonstrated acceptable acute risk to ~~mammals~~ **birds** from the intended uses of ADM.3304.H.1.A in cereals.

For the intended uses in grassland an acceptable risk could be concluded for large herbivorous species from 2,4-D (acid and ester) and formulation, but unacceptable risk was concluded for small herbivorous mammals based on Tier 1 calculations. The field population study provided by the Applicant to address the acute risk to small herbivores (common vole) from 2,4-D formulations was not agreed by the zRMS for the following reasons:

- The study was performed with two formulations, each containing 2,4-D in a form of DMA salt, while ADM.3304.H.1.A contains 2,4-D EHE. Behaviour of DMA salt and an ester is different and extrapolation between these two forms is not possible, so results of studies performed with 2,4-D DMA salt are not relevant to address the acute and long-term risk from formulations containing 2,4-D EHE, especially in case of ADM.3304.H.1.A the acute risk to small herbivorous mammals is unacceptable also for 2,4-D EHE and the formulated product itself.
- The study was already evaluated in the course of the 2,4-D EU renewal and rejected during the peer-review as unreliable due to numerous uncertainties described in EFSA Journal 2014;12(9):3812. The zRMS was not in the position to challenge the decision of the MS experts and EFSA on the study reliability, as this obviously was intensively discussed and all potential arguments in favour of keeping the study for refinement of the risk were already taken into account in the course of the peer-review and rejected.

Overall, the acute dietary risk from 2,4-D EHE, 2,4-D acid and formulation to small herbivorous mammals in grassland remains unresolved and further data must be submitted by the Applicant to support authorisation in this crop.

Acceptable acute dietary risk from all active compounds and the formulations could be concluded for uses in cereals.

~~Acute screening/first tier risk assessments for 2,4-D (the toxicity “driver”) were conducted. All the  $TER_{\alpha}$  values for the active substance exceed the trigger value of 10, indicating an acceptable acute risk to mammals following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use patterns proposed.~~

#### Reproductive risk assessment to mammals

Initially the Applicant performed the long-term risk assessment for 2,4-D only, explaining that this substance is a toxicity driver. However, 2,4-D was identified as a toxicity driver of the acute risk and not the long-term risk, which should have been performed for the active compounds and the mixture.

Performed evaluation demonstrated acceptable reproductive risk to mammals from clopyralid and fluroxypyr from all intended uses of ADM.3304.H.1.A.

For 2,4-D acceptable risk could be demonstrated for uses in cereals, however unacceptable risk was demonstrated for uses in grassland for both indicator species (large and small herbivore). The risk was refined using the EU agreed residue decline data and acceptable risk could be demonstrated for large herbivore, but the risk to small herbivorous species remained unacceptable. There were no other refinement options provided by the Applicant with exception of the field population study, which was, however, rejected **already at the EU level** due to reasons highlighted above in paragraph referring to the acute risk assessment.

The combined long-term mixture risk assessment **was deemed not necessary since the Toxic Units calculated following the commenting period indicated that 2,4-D (regardless of its form) clearly drives the long-term toxicity of the mixture. Taking this into account, the long-term risk from the mixture is considered to be covered by evaluation performed for 2,4-D.** ~~performed using simplified approach via calculation of  $TER_{mix}$  values. Based on performed calculations, an acceptable risk from the mixture could be concluded for the intended uses of ADM.3304.H.1.A in cereals, but unacceptable Tier 1 risk was concluded for both generic focal species following uses in grassland, but acceptable risk could be demonstrated to large herbivorous mammal when the refined  $TER_{LT}$  value for 2,4-D was considered. However, the long-term risk to small herbivore remained unresolved.~~

Overall, the long-term dietary risk from 2,4-D acid ~~and the mixture~~ to small herbivorous mammals in grassland remains unresolved and further data must be submitted by the Applicant to support authorisation in this crop.

Acceptable long-term dietary risk from all active compounds and the mixture could be concluded for uses in cereals.

~~The  $TER_{\alpha}$  values for 2,4-D to mammals in cereals exceed the Annex VI trigger value of 5, indicating that the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) applied according to the intended use do not pose a potential reproductive risk to birds.~~

~~Based on the refined  $TER_{\alpha}$  values for 2,4-D, a chronic risk to mammals in grassland can be excluded.~~

#### Secondary poisoning to mammals and risk from drinking water

No risk to mammals is expected via the consumption of water contaminated with the active substances **and their pertinent soil metabolites** from puddles on soil. A risk of secondary poisoning in terrestrial environments can be also excluded.

## **9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)**

No data are available regarding the potential effects of ADM.3304.H.1.A (old code AG-CDF1-480 EC) to reptiles and amphibians.

### **zRMS comments:**

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

## **9.5 Effects on aquatic organisms (KCP 10.2)**

### **9.5.1 Toxicity data**

Studies on the toxicity to aquatic organisms have been carried out with active substances Clopyralid, Fluroxypyr acid and its variant Fluroxypyr meptyl, 2,4-D acid and its variant ester 2,4-D EHE. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) were not evaluated as part of the EU assessment of the active substances. Data for the formulation AG-CDF1-480 EC was already submitted in 2019 (Falk, 2015) to the competent authority and new data with formulation ADM.3304.H.1.A were already submitted in 2021 for the composition change (Eser, 2019) are listed in **Appendix 1** and summarised in **Appendix 2**.

The endpoints selected for the risk assessments of active substances, their variants and metabolites are in line with the values used in the EU review processes. Additionally, risk assessments are presented based on data for the formulation. Justification for new endpoints is provided below.

**Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – 2,4-D acid / variant and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Pimephales promelas</i>	2,4-D acid	96 hr, f	LC <sub>50</sub> = 100 mg/L <sub>nom</sub>	EFSA Journal 2014; 12(9): 3812
<i>Pimephales promelas</i>	2,4-D acid	32 d ELS, f	NOEC = 63.4 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	2,4-D acid	48 hr, s	EC <sub>50</sub> = 134.2 mg/L <sub>nom</sub>	
<i>Daphnia magna</i>	2,4-D acid	21 d, ss	NOEC = 38.4 mg/L <sub>nom</sub>	
<i>Daphnia magna</i>	2,4-D acid	21 d, f	NOEC = 79 mg/L <sub>mm</sub>	
<i>Pseudokircheriella subcapitata</i>	2,4-D acid	72 hr, s	EyC <sub>50</sub> > 78 mg/L <sub>mm</sub> ErC <sub>50</sub> > 78 mg/L <sub>mm</sub>	
<i>Navicula pelliculosa</i>	2,4-D acid	72 hr, s	EyC <sub>50</sub> > 100 mg/L <sub>nom</sub> ErC <sub>50</sub> > 100 mg/L <sub>nom</sub>	
<i>Desmodesmus subspicatus</i>	2,4-D acid	72 hr, s	EyC <sub>50</sub> > 582.2 mg/L <sub>mm</sub> ErC <sub>50</sub> > 582.2 mg/L <sub>mm</sub>	
<i>Skeletonema costatum</i> (marine species)	2,4-D acid	72 hr, s	EyC <sub>50</sub> = 0.68 mg/L <sub>nom</sub> ErC <sub>50</sub> = 4.58 mg/L <sub>nom</sub>	
<i>Lemna minor</i>	2,4-D acid	7 d, s	EyC <sub>50</sub> fronds = 10.66 mg/L <sub>nom</sub> ErC <sub>50</sub> fronds = 17.51 mg/L <sub>nom</sub> EyC <sub>50</sub> dry weight = 18.50 mg/L <sub>nom</sub> ErC <sub>50</sub> dry weight > 100 mg/L	
<i>Myriophyllum spicatum</i>	2,4-D acid	14 d	EC <sub>50</sub> total root length = 0.011 mg a.s./L <sub>nom</sub> <sup>#</sup> NOEC <sub>total root length</sub> = 0.0047 mg/L <sup>#</sup>	
<i>Oncorhynchus mykiss</i>	2,4-DCA	96 hr, f	LC <sub>50</sub> > 1.4 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	2,4-DCA	48 hr, s	EC <sub>50</sub> = 6.4 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	2,4-DCP	48 hr, s	EC <sub>50</sub> = 2.8 mg/L <sub>mm</sub>	
<i>Pseudokircheriella subcapitata</i>	2,4-DCA	72 hr, s	EyC <sub>50</sub> = 2.2 mg/L <sub>mm</sub> ErC <sub>50</sub> = 4.3 mg/L <sub>mm</sub>	
<i>Pseudokircheriella subcapitata</i>	2,4-DCP	72 hr, s	EyC <sub>50</sub> = 1.13 mg/L <sub>mm</sub> ErC <sub>50</sub> = 3.44 mg/L <sub>mm</sub>	
<i>Lemna gibba</i>	2,4-DCA	7 d, ss	EyC <sub>50</sub> fronds = 2.1 mg/L <sub>mm</sub>	
<i>Lemna gibba</i>	2,4-DCP	10 d, s	EyC <sub>50</sub> fronds = 1.5 mg/L <sub>mm</sub>	
<i>Myriophyllum spicatum</i>	2,4-DCA	10 d, s	EC <sub>50</sub> shoot length = 1.16 mg/L <sub>mm</sub>	KCP 10.2.1/10 Gonsior, 2015 S15-00666
<i>Myriophyllum spicatum</i>	2,4-DCP	10 d, s	EC <sub>50</sub> fresh weight = 12.4 mg/L <sub>mm</sub>	
<i>Myriophyllum spicatum</i>	4-CP	14 d, static	Total shoot length ErC <sub>50</sub> : 13.1 mg pm/L <sub>mm</sub> Fresh weight ErC <sub>50</sub> : 48.0 mg pm/L <sub>mm</sub> Dry weight ErC <sub>50</sub> : 56.7 mg pm/L <sub>mm</sub>	Bridging report 2018 SANCO 7599/VL/97-final (1 October 2001)
<i>Menidia beryllina</i>	2,4-D EHE	96 hr, f	LC <sub>50</sub> > 0.24 mg/L <sub>mm</sub>	
<i>Pimephales promelas</i>	2,4-D EHE	32 d ELS, f	NOEC = 0.12 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	2,4-D EHE	48 hr, f	EC <sub>50</sub> = 0.25 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	2,4-D EHE	21 d, f	NOEC = 0.015 mg/L <sub>mm</sub> <sup>1)</sup>	Bridging report 2018 SANCO 7599/VL/97-final (1 October 2001)

<i>Skeletonema costatum</i>	2,4-D EHE	120 hr, s	ErC <sub>50</sub> = 0.23 mg/L <sub>nom</sub>	Bridging report 2018 SANCO 7599/VI/97 final (1 October 2001)
<i>Lemna gibba</i>	2,4-D EHE	7 d, s	EC <sub>50</sub> = 0.50 mg/L <sub>nom</sub>	Bridging report 2018 SANCO 7599/VI/97 final (1 October 2001)
<i>Myriophyllum spicatum</i>	GF-1387 (901 g/L 2,4-D EHE)	14 d, s, spiked water	Total shoot length ErC <sub>50</sub> : 0.202 mg a.s./L <sub>nom</sub> Fresh weight ErC <sub>50</sub> : 0.233 mg a.s./L <sub>nom</sub> Dry weight ErC <sub>50</sub> : 0.313 mg a.s./L <sub>nom</sub>  <del>Total shoot length: ErC<sub>50</sub> = 0.247 mg formulated product/L<sub>nom</sub> equivalent to 0.202 mg 2,4-D EHE/L</del> <del>Fresh weight: ErC<sub>50</sub> = 0.131 mg formulated product/L<sub>nom</sub> equivalent to 0.107 mg 2,4-D EHE/L</del>	Bridging report 2018 <del>Test provided with EU Bridging report 2018, Gonsior, G. 2014, S14-03289</del>
<i>Myriophyllum spicatum</i>	2,4-D	14 d, static, spiked water	Total shoot length: ErC <sub>50</sub> = 0.346 mg/L <sub>nom</sub> Fresh weight ErC <sub>50</sub> : 0.373 mg a.s./L Dry weight ErC <sub>50</sub> : 0.499 mg a.s./L  Fresh weight: ErC <sub>50</sub> = 0.373 mg/L <sub>nom</sub>	Test provided with EU Bridging report 2018, Gonsior, G. 2014, S14-03290

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; imm: based on initial mean measured concentrations.

# Endpoint agreed at the Pesticides Peer Review Meeting 111 (04 – 07 February 2013) and it is the geometric mean value for root length from the available 6 ring test studies with *Myriophyllum*. Endpoint not considered reliable; only useful for screening of toxicity for comparative hazard assessment. New water-sediment exposure study provides a more reliable and realistic exposure scenario for rooted aquatic macrophytes.

<sup>1)</sup> In the LoEP from the Bridging Report (2018) the NOEC of 0.13 mg a.s./L is reported, being, however, an EC<sub>50</sub> value. In the study summary presented in the Bridging Report in area of ecotoxicology (2018) the NOEC of 0.015 mg a.s./L is provided as relevant for the risk assessment purposes.

#### zRMS comments:

Aquatic toxicity data presented in Table 9.5-1 for 2,4-D acid and its metabolites 2,4-DCA and 2,4-DCP are in line with EU agreed endpoints reported in EFSA Journal 2014;12(9):3812.

The toxicity data for 2,4-D EHE are in line with endpoints presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Since the SANCO 7599/VI/97 final is not applicable anymore, reference to this document has been struck through in Table 9.5-1 and the Bridging Report has been referenced as being the relevant document where the currently agreed EU data for 2,4-D 2-EHE may be found.

It is noted that the Applicant reported also endpoints from the study on toxicity of 2,4-D to *Myriophyllum spicatum* by Gonsior (2014), which was evaluated and agreed by the RMS (Greece) in the course of evaluation of the additional data for 2,4-D 2-EHE and generating the Bridging Report (2018), where the study summary may be found. Applicability of the endpoint derived from this study is discussed in the zRMS commenting box in point 9.5.1.1 below.

In support of authorisation process of ADM.3304.H.1.A the Applicant submitted new aquatic toxicity studies performed with 2,4-D metabolites: 1,2,4-benzenetriol and 4-CP. Although the new active substance data should not be taken into account at the zonal level, it is justified to include the new endpoints for 1,2,4-benzenetriol and 4-CP since they were identified as a data gaps in EFSA Journal 2014;12(9):3812. Studies with metabolite 1,2,4-benzenetriol were evaluated and by the zRMS and considered not valid, since no reliable endpoints could be derived due to the test item measured concentrations dropping <LOD already after 1 hour after the test initiation. Since the studies were performed under the static exposure regime, it was not possible to calculate the mean measured concentrations, relevant for not stable substance for endpoints derivation. Nevertheless, the studies provided clear information that 1,2,4-benzenetriol is a transient metabolite of 2,4-D and for this reason no significant exposure from this compound is expected. Taking this into account, the risk assessment performed for the parent compound is deemed sufficient to cover the risk from this transient metabolite. The issue of the hazard assessment for 1,2,4-benzenetriol should be further dealt with at the next 2,4-D renewal. The study on toxicity of 4-Chlorophenol to *Myriophyllum spicatum* was evaluated and agreed by the zRMS. Details of the evaluation together with the study

summaries may be found in Appendix 3. Respective endpoints as agreed by the zRMS were added in Table 9.5-1 for completeness.

Some additional information has been added in Table 9.5-1 for informative purposes.

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Clopyralid**

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Clopyralid	96 hr, s	LC <sub>50</sub> > 99.9 mg/L <sub>mm</sub>	EFSA Journal 2018;16(8):5389
<i>Pimephales promelas</i>	Clopyralid	34 d, f	NOEC = 10.8 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	Clopyralid	96 hr, s	EC <sub>50</sub> > 99.0 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	Clopyralid	21 d, ss	NOEC = 17 mg/L <sub>mm</sub> EC <sub>10</sub> = 23.5 mg/L <sub>mm</sub>	
<i>Chironomus riparius</i>	Clopyralid	28 d, s, water spiked test	NOEC <sub>emergence</sub> = 50 mg/L <sub>mm</sub>	
<i>Scenedesmus capricornutum</i>	Clopyralid	72 hr, s	E <sub>b</sub> C <sub>50</sub> = 30.9 mg/L <sub>mm</sub> E <sub>r</sub> C <sub>50</sub> = 30.0 mg/L <sub>mm</sub>	
<i>Navicula pelliculosa</i>	Clopyralid	72 hr, s	E <sub>b</sub> C <sub>50</sub> = 31.5 mg/L <sub>nom,mm</sub> E <sub>r</sub> C <sub>50</sub> = 31.3 mg/L <sub>mm</sub>	
<i>Lemna gibba</i>	Clopyralid	14 d, s	EC <sub>50fronds</sub> = 89 mg/L <sub>mm,mm</sub>	
<i>Myriophyllum spicatum</i>	Clopyralid	14 d, s	E <sub>r</sub> C <sub>50</sub> > 3.0 mg/L <sub>mm</sub>	

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations.

**zRMS comments:**

Aquatic toxicity data presented in Table 9.5-2 for clopyralid are in line with EU agreed endpoints reported in EFSA Journal 2018;16(8):5389.

**Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Fluroxypyr acid / variant and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Lepomis macrochirus</i>	Fluroxypyr acid	96 hr, s	LC <sub>50</sub> = 14.3 mg/L <sub>mm</sub>	EFSA Journal 2011;9(3):2091
<i>Oncorhynchus mykiss</i>	Fluroxypyr acid	21 d, ss	NOEC <sub>mortality</sub> = 100 mg/L <sub>mm</sub>	
<i>Daphnia magna</i>	Fluroxypyr acid	48 hr, s	EC <sub>50</sub> > 100 mg/L <sub>nom</sub>	
<i>Daphnia magna</i>	Fluroxypyr acid	21 d, f	NOEC <sub>reproduction</sub> = 56 mg/L <sub>nom</sub>	
<i>Navicula pelliculosa</i>	Fluroxypyr acid	96 hr, s	72 h E <sub>b</sub> C <sub>50</sub> = 26 mg/L <sub>mm</sub> 72 h E <sub>r</sub> C <sub>50</sub> = 35.3 mg/L <sub>mm</sub> 96 h EC <sub>50</sub> = 36.2 mg/L <sub>mm</sub>	
<i>Lemna gibba</i>	Fluroxypyr acid	14 d, s	EC <sub>50fronds</sub> = 12.3 mg/L <sub>mm</sub>	
<i>Oncorhynchus mykiss</i>	Fluroxypyr-meptyl	96 hr, ss	LC <sub>50</sub> > 0.225 mg/L (maximum solubility)	
<i>Oncorhynchus mykiss</i>	Fluroxypyr-meptyl	21 d, f 96 hr, f	NOEC <sub>behaviour</sub> = 0.2 mg/L (actual)	
<i>Daphnia magna</i>	Fluroxypyr-meptyl	48 h, ss 96 hr, ss	EC <sub>50</sub> > 0.183 mg/L (maximum solubility)	
<i>Daphnia magna</i>	Fluroxypyr-meptyl	21 d, f 96 hr, f	NOEC <sub>reproduction</sub> = 0.0605 mg/L <sub>mm</sub> (actual)	
<i>Chironomus riparius</i>	Fluroxypyr-meptyl	28 d, s, water spiked test	NOEC <sub>development rate</sub> = 0.13 mg/L <sub>nom</sub>	
<i>Skeletonema costatum</i>	Fluroxypyr-meptyl	120 h, s	EC <sub>50</sub> = 0.208 mm (E <sub>r</sub> C <sub>50</sub> and E <sub>b</sub> C <sub>50</sub> were not calculated)	
<i>Lemna gibba</i>	Fluroxypyr-meptyl	14 d, s	EC <sub>50fronds</sub> > 2.31 mg/L <sub>mm</sub>	

<i>Anabeana flosaquae</i>	Methoxypyridine	120 hr, s	72 h EC <sub>50</sub> cell density < 1.12 mg/L imm 72 h ErC <sub>50</sub> = 3.16 mg/L imm 120 h EC <sub>50</sub> cell density = 1.80 mg/L imm 120 h ErC <sub>50</sub> = 2.23 mg/L imm	
<i>Lemna gibba</i>	Methoxypyridine	14 d, s	EC <sub>50</sub> fronds = 10.6 mg/L imm	
<i>Oncorhynchus mykiss</i>	Pyridinol	96 hr, ss	LC <sub>50</sub> = 39 mg/L mm	
<i>Daphnia magna</i>	Pyridinol	48 hr, s	EC <sub>50</sub> > 49 mg/L mm	
<i>Navicula pelliculosa</i>	Pyridinol	120 hr, s	72 h EC <sub>50</sub> = 0.640 mg/L mm (cell density) 72 h ErC <sub>50</sub> = 2.7 mg/L mm	
<i>Lemna gibba</i>	Pyridinol	14 d, s	EC <sub>50</sub> > 3.2 mg/L imm	
<i>Oncorhynchus mykiss</i>	3-CP	96 hr, ss	LC <sub>50</sub> = 95.1 mg/L mm	
<i>Daphnia magna</i>	3-CP	48 hr, s	EC <sub>50</sub> = 7.56 mg/L mm	
<i>Selenastrum capricornutum</i>	3-CP	96 hr, s	72 h EC <sub>50</sub> cell density = 35 mg/L mm 72 h EbC <sub>50</sub> = 46.3 mg/L mm 96 h EC <sub>50</sub> cell density = 35.8 mg/L mm 72 h EbC <sub>50</sub> = 42.6 mg/L mm	
<i>Myriophyllum spicatum</i>	Methoxypyridine	14 d, static	All parameters ErC <sub>50</sub> : >7700 µg pm/kg dw sediment	KCP 10.2.1/12 Gonsior, 2012 S12-00026
<i>Myriophyllum spicatum</i>	Fluroxypyr acid	14 d	ErC <sub>50</sub> = 0.276 mg /L	EFSA supporting publication 2015:EN-857 <sup>6</sup> , confirmatory data
<i>Myriophyllum spicatum</i>	Fluroxypyr-meptyl	14 d	ErC <sub>50</sub> = 0.0536 mg/L	EFSA supporting publication 2015:EN-857 <sup>7</sup> , confirmatory data

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; imm: based on initial mean measured concentrations.

#### zRMS comments:

Aquatic toxicity data presented in Table 9.5-3 for fluroxypyr and its metabolites are in line with EU agreed endpoints reported in EFSA Journal 2011;9(3):2091.

Studies on toxicity of fluroxypyr acid and meptyl to *Myriophyllum spicatum* were evaluated by the RMS (Ireland) and are presented in Fluroxypyr Addendum to Vol. 3: Confirmatory information (December 2014).

In support of authorisation process of ADM.3304.H.1.A the Applicant submitted new studies on toxicity of fluroxypyr acid as well as metabolite methoxypyridine to *Myriophyllum spicatum*. New study performed with active substance was not evaluated by the zRMS as sufficient toxicity data are already available from the EU review and the Applicant has the access to the EU agreed data via the LoA issued by the authorisation holder. No EU agreed endpoint was available for metabolite methoxypyridine and the study was thus considered relevant for authorisation process of ADM.3304.H.1.A, especially *Myriophyllum spicatum* turned out to be more sensitive to fluroxypyr comparing to *Lemna gibba*. The study was evaluated and agreed by the zRMS. Details of the evaluation together with the study summary may be found in Appendix 3. Respective endpoints as agreed by the zRMS were added in Table 9.5-3 for completeness.

<sup>6</sup> EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for fluroxypyr in light of confirmatory data. EFSA supporting publication 2015:EN-857. 43 pp.

<sup>7</sup> EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for fluroxypyr in light of confirmatory data. EFSA supporting publication 2015:EN-857. 43 pp.



**Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – AG-CDF1-480 EC**

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	AG-CDF1-480 EC	96 h, static	LC <sub>50</sub> > 100 mg/L	KCP 10.2.1/01 ... (2014) Study code: ...
<i>Daphnia magna</i>	AG-CDF1-480 EC	48 h, static	EC <sub>50</sub> > 100 mg/L	KCP 10.2.1/02 Hermes & Wydra (2014) Study code: 90311220
<i>Pseudokirchneriella subcapitata</i>	AG-CDF1-480 EC	72 h, static	ErC <sub>50</sub> > 100 mg/L EyC <sub>50</sub> = 55.9 mg/L NOErC = 1.0 mg/L	KCP 10.2.1/03 Hermes & Wydra (2015) Study code: 90311210
<i>Lemna gibba</i>	AG-CDF1-480 EC	7 d, static	EyC <sub>50</sub> = 2.9 mg/L (frond number) EyC <sub>50</sub> = 37.4 mg/L (dry weight) ErC <sub>50</sub> = 82.1 mg/L (frond number) ErC <sub>50</sub> > 100 mg/L (dry weight) NOErC = 0.1 mg/L	KCP 10.2.1/04 Hermes & Wydra (2015) Study code: 90311240
<i>Myriophyllum spicatum</i>	AG-CDF1-480 EC	14 d, spiked water	Lowest endpoints (based on mean measured concentrations): ErC <sub>50</sub> = 0.306 mg/L (total shoot length) EyC <sub>50</sub> = 0.121 mg/L (dry weight) NOErC = 0.0214 mg/L <del>ErC<sub>50</sub> = 0.381 mg/L (total shoot length)</del> <del>EyC<sub>50</sub> = 0.161 mg/L (dry weight)</del>	KCP 10.2.1/05 Falk (2015) Study code: S15-00056
<i>Myriophyllum spicatum</i>	ADM.3304.H.1.A	14 d, spiked water	Lowest endpoints (based on mean measured concentrations): ErC <sub>50</sub> = 0.054 mg/L (fresh weight) EyC <sub>50</sub> = 0.023 mg/L (fresh weight) NOErC = 0.004 mg/L	<b>KCP 10.2.1/17</b> <b>Eser (2019)</b> <b>Study code: S19-03357</b> KCP 10.2.1/05 Falk (2015) Study code: S15-00056

**zRMS comments:**

Studies on toxicity of formulation AF-CDF1-480 EC were evaluated and agreed by the zRMS. Summaries of the studies together with zRMS evaluation may be found in Appendix 2. Endpoints presented in Table 9.5-4 are confirmed to be in general correct with some amendments of endpoints derived from *Myriophyllum* study, since due to fluroxypyr measured concentrations dropping <80% of nominal, the endpoints should be based on mean measured concentrations.

Due to change of the composition of the formulated product the Applicant submitted the bridging study on toxicity of the new variant of the formulation (ADM.3304.H.1.A) to *Myriophyllum spicatum*, being the most sensitive species to the active compounds. The study was evaluated and agreed by the zRMS and the endpoints have been added to Table 9.5-4. The summary of the study together with the zRMS evaluation are presented in Appendix 2.

**Toxicity comparison for formulations AG-CDF1-480 EC and ADM.3304.H.1.A**

As a change of composition was requested in 2021 (formulation AG-CDF1-480 EC to ADM.3304.H.1.A), additional information is provided.

Toxicity tests with the formulations AG-CDF1-480 EC and ADM.3304.H.1.A on aquatic plants are available (Falk, 2015 and Eser, 2019). The studies of two formulations were conducted according to the OECD test guideline No. 239 using the same aquatic macrophytes (*Myriophyllum spicatum*) and under the same conditions/test design (14 days exposure and spiked water). Both studies are summarized in **Appendix 2**, see KCP 10.2.1/05 and KCP 10.2.1/17 for further details. Endpoints for each formulation are presented in the table below.

**Table 9.5-5: Toxicity values for AG-CDF1-480 EC and ADM.3304.H.1.A on aquatic plants**

Species	AG-CDF1-480 EC	ADM.3304.H.1.A
<i>Myriophyllum spicatum</i> (14 d, spiked water)	ErC <sub>50</sub> = 0.306 mg/L (shoot length)	ErC <sub>50</sub> = 0.119 mg/L (shoot length)
	ErC <sub>50</sub> = 0.371 mg/L (fresh weight)	ErC <sub>50</sub> = 0.054 mg/L (fresh weight)
	ErC <sub>50</sub> = 0.329 mg/L (dry weight)	ErC <sub>50</sub> = 0.278 mg/L (dry weight)
	ErC <sub>50</sub> = 0.381 mg/L (shoot length)	ErC <sub>50</sub> = 0.165 mg/L (shoot length)
	ErC <sub>50</sub> = 0.161 mg/L (dry weight)	ErC <sub>50</sub> = 0.0954 mg/L (dry weight)
	ErC <sub>50</sub> = 0.404 mg/L (dry weight)	ErC <sub>50</sub> = 0.403 mg/L (dry weight)

Comparing the toxicity data achieved for each formulation based on shoot length and dry weight, it is shown that both formulations present very similar toxicity to aquatic plants. Therefore, the risk for aquatic organisms posed by formulation ADM.3304.H.1.A can be assessed through the risk estimated by formulation AG-CDF1-480 EC.

**zRMS comments:**

The zRMS agrees that the endpoints for dry weight and possibly for shoot length are comparable for both variants of formulation, but the endpoint for fresh weight is clearly lower for ADM.3304.H.1.A and should be thus used for the risk assessment purposes, especially ADM.3304.H.1.A is the variant that will be placed on the market.

With regard to other aquatic species, no bridging studies are deemed necessary, since the most sensitive species has been tested with the new variant of the formulation and even if ADM.3304.H.1.A was slightly more toxic it would have no impact on the outcome of the risk assessment since the risk is driven by the *Myriophyllum spicatum* endpoints.

**Toxicity of mixture**

In addition to the risk assessment for the single active substances, the mixture toxicity and the risk assessment of 2,4-D acid, Clopyralid and Fluroxypyr meptyl were evaluated according to *Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters* (EFSA Journal 2013; 11 (7): 3290).

The ETR is determined via a step-wise approach:

**Step 1:** the measured formulation toxicity (EC<sub>x</sub> PPP) must be checked against the calculated toxicity (EC<sub>x</sub> mix-CA). The Concentration Addition (CA) is determined via the following equation:

$$EC_{x \text{ mix-CA}} = (\sum p_i / EC_{xi})^{-1}$$

where:

p = the component as a relative fraction of the mixture composition

EC<sub>xi</sub> = concentration on the component provoking x % effect

**Step 2:** the EC<sub>x</sub> PPP and EC<sub>x</sub> mix-CA are compared to give a model deviation ratio (MDR). If the MDR is 0.2 – 5.0 then it can be concluded that the predicted toxicity is comparable to the measured toxicity, allowing the use of measured toxicity values in the formulation risk assessment. If the MDR is < 0.2 it can be concluded that the measured toxicity is less toxic than the EC<sub>x</sub> mix-CA, and the EC<sub>x</sub> mix-CA should be used in the risk assessment.

$$MDR = EC_{x \text{ mix-CA}} / EC_{x \text{ PPP}}$$

**Table 9.5-6: Calculation of EC<sub>50mix-CA</sub> and Model deviation ration (MDR) using the acute toxicity data for aquatic species.**

aquatic species.						
Active substance	EC <sub>50i</sub>	Concentration in formulation [g a.s./L]	p <sub>i</sub>	EC <sub>50mix-CA</sub> [mg/L]	EC <sub>50product</sub> measured – corrected for active substance content and density of product (1.098 g/cm <sup>3</sup> ) [mg a.s./L]	MDR
Fish						
2,4-D acid	100	375	0.73	1.1	>47.1 ≥ 46.8	0.02
Clopyralid	> 99.9	30	0.06			
Fluroxypyr-meptyl	0.225	108	0.21			
Aquatic invertebrate						
2,4-D acid	134.2	375	0.73	0.9	>47.1 ≥ 46.8	0.02
Clopyralid	99.0	30	0.06			
Fluroxypyr-meptyl	> 0.183	108	0.21			
Algae						
2,4-D acid	4.58	375	0.73	0.9	>47.1 ≥ 46.8	0.02
Clopyralid	30.0	30	0.06			
Fluroxypyr-meptyl	0.208	108	0.21			
Higher plants ( <i>Lemna</i> )						
2,4-D acid	17.51	375	0.73	7.5	38.7 38.5	0.2
Clopyralid	89	30	0.06			
Fluroxypyr-meptyl	> 2.31	108	0.21			
Higher plants ( <i>Myriophyllum</i> )						
2,4-D acid	0.346	375	0.73	0.17	0.024 0.2 0.9	7.1 0.9
Clopyralid	> 3.0	30	0.06			
Fluroxypyr meptv]	0.0536	108	0.21			

For fish, aquatic invertebrates, algae and Lemna the MDR is < 0.2, thereby the EC<sub>50mix-CA</sub> should be used in the combined toxicity. For *Myriophyllum*, the MDR is above 0.2 (i.e. 0.9), therefore the measured EC<sub>50product</sub> can be used for the risk assessment.

**Step 3:** the EC<sub>xPPP</sub> is checked against the mixture composition at the PEC<sub>mix</sub>. If EC<sub>xmix-CA</sub> (a.s. in PPP)/EC<sub>xmix-CA</sub> (a.s. in PEC<sub>mix</sub>) = 0.8 – 1.2 (mixture similar).

$$EC_{xmix-CA} / EC_{xmix-CA} = 0.8 - 1.2$$

**Step 4:** where it has been determined that the mixture is similar, i.e. EC<sub>xmix-CA</sub> (a.s. in PPP)/EC<sub>xmix-CA</sub> (a.s. in PEC<sub>mix</sub>) = 0.8 – 1.2, then the ETR (estimated toxicity ratio) for the formulation is calculated as follows:

$$ETR_{ppp} = PEC_{mix} / EC_{xPPP}$$

Where no formulation data is available to compare with the calculated toxicity to generate an MDR, the calculated toxicity (EC<sub>x mix-CA</sub>) is used directly with the PEC<sub>mix</sub> to determine the ETR.

$$ETR = PEC_{mix} / EC_{x CA-mix}$$

As already indicated, the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) contains in its composition the variant 2-ethylhexyl ester of 2,4-D (i.e. 2,4-D EHE). This compound is rapidly converted to 2,4-D acid in the environment. Non-target aquatic organisms can be exposed to 2,4-D EHE via spray for a few hours immediately after application. Exposure via drainflow or run-off will occur to 2,4-D acid only. Mixture toxicity with acid has been already evaluated above.

The mixture toxicity of 2,4-D EHE, Clopyralid and Fluroxypyr meptyl is shown below.

It is important to note that 2,4-D EHE is a poorly water-soluble substance and the endpoints obtained in toxicity tests for ester were above its maximum water solubility (please refer to next Point 9.5.1.1, **Table 9.5-7**).

**Table 9.5-7: Calculation of EC<sub>50mix-CA</sub> and Model deviation ration (MDR) using the acute toxicity data for aquatic species**

Active substance	EC <sub>50i</sub>	Concentration in formulation [g a.s./L]	p <sub>i</sub>	EC <sub>50mix-CA</sub> [mg/L]	EC <sub>50product</sub> measured – corrected for active substance content and density of product* [mg a.s./L]	MDR
Fish						
2,4-D EHE	0.24	562.5	0.80	0.248	> 64.0	0.004
Clopyralid	> 99.9	30	0.04			
Fluroxypyr-meptyl	0.225	108	0.15			
Aquatic invertebrate						
2,4-D EHE	0.25	562.5	0.80	0.247	> 64.0	0.004
Clopyralid	99.0	30	0.04			
Fluroxypyr-meptyl	> 0.183	108	0.15			
Algae						
2,4-D EHE	0.23	562.5	0.80	0.236	> 64.0	0.004
Clopyralid	30.0	30	0.04			
Fluroxypyr-meptyl	0.208	108	0.15			
Higher plants ( <i>Lemna</i> )						
2,4-D EHE	0.50	562.5	0.80	0.598	> 52.5	0.011
Clopyralid	89	30	0.04			
Fluroxypyr-meptyl	> 2.31	108	0.15			
Higher plants ( <i>Myriophyllum</i> )						
2,4-D EHE	0.202	562.5	0.80	0.146	0.034 0.2	4.3 0.599
Clopyralid	> 3.0	30	0.04			
Fluroxypyr meptyl	0.0536	108	0.15			

\* Density of the product: 1.098 g/cm<sup>3</sup>.

As it can be concluded from the above table, it can also excluded 2,4-D EHE combined with Clopyralid and Fluroxypyr meptyl will cause synergistic effects on non-target aquatic organisms.

#### **zRMS comments:**

Combined toxicity assessment presented by the Applicant above was amended by the zRMS with consideration of the formulation endpoint for *M. spicatum* as agreed by the zRMS.

In addition to that, the EC<sub>50mix-CA</sub> values were recalculated with consideration of the active substance concentrations and density of formulations that were actually tested (information for calculations taken from the study reports).

Provided amendments had only marginal (if any) impact on the combined risk assessment performed for fish, aquatic invertebrates, algae and *Lemna*. Based on the performed calculations it may be concluded that, regardless of the form of 2,4-D considered, the formulation is less toxic than expected based on the active substance data and for this reason for these groups aquatic organisms the risk is considered to be covered by evaluation performed for particular active compounds.

Consideration of the endpoint for *M. spicatum* derived with formulation ADM.3304.H.1.A (variant which will be placed on the marked) had significant impact on the combined toxicity assessment when 2,4-D in an acid form is taken into account in calculations, since MDR value is >5, indicating that the formulation is more toxic to this species than expected based on the active substance data. In case 2,4-D in an ester form is taken into account, the toxicity of the formulation is comparable with mixture toxicity expected based on the active substance data. In either case, the measured formulation endpoint is considered relevant for the risk assessment purposes. Respective calculations are presented in point 9.5.2 below.

### 9.5.1.1 Justification for new endpoints

Since Annex I inclusion new studies on the active substances/metabolites have been performed. Summaries of new tests are included in **Appendix 3**. The new endpoints have been considered in the risk assessment.

Data from a literature research conducted for metabolite 4-Chlorophenol are presented in **Appendix 4**.

### 2,4-D

**Table 9.5-8: New endpoints and effect values relevant for the risk assessment for aquatic organisms**

Species	Substance	Exposure System	Results	Reference
<i>Myriophyllum spicatum</i>	2,4-D	14 d, static, spiked water	EC <sub>50</sub> = 0.346 mg/L	Test provided with EU Bridging report 2018, Gonsior, G. 2014, S14-03290
<i>Myriophyllum spicatum</i>	724 g/L 2,4-D DMA (60 % w/w); equivalent to 600 g/L 2,4-D (50 % w/w)	14 d, static, spiked water	EC <sub>50</sub> > 0.499 mg 2,4-D/L	KCP 10.2.1/06 Gonsior, G. 2014, S14-03291
<i>Oncorhynchus mykiss</i>	1,2,4 Benzenetriol	96 h, static	LC <sub>50</sub> > 29.5 mg/L	KCP 10.2.1/07 ---
<i>Daphnia magna</i>	1,2,4 Benzenetriol	48 h, static	EC <sub>50</sub> = 3.66 mg/L	KCP 10.2.1/08 Zawadsky (2015) Study code: S15-00612
<i>Myriophyllum spicatum</i>	1,2,4 Benzenetriol	14 d, static, spiked water	Lowest endpoints: EC <sub>50</sub> = 19.0 mg/L (dry weight) EC <sub>50</sub> = 11.7 mg/L (dry weight)	KCP 10.2.1/09 Gonsior (2015) Study code: S15-00667
<i>Oncorhynchus mykiss</i>	4-Chlorophenol	96 h, ip	LC <sub>50</sub> = 1.9 mg/L	KCP 10.2.1/13 Peer reviewed publication Hodson <i>et al</i> (1984)
<i>Daphnia magna</i>	4-Chlorophenol	48 h, static	EC <sub>50</sub> = 2.5 mg/L	KCP 10.2.1/14 Peer reviewed publication Kühn <i>et al</i> (1989)
<i>Skeletonema costatum</i>	4-Chlorophenol	120 h	EC <sub>50</sub> = 13.8 mg/L	KCP 10.2.1/15 Peer reviewed publication Cowgill <i>et al</i> (1989)
<i>Myriophyllum spicatum</i>	4-Chlorophenol	14 d, s, spiked water	EC <sub>50</sub> = 13.1 mg/L EC <sub>50</sub> = 10.3 mg/L	KCP 10.2.1/10 Gonsior (2015) Study code: S15-00666

ip = intraperitoneal injection

At the time of Annex I renewal of 2,4-D, the selection of the aquatic plant endpoint, upon which the EFSA risk assessment was based, was decided during the EFSA Peer Review Meeting 111 (04 – 07 February 2014). In this Peer Review Meeting, in the absence of a GLP, guideline study, the aquatic plant endpoint was selected based on reported effects on root length determined in a non-GLP ring-test using a water-only screening level test method (Maletzki, 2011)<sup>8</sup>. This is despite significant reservations about the suitability of this test method, and the use of a root length endpoint, which are summarized below:

- *Myriophyllum sp.* cuttings, without roots, were used resulting in an unrealistic exposure scenario for assessing exposure and risk to rooted aquatic plants since subsequent root development and growth is unnatural due to the lack of a sediment substrate.

<sup>8</sup> Maletzki, D. (2011): *Myriophyllum spicatum* toxicity test: Results of an inter-laboratory ring test using a sediment-free test system. Final report FKZ:36301294, Federal Environment Agency, Dessau, Germany.

- The exposure of roots to light results in atypical root development, and the addition of sucrose to the test media will interfere with photosynthesis and hence the normal physiology and metabolism of the plants.
- The use of sterile test conditions in this test system means that important biotic degradation processes for 2,4-D were omitted. Thus, the ecological relevance of any effects on root endpoints will be highly questionable.

In addition to these reservations, the ring test (from which the endpoint was selected) was not conducted to GLP, certified test material was not used, and exposure concentrations were not verified during the test. So, as well as the conditions not being ecologically relevant (no roots at test start, exposure of roots to light, addition of sucrose, and no sediment), the reliability, reproducibility and repeatability of this test system is in question.

Since Annex I renewal, new GLP compliant studies with 2,4-D conducted for the aquatic plant *Myriophyllum spicatum* in accordance with OECD Test Guideline No. 239 (water-sediment exposure) and the latest EFSA Aquatic Guidance, using certified test material, and with analytical verification of exposure (Gonsior 2014 (report S14-03291) (KCP 10.2.1/06) and Gonsior 2014 (report S14-03290)).

The biomass (fresh and dry weight) measurements were performed with whole plants so that any significant effects on root development were implicitly measured with the biomass endpoints. These new studies are more reliable, and environmentally relevant, than the screening level value evaluated for Annex I Renewal. In addition, it should be noted that the OECD Test Guideline No. 238 (water-only exposure) for testing aquatic plants states that ‘the inclusion of root endpoints is questionable’ for molecules with an auxin-type mode of action. Thus, the critical endpoint for aquatic plants provided in the EFSA Conclusion Report has been revised with the relevant growth rate endpoint taken from the new water-sediment studies with 2,4-D (Gonsior, 2014, S14-03290). Further, this study was considered to be acceptable by RMS Greece and is now referred to in the Bridging report (2018), providing a valid endpoint for 2,4-D acid.

Therefore, the more appropriate endpoint for *Myriophyllum spicatum* has been used in the risk assessment based on the recent findings in these GLP compliant studies with 2,4-D acid conducted in laboratory sediment-water test systems.

Furthermore, since Annex I renewal process, additional data have been considered to evaluate the effects of the metabolites 1,2,4-Benzenetriol and 4-Chlorophenol to aquatic non-target organisms. For metabolite 4-Chlorophenol peer reviewed literature has been taken into account. Since *Myriophyllum* was the most sensitive species for the active substance, an OECD 239 study has been conducted with 4-Chlorophenol.

**zRMS comments:**

2,4-D *Myriophyllum* endpoint

Although, in general, the EU agreed data should be used in the risk assessment at the zonal level and the discussion on endpoints discussed at the EU level should not be re-opened, there are situations when this seems to be necessary, like in case of 2,4-D *Myriophyllum* endpoint. It has to be emphasised that due to the MoA of 2,4-D *Lemna* sp. is not expected to be the most sensitive and for this reason the toxicity data for other macrophytes was necessary to derive the toxicity endpoint to be used in the risk assessment. However, no valid *Myriophyllum* study was available at the time of the 2,4-D renewal process and this was the only reason why the results of the ring studies were considered. The EC<sub>50</sub> of 0.011 mg a.s./L based on the effects on the root length was considered to be most relevant based on the available data, despite significant deficiencies already described in Vol. 3, B.9 (February 2019). It should be also pointed out that the EU agreed endpoint is not based on growth rates, although in line with EFSA (2013) growth rate endpoints should be used in the risk assessment. Furthermore, as already stated by the Applicant above, the ring studies were performed without sediment present, so indications set by OECD TG 238 should be followed in evaluation of the studies, while OECD TG 238 clearly indicates that inclusion of the root endpoints is questionable especially in case of substances with auxin-type MoA (such as 2,4-D).

Taking into account that currently valid EU agreed endpoint for *Myriophyllum spicatum* is available for the OECD TG 239 study evaluated in the course of preparation of the Bridging Report for 2,4-D 2-EHE in 2018, the zRMS is of the opinion that this endpoint, originating from the study performed in line with data requirements, should

supersede the not fully reliable endpoint derived from ring testing.

Overall, consideration of  $E_rC_{50}$  of 0.346 mg a.s./L as presented in the Bridging Report (2018) is agreed by the zRMS.

#### 1,2,4-benzenetriol studies

As already indicated in point 9.5.1 above, studies performed with 1,2,4-benzenetriol were evaluated by the zRMS and considered no valid since the measured concentrations of the test item in test solutions were <LOD already 1 hour after test initiation and due to the static test design the mean measured concentrations over the test period could not be calculated. Endpoints for 1,2,4-benzenetriol are thus struck through in Table 9.5-8 above. For more detailed discussion, please refer to zRMS commenting box in point 9.5.1 above.

#### 4-Chlorophenol studies

As already indicated in point 9.5.1 above, study on toxicity of 4-Chlorophenol to *Myriophyllum spicatum* was evaluated and agreed by the zRMS. Details of the evaluation together with the study summaries may be found in Appendix 3.

The literature data submitted for other aquatic species (fish, *Daphnia magna* and algae) were not evaluated by the zRMS since In line with EFSA (2013) it is sufficient to perform the metabolite study with the species that turned out to be most sensitive to the parent. Since available data demonstrate that *Myriophyllum spicatum* is species most sensitive to 2,4-D, inclusion of other species is not necessary, especially no studies were performed according to current standards. Endpoints from the literature studies were struck through in Table 9.5-8 above.

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

Due to rapid degradation of 2,4-D EHE in the environment, aquatic organisms will only be exposed for a short period to 2,4-D EHE (half-life = 6.2 hr in natural water) as a result of entry via spray drift immediately after application. Exposure via drainflow or run-off will occur to 2,4-D acid only. Studies with aquatic organisms are available for 2,4-D EHE, although the assessment of acute and chronic ecotoxicity must be treated with caution due to its low water solubility (0.0867 mg/L), and rapid degradation in the test systems, especially when tested under static and static-renewal conditions.

Generally, in aquatic tests 2,4-D EHE was not toxic at maximum water solubility level. Low endpoints derived from long-term studies (c.f. NOEC = 0.015 mg 2,4-D EHE/L from a 21-day *Daphnia* study) can be attributed to the flow through study design, which represents an unrealistic long environmental exposure duration.

For algae and aquatic plants, in which static test designs are typical, the endpoints reflect exposure to both 2,4-D EHE (in the first hours) and 2,4-D acid (for the remainder of the test) due to the rapid degradation occurring during the course of the study. Since both 2,4-D EHE and 2,4-D acid are herbicidal actives, the endpoint cannot be attributed only to the initial 2,4-D EHE exposure.

**Table 9.5-9: Comparison of 2,4-D acid and 2,4-D EHE endpoints derived from aquatic studies (Bridging report 2018)**

Test species/system	Type	Lowest Endpoint <sup>1</sup>		Comment
		Acid <sup>2</sup>	Ester <sup>3</sup>	
Acute toxicity to fish	LC <sub>50</sub> (mg/L)	100 (nom)	> 0.24 (mm)	The ester study was conducted under flow-through conditions; the LC <sub>50</sub> was above the maximum water solubility.
Long-term toxicity to fish	NOEC (mg/L)	63.4 (mm)	0.12 (mm)	The ester has low water solubility, and quickly degrades to the acid form; low persistence in natural systems is expected. However from a 32 day embryo larval test the overall NOEC was 0.12 mg/L which is slightly above the reported maximum water solubility (physical effect of the undissolved material).
Acute toxicity to <i>Daphnia magna</i>	EC <sub>50</sub> (mg/L)	134.2 (mm)	0.25 (mm)	For the ester a new study is available (Palmer et al., 2010). The study was conducted under flow-through conditions; the EC <sub>50</sub> was above the reported maximum water solubility.
Long-term toxicity to <i>Daphnia magna</i>	NOEC (mg/L)	38.4 (nom)	0.015 (mm)	Ester study was performed under flow-through conditions which represent an unrealistic scenario. The NOEC based on nominal concentrations was 0.11 mg/L (i.e. above limit of solubility) as indicated by the lower mean measured concentration of 0.015 mg/L.
Toxicity to algae	E <sub>r</sub> C <sub>50</sub> <del>EC<sub>50</sub></del> (mg/L)	4.58 (nom) <del>0.68 (nom)</del>	0.23 (nom)	<i>Skeletonema costatum</i> . Low measured concentrations of ester were considered to be due the poor solubility of the test substance in water.
Long-term toxicity to aquatic macrophyte	E <sub>r</sub> C <sub>50</sub> (mg/L)	17.51 (nom)	0.5 (nom)	Floating monocot, <i>Lemna spp.</i> Endpoint for the acid is the E <sub>r</sub> C <sub>50</sub> from a study with <i>Lemna minor</i> ; and for ester is the EC <sub>50</sub> from a study with <i>Lemna gibba</i> . The EC <sub>50</sub> for the ester was above the reported maximum water solubility, and so the E <sub>r</sub> C <sub>50</sub> would also exceed the limit of solubility.
	E <sub>r</sub> C <sub>50</sub> (mg/L)	0.346 (nom)	0.202 (nom)	Rooted dicot, <i>Myriophyllum spp.</i> Endpoint for acid is taken from Gonsior, G. (2014a). Endpoint for ester is taken from study conducted with the EC formulation GF-1387 <sup>4</sup> ; Gonsior, G. (2014b). Rapid conversion of the ester to the acid was measured in the test system.

<sup>1</sup> Endpoint based on nominal concentrations (nom) or mean measured concentrations (mm).

<sup>2</sup> EFSA Journal 2014;12(9):3812.

<sup>3</sup> SANCO 7599/VI/97-final (1 October 2001).

<sup>4</sup> GF-1387 contains 905 g/L (81.68%) 2,4-D EHE.

#### zRMS comments:

The zRMS agrees that due to rapid degradation in soil with DT<sub>50</sub> of 0.1 days, 2,4-D in an ester form is not expected to be subject of drainage and/or run-off. For more detailed discussion on this issue, please refer to the Core Assessment, Part B, Section 8.

Reason for comparison of aquatic toxicity endpoints for both forms of 2,4-D is not fully clear to the zRMS, nevertheless endpoints reported in Table 9.5-9 are confirmed to be correct with exception of *Skeletonema costatum* endpoint for 2,4-D acid (E<sub>y</sub>C<sub>50</sub> value was reported for this compound instead of E<sub>r</sub>C<sub>50</sub>). Respective correction was made by the zRMS in the table above.



## Fluroxypyr

Spiked sediment studies were conducted with the rooted aquatic macrophytes for Fluroxypyr acid (20 % maximum occurrence in sediment) and Methoxypyridine (soil metabolite). The endpoints are summarized in the following table.

**Table 9.5-10: New endpoints and effect values relevant for the risk assessment for aquatic organisms**

Species	Substance	Exposure System	Results	Reference
<i>Myriophyllum spicatum</i>	Fluroxypyr acid	14 d, static, spiked sediment	$E_y C_{50} > 1140 \mu\text{g/kg d.w.}$ $E_r C_{50} > 1140 \mu\text{g/kg d.w.}$	Included as KCP 10.2.1/11 Gonsior (2012a) Study code: 90015211
<i>Myriophyllum spicatum</i>	Methoxypyridine	14 d, static, spiked sediment	$E_y C_{50} > 7700 \mu\text{g/kg d.w.}$ $E_r C_{50} > 7700 \mu\text{g/kg d.w.}$	Included as KCP 10.2.1/12 Gonsior (2012b) Study code: 90015185

### zRMS comments:

As already indicated in point 9.5.1 above, new study performed with active substance was not evaluated by the zRMS as sufficient toxicity data are already available from the EU review and the Applicant has the access to the EU agreed data via the LoA issued by the authorisation holder.

Study on toxicity of methoxypyridine to *Myriophyllum spicatum* was evaluated and agreed by the zRMS. Endpoints reported in Table 9.5-10 above are confirmed to be correct.

## 9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2, 3  $PEC_{SW}$  for risk assessments of each active substance, their variants and metabolites covering the proposed use pattern and the resulting PEC/RAC ratios for each taxonomic group are presented in the tables below. The risk assessment for the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) has also been estimated.

### zRMS comments:

~~Since Poland is the only eMS indicated in GAP for ADM.3304.H.1.A, only scenarios representative for Poland were taken into account in the below evaluation (North Europe at Step 2 and D3, D4 and R1 at Step 3). Risk assessment for scenarios not representative for Poland is displayed in grey font and was not validated.~~

The risk assessment presented below was amended where necessary (e.g. with other endpoints agreed by the zRMS or new exposure agreed in area of Section 8). Evaluation based on Step 2 results for the Southern Europe was struck through as not relevant for the Central Zone.

Please note that results for R1 scenario in winter cereals may be used to address the risk in this scenario following uses in grassland and spring cereals, for which scenario R1 is not defined in FOCUS models.

In case of mutual recognition process additional simulations may be requested by Member States considering other scenarios as representative or not accepting FOCUS modelling at all.

## RISK ASSESSMENTS FOR AQUATIC ORGANISMS

### 2,4-D

**Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 2,4-D for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Pimephales promelas</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	--	<i>Skeletonema costatum</i>	<i>Myriophyllum spicatum</i>
Endpoint			LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	--	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)			100000	63400	134200 143200	38400	-	4580	346
AF			100	10	100	10	-	10	10
RAC (µg/L)			1000	6340	1342 1432	3840	--	458	34.6
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	238.78	135.88	0.24	0.04	0.18 0.17	0.06	-	0.52	<b>6.90</b>
<b>FOCUS Step 2</b>									
EU-N	64.97	36.97	0.06	0.01	0.05	0.02	-	0.14	<b>1.88</b>
EU-S	53.10	30.02	0.053	0.01	0.04	0.01	-	0.12	<b>1.53</b>
<i>Grassland – 01.March, 750 g a.s./ha, BBCH 21-39</i>									
FOCUS Step 3			PEC/RAC ratio						
D3_Ditch	4.748	0.8613	0.005	0.001	0.004 0.003	0.001	-	0.010	0.137
D4_Pond	0.164	0.2324	0.00016	0.00003	0.0012 0.00011	0.00004	-	0.00036	0.00474
D4_Stream	3.831	0.1659	0.004	0.001	0.003	0.001	-	0.008	0.111
D5_Pond	0.164	0.2138	0.00016	0.00003	0.00011	0.00004	-	0.00036	0.00474
D5_Stream	3.89	0.107	0.004	0.001	0.003	0.001	-	0.008	0.112
R3_Stream	4.407	0.4722	0.004	0.001	0.003	0.001	-	0.010	0.127
<i>Grassland – 01.August, 750 g a.s./ha, BBCH 21-39</i>									
FOCUS Step 3			PEC/RAC ratio						
D3_Ditch	4.782 4.748	0.8613	0.005	0.001	0.004 0.003	0.001	-	0.010	0.138 0.137
D4_Pond	0.164	0.2324	0.00016	0.00003	0.0012 0.00011	0.00004	-	0.00036	0.00474
D4_Stream	4.111 3.831	0.1659	0.004	0.001	0.003	0.001	-	0.009 0.008	0.119 0.111
D5_Pond	0.164	0.2138	0.00016	0.00003	0.00011	0.00004	-	0.00036	0.00474

D5_Stream	3.89	0.107	0.004	0.001	0.003	0.001	-	0.008	0.112
R3_Stream	<b>4.433</b> <sup>1</sup> 4.407	<b>0.5714</b> 0.4722	0.004	0.001	0.003	0.001	-	0.010	<b>0.128</b> 0.127
<i>Winter cereals – 750 g a.s./ha, BBCH 21-39</i>									
<b>FOCUS Step 3</b>			<b>PEC/RAC ratio</b>						
D3_Ditch	4.756	0.9391	0.005	0.001	0.003	0.001	-	0.010	0.137
D4_Pond	0.164	0.1771	0.00016	0.00003	0.00011	0.00004	-	0.00036	0.00474
D4_Stream	3.965	0.2435	0.004	0.001	0.003	0.001	-	0.009	0.115
D5_Pond	0.164	0.2033	0.00016	0.00003	0.00011	0.00004	-	0.00036	0.00474
D5_Stream	3.793	0.09078	0.004	0.001	0.003	0.001	-	0.008	0.110
R1_Pond	0.1751	0.2763	0.00018	0.00003	0.00012	0.00005	-	0.00038	0.00506
R1_Stream	4.288	0.6655	0.004	0.001	0.003	0.001	-	0.009	0.124
R3_Stream	<b>8.400</b> <sup>1</sup> 4.398 <sup>2</sup>	<b>1</b> 0.764	<b>0.008</b> 0.004	<b>0.001</b> 0.001	<b>0.006</b> 0.003	<b>0.002</b> 0.001	- -	<b>0.018</b> 0.010	<b>0.24</b> 0.127
<i>Spring cereals – 750 g a.s./ha, BBCH 21-39</i>									
<b>FOCUS Step 3</b>			<b>PEC/RAC ratio</b>						
D3_Ditch	4.766	1.062	0.005	0.001	0.003	0.001	-	0.010	0.138
D4_Pond	0.1641	0.1603	0.00016	0.00003	0.00011	0.00004	-	0.00036	0.00474
D4_Stream	4.099	0.4111	0.004	0.001	0.003	0.001	-	0.009	0.118
D5_Pond	0.1642	0.1795	0.00016	0.00003	0.00011	0.00004	-	0.00036	0.00475
D5_Stream	4.149	0.1912	0.004	0.001	0.003	0.001	-	0.009	0.120

<sup>1)</sup> PEC<sub>sw</sub> obtained in zRMS surface water modelling performed for application windows suggested by AppDate

<sup>2)</sup> PEC<sub>sw</sub> obtained in Applicants' surface water modelling performed for later application windows (Member States must decide which application windows are more relevant in their countries)

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

#### zRMS comments:

The risk assessment for 2,4-D acid is agreed by the zRMS with some minor corrections.

Based on the above calculations, acceptable risk to aquatic organisms from 2,4-D may be concluded for all intended Central Zone uses of ADM.3304.H.1.A with no need for risk mitigation measures.

## 2,4-D EHE

Due to the low DT<sub>90</sub> value (water) of 2,4-D EHE of less than 24 hrs, no long-term risk evaluation for fish and *Daphnia* is deemed necessary. Therefore, only acute risk assessments for fish and *Daphnia* together with risk assessments to algae and aquatic macrophytes are presented below assuming exposure from spray drift into an adjacent water body. For the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) (containing 2,4-D EHE), an initial concentration in surface water via spray drift was calculated considering published spray drift data (Rautmann, 2001).

It is also important to note the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) is only applied once per season.

**Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 2,4-D EHE for each organism group following entry via spray drift after the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Algae	Aquatic macrophytes	Aquatic macrophytes
Test species		<i>Menidia beryllina</i> <i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Skeletonema costatum</i>	<i>Lemna gibba</i>	<i>Myriophyllum aquaticum</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)		> 240	120	250	15	230	500	202
AF		100	10	100	10	10	10	10
RAC (µg/L)		2.4	12	2.5	1.5	23	50	20.2
Entry via spray drift	PEC <sub>sw</sub> (µg/L)	PEC/RAC ratio						
1 m drift buffer	10.388	4.328	0.866 0.45	4.155	6.93	0.452	0.208	0.514
1 m drift buffer + 90% drift reduction	1.039	0.433	-	0.416	0.69	-	-	-
5 m drift buffer (protective for scenarios D3, D4, D5 and R1)	2.138	0.891	-	0.855	1.43	0.093	0.043	0.106
5 m buffer (R3 scenario)	2.410	1.004	-	0.964	1.61	-	-	-
10 m drift buffer (protective for scenarios D3, D4, D5 and R1)	1.088	-	-	-	0.73	-	-	-

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Algae	Aquatic macrophytes	Aquatic macrophytes
10 m buffer (R3 scenario)	1.278	0.533	█	█	0.852	█	█	█

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

#### zRMS comments:

The risk assessment for 2,4-D 2-EHE has been amended by the zRMS with consideration of the chronic toxicity data. Although long-term exposure to the ester form of 2,4-D is not expected, the prolonged effects of the short-term exposure cannot be completely ruled out and in line with indications of EFSA (2013), the long-term risk assessment is required for all substances, also those rapidly degrading.

Following the commenting period additional surface water modelling according to FOCUS methods was performed in order to check if the approach taken by the Applicant (consideration of the spray drift only) is protective also for scenarios D5 and R3. Obtained results confirmed that the Applicants' approach is protective for scenario D5, however PEC<sub>sw</sub> calculated at Step 4 with assumption of 5 and 10 m buffer zone for R3 scenario were higher than the exposure resulting from the spray drift. Taking this into account initially performed calculations were retained as being protective for scenarios D3, D4, D5 and R1, while for scenario R3 separate calculations were included in Table 9.5-12 above. Surface water exposure calculated using FOCUS methods at Step 4 with assumption of drift reduction was lower in all scenarios comparing to results obtained using the Applicants' approach. No separate calculations were thus necessary for any of the scenarios when simple mitigation using drift reducing nozzles is assumed.

Based on the above calculations, acceptable risk to aquatic organisms from 2,4-D 2-EHE may be concluded for all intended Central Zone uses of ADM.3304.H.1.A provided that 10 m unsprayed buffer zone to surface water bodies is respected or the spray drift is reduced by 90% using appropriate drift reducing techniques.

## Clopyralid

**Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Clopyralid for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Selenastrum capricornutum (P. subcapitata)</i>	<i>Myriophyllum spicatum</i>
Endpoint			LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	NOEC	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)			> 99900	10800	> 99000	17000	50000	30000	3000
AF			100	10	100	10	10	10	10
RAC (µg/L)			999	1080	990	1700	5000	3000	300
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	20.5	0.282	0.021	0.019	0.021	0.012	0.004	0.007	0.068

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

### **zRMS comments:**

The risk assessment for clopyralid is agreed by the zRMS.

Based on the above calculations, acceptable risk to aquatic organisms from clopyralid may be concluded for all intended Central Zone uses of ADM.3304.H.1.A with no need for risk mitigation measures.

## Fluroxypyr-meptyl

**Table 9.5-14:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Fluroxypyr-meptyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Skeletonema costatum</i>	<i>Myriophyllum spicatum</i>
Endpoint			LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	NOEC	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)			> 225	200	> 183	60.5	130	208	53.6
AF			100	10	100	10	10	10	10
RAC (µg/L)			2.25	20	1.83	6.05	13	20.8	5.36
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
<b>FOCUS Step 1</b>			<b>PEC/RAC ratio</b>						
	4.60	525.15	<b>2.04</b>	0.23	<b>2.51</b>	0.76	0.35	0.22	0.86
<b>FOCUS Step 2</b>									
EU-N	1.99	29.24	0.88	0.10	<b>1.09</b>	0.33	0.15	0.10	0.37
EU-S	<del>1.99</del>	<del>25.98</del>	<del>0.88</del>	<del>0.10</del>	<del>1.09</del>	<del>0.33</del>	<del>0.15</del>	<del>0.10</del>	<del>0.37</del>
<i>Grassland – 01.March, 750 g a.s./ha, BBCH 21-39</i>									
<b>FOCUS Step 3</b>			<b>PEC/RAC ratio</b>						
D3_Ditch	1.346	0.8807	0.598	0.067	0.736	0.222	0.104	0.065	0.251
D4_Pond	0.0465	0.4682	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D4_Stream	1.086	0.06321	0.483	0.054	0.593	0.180	0.084	0.052	0.203
D5_Pond	0.04649	0.4551	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D5_Stream	1.103	0.03816	0.490	0.055	0.603	0.182	0.085	0.053	0.206
R3_Stream	1.25	0.2831	0.556	0.063	0.683	0.207	0.096	0.060	0.233
<i>Grassland – 01.August, 750 g a.s./ha, BBCH 21-39</i>									
<b>FOCUS Step 3</b>			<b>PEC/RAC ratio</b>						
D3_Ditch	1.356	2.052	0.603	0.068	0.741	0.224	0.104	0.065	0.253
D4_Pond	0.04652	0.4235	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D4_Stream	1.166	0.2544	0.518	0.058	0.637	0.193	0.090	0.056	0.218
D5_Pond	0.04653	0.3904	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D5_Stream	1.258	0.0988	0.559	0.063	0.687	0.208	0.097	0.060	0.235
R3_Stream	1.257	0.3484	0.559	0.063	0.687	0.208	0.097	0.060	0.235

<i>Winter cereals – 750 g a.s./ha, BBCH 21-39</i>									
<b>FOCUS Step 3</b>			<b>PEC/RAC ratio</b>						
D3_Ditch	1.348	1.035	0.599	0.067	0.737	0.223	0.104	0.065	0.251
D4_Pond	0.04651	0.4066	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D4_Stream	1.124	0.1054	0.500	0.056	0.614	0.186	0.086	0.054	0.210
D5_Pond	0.04648	0.4427	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D5_Stream	1.076	0.03131	0.478	0.054	0.588	0.178	0.083	0.052	0.201
R1_Pond	0.04648	0.4115	0.021	0.002	0.025	0.008	0.004	0.002	0.009
R1_Stream	0.8879	0.1255	0.395	0.044	0.485	0.147	0.068	0.043	0.166
R3_Stream	1.247	0.2631	0.554	0.062	0.681	0.206	0.096	0.060	0.233
<i>Spring cereals – 750 g a.s./ha, BBCH 21-39</i>									
<b>FOCUS Step 3</b>			<b>PEC/RAC ratio</b>						
D3_Ditch	1.351	1.292	0.600	0.068	0.738	0.223	0.104	0.065	0.252
D4_Pond	0.04651	0.3842	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D4_Stream	1.162	0.2297	0.516	0.058	0.635	0.192	0.089	0.056	0.217
D5_Pond	0.04651	0.4129	0.021	0.002	0.025	0.008	0.004	0.002	0.009
D5_Stream	1.177	0.07643	0.523	0.059	0.643	0.195	0.091	0.057	0.220

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

#### **zRMS comments:**

The risk assessment for fluroxypyr-meptyl is agreed by the zRMS.

Based on the above calculations, acceptable risk to aquatic organisms from fluroxypyr-meptyl may be concluded for all intended Central Zone uses of ADM.3304.H.1.A with no need for risk mitigation measures.



## Fluroxypyr acid

**Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Fluroxypyr acid for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants		
Test species			<i>Lepomis macrochirus</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	-	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>	
Endpoint (µg/L)			LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	-	E <sub>r</sub> C <sub>50</sub>	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>	
AF			14300	100000	100000	56000	-	35300	123000	276	> 1140 (µg/kg)
RAC (µg/L)			100	10	100	10	-	10	10	10	40
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)	143	10000	1000	5600	-	3530	12300	27.6	> 114 (µg/kg)
Grassland and cereals											
FOCUS Step 1			PEC/RAC ratio								
	47.22	31.46	0.330	0.005	0.047	0.008	-	0.013	0.004	<b>1.71</b>	0.276
FOCUS Step 2			PEC/RAC ratio								
EU-N	19.99	13.56	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.724	n.r.
EU-S	16.23	11.01	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.588	n.r.

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**  
 n.r. = not required

### zRMS comments:

The risk assessment for fluroxypyr acid is in general agreed by the zRMS. Calculations based on newly submitted sediment-spiked study on toxicity of fluroxypyr acid to *Myriophyllum spicatum* were struck through since sufficient EU agreed data were available and there was no need for further testing at the zonal/national level.

Based on the above calculations, acceptable risk to aquatic organisms from fluroxypyr acid may be concluded for all intended Central Zone uses of ADM.3304.H.1.A with no need for risk mitigation measures.

### Metabolites of 2,4-D: 2,4-DCA, 2,4-DCP and 1,2,4-Benzenetriol

**Table 9.5-16:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 2,4-DCA for each organism group based on FOCUS Steps 1, 2 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	-	<i>Daphnia magna</i>	-	-	<i>Pseudokircheriella subcapitata</i>	<i>Myriophyllum aquaticum</i>
Endpoint			LC <sub>50</sub>	-	EC <sub>50</sub>	-	-	E <sub>r</sub> C <sub>50</sub>	EC <sub>50</sub>
(µg/L)			> 1400	-	6400	-	-	4300	1160 <del>116</del>
AF			100	-	100	-	-	10	10
RAC (µg/L)			14	-	64	-	-	430	116
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	17.44	177.40	<b>1.246</b>	-	0.273	-	-	0.041	0.150
FOCUS Step 2			PEC/RAC ratio						
EU-N	6.15	62.88	0.439	-	0.096	-	-	0.014	0.053
<del>EU-S</del>	<del>4.95</del>	<del>50.56</del>	<del>0.353</del>	-	<del>0.077</del>	-	-	<del>0.012</del>	<del>0.043</del>

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

**Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 2,4-DCP for each organism group based on FOCUS Steps 1, 2 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			-	-	<i>Daphnia magna</i>	-	-	<i>Pseudokircheriella subcapitata</i>	<i>Lemna gibba</i>
Endpoint			-	-	EC <sub>50</sub>	-	-	ErC <sub>50</sub>	EC <sub>50</sub>
(µg/L)			-	-	2800	-	-	3440	1500
AF			-	-	100	-	-	10	10
RAC (µg/L)			-	-	28	-	-	344	150
FOCUS Scenario	PEC <sub>SW</sub> (µg/L)	gl max	PEC <sub>SED</sub> (µg/kg)	gl max					
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	46.34	232.33	-	-	1.655	-	-	0.135	0.309
FOCUS Step 2			PEC/RAC ratio						
EU-N	13.31	67.34	-	-	0.475	-	-	0.039	0.089
EU-S	<del>10.87</del>	<del>54.85</del>	-	-	<del>0.388</del>	-	-	<del>0.032</del>	<del>0.072</del>

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

**Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 1,2,4-Benzenetriol for each organism group based on FOCUS Steps 1, 2 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish-acute	Fish-chronic	Invertebrate-acute	Invertebrate-chronic	Sediment-dwellers	Algae	Aquatic-plants
Test-species			<i>Oncorhynchus mykiss</i>	-	<i>Daphnia magna</i>	-	-	-	<i>Myriophyllum aquaticum</i>
Endpoint			LC <sub>50</sub>	-	EC <sub>50</sub>	-	-	-	EC <sub>50</sub>
(µg/L)			> 29500	-	3660	-	-	-	19000
AF			100	-	100	-	-	-	10
RAC (µg/L)			295	-	36.6	-	-	-	1900
FOCUS-Scenario	PEC <sub>SW-max</sub> (µg/L)	PEC <sub>SED-max</sub> (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS-Step 1			PEC/RAC ratio						
	43.2	-	0.146	-	<b>1.180</b>	-	-	-	0.015
FOCUS-Step 2			PEC/RAC ratio						
EU-N	11.7	-	0.040	-	0.320	-	-	-	0.006
EU-S	9.6	-	0.033	-	0.262	-	-	-	0.005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

**Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 4-CP for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	-	<i>Daphnia magna</i>	-	-	<i>Skeletonema costatum</i>	<i>Myriophyllum spicatum</i>
Endpoint			EC <sub>50</sub>	-	EC <sub>50</sub>	-	-	EC <sub>50</sub>	EC <sub>50</sub>
(µg/L)			1900	-	2500	-	-	13800	13100 10300
AF			100	-	100	-	-	10	10
RAC (µg/L)			19	-	25	-	-	1380	1310 1030
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	49.43 46.99	76.21 85.36	2.47	-	1.88	-	-	0.03	0.04 0.05
FOCUS Step 2			PEC/RAC ratio						
EU-N	5.23 2.31	8.0 4.16	0.12	-	0.09	-	-	0.002	0.002
EU-S	1.89	3.41	0.099	-	0.076	-	-	0.015	0.002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

#### zRMS comments:

The risk assessment for 2,4-D metabolites 2,4-DCA and 2,4-DCP is agreed by the zRMS.

Calculations for 1,2,4-benzenetriol were struck through since no reliable endpoints could be derived from the available aquatic toxicity studies. For discussion on this issue, please, refer to point 9.5.1 of this document. Nevertheless, the studies provided clear information that 1,2,4-benzenetriol is a transient metabolite of 2,4-D and for this reason no significant exposure from this compound is expected. Taking this into account, the risk assessment performed for the parent compound is deemed sufficient to cover the risk from this transient metabolite. The issue of the hazard assessment for 1,2,4-benzenetriol should be further dealt with at the next 2,4-D renewal.

With regard to 4-Chlorophenol, the risk assessment for fish, *Daphnia magna* and algae based on endpoints derived from the literature was struck through sine neither of these studies was validated by the zRMS due to the fact that the endpoint for species most sensitive to the parent compound was available and further data were deemed not necessary. Calculations in Table 9.5-19 above were corrected using the relevant endpoint and exposure estimates as agreed by the zRMS.

Based on the above calculations, acceptable risk to aquatic organisms from 2,4-D metabolites may be concluded for all intended Central Zone uses of ADM.3304.H.1.A with no need for risk mitigation measures.

### Metabolites of Fluroxypyr: Pyridinol, Methoxypyridine and 3-CP

**Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Pyridinol for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	-	<i>Daphnia magna</i>	-	-	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>
Endpoint			LC <sub>50</sub>	-	EC <sub>50</sub>	-	-	E <sub>r</sub> C <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)			39000	-	> 49000	-	-	2700	> 3200
AF			100	-	100	-	-	10	10
RAC (µg/L)			390	-	490	-	-	270	320
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	28.69	19.25	0.074	-	<b>0.059</b>	-	-	0.106	0.090

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

**Table 9.5-21:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite Methoxypyridine for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants	
Test species			-	-	-	-	-	<i>Anabaena flosaquae</i>	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>
Endpoint			-	-	-	-	-	EC <sub>50</sub>	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)			-	-	-	-	-	3160	10600	> 7700 (µg/kg)
AF			-	-	-	-	-	10	10	10
RAC (µg/L)			-	-	-	-	-	316	1060	770 (µg/kg)
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)								
<i>Grassland and cereals</i>										
FOCUS Step 1			PEC/RAC ratio							
	11.17	34.74	-	-	-	-	-	0.035	0.011	0.045

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

**Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite 3-CP for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	-	<i>Daphnia magna</i>	-	-	<i>Selenastrum capricornutum</i>	-
Endpoint			LC <sub>50</sub>	-	EC <sub>50</sub>	-	-	EC <sub>50</sub>	-
(µg/L)			95100 <del>951000</del>	-	7560	-	-	35000	-
AF			100	-	100	-	-	10	-
RAC (µg/L)			951 <del>9510</del>	-	75.6	-	-	3500	-
FOCUS Scenario	PEC <sub>SW</sub> gl max (µg/L)	PEC <sub>SED</sub> gl max (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	5.84	0.06	0.006	-	0.077	-	-	0.002	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown **in bold**

**zRMS comments:**

The risk assessment for fluroxypyr metabolites is agreed by the zRMS with some minor corrections.

Based on the above calculations, acceptable risk to aquatic organisms from fluroxypyr metabolites may be concluded for all intended Central Zone uses of ADM.3304.H.1.A with no need for risk mitigation measures.



### Formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC)

For the formulation AG-CDF1-480 EC, an initial concentration in surface water via spray drift was calculated considering published spray drift data (Rautmann, 2001). No chronic toxicity studies with fish and daphnids are available for the product. Based on the toxicity data of the formulation on the most sensitive species (macrophytes), there is no indication of chronic synergistic interactions (MDR are not larger than 5). Thus, a chronic risk assessment to fish and aquatic invertebrates is considered unnecessary.

**Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for AG-CDF1-480 EC for each organism group based on FOCUS Steps 1 calculations for the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in cereals / grassland with 2.0 L/ha.**

Group			Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Sediment dwellers	Algae	Aquatic plants
Test species			<i>Oncorhynchus mykiss</i>	-	<i>Daphnia magna</i>	-	-	<i>Pseudokirchneriella subcapitata</i>	<i>Myriophyllum spicatum</i>
Endpoint			LC <sub>50</sub>	-	EC <sub>50</sub>	-	-	ErC <sub>50</sub>	ErC <sub>50</sub>
(µg/L)			> 100000	-	> 100000	-	-	> 100000	38.1
AF			100	-	100	-	-	10	10
RAC (µg/L)			1000	-	1000	-	-	10000	38.1
FOCUS Scenario	PEC <sub>SW-gl-max</sub> (µg/L)	PEC <sub>SED-gl-max</sub> (µg/kg)							
<i>Grassland and cereals</i>									
FOCUS Step 1			PEC/RAC ratio						
	20.22	-	0.020	-	0.020	-	-	0.002	0.531

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

#### zRMS comments:

The risk assessment for aquatic organisms presented above is not agreed by the zRMS for the following reasons:

1. PEC/RAC values were calculated using the formulation PEC<sub>SW</sub> derived for spray drift, while this approach is not foreseen in EFSA (2013) which clearly states that the risk assessment for the formulated product should be based on PEC<sub>mix</sub> (being the sum of PEC<sub>SW</sub> for particular active compounds) compared with endpoints expressed in terms of the sum of active substances.
2. For *Myriophyllum spicatum* endpoint derived for old variant of formulation (AG-CDF1-480 EC) was considered, while significantly lower endpoint was derived from study performed with the new variant (ADM.3304.H.1.A). Furthermore, evaluation should be performed using data for the formulated product that will be placed on the marked, in case relevant data are available.

The Applicants' risk assessment above was thus struck through as being not performed in line with EFSA (2013) and respective evaluation was performed by the zRMS. In calculations sum of Step 3 and 4 PEC<sub>SW</sub> values for particular compounds as agreed in area of Section 8 was used. The evaluation was focused on *Myriophyllum spicatum*, which clearly drives the risk. Calculations are thus protective also for other species, for which low toxicity of the formulated product was observed. For uses in grassland surface water

exposure derived for winter cereals was considered since not all scenarios representative for **the Central Zone** Poland are defined for grassland. The same is applicable for scenario R1 **and R3** for spring cereals.

Species	<i>Myriophyllum spicatum</i>						
ErC <sub>50</sub> [µg/L]	24.0 (expressed as sum of active substances in the tested formulation and density of 1.08 g/L)						
AF	10						
RAC [µg/L]	2.4						
Substance	Application pattern	Step / mitigation	Scenario	PEC <sub>SW,MIX</sub> [µg/L]	RAC [µg/L]	PEC/RAC	Trigger
ADM.3304.H.1.A	Winter cereals and grassland BBCH 21-39 750 g a.s./ha	3	D3 ditch	6.485	2.4	2.7	1
			D4 pond	0.225		0.1	
			D4 stream	5.371		2.2	
			D5 pond	0.223		0.1	
			D5 stream	5.169		2.2	
			R1 pond	0.238		0.1	
			R1 stream	5.693		2.4	
			R3 stream	10.091		4.2	
	Spring cereals BBCH 21-39 750 g a.s./ha	3	D3 ditch	6.499	2.4	2.7	1
			D4 pond	0.224		0.1	
			D4 stream	5.572		2.3	
			D5 pond	0.224		0.1	
			D5 stream	5.628		2.3	
ADM.3304.H.1.A	Winter cereals and grassland BBCH 21-39 750 g a.s./ha	4 10 m buffer	D3 ditch	0.929 0.936	2.4	0.4	1
			D4 pond	0.139 0.14		0.1	
			D4 stream	0.925 0.932		0.4	
			D5 pond	0.138		0.1	
			D5 stream	0.987		0.4	
			R1 pond	0.184 0.185		0.1	
			R1 stream	5.052 5.059		2.1	
			R3 stream	9.081		3.8	
		4 10 m VFS <sub>mod</sub> (including 10 m buffer)	D3 ditch	0.929 0.936	2.4	0.4	1
			D4 pond	0.139 0.14		0.1	
			D4 stream	0.925 0.932		0.4	
			D5 pond	0.138		0.1	
			D5 stream	0.987		0.4	
			R1 pond	0.142 0.143		0.1	
			R1 stream	1.292 1.299		0.5	
			R3 stream	4.464		1.9	
	Spring cereals	4	D3 ditch	0.930 0.936	2.4	0.4	1

	BBCH 21-39 750 g a.s./ha	10 m buffer	D4 pond	<b>0.138</b> 0.139		0.1	
			D4 stream	<b>0.835</b> 1.016		<b>0.3</b> 0.4	
			D5 pond	<b>0.138</b>		0.1	
			D5 stream	<b>0.993</b>		0.4	

Values in **bold** indicate unacceptable risk

For purposes of the potential mutual recognition process, the risk assessment above was amended with consideration of the additional scenarios (D5 and R3). Furthermore, the exposure calculated for scenarios D3 D4 and R1 was amended using  $PEC_{SW}$  corrected following the additional comments provided by the Applicant. Performed evaluation demonstrated acceptable risk to aquatic organisms from uses of ADM.3304.H.1.A, provided that:

- 10 m unsprayed buffer zone to surface water bodies is respected in scenarios D3, D4 and D5.
- 10 m vegetated filter strip to surface water bodies is respected in scenario R1.

**No acceptable risk could be concluded in scenario R3 and further assessment will be necessary at the national level in the course of the mutual recognition process.**

The above conclusion is based on the outcome of calculations for winter cereals, but is applicable for all intended uses, since scenario R1 is not defined for grassland and spring cereals and winter cereals are used as surrogate.

### 9.5.3 Overall conclusions

The risk to aquatic organisms was evaluated for the active substances 2,4-D, Clopyralid, Fluroxypyr, their variants and its degradation products in consideration of the GAP uses envisaged for ADM.3304.H.1.A (old code AG-CDF1-480 EC). The risk of the formulated product itself was also evaluated.

Performed evaluation demonstrated acceptable risk to aquatic organisms from 2,4-D acid, clopyralid and fluroxypyr (meptyl and acid) and their metabolites with no need for risk mitigation measures.

For 2,4-D EHE acceptable risk could be demonstrated provided that the unsprayed buffer zone of 10 m to surface water bodies is respected or the spray drift is reduced by 90%.

The combined toxicity assessment demonstrated that the formulated product is more toxic to *Myriophyllum spicatum* than expected based on the active substance data. Measured toxicity to other species (fish, aquatic invertebrates, algae and *Lemna*) was either comparable or lower than the predicted mixture toxicity. The risk assessment performed for the formulation ADM.3304.H.1.A using the lowest relevant endpoint from the study performed with the most sensitive species (*Myriophyllum spicatum*) demonstrated acceptable risk from all intended Central Zone uses provided that

- 10 m unsprayed buffer zone to surface water bodies is respected in scenarios D3, D4 and D5,
- 10 m vegetated filter strip to surface water bodies is respected in scenario R1.

No acceptable risk could be concluded in scenario R3 and further assessment will be necessary at the national level in the course of the mutual recognition process.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.3304.H.1.A AG-E1-500-SC1, which was performed in line with the EU agreed methodology.

*“The endpoint  $E_rC_{50}$  is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”*

~~Following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use pattern proposed only an acute risk to fish and aquatic invertebrates is identified for 2,4-D EHE. Thus, risk mitigation measures are necessary in to prevent the risk to aquatic non target organisms:~~

~~“To protect aquatic organisms respect an unsprayed buffer zone of 5 m or to use 90 % drift reducing nozzles”.~~

## 9.6 Effects on bees (KCP 10.3.1)

### 9.6.1 Toxicity data

Acute toxicity tests on bees with the active substances and their variants are available. Full details of these studies are provided in the respective EU DAR and related documents.

The effects on bees of ADM.3304.H.1.A (old code AG-CDF1-480 EC) were not evaluated as part of the EU review of 2,4-D acid, Clopyralid and Fluroxypyr acid.

The endpoints selected for the risk assessments of active substances and their variants are in line with the values used in the EU review processes. Additionally, risk assessments are presented based on acute data for the formulation. Justification for new endpoints is provided below.

**Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees**

Species	Test item	Exposure System	Results	Reference
<i>Apis mellifera</i> adults	2,4-D acid	Acute, oral, 48 h	LD <sub>50</sub> = 94 µg a.s./bee	EFSA Journal 2014;12(9):3812
<i>Apis mellifera</i> adults	2,4-D acid	Acute, contact, 48 h	LD <sub>50</sub> > 100 µg a.s./bee	EFSA Journal 2014;12(9):3812
<i>Apis mellifera</i> adults	2,4-D EHE	Acute, oral, 48 h	LD <sub>50</sub> > 100 µg a.s./bee	<del>Bridging report 2018 SANCO 7599/VI/97 final (1 October 2004)</del>
<i>Apis mellifera</i> adults	2,4-D EHE	Acute, contact, 48 h	LD <sub>50</sub> > 100 µg a.s./bee	<del>Bridging report 2018 SANCO 7599/VI/97 final (1 October 2004)</del>
<i>Apis mellifera</i> adults	Clopyralid	Acute, oral, 48 h	LD <sub>50</sub> > 100 µg a.s./bee	EFSA Journal 2018;16(8):5389
<i>Apis mellifera</i> adults	Clopyralid	Acute, contact, 48 h	LD <sub>50</sub> > 98.1 µg a.s./bee	EFSA Journal 2018;16(8):5389
<i>Apis mellifera</i> adults	Clopyralid	Chronic, 10 d	LDD <sub>50</sub> > 71.2 µg a.s./bee/day	EFSA Journal 2018;16(8):5389
<i>Apis mellifera</i> larvae	Clopyralid	Chronic, 22 d	LDD <sub>50</sub> = 12.5 µg a.s./larva	EFSA Journal 2018;16(8):5389
<i>Apis mellifera</i> adults	Fluroxypyr acid	Acute, oral, 48 h	LD <sub>50</sub> > 100 µg a.s./bee	EFSA Journal 2011;9(3):2091
<i>Apis mellifera</i> adults	Fluroxypyr acid	Acute, contact, 48 h	LD <sub>50</sub> > 100 µg a.s./bee	EFSA Journal 2011;9(3):2091
<i>Apis mellifera</i> adults	Fluroxypyr-meptyl	Acute, oral, 48 h	LD <sub>50</sub> = 37.1 µg a.s./bee	EFSA Journal 2011;9(3):2091
<i>Apis mellifera</i> adults	Fluroxypyr-meptyl	Acute, contact, 48 h	LD <sub>50</sub> > 180 µg a.s./bee	EFSA Journal 2011;9(3):2091

#### zRMS comments:

Endpoints presented in Table 9.6-1 for 2,4-D, clopyralid, and fluroxypyr are in line with EU agreed endpoints reported in EFSA Journal 2014;12(9):3812, EFSA Journal 2018;16(8):5389, and EFSA Journal 2011;9(3):2091, respectively.

The toxicity data for 2,4-D EHE are in line with endpoints presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Since the SANCO 7599/VI/97 final is not applicable anymore, reference to this document has been struck through in Table 9.6-1 and the Bridging Report has been referenced as being the relevant document where the currently agreed EU data for 2,4-D 2-EHE may be found.

**Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees**

Species	Test item	Exposure System	Results	Reference
<i>Apis mellifera</i> adults	AG-CDF1-480 EC	Acute, oral, 48 h	LD <sub>50</sub> > 684 µg product/bee	KCP 10.3.1.1/01 Franke (2015) Study code: 14 10 48 114 B
<i>Apis mellifera</i> adults	AG-CDF1-480 EC	Acute, contact, 48 h	LD <sub>50</sub> > 911.6 µg product/bee	
<i>Apis mellifera</i> adults	AG-CDF1-480 EC	Chronic, oral, 10 days	LDD <sub>50</sub> = 85.56 µg product/bee	KCP 10.3.1.2/01 Noël (2016) Stude code: 307SRFR15C05
<i>Apis mellifera</i> development	AG-CDF1-480 EC1	Chronic, oral, 22 days	NOED = 38.5 <del>77.0</del> µg product/larva <del>bee</del>	KCP 10.3.1.3/01 Wilkins (2018) Study code: FR/000764

**zRMS comments:**

Studies on acute, chronic and larvae toxicity of ADM.3304.H.1.A to bees were evaluated and agreed by the zRMS. The summaries of studies together with the zRMS evaluation may be found in Appendix 2. The endpoints reported in Table 9.6-2 are confirmed with exception of the NOED for larvae which was changed to 38.5 µg product/larva due to >10% effects observed at 77.0 µg product/larva, which could be of biological relevance. Alternatively, ED<sub>10</sub> value could be used, but ECx values were not available in the study report.

### 9.6.1.1 Justification for new endpoints

Acute oral and contact risk assessments are based on the EU agreed toxicity endpoints for honeybees with the active substances (and its variants) as well as based on the data available for AG-CDF1-480 EC. It is important to note the formulation exhibits a low acute toxicity, since LD<sub>50</sub> values are much higher than 100 µg/bee (see **Table 9.6-2**). Chronic endpoints with formulation are also available for adults and larvae of honeybees.

### 9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

#### 9.6.2.1 Hazard quotients for bees

The risk assessments for the uses of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in grassland and cereals are presented in the following tables. As the same application pattern is used for both crops (1 × 2 L product/ha), these uses were assessed together.

**Table 9.6-3: First-tier assessment of the risk for bees due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals**

Intended use	Grassland and cereals (BBCH 21 – 39)		
Active substance	2,4-D acid		
Application rate (g/ha)	750		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	94	750	8.0
Contact toxicity	> 100		< 7.5
Active substance	2,4-D EHE (variant of 2,4-D acid)		
Application rate (g/ha)	1125		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 100	1125	< 11.3
Contact toxicity	> 100		< 11.3
Active substance	Clopyralid		
Application rate (g/ha)	60		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 100	60	< 0.5
Contact toxicity	> 98.1		< 0.6
Active substance	Fluroxypyr acid		
Application rate (g/ha)	150		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 100 <del>37.1</del>	150	< 1.5 <del>4.0</del>
Contact toxicity	> 100 <del>180</del>		< 1.5 <del>0.7</del>
Active substance	Fluroxypyr-meptyl		
Application rate (g/ha)	216		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	37.1 <del>&gt;100</del>	216	5.8 <del>&lt;2.2</del>
Contact toxicity	> 180 <del>100</del>		< 1.2 <del>2.2</del>
Product	AG-CDF1-480 EC		
Application rate (g/ha)	2144*		
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 684.0	2190* <del>2144*</del>	< 3.2 <del>3.0</del>
Contact toxicity	> 911.6		< 2.4

Q<sub>HO</sub>, Q<sub>HC</sub>: Hazard quotients for oral and contact exposure. Q<sub>H</sub> values shown in **bold** breach the relevant trigger.

\* Assuming formulation density of ~~1.095~~ ~~1.072~~ g/mL and the max. application of 2 L formulation/ha.

All the hazard quotients (HQs) are lower than the trigger value of 50, indicating that the active substances, their variants and the formulation AG-CDF1-480 EC pose a low risk to honeybees following the application of the formulation according to the use pattern proposed.

Furthermore, the results of the chronic feeding studies to adult bees and bee larvae from AG-CDF1-480 EC do not give rise to a specific concern.

In conclusion, it is reasonable to conclude that the acute and chronic risk for bees can be considered as acceptable.

**zRMS comments:**

The risk assessment for bees presented in Table 9.6-3 above is in general agreed by the zRMS. Performed calculations cover both intended uses of ADM.3307.H.1.A in grassland and cereals.

It is noted that the endpoints for fluroxypyr acid and fluroxypyr-meptyl were switched. Furthermore, for conversion of the rate of the formulated product from mL/ha to g/ha the relative density of 1.072 g/mL was assumed, while in both, Part B1 and Part C, the relative density of 1.095 g/mL is reported. The risk assessment was amended accordingly.

Overall, acceptable risk to bees may be concluded from all intended uses of ADM.3307.H.1.A in the Central Zone.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Therefore risk assessment based on indications of EFSA (2013) must be performed at the national level by the Member States that do require such evaluation.

### **9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)**

No data available.

### **9.6.3 Effects on bumble bees**

No data available.

### **9.6.4 Effects on solitary bees**

No data available.

### **9.6.5 Overall conclusions**

The acute risk assessments for the individual active substances as well as for the formulated product with Hazard Quotients well below the trigger for acceptability of effects indicates an acceptable risk for bees exposed in accordance with the intended Central Zone uses of ADM.3304.H.1.A (old code AG-CDF1-480 EC).



## 9.7 Effects on arthropods other than bees (KCP 10.3.2)

### 9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods (NTAs) have been carried out with representative formulations of the active substances. Full details of these studies are provided in the respective EU DARs and related documents.

Data with a representative formulation of 2,4-D EHE (variant of 2,4-D) on NTAs have been provided with EU Bridging report (2018).

Effects on non-target arthropods of the formulations AG-CDF1-480 EC and ADM.3304.H.1.A were not evaluated as part of the EU assessment of active substances. Data for the formulation AG-CDF1-480 EC was already submitted in 2019 (Röhlig, 2015) and new data with formulation ADM.3304.H.1.A were already submitted in 2021 for the composition change (Walter, 2019 a,b) are listed in **Appendix 1** and summarised in **Appendix 2**.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

**Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods**

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i>	AG-CDF1-480 EC	Extended laboratory test, barley seedling (3D)	LR <sub>50</sub> > 2000 mL product/ha ER <sub>50</sub> > 2000 mL product/ha	KCP 10.3.2.2/01 Großmann, (2014) Study code: 90311002
<i>Typhlodromus pyri</i>	AG-CDF1-480 EC	Extended laboratory test, detached maize leaves (2D)	LR <sub>50</sub> = 279.7 mL product/ha  Reduction of reproduction: 15 % at 51.2 mL product/ha 28.4 % at 128 mL product/ha 77.4 % at 320 mL product/ha  ER <sub>50</sub> > 128 mL product/ha	KCP 10.3.2.2/02 Großmann, (2014) Study code: 90311062
<i>Chrysoperla carnea</i>	AG-CDF1-480 EC	Extended laboratory test, detached vines leaves (2D)	LR <sub>50</sub> > 2000 mL product/ha ER <sub>50</sub> > 2000 mL product/ha	KCP 10.3.2.2/03 Großmann, (2014) Study code: 90311047
<i>Typhlodromus pyri</i>	AG-CDF1-480 EC	Aged residue test under semi-field conditions - extended laboratory test (exposure phase), detached maize leaves (from maize plants –3D)	Bioassay initiated on DAT 0: Mortality: 0 % at 2000 mL product/ha Reproduction: +7.9 % at 2000 mL product/ha  Bioassay initiated on DAT 7 (aged residue): Mortality: 0 % at 2000 mL product/ha Reproduction: -0.3 % at 2000 mL product/ha	KCP 10.3.2.2/04 Röhlig, (2015) Study code: 14 10 48 070 A

### Toxicity comparison for formulations AG-CDF1-480 EC and ADM.3304.H.1.A

As a change of composition was requested in 2021 (formulation AG-CDF1-480 EC to ADM.3304.H.1.A), additional information is provided.

Toxicity tests with the formulations AG-CDF1-480 EC and ADM.3304.H.1.A on non-target arthropods are available (Röhlig, 2015 and Walter, 2019a). The studies of two formulations were conducted according to the test guideline Blümel et al. (2000) using the same arthropod species (*Typhlodromus pyri*)

and under the same conditions/test design (exposure aged residue test under semi-field condition). Both studies are summarized in **Appendix 2**, see KCP 10.3.2.2/04 for further details. Endpoints for each formulation are presented in the table below.

**Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods**

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i>	AG-CDF1-480 EC	Aged residue test under semi-field conditions - extended laboratory test (exposure phase), detached maize leaves (from maize plants –3D)	Bioassay initiated on DAT 0: Mortality: 0 % at 2000 mL product/ha Reproduction: +7.9 % at 2000 mL product/ha  Bioassay initiated on DAT 7 (aged residue): Mortality: 0 % at 2000 mL product/ha Reproduction: -0.3 % at 2000 mL product/ha	KCP 10.3.2.2/04 Röhlig, (2015) Study code: 14 10 48 070 A
<i>Typhlodromus pyri</i>	ADM.3304.H.1.A	Aged residue study with a single semi-field application (L1) on potted maize plants and two laboratory applications for the reference item (L2 – L3)	L1 (DAT 0) Mortality: 25.0 % mortality at 2 L prod./ha Reproduction: -7.7%  L2 (DAT 7) Mortality: 10.0 % mortality at 2 L prod./ha Reproduction: 3.8%  L3 (DAT 14) Mortality: 20.0 % mortality at 2 L prod./ha Reproduction: 23.5%	KCP 10.3.2.2/05 Walter (2019a) Study code: S19-03574
<i>Coccinella septempunctata</i> *	ADM.3304.H.1.A	Extended laboratory test, detached bean leaves	LR <sub>50</sub> > 2000 mL product/ha ER <sub>50</sub> = no effect determined at 2000 mL product/ha	KCP 10.3.2.2/06 Walter (2019b) Study code: S19-01799

\*This test on *Coccinella septempunctata* was only performed to cover a formal gap, not for comparison reasons of compositions.

Comparing the toxicity data achieved for each formulation based on mortality and reproduction, it is shown that both formulations does not achieve 50% effect at 2 L product/ha. Due to the similar toxicity presented by both formulations, the risk posed for non-target arthropods by formulation ADM.3304.H.1.A can be assessed through the risk estimated by formulation AG-CDF1-480 EC.

**zRMS comments:**

Studies on effects of AG-CDF1-480 EC and ADM.3304.H.1.A were evaluated and agreed by the zRMS. The summaries of studies together with zRMS evaluation may be found in Appendix 2. Endpoints reported in Tables 9.7-1 and 9.7-2 are confirmed to be correct.

It is noted that part of the studies has been performed with the old version of the formulation (AG-CDF1-480 EC) while the authorisation is sought for formulation ADM.3304.H.1.A (AG-CDF1-480 EC1). The change of the composition included removal of <3% of one solvent and addition of the same amount of the other solvent. The removed solvent was toxic to aquatic organisms with EC<sub>50</sub> for algae <1.0 mg/L, while the added solvent is not toxic to aquatic species with L(E)C<sub>50</sub> values for fish, *Daphnia magna* and algae being all >100 mg/L. Based on that, no change of the ecotoxicological profile of the new version of the formulation is expected.

A bridging aged residue study was performed with *Typhlodromus pyri*, which turned out to be most sensitive species in the extended laboratory studies performed with AG-CDF1-480 EC. The mortality (25%) of *T.pyri* exposed to fresh residues on day 0 was higher in test performed with ADM.3304.H.1.A (AG-CDF1-480 EC1) comparing to AG-CDF1-480 EC (0% mortality). Exposure to residues aged for 7 days also resulted in higher mortality rate (10%) in test performed with ADM.3304.H.1.A (AG-CDF1-480 EC1) comparing to AG-CDF1-480 EC. Nevertheless, in both studies mortality and effects on reproduction were considerably <50%. It is noted that the study with

ADM.3304.H.1.A (AG-CDF1-480 EC1) was performed 4 years later in different laboratory, so different batch of the test species was used and the zRMS is of the opinion that increased mortality of the tested species could be due to the inter-laboratory variability and higher sensitivity of the batch of *T. pyri* used for testing.

Overall, the zRMS is of the opinion that toxicity of both variants of the formulations is comparable since effects in the study performed with the new version of the formulated product were considerably <50%, the change in the composition was only slight and the added co-formulant is of lower toxicity than the partially removed co-formulant (only aquatic toxicity data available, but testing for other non-target species is not mandatory). Hence, results of studies performed with AG-CDF1-480 EC may be used for purposes of the risk assessment performed for ADM.3304.H.1.A (AG-CDF1-480 EC1).

### 9.7.1.1 Justification for new endpoints

Risk assessments are most adequately conducted based on the available data for formulations (AG-CDF1-480 EC and ADM.3304.H.1.A), since contact exposure of non-target arthropods is considered to be mainly to the formulation (mixture) rather than to the single active substances. Extended tests on the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi* are available together with data on one additional foliar-dwelling arthropod, *Chrysoperla carnea* for formulation AG-CDF1-480 EC. An aged residue test performed on *T. pyri* is also available with AG-CDF1-480 EC. An aged residue test performed on *T. pyri* is available with formulation ADM.3304.H.1.A, together with an extended laboratory test with *Coccinella septempunctata*.

### 9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

No changes with respect to the previous formulation AG-CDF1-480 EC dossier submitted is expected. The additional test on *Coccinella septempunctata* (Walter 2019b) supports the results previously achieved as the product does not have any toxicological effect up to the highest intended rate (2 L product/ha).

#### 9.7.2.1 Risk assessment for in-field exposure

**Table 9.7-3: First-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals**

Intended use	Grassland – Cereals (BBCH 21 – 39)		
Active substance/product	AG-CDF1-480 EC		
Application rate (mL/ha)	1 × 2000		
MAF	1		
Test species Tier II	L/ER <sub>50</sub> (lab.) (mL/ha)	PER <sub>in-field</sub> (mL/ha)	HQ <sub>in-field</sub> criterion: HQ ≤ 1
<i>Aphidius rhopalosiphi</i>	> 2000	2000	< 1.0
<i>Typhlodromus pyri</i>	>128 <del>279.7</del>		<15.6 <del>7.1</del>
<i>Chrysoperla carnea</i> <i>Coccinella septempunctata</i>	> 2000		< 1.0

MAF: Multiple application factor (based on default for leaf substrate from ESCORT 2 guidance); PER: Predicted environmental rate; HQ: Hazard quotient; Criteria values shown in **bold** breach the relevant trigger.

The above results show that the Hazard Quotient for *T. pyri* exceeds the trigger level of 1, indicating a need for a refinement of the risk.

## Refined in-field risk assessment

**Table 9.7-4: Refined assessment of the in-field risk for non-target arthropods due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals**

Intended use	Grassland – Cereals (BBCH 21 – 39)		
Active substance/product	AG-CDF1-480 EC		
Application rate (mL/ha)	1 × 2000		
MAF	1		
Test species Tier II	L/ER <sub>50</sub> (lab.) (mL/ha)	PER <sub>in-field</sub> (mL/ha)	HQ <sub>in-field</sub> criterion: HQ ≤ 1
<i>T. pyri</i> (aged residue test – 7 days)	> 2000	2000	< 1.0

MAF: Multiple application factor (based on default for leaf substrate from ESCORT 2 guidance); PER: Predicted environmental rate; HQ: Hazard quotient; Criteria values shown in **bold** breach the relevant trigger.

The outcome of the refined risk assessment indicated that there is an acceptable risk for non-target arthropods in-field area 7 days after the application of AG-CDF1-480 EC. Thus, a potential recolonization from off-field area is expected within ecologically relevant period (less than 1 year).

### zRMS comments:

The risk assessment provided by the Applicant in Tables 9.7-3 and 9.7-4 above is in general agreed by the zRMS. Since evaluation was based on Tier II studies, the sub-lethal endpoints should be also considered and for this reason the HQ calculated with estimated ER<sub>50</sub> for *T. pyri* was included by the zRMS in Table 9.7-3 (no definite value determined in the study, but effects at 128 mL/ha were <50%), since this endpoint was lower than LR<sub>50</sub> taken into account by the Applicant. For remaining species LR<sub>50</sub> and ER<sub>50</sub> values were the same.

The additional species (*Coccinella septempunctata*) tested with ADM.3304.H.1.A has been included in Table 9.7-3 for completeness in a row presenting risk assessment for *Chrysoperla carnea* for which the same endpoint was derived.

For *Aphidius rhopalosiphi*, *Chrysoperla carnea* and *Coccinella septempunctata* acceptable in-field risk could be concluded based on results of the extended lab studies. For *Typhlodromus pyri* the in-field risk was resolved based on results of the aged residue studies which demonstrated that after 7 days of aging the residues of ADM.3304.H.1.A decline to level that poses no unacceptable risk to this species confirming that recolonisation of the treated field is possible within <1 year.

## 9.7.2.2 Risk assessment for off-field exposure

**Table 9.7-5: First-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals**

Intended use	Grassland – Cereals (BBCH 21 – 39)					
Active substance/product	AG-CDF1-480 EC					
Application rate (mL/ha)	1 × 2000					
MAF	1					
vdf	10					
Test species Tier II	L/ER <sub>50</sub> (lab.) (mL/ha)	Drift percentile (%)	Drift rate (mL/ha)	PER <sub>off-field</sub> (mL/ha)	CF	HQ <sub>off-field</sub> criterion: HQ ≤ 1
<i>Aphidius rhopalosiphi</i>	> 2000	2.77	55.4	277	5	< 0.14
<i>Typhlodromus pyri</i>	>128 <del>279.7</del>	2.77	55.4	27.7	5	< 0.22 <del>0.1</del>
<i>Chrysoperla carnea</i> <i>Coccinella septempunctata</i>	> 2000	2.77	55.4	27.7	5	< 0.014

MAF: Multiple application factor (based on default for leaf substrate from ESCORT 2 guidance); vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

The above results show that the Hazard Quotients are well below the trigger level of 1 at the standard buffer distance. Thus, an acceptable off-field risk for non-target arthropods from the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) can be concluded.

**zRMS comments:**

The risk assessment provided by the Applicant in Tables 9.7-5 above is in general agreed by the zRMS. Since evaluation was based on Tier II studies, the sub-lethal endpoints should be also considered and for this reason the HQ calculated with estimated ER<sub>50</sub> for *T. pyri* was included by the zRMS in Table 9.7-5 (no definite value determined in the study, but effects at 128 mL/ha were <50%), since this endpoint was lower than LR<sub>50</sub> taken into account by the Applicant. For remaining species LR<sub>50</sub> and ER<sub>50</sub> values were the same.

The additional species (*Coccinella septempunctata*) tested with ADM.3304.H.1.A has been included in Table 9.7-5 for completeness in a row presenting risk assessment for *Chrysoperla carnea* for which the same endpoint was derived.

Based on the performed calculations, acceptable off-field risk to non-target arthropods may be concluded from both intended uses of ADM.3304.H.1.A in the Central Zone with no need for risk mitigation measures.

### **9.7.2.3 Additional higher-tier risk assessment**

Based on the outcome of the risk assessments, no higher-tier risk assessment is necessary.

### **9.7.2.4 Risk mitigation measures**

No risk mitigation required.

### **9.7.3 Overall conclusions**

For the intended **Central Zone** uses in grassland and cereals, an acceptable in- and off-field risk for terrestrial non-target arthropods other than bees is determined following the application of the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) **with no need for risk mitigation measures.** ~~according to the use pattern proposed.~~

## 9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

### 9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with Clopyralid, 2,4-D acid and its variant 2,4-D EHE, Fluroxypyr acid and its variant Fluroxypyr meptyl (**Tables** from **9.8-1** to **9.8-4**). Toxicity data for the relevant metabolites in soil compartment were also available. Full details of all above studies are provided in the respective EU DAR and related documents.

Effects on meso- and macrofauna of ADM.3304.H.1.A (old code AG-CDF1-480 EC) were not evaluated as part of the EU assessment of the active substances. New data submitted with this application are listed in **Appendix 1** and summarised in **Appendix 2**.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. Justifications are provided below.

**Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna), 2,4-D, its variant and metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia foetida</i>	2,4-D	Acute	$LC_{50} = 350 \text{ mg/kg dw soil}$	EFSA Journal 2014;12(9):3812
<i>Eisenia foetida</i>	2,4-D	Chronic	NOEC = 62.5 mg/kg dw soil	EFSA Journal 2014;12(9):3812
<i>Eisenia foetida</i>	2,4-DCA (metabolite)	Acute	$LC_{50} > 101.8 \text{ mg/kg soil}$ $LC_{50\text{corr.}} > 50.9 \text{ mg/kg soil}$	EFSA Journal 2014;12(9):3812
<i>Eisenia foetida</i>	2,4-DCA (metabolite)	Chronic	NOEC = 10 mg/kg soil NOEC <sub>corr.</sub> = 5 mg/kg soil	EFSA Journal 2014;12(9):3812
<i>Hypoaspis aculeifer</i>	2,4-DCA (metabolite)	Chronic	NOEC = 10 mg/kg dw soil NOEC <sub>corr.</sub> = 5 mg/kg soil	EFSA Journal 2014;12(9):3812
<i>Folsomia candida</i>	2,4-DCA (metabolite)	Chronic	NOEC = 10 mg/kg dw soil NOEC <sub>corr.</sub> = 5 mg/kg soil	EFSA Journal 2014;12(9):3812
<i>Eisenia foetida</i>	2,4-DCP (metabolite)	Chronic	NOEC = 10 mg/kg soil NOEC <sub>corr.</sub> = 5 mg/kg soil	EFSA Journal 2014;12(9):3812
<i>Hypoaspis aculeifer</i>	2,4-DCP (metabolite)	Chronic	NOEC = 5 mg/kg dw soil NOEC <sub>corr.</sub> = 2.5 mg/kg soil	EFSA Journal 2014;12(9):3812
<i>Folsomia candida</i>	2,4-DCP (metabolite)	Chronic	NOEC = 1.25 mg/kg dw soil NOEC <sub>corr.</sub> = 0.625 mg/kg soil	EFSA Journal 2014;12(9):3812
<i>Eisenia foetida</i>	4-Chlorophenol (metabolite)	Chronic	NOEC = 10 mg/kg soil NOEC <sub>corr.</sub> = 5.0 mg/kg soil EC <sub>10</sub> could not be calculated	KCP 10.4.1/02 Wagenhoff (2015) Study code: S15-00154

Corr. = corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 (where the log Kow of the active substance is above 2 the endpoint has to be corrected by the factor of 2 if the organic carbon content of the substrate is 10 %. If the test was conducted with 5 % organic carbon, a correction of the endpoint is not necessary).

**Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia foetida</i>	2,4-D EHE	Acute	$LC_{50} > 98 \text{ mg/kg dw soil}^{1)}$	SANCO 7599/VI/97 final (1 October 2001)
<i>Eisenia foetida</i>	2,4-D EHE	Chronic	NOEC = 98 mg/kg dw soil <sup>1)</sup> NOEC <sub>corr.</sub> = 49.0 mg/kg soil	Bridging report 2018 SANCO 7599/VI/97 final (1 October 2001)

<sup>1)</sup> Formulation endpoint expressed as 2,4-D EHE; formulation study covers 2,4-D EHE toxicity.

**Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) - Clopyralid**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia foetida</i>	Clopyralid	Acute	LC <sub>50</sub> > 1000 mg/kg	EFSA Scientific Report (2005) 50, 1-65
<i>Eisenia foetida</i>	Clopyralid	Chronic	NOEC (reproduction) 1.97 mg a.s./kg <sup>1)</sup>	EFSA Journal 2018;16(8):5389

<sup>1)</sup> Formulation endpoint expressed as active ingredient; formulation study covers Clopyralid toxicity.

**Table 9.8-4: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – Fluroxypyr, its variant and metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia foetida</i>	Fluroxypyr-meptyl	Acute	LC <sub>50</sub> > 1000 mg/kg dw soil LC <sub>50corr.</sub> > 500 mg/kg dw soil	EFSA Journal 2011;9(3):2091
<i>Eisenia foetida</i>	Fluroxypyr-meptyl	Chronic	NOEC = 3.92 mg/kg dw soil <sup>1)</sup> NOEC <sub>corr.</sub> = 1.96 mg/kg dw soil	EFSA Journal 2011;9(3):2091
<i>Eisenia foetida</i>	Fluroxypyr acid	Acute	LC <sub>50</sub> = 64.8 mg/kg dw soil <sup>2)</sup>	EFSA Journal 2011;9(3):2091
<i>Eisenia foetida</i>	Fluroxypyr acid	Chronic	NOEC = 3.05 mg/kg dw soil <sup>2)</sup>	EFSA Journal 2011;9(3):2091
<i>Eisenia foetida</i>	Pyridinol (metabolite)	Acute	LC <sub>50</sub> = 79 mg/kg dw soil	EFSA Journal 2011;9(3):2091
<i>Eisenia foetida</i>	Pyridinol (metabolite)	Chronic	NOEC = 0.720 mg/kg dw soil	EFSA supporting publication 2015:EN-857 <sup>9</sup> , confirmatory data
<i>Eisenia foetida</i>	Methoxypyridine (metabolite)	Acute	LC <sub>50</sub> = 313 mg/kg dw soil LC <sub>50corr.</sub> = 156.6 mg/kg dw soil	EFSA Journal 2011;9(3):2091
<i>Eisenia foetida</i>	Methoxypyridine (metabolite)	Chronic	NOEC = 1.17 mg/kg dw soil <sup>a</sup> NOEC <sub>corr.</sub> = 0.585 mg/kg soil	EFSA supporting publication 2015:EN-857 <sup>10</sup> , confirmatory data <sup>c</sup>
<i>Folsomia candida</i>	Methoxypyridine (metabolite)	Chronic	NOEC = 1.0 mg/kg dw soil <sup>a</sup> NOEC <sub>corr.</sub> = 0.5 mg/kg soil	EFSA supporting publication 2015:EN-857 <sup>7</sup> , confirmatory data <sup>c</sup>
<i>Hypoaspis aculeifer</i>	Methoxypyridine (metabolite)	Chronic	NOEC = 0.25 mg/kg dw soil <sup>a</sup> NOEC <sub>corr.</sub> = 0.125 mg/kg soil	EFSA supporting publication 2015:EN-857 <sup>7</sup> , confirmatory data <sup>c</sup>
<i>Hypoaspis aculeifer</i>	Methoxypyridine (metabolite)	Chronic	NOEC = 2.5 mg/kg dw soil <sup>b</sup> NOEC <sub>corr.</sub> = 1.25 mg/kg soil	EFSA supporting publication 2015:EN-857 <sup>7</sup> , confirmatory data <sup>c</sup>

<sup>1)</sup> Formulation endpoint expressed as Fluroxypyr-meptyl; formulation study covers Fluroxypyr-meptyl toxicity.

<sup>2)</sup> Formulation endpoint expressed as acid equivalent; formulation study covers Fluroxypyr acid toxicity.

Corr. = corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 (where the log K<sub>ow</sub> of the active substance is above 2 the endpoint has to be corrected by the factor of 2 if the organic carbon content of the substrate is 10 %. If the test was conducted with 5 % organic carbon, a correction of the endpoint is not necessary).

<sup>a</sup> NOEC from study conducted under worst-case conditions, i.e. artificial soil containing 5% peat.

<sup>b</sup> NOEC from study conducted under more realistic conditions, i.e. natural LUFA 2.4 soil containing 2.42 % organic matter.

<sup>c</sup> During the EU-evaluation of Fluroxypyr a data gap was identified to address the long-term risk for earthworms and for soil macroorganisms for the metabolite Fluroxypyr Methoxypyridine. Therefore, additional confirmatory studies with the metabolite Methoxypyridine investigating chronic effects on *Eisenia foetida* and *Folsomia candida* were submitted by the Notifier (Dow).

<sup>9</sup> EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for fluroxypyr in light of confirmatory data. EFSA supporting publication 2015:EN-857. 43 pp.

<sup>10</sup> EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for fluroxypyr in light of confirmatory data. EFSA supporting publication 2015:EN-857. 43 pp.

**zRMS comments:**

Toxicity data for 2,4-D (acid form), clopyralid, and fluroxypyr reported in Table 9.8-1, 9.8-3 and 9.8-4 are in line with the EU agreed endpoints reported in EFSA Journal 2014;12(9):3812, EFSA Journal 2018;16(8):5389, and EFSA Journal 2011;9(3):2091, respectively.

Studies on toxicity of fluroxypyr metabolites to soil macro- and meso-fauna were evaluated by the RMS (Ireland) and are presented in Fluroxypyr Addendum to Vol. 3: Confirmatory information (December 2014).

The toxicity data for 2,4-D EHE reported in Table 9.8-2 are in line with endpoints presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Since the SANCO 7599/VI/97 final is not applicable anymore, reference to this document has been struck through in Table 9.2-1 and the Bridging Report has been referenced as being the relevant document where the currently agreed EU data for 2,4-D 2-EHE may be found.

In line with indications of EFSA Supporting publication 2015: EN-924, all endpoints for substances with log Pow >2 were corrected by factor of 2, regardless of the peat content in the study.

It should be noted that with exception of 2,4-D, the chronic toxicity of clopyralid and fluroxypyr to earthworms was addressed in studies performed with the representative solo formulations and for this reason derived endpoints may be not necessarily representative for the active substances due to presence of co-formulants in the test item. Nevertheless, in absence of the toxicity data for the both active compounds, endpoints from studies with representative formulations were agreed as a surrogate solution. Risk from clopyralid and fluroxypyr in ADM.3304.H.1.A is covered by the risk assessment performed for the formulated product.

No studies on toxicity of 2,4-D, clopyralid and fluroxypyr to *Folsomia candida* and *Hypoaspis aculeifer* were performed in the course of the EU review of these active compounds. However, respective studies are available for ADM.3304.H.1.A which address the toxicity from all three active compounds in the formulation and are deemed sufficient to finalise the risk assessment, especially in line with indications of the Commission Regulation (EU) No 283/2013, in case of soil organisms testing of the formulation is more appropriate than testing of the active compound.

Acute toxicity data are no longer a data requirement and were thus struck through in tables above.

### **9.8.1.1 Justification for new endpoints**

Risk assessments are presented based on the EU agreed and relevant data for the individual active substances (**Tables** from **9.8-1** to **9.8-4**) as well as based on new chronic data for soil meso- and macrofauna (earthworms, collembolans and soil mites) provided for the formulation AG-CDF1-480 EC (see **Table 9.8-5**), which are considered relevant for the intended uses.

In addition, the risk to different environmental compartments from the formation of the anaerobic soil metabolite 4-Chlorophenol was identified by EFSA for those situations and member states where anaerobic soil conditions are expected to occur.

Therefore, new information on the toxicity of 4-Chlorophenol to representative soil taxa (earthworms) is also provided here (please refer to **Appendix 5**). The new study, which was conducted after the EU review, and was therefore not peer reviewed during the Annex I Renewal of 2,4-D, is listed below.



**Table 9.8-5: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	AG-CDF1-480 EC	Chronic	NOEC = 62.5 mg/kg soil NOEC <sub>corr.</sub> = 31.25 mg/kg soil  EC <sub>10</sub> = 60.2 mg/kg soil EC <sub>10,corr.</sub> = 30.1 mg/kg soil	KCP 10.4.1/01 Friedrich (2014) Study code: 14 10 48 131 S
<i>Folsomia candida</i>	AG-CDF1-480 EC	Chronic	NOEC <sub>repro</sub> = 62.5 mg/kg soil NOEC <sub>corr.</sub> = 31.25 mg/kg soil  NOEC <sub>mortality</sub> = 31.25 mg/kg soil NOEC <sub>corr.</sub> = 15.63 mg/kg soil  EC <sub>10</sub> not reliable 49.2 mg/kg soil	KCP 10.4.2/01 Friedrich (2014) Study code: 14 10 48 129 S
<i>Folsomia candida</i>	AG-CDF1-480 EC*	Chronic	NOEC = 88.8 mg/kg soil NOEC <sub>corr.</sub> = 44.4 mg/kg soil  EC <sub>10</sub> = 103.0 mg/kg soil	KCP 10.4.2/02 Friedrich (2015) Study code: 15 10 48 133 S
<i>Hypoaspis aculeifer</i>	AG-CDF1-480 EC	Chronic	NOEC ≥ 1000 mg/kg soil NOEC <sub>corr.</sub> ≥ 500 mg/kg soil  EC <sub>10</sub> could not be calculated	KCP 10.4.2/03 Schulz (2014) Study code: 14 10 48 130 S
<i>Eisenia fetida</i>	4-Chlorophenol (metabolite)	Chronic	NOEC = 10 mg/kg soil NOEC <sub>corr.</sub> = 5.0 mg/kg soil  EC <sub>10</sub> could not be calculated	KCP 10.4.1/02 Wagenhoff (2015) Study code: S15-00154
<i>Eisenia fetida</i>	Methoxy pyridine (metabolite)	Chronic	NOEC = 36 mg/kg soil NOEC <sub>corr.</sub> = 18 mg/kg soil	KCP 10.4.1/03 Witte (2014) Study code: 92411022
<i>Folsomia candida</i>	Methoxy pyridine (metabolite)	Chronic	LOEC <sub>reproduction</sub> = 50 mg/kg soil  NOEC <sub>corr.</sub> = 22 mg/kg soil  EC <sub>10</sub> = 57.9 mg/kg soil	KCP 10.4.2/04 Höhn (2012) Study code: S12-00021  Amendment to KCP 10.4.2/04 Wagenhoff (2015)  Amendment No. 2 to KCP 10.4.2/04 Wagenhoff, E. (2017)
<i>Folsomia candida</i>	Methoxy pyridine (metabolite)	Chronic	NOEC <sub>mort</sub> = 15.6 mg/kg soil <sup>±</sup> NOEC <sub>repro</sub> = 31.3 mg/kg soil <sup>±</sup> EC <sub>10</sub> = 11.2 mg/kg soil <sup>±</sup>	KCP 10.4.2/06 Geary, N. (2016) Sponsor ID: 90019202

<sup>±</sup> Study performed on LUFA 2.2 (natural soil).

#### Discussion of the most relevant endpoint for the metabolite Methoxy pyridine used in the risk assessment

Taking into account confirmatory data for Methoxy pyridine the lowest endpoint is the *Hypoaspis aculeifer* NOEC of 0.25 mg/kg soil performed in artificial soil. As higher tier the NOEC of 2.5 mg/kg soil performed under more realistic conditions using natural soil is available and will be used in the risk assessment. Therefore the most relevant species is *Folsomia candida* with a NOEC of 1.0 mg/kg soil performed with artificial soil (Witte, 2010).

A chronic study on *Folsomia candida* conducted with reduced peat content is available (KCP 10.4.2/04, Höhn 2012) for Methoxy pyridine. The summary of this test is presented in **Appendix 5**.

In order to address the reproductive risk for soil mesofauna, an EC<sub>10</sub> was extrapolated from the dose-response curve in the study by Höhn (2012). The report was amended accordingly (Wagenhoff 2015, 2017). As the extrapolated EC<sub>10</sub> for reproduction is greater than the rate at which 14.9 % effect on reproduction was observed, conservatively the lower limit of the 95<sup>th</sup> confidence interval of 44 mg Methoxy pyridine/kg d.w. soil is selected for risk assessment (NOEC<sub>corrected</sub> = 22 mg/kg).

Remarkably, a considerable discrepancy occurs between the studies of Höhn (2012) and Witte (2010) as shown in the following table:

**Table 9.8-6: Comparison of effect data on *Folsomia* in the studies by Witte (2010) and Höhn (2012)**

Study (Author, Year)	Test item concentration [mg Methoxy pyridine/kg d.w. soil]	Mean mortality <sup>a)</sup> [%]	Corrected mortality <sup>b)</sup> [%]	Mean number of juveniles <sup>a)</sup>	Reduced reproduction [%]
Witte, 2010	0.125	20	1.2	638	-7
	0.25	18	-1.2	704	-18
	0.50	20	1.2	577	3
	1.0	13	-7.4	589	1
	2.0	18	-1.2	228	62
Höhn, 2012 (amended by Wagenhoff, 2015, 2017)	50	2.5	-4.7	802.8	14.9
	100	17.5	-11.4	620.4	34.2
	200	77.5	75.8	0.0	100.0
	400	90.0	89.3	0.0	100.0
	800	95.0	94.6	0.0	100.0

<sup>a)</sup> Mean control mortality was 19 % and 6.9 % (combined water and acetone control) in the studies by Witte and Höhn and mean number of juveniles was 595 and 942.8 in the studies by Witte and Höhn, respectively.

<sup>b)</sup> The mortality was corrected for control mortality according to Schneider Orelli (1947); corrected mortality calculated by the applicant based on the data provided in the confirmatory information on the study by Witte. Negative values indicate lower mortality or increased reproduction compared to controls.

Whereas no mortality was observed in the combined data up to and including the concentration of 50 mg/kg soil, no consistent results are indicated for the reproductive output of collembolans. In the study by Witte (2010), an effect was observed only at the highest test concentration of 2.0 mg/kg soil (-62 %) compared to no effect (-1 %) at 1.0 mg/kg soil. Other than this steep concentration response, the data do not show concentration dependence over the tested range of concentrations. In contrast, the data presented by Höhn (2012) show a clear concentration response relationship allowing for an extrapolation to the EC<sub>10</sub> applied for revised risk assessments. Even at a concentration of 100 mg/kg soil, the reduction in reproductive output was recorded to be lower than at 2 mg/kg soil in the study by Witte (2010).

The observed concentration effect relationship in the study by Höhn (2012) is argued to give evidence for the validity of data. It is further noted that the low effect level of 14.9 % could be considered not biologically relevant. In this context it is further noted that according to OECD test guideline No. 232, a coefficient of variation of up to 30 % is allowed in the controls.

The low effect magnitude at the lowest test rate of 50 mg/kg soil (see Höhn 2012), suggests that the effect observed by Witte (2010) is an artifact. The applicant accordingly considers the NOEC of 1.0 mg/kg soil as not reliable.

In addition, new *Folsomia* study is available using natural soil (LUFA 2.2) containing 1.61 ± 0.15% organic carbon (KCP 10.4.2/06, Geary 2016). Under these more realistic conditions, the following endpoints were derived: NOEC for mortality and NOEC for reproductive were determined to be 15.6 mg/kg soil d.w. and 31.3 mg/kg soil d.w., respectively. The EC<sub>10</sub> value for effects on reproduction was 11.2 mg/kg soil d.w.). A study summary is also presented in **Appendix 5**.

The endpoints from acute and long term studies with 2,4-D EHE on earthworms were determined to be greater than (>) the highest concentration tested. Furthermore, as stated above, degradation to the acid in the soil is very rapid. Therefore, the potential for exposure to the ester is low, except directly after spray application. Further, the endpoints for 2,4-D EHE and 2,4-D acid can be considered ecotoxicologically equivalent.

**Table 9.8-7: Comparison of 2,4-D and 2,4-D EHE endpoints derived from earthworm studies (Bridging report 2018)**

Test species/system	Type	Lowest Endpoint		Comment
		Acid <sup>1</sup>	Ester <sup>2</sup>	
Acute toxicity to earthworm	LC <sub>50</sub> (mg/kg d.w. soil)	350	>98	Endpoint for Ester is taken from study conducted with the EC formulation GF-1387 <sup>3</sup>
Long-term toxicity to earthworm	NOEC (mg/kg d.w. soil)	62.5	98 (68 mg acid equivalents)	Endpoint for Ester is taken from study conducted with the EC formulation GF-1387 <sup>3</sup>

<sup>1</sup> EFSA Journal 2014;12(9):3812.

<sup>2</sup> SANCO 7599/VI/97-final (1 October 2001).

<sup>3</sup> GF-1387 contains 905 g/L (81.68%) 2,4-D EHE.

#### **zRMS comments:**

##### Formulation toxicity data

Studies on effects of ADM.3304.H.1.A to soil macro- and meso-fauna were evaluated and agreed by the zRMS. Summaries of the studies together with zRMS evaluation may be found in Appendix 2. Endpoints reported in Table 9.8-5 were amended in line with the outcome of the studies evaluation.

Although not indicated by the Applicant, it is noted that part (or all) of the studies with the formulation have been performed with the old version of the formulation (AG-CDF1-480 EC) while the authorisation is sought for formulation ADM.3304.H.1.A (AG-CDF1-480 EC1). The change of the composition included removal of <3% of one solvent and addition of the same amount of the other solvent. The removed solvent was toxic to aquatic organisms with EC<sub>50</sub> for algae <1.0 mg/L, while the added solvent is not toxic to aquatic species with L(E)C<sub>50</sub> values for fish, *Daphnia magna* and algae being all >100 mg/L. Based on that, no change of the ecotoxicological profile of the new version of the formulation is expected. Please note that only aquatic toxicity data are available for co-formulants, but in line with the current legislation, no further studies with other non-target species are required.

In line with indications of EFSA Supporting publication 2015: EN-924, all endpoints for the formulated product were corrected by factor of 2, regardless of the peat content in the study, since the formulation contains 2,4-D EHE and fluroxypyr-meptyl, both with log Pow >2.

##### Methoxyipyridine toxicity data

In support of evaluation for ADM.3304.H.1.A the Applicant provided additional studies on toxicity of methoxyipyridine (fluroxypyr metabolite) to earthworms and *Folsomia candida*. These studies were, however, not evaluated by the zRMS since the risk assessment could be finalised using EU agreed toxicity data (see Addendum for confirmatory information of 2014) to which the Applicant for ADM.3304.H.1.A has access via the LoA. In addition to that, the new toxicity studies do not generate adverse data (the endpoints are actually higher comparing to these agreed at the EU level). Taking this into account, discussion regarding methoxyipyridine endpoint has been struck through above and the EU agreed endpoints are considered relevant for purposes of the risk assessment.

##### 4-chlorophenol (4-CP) toxicity data

Although the study with 4-CP (2,4-D metabolite) is considered to be the new active substance data, it was necessary to finalise the risk assessment since no respective endpoints were available from the EU review of 2,4-D and lack of toxicity studies with 4-CP was identified to be a data gap in EFSA Journal 2014;12(9):3812. Taking this into account, the study on toxicity of 4-CP (2,4-D metabolite) to earthworms was evaluated and agreed by the zRMS. Study summary together with zRMS evaluation may be found in Appendix 2. Endpoint reported in Table 9.8-5 is confirmed. No studies on toxicity of 4-CP to *Folsomia candida* and *Hypoaspis aculeifer* were submitted and the risk assessment for these species will be performed assuming 10 times toxicity of the parent in ADM.3304.H.1.A.

##### 2,4-D EHE toxicity data

As already indicated in point 9.8.1 above, endpoints reported in Table 9.8-7 are in line with endpoints presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). When the 2,4-D EHE endpoints are

expressed in acid equivalents, the endpoints for ester and acid may be considered equivalent. Acute toxicity data are no longer a data requirement and were thus struck through in Table 9.8-7 above.

## 9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

### 9.8.2.1 First-tier risk assessment

The relevant  $PEC_{soil}$  for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate).

**Table 9.8-8: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals**

Intended use	Grassland — Cereals (BBCH 21 — 39)		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>dt</sub> (criterion TER ≥ 5)
2,4-D	62.5	0.8000	78.1
2,4-DCA (metabolite)	5	0.0876	57.1
4-Chlorophenol (metabolite)	≥ 5	0.192	≥ 26.0
2,4-D-EHE	98	1.200	81.7
Clopyralid	1.97	0.064	30.8
Fluroxypyr-meptyl	1.96	0.2304	8.5
Fluroxypyr acid	3.05	0.1600	19.1
Pyridinol (metabolite)	0.72	0.0197	36.5
Methoxypyridine (metabolite)	1.17	0.0900	13.0
AG-CDF1-480-EC	62.5	2.336 <sup>a</sup>	26.8
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC/EC <sub>10</sub> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>dt</sub> (criterion TER ≥ 5)
<i>Folsomia candida</i>			
2,4-DCA (metabolite)	10	0.0876	114.2
2,4-DCP (metabolite)	1.25	0.0839	14.9
Methoxypyridine (metabolite)	22 (study performed on artificial soil)	0.0900	244.3
Methoxypyridine (metabolite)	11.2 <sup>b</sup> (EC <sub>10</sub> )	0.0900	124.4
AG-CDF1-480-EC	49.2 (study performed on artificial soil)	2.336 <sup>a</sup>	21.1
AG-CDF1-480-EC	103.0 <sup>b</sup>	2.336 <sup>a</sup>	44.1
<i>Hypoaspis aculeifer</i>			
2,4-DCA (metabolite)	10	0.0876	114.2
2,4-DCP (metabolite)	5	0.0839	59.6
AG-CDF1-480-EC	≥ 1000	2.336 <sup>a</sup>	≥ 428.1
Methoxypyridine (metabolite)	0.25 (study performed on artificial soil)	0.0900	2.8
Methoxypyridine (metabolite)	2.5 <sup>c</sup>	0.0900	27.8 (refinement of the risk)

TER values shown in **bold** fall below the relevant trigger.

<sup>a</sup> Formulation  $PEC_{soil}$ , calculated by combining the application of 2 L/ha with the density of 1.095 g/mL of the formulation with 20 % crop interception.

<sup>b</sup> Study performed on LUFA 2.2 (natural soil).

<sup>c</sup> Study performed on LUFA 2.4 (natural soil).

The TER values indicate an acceptable risk to soil meso- and macroorganisms following the application of the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use pattern proposed. For the metabolite Methoxypyridine, an acceptable risk to soil mites is also expected under realistic field conditions (natural soils).

#### zRMS comments:

The risk assessment to soil macro- and meso-fauna provided by the Applicant in Table 9.8-8 above was partially correct, however for some compounds and the formulated products different endpoints were agreed by the zRMS and correction of the Table 9.8-8 would make it no transparent. Taking this into account, Table 9.8-8 was struck through and the risk assessment for soil macro- and meso-fauna was performed by the zRMS in table below with consideration of the agreed endpoints and soil exposure. In the risk assessment the lower of the NOEC and EC<sub>10</sub> values were used.

For substances with log Pow >2 the endpoints were corrected regardless of the peat content in the study as being required by EFSA Supporting publication 2015: EN-924. The same procedure has been applied to endpoints from studies performed in natural soil, since EFSA (2015) indicates that for natural soil the correction factor could be lower, but does not provide any specific value.

In absence of respective toxicity data, the risk assessment for *F. candida* and *H. aculeifer* from 4-Chlorophenol was performed with assumption of 10 times toxicity of the parent (2,4-D acid) in ADM.3304.H.1.A.

Intended use	Grassland – Cereals (BBCH 21 – 39)		
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
2,4-D	62.5	0.800	78.1
2,4-DCA (metabolite)	5.0 *	0.088	56.8
2,4-DCP (metabolite)	5.0 *	0.084	59.5
4-Chlorophenol (metabolite)	5.0 *	0.192	26.0
2,4-D EHE	49.0 *	1.200	40.8
Clopyralid	1.97	0.064	30.8
Fluroxypyr-meptyl	1.96 *	0.230	8.5
Fluroxypyr acid	3.05	0.160	19.1
Pyridinol (metabolite)	0.72	0.021	34.3
Methoxypyridine (metabolite)	0.585 *	0.090	6.5
AG-CDF1-480 EC	30.1 *	2.336	12.9
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC/EC <sub>10</sub> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
<i>Folsomia candida</i>			
2,4-DCA (metabolite)	5.0 *	0.088	56.8
2,4-DCP (metabolite)	0.625 *	0.084	7.4
4-Chlorophenol (metabolite)	0.53 ** (study performed on artificial soil)	0.192	<b>2.8</b>
	1.51 ** (study performed on natural soil)		7.9 (refinement of the risk)
Methoxypyridine (metabolite)	0.5 *	0.090	5.6
AG-CDF1-480 EC	15.63 * (study performed on artificial soil)	2.336	6.7
	44.4 * (study performed on natural soil)		19.0
<i>Hypoaspis aculeifer</i>			
2,4-DCA (metabolite)	5.0 *	0.088	56.8
2,4-DCP (metabolite)	2.5 *	0.084	29.8
4-Chlorophenol (metabolite)	170.4 **	0.192	887.5
Methoxypyridine (metabolite)	0.125 * (study performed on artificial soil)	0.090	<b>1.4</b>
	1.25 * (study performed on natural soil)		13.9 (refinement of the risk)
AG-CDF1-480 EC	500 *	2.336	214

\* Endpoints corrected by a factor of 2 due to log Pow >2

\*\* 10 times toxicity of the parent in the formulation

Based on the performed above calculations, acceptable risk to earthworms may be concluded for all considered compounds and the formulated product from the intended Central Zone uses of ADM.3304.H.1.A.

For *Folsomia candida* acceptable risk could be concluded for most of the compounds and the formulated product with exception of 4-Chlorophenol, for which the TER value was below the trigger of 5 when calculated with assumption of 10 times toxicity of the parent (2,4-D acid) in ADM.3304.H.1.A used in the standard toxicity study in artificial soil. When results of the study performed in natural soil were considered, the risk from the metabolite 4-Chlorophenol was acceptable.

For *Hypoaspis aculeifer* acceptable risk could be concluded for most of the compounds and the formulated product with exception of methoxypyridine, for which the TER value was below the trigger of 5 when calculated with consideration of the endpoint derived from the standard toxicity study in artificial soil. When results of the study performed in natural soil were considered, the risk from the metabolite methoxypyridine was acceptable.

Overall, acceptable risk to soil macro- and meso-fauna may be concluded from all intended uses of ADM.3304.H.1.A in the Central Zone.

### 9.8.2.2 Higher-tier risk assessment

Based on the risk assessments shown above, a potential risk to the soil meso- and macrofauna can be excluded.

### 9.8.3 Overall conclusions

The risk for soil macro- and mesofauna from ADM.3304.H.1.A (old code AG-CDF1-480 EC) as well as the single active substances, their variants and metabolites is acceptable for the intended Central Zone uses in grassland and cereals.

## 9.9 Effects on soil microbial activity (KCP 10.5)

### 9.9.1 Toxicity data

Studies on the toxicity to soil microorganisms have been carried out with Clopyralid, 2,4-D acid and its variant 2,4-D EHE. Toxicity data for the relevant metabolites in soil compartment were also available (see **Table 9.9-1**). Full details of all above studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of ADM.3304.H.1.A (old code AG-CDF1-480 EC) were not evaluated as part of the EU assessment of the active substances. Data submitted with this application are listed in **Appendix 1** and summarised in **Appendix 2**.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. Justifications are provided below.

**Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms**

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation and Carbon transformation	2,4-D	-	No effects at 3.0 mg/kg dw soil	EFSA Journal 2014;12(9):3812
N-mineralisation and Carbon transformation	2,4-DCA (metabolite)	28 d, aerobic soil type	No effects at 5.0 mg/kg dw soil after 28 days	EFSA Journal 2014;12(9):3812
N-mineralisation and Carbon transformation	2,4-DCP (metabolite)	42 d, aerobic soil type	No effects on N-mineralization at 5.0 mg/kg dw soil after 42 days No effects on Carbon transformation at 5 mg/kg dw soil after 28 days	EFSA Journal 2014;12(9):3812
N-mineralisation and Carbon transformation	Clopyralid	56 d, aerobic soil type	<25% effect at 0.417 and 209 mg a.s./kg d.w. soil after 56 day	EFSA Journal 2018;16(8):5389
N-mineralisation	Pyridinol (metabolite)	28 d, aerobic soil type	<25% effect at 0.240 mg a.s./kg d.w. soil after 56 day	Fluroxypyr Addendum to Vol. 3: Confirmatory information, 2014
N-mineralisation and Carbon transformation	Methoxypyridine (metabolite)	-	No effects at 0.66 mg/kg dw soil	EFSA Journal 2011;9(3):2091
Carbon transformation	Pyridinol (metabolite)	28 d, aerobic soil type	No effect at 0.441 mg/kg dw soil after 28 days	EFSA Journal 2011;9(3):2091

#### zRMS comments:

Endpoints presented in Table 9.99-2 are in line with EU agreed endpoints for 2,4-D, clopyralid and fluroxypyr and their metabolites reported in EFSA Journal 2014;12(9):3812, EFSA Journal 2018;16(8):5389, and EFSA Journal 2011;9(3):2091, respectively.

Since Annex I inclusion additional studies on effects of fluroxypyr metabolite, pyridinol, were evaluated in Fluroxypyr Addendum to Vol. 3: Confirmatory information (December 2014) indicating that at 0.240 mg pm/kg dws the effect of pyridinol on nitrogen transformation in soil is <25%. Respective information has been added by the zRMS in Table 9.9-1.

Endpoints for effects on C-mineralisation were struck through as being no longer a data requirement.

#### 9.9.1.1 Justification for new endpoints

The risk assessments are based on the data on nitrogen transformation for the formulation AG-CDF1-480 EC, which are considered to be most relevant for the combined exposure of the three active substances. For completeness reasons, the risk assessments of the individual active substances, variant(s) and metabolites are also presented.

**Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms**

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation and Carbon transformation	AG-CDF1-480 EC	28 d, aerobic soil type	No adverse effects at 14.47 mg/kg dw soil after 28 days	KCP 10.5/01 Schulz (2015) Study code: 15 10 48 049 C/N
N-mineralization	Pyridinol (metabolite)	28 d, aerobic soil type	No effect at 0.09 mg/kg dw soil after 28 days	KCP 10.5/02 Schöbinger (2012) Study code: S12-00189*

\* The summary of this test is presented in **Appendix 5**

The variant 2,4-D EHE is rapidly converted to the 2,4-D acid in soil. Thus, it is not surprising that in a N-transformation study performed with 2,4-D EHE no effects were reported at the maximum tested concentration of 10.6 mg/kg dry soil, which is twice the maximum amount tested for 2,4-D acid when the ester endpoints are expressed as acid equivalents (a.e.). Thus, the endpoints for 2,4-D EHE and 2,4-D acid can be considered ecotoxicologically equivalent.

**Table 9.9-3: Comparison of 2,4-D and 2,4-D EHE endpoints derived from N-transformation studies**

Test species/system	Type	Lowest Endpoint		Comment
		Acid <sup>1</sup>	Ester <sup>2</sup>	
Nitrogen mineralisation	No effect at (mg a.s./kg soil)	3	> 10.6	Endpoint for Ester is taken from study conducted with 2,4-D EHE; Feil, N. (2010) (please refer to the Bridging report, 2018)

<sup>1</sup> EFSA Journal 2014;12(9):3812.

<sup>2</sup> Bridging Report (2018) ~~SANCO 7599/VI/97 final (1 October 2001).~~

#### **zRMS comments:**

##### Formulation toxicity data

Study on effects of ADM.3304.H.1.A on soil microbial activity was evaluated and agreed by the zRMS. Summary of the study together with zRMS evaluation may be found in Appendix 2. Endpoint reported in Table 9.9-2 is confirmed to be correct.

Although not indicated by the Applicant, it is noted that the study with the formulation has been performed with the old version of the formulation (AG-CDF1-480 EC) while the authorisation is sought for formulation ADM.3304.H.1.A (AG-CDF1-480 EC1). The change of the composition included removal of <3% of one solvent and addition of the same amount of the other solvent. The removed solvent was toxic to aquatic organisms with EC<sub>50</sub> for algae <1.0 mg/L, while the added solvent is not toxic to aquatic species with L(E)C<sub>50</sub> values for fish, *Daphnia magna* and algae being all >100 mg/L. Based on that, no change of the ecotoxicological profile of the new version of the formulation is expected. Please note that only aquatic toxicity data are available for co-formulants, but in line with the current legislation, no further studies with other non-target species are required.

##### Pyridinol toxicity data

In support of evaluation for ADM.3304.H.1.A the Applicant provided additional study on effects of pyridinol (fluroxypyr metabolite) on soil microbial activity. The study was, however, not evaluated by the zRMS since the risk assessment could be finalised using EU agreed toxicity data (see Addendum for confirmatory information of 2014) to which the Applicant for ADM.3304.H.1.A has access via the LoA. In addition to that, the new toxicity studies do not generate adverse data (the lower endpoint is a result of selection of the concentrations tested and not more severe effects observed in the new study). Taking this into account, discussion regarding pyridinol endpoint has been struck through above and the EU agreed endpoint is considered relevant for purposes of the risk assessment.

##### 2,4-D EHE toxicity data

Endpoint reported in Table 9.9-3 is in line with value presented in the 2,4-D 2-EHE Bridging Report in area of ecotoxicology (October 2018). Since the SANCO 7599/VI/97 final is not applicable anymore, reference to this document has been struck through under the Table 9.9-3 and the Bridging Report has been referenced as being the relevant document where the currently agreed EU data for 2,4-D 2-EHE may be found.



## 9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The risk assessments for the active substances and the formulation are shown below. The relevant PEC<sub>soil</sub> for risk assessments, covering the proposed use pattern, are taken from Section B8 (Environmental Fate) and were already used in the risk assessment for meso- and macrofauna organisms (please refer to Point 0).

**Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland and Cereals**

Intended use	Grassland – Cereals (BBCH 21 – 29)		
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable (margin of safety)?
2,4-D	3.0	0.8000	Yes (margin of safety: 3.8)
2,4-DCA (metabolite)	5.0	0.0876	Yes (margin of safety: 57.1)
2,4-DCP (metabolite)	5.0	0.0839	Yes (margin of safety: 59.6)
4-Chlorophenol	0.3 **	0.192	Yes (margin of safety: 1.6)
2,4-D EHE	>10.6	1.2000	Yes (margin of safety: > 8.8)
Clopyralid	209	0.064	Yes (margin of safety: 3265.6)
Pyridinol (metabolite)	0.240 0.09	0.021 0.0408	Yes (margin of safety: 11.4 2.2)
Methoxypyridine (metabolite)	0.66	0.0900	Yes (margin of safety: 7.3)
AG-CDF1-480 EC	14.47	2.3360 *	Yes (margin of safety: 6.2)

\* Formulation PEC<sub>s</sub> calculated by combining the application of 2 L/ha with the density of 1.095 g/mL of the formulation and 20 % crop interception.

\*\* 10 times toxicity of the parent assumed as a worst case

The margins of safety shown in the above table indicate that PEC<sub>soil</sub> values do not exceed the trigger value for acceptable functional effects (adverse effects ≤ 25 % on N-transformation). Thus, it can be concluded that the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) at the proposed rates do not poses a risk to soil micro-organisms.

### zRMS comments:

The risk assessment to soil micro-organisms provided by the Applicant in Table 9.9-4 above is in general agreed by the zRMS with some minor corrections resulting from different soil exposure agreed in area of Section 8 for pyridinol and higher EU agreed endpoint for this compound. Justification for selection of this higher endpoint may be found in commenting box point 9.9.1.1 above.

In absence of respective toxicity data, the risk assessment for 4-Chlorophenol was performed with assumption of 10 times toxicity of the parent (2,4-D acid).

Overall, based on the above calculations no unacceptable effects on soil microbial activity are expected from the intended uses of ADM.3304.H.1.A in the Central Zone.

## 9.9.3 Overall conclusions

An acceptable risk for soil microbial function (N-transformation) is determined from for intended Central Zone uses of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in grassland and cereals. following the application of the formulation ADM.3304.H.1.A (old code AG-CDF1-480 EC).

## 9.10 Effects on non-target terrestrial plants (KCP 10.6)

### 9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants (NTTPs) have been carried out with representative formulations during EU review of the active substances Clopyralid, Fluroxypyr acid and 2,4-D acid. Full details of these studies are provided in the respective EU DAR and related documents.

Data with a representative formulation of 2,4-D EHE (variant of 2,4-D) on NTTPs have been provided with EU Bridging Report (2018).

Effects on non-target terrestrial plants of the formulations AG-CDF1-480 EC and ADM.3304.H.1.A were not evaluated as part of the EU assessment of active substances. Data for the formulation AG-CDF1-480 EC was already submitted in 2019 (Marquardt & Braje, 2014) and new data with formulation ADM.3304.H.1.A was already submitted in 2021 for the composition change (Duffner, 2019 a,b) are listed in **Appendix 1** and summarised in **Appendix 2**.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

**Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants**

Species (most sensitive)	Substance	Exposure System	Results	Reference
Lettuce <sub>d</sub>	AG-CDF1-480 EC	21 d, vegetative vigour test, 10 species	ER <sub>50</sub> = 43 mL/ha	KCP 10.6.2/01 Marquardt & Braje (2014) Study code: AS353
Lettuce <sub>d</sub>		21 d, Seedling emergence test, 10 species	ER <sub>50</sub> = 39 mL/ha	KCP 10.6.2/02 Marquardt & Braje (2014) Study code: AS352
Lettuce <sub>d</sub>	ADM.3304.H.1.A	21 d, vegetative vigour test, 10 species	ER <sub>50</sub> = 24.8 mL/ha (corresponding to 17.69 g sum of a.s./ha) <sup>1)</sup>	KCP 10.6.2/03 Duffner (2019a) Study code: S19-03359
Lettuce <sub>d</sub>		21 d, Seedling emergence test, 10 species	ER <sub>50</sub> = 341 mL/ha (corresponding to 243.3 g sum of a.s./ha) <sup>1)</sup>	KCP 10.6.2/04 Duffner (2019b) Study code: S19-03358

<sup>1)</sup> Calculated on the basis of the analysed content of active compounds in the tested formulation, i.e. 573.9 2,4-D EHE/L, 30.6 g clopyralid/L and 109 g fluroxypyr-meptyl/L.

m: monocotyledonous; d: dicotyledonous.

### Toxicity comparison for formulations AG-CDF1-480 EC and ADM.3304.H.1.A

As a change of composition was requested in 2021 (formulation AG-CDF1-480 EC to ADM.3304.H.1.A), additional information is provided.

Toxicity tests with the formulations AG-CDF1-480 EC and ADM.3304.H.1.A on non target plants are available. Two studies assessing vegetative vigour (Marquardt & Braje, 2014 and Duffner, 2019a) and two studies assessing seedling emergence (Marquardt & Braje, 2014 and Duffner, 2019b). The studies of two formulations were conducted according to the OECD test guidelines No. 227 and 208 (respectively), and under same conditions/test design (21 days exposure and 10 species), both presenting Lettuce as the most sensitive species. Studies are summarized in **Appendix 2**, see KCP 10.6.2/01 and KCP 10.6.2/02 (presented on to the old formulation AG-CDF1-480 EC dossier) and KCP 10.6.2/03 and KCP 10.6.2/04 (presented in this dossier) for further details. Endpoints for each formulation are presented in the table below.

**Table 9.10-2: Toxicity values for AG-CDF1-480 EC and ADM.3304.H.1.A on Non target Terrestrial Plants**

	AG-CDF1-480 EC	ADM.3304.H.1.A
Vegetative vigour (Lettuce)	ER <sub>50</sub> = 43 mL/ha (wet weight)	ER <sub>50</sub> = 24.8 mL/ha (dry weight)
Seedling emergence (Lettuce)	ER <sub>50</sub> = 39 mL/ha (wet weight)	ER <sub>50</sub> = 341 mL/ha (dry weight)

It is important to note that the two studies performed on NTTPs with AG-CDF1-480 EC reported toxicity values based on fresh weight while those studies with ADM.3304.H.1.A gave the endpoints as dry weight. The endpoints from vegetative vigour tests shows a similar toxicity data for both formulations. Based on the ER<sub>50</sub> seedling emergence it can be concluded that the formulation ADM.3304.H.1.A is less toxic than AG-CDF1-480 EC. Therefore, the risk for non-target plants posed by formulation ADM.3304.H.1.A can be assessed through the risk estimated by formulation AG-CDF1-480 EC.

**zRMS comments:**

Studies on toxicity of both variants of the formulation (previous: AG-CDF1-480 EC and current: ADM.3304.H.1.A) to non-target terrestrial plants were evaluated by the zRMS and considered acceptable. For summaries of the studies and details of evaluation, please refer to Appendix 2. Endpoints reported in Tables 9.10-1 and 9.10-2 are confirmed to be correct.

The zRMS agrees that in case of the seedling emergence, the previous version of the formulated product was more toxic. However, the vegetative vigour was more sensitive to the new variant of the formulation with lower endpoint, which is considered by the zRMS as most suitable for the risk assessment. Furthermore, in case studies with the current variant are available, they should supersede endpoints for the old version of the formulation, which will be not placed on the market.

The phytotoxicity endpoints were not calculated by the Applicant, however they are not required for purposes of the risk assessment performed in the scope of the authorisation process of the plant protection products in Poland, being the only cMS in the GAP table for ADM.3304.H.1.A.

Due to different approach in calculation of the off-field exposure proposed by the Applicant in the course of the commenting period (see point 9.10.2.4 for details), the endpoints for ADM.3304.H.1.A were recalculated by the zRMS to express them in terms of the sum of active substances (based on the content of the active compounds in the formulation tested).

### 9.10.1.1 Justification for new endpoints

Assessments are presented based on data for the formulation AG-CDF1-480 EC, which are most appropriate to address a potential risk for the simultaneous exposure of three active substances.

### 9.10.2 Risk assessment

#### 9.10.2.1 Tier-1 risk assessment (based on screening data)

Not relevant for herbicides. The risk assessments are based on available toxicity tests performed in a rate-response design (see Point 9.10.2.2 below).

#### 9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area, where they may be exposed to spray drift. The amount of

spray drift reaching off-crop habitats is calculated using the 90<sup>th</sup> percentile estimates derived by the BBA (2000)<sup>11</sup> from the spray-drift predictions of Ganzelmeier & Rautmann (2000)<sup>12</sup>.

**Table 9.10-3: Assessment of the risk for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals**

Intended use	Grassland – Cereals (BBCH 21 – 39)			
Active substance/product	AG-CDF1-480 EC			
Application rate (mL/ha)	1 × 2000			
MAF (foliar)	1.0			
Test species (most sensitive)	ER <sub>50</sub> (g sum of a.s./ha) (mL/ha)	Drift percentile (%)	PER <sub>off-field</sub> <sup>1)</sup> (g sum of a.s./ha) (mL/ha)	TER criterion: TER ≥ 5
Lettuce (vegetative vigour test)	17.69 24.8 43	2.77 (for 2,4-D EHE and fluroxypyr meptyl) 2.99 (for clopyralid)	38.9 60.0 55.4	0.45 0.41 0.8
Lettuce (seedling emergence test)	243.3 341 39	2.77 (for 2,4-D EHE and fluroxypyr meptyl) 2.99 (for clopyralid)	38.9 60.0 55.4	6.2 5.7 0.7

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

<sup>1)</sup> PER<sub>off-field</sub> including deposition of clopyralid due to volatilisation (see point 9.10.2.4 for details)

The TER value for vegetative vigour is **are** below the relevant trigger of 5, indicating an unacceptable risk to non-target terrestrial plants following the application of ADM.3304.H.1.A (old code AG-CDF1-480 EC) according to the use pattern proposed. The TER for seedling emergence is above the trigger indicating acceptable risk for this parameter.

#### **zRMS comments:**

The risk assessment presented in table 9.10-3 above has been amended by the zRMS with consideration of results of studies performed with the new variant of the formulation (ADM.3304.H.1.A), for which authorisation is being sought. Since the old variant will be not placed on the market, endpoints derived from studies performed with this variant are not relevant for the risk assessment.

In addition to that, the off-field exposure calculated with consideration of deposition of clopyralid due to volatilisation was used, similarly as in case of the risk assessment performed with consideration of risk mitigation measures (see point 9.10.2.4 for details).

Based on the performed calculations, acceptable risk from the intended uses of ADM.3304.H.1.A could be concluded for seedling emergence. However, the TER calculated for vegetative vigour was below the trigger of 5 and further evaluation has been performed in point 9.10.2.4 below.

Due to different approach in calculation of the off-field exposure proposed by the Applicant in the course of the commenting period (see point 9.10.2.4 for details), the endpoints for ADM.3304.H.1.A were recalculated by the zRMS to express them in terms of the sum of active substances (based on the content of the active compounds in the formulation tested) and the risk assessment was thus also amended accordingly. Recalculation has no impact on the conclusions derived when endpoints and exposure were expressed in terms of the formulated product. The risk for seedling emergence is acceptable, while for vegetative vigour further assessment is deemed necessary and is presented in point 9.10.2.4 below.

<sup>11</sup> BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

<sup>12</sup> Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

### 9.10.2.3 Higher-tier risk assessment

Based on the outcome of the risk assessments, a refinement of the risk to non-target terrestrial plants is required. The refined risk assessments are presented in the Point 9.10.2.4 below.

### 9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following tables.

**Table 9.10-4:** ~~Risk assessment for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – vegetative vigour~~

<b>Intended use</b>		Grassland – Cereals (BBCH 21 – 39)			
<b>Active substance/product</b>		AG-CDF1-480 EC			
<b>Application rate (mL/ha)</b>		1 × 2000			
<b>MAF</b>		1.0			
<b>Buffer strip (m)</b>	<b>Drift rate (%)</b>	<b>PER<sub>off-field</sub> (mL/ha)</b>	<b>PER<sub>off-field</sub> 50 % drift red. (mL/ha)</b>	<b>PER<sub>off-field</sub> 75 % drift red. (mL/ha)</b>	<b>PER<sub>off-field</sub> 90 % drift red. (mL/ha)</b>
1	2.77	55.4	27.7	13.9	5.4
3	0.95	19.0	9.5	4.8	-
5	0.57	11.4	5.7	-	-
10	0.29	5.8	-	-	-
<b>Toxicity value</b> ER <sub>50</sub> = 24.843 g/ha		<b>TER</b> <b>criterion: TER ≥ 5</b>			
1		0.8	1.6	3.1	7.8
3		2.3	4.5	9.0	-
5		3.8	7.8	-	-
10		7.2	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in **bold** breach the relevant trigger.

**Table 9.10-5:** ~~Risk assessment for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) – seedling emergence~~

<b>Intended use</b>		Grassland – Cereals (BBCH 21 – 39)			
<b>Active substance/product</b>		AG-CDF1-480 EC			
<b>Application rate (mL/ha)</b>		1 × 2000			
<b>MAF</b>		1.0			
<b>Buffer strip (m)</b>	<b>Drift rate (%)</b>	<b>PER<sub>off-field</sub> (mL/ha)</b>	<b>PER<sub>off-field</sub> 50 % drift red. (mL/ha)</b>	<b>PER<sub>off-field</sub> 75 % drift red. (mL/ha)</b>	<b>PER<sub>off-field</sub> 90 % drift red. (mL/ha)</b>
1	2.77	55.4	27.7	13.9	5.4
3	0.95	19	9.5	4.8	-
5	0.57	11.4	5.7	-	-
10	0.29	5.8	-	-	-
<b>Toxicity value</b> ER <sub>50</sub> = 39 g/ha		<b>TER</b> <b>criterion: TER ≥ 5</b>			
1		0.7	1.4	2.8	7.1
3		2.1	4.1	8.1	-
5		3.5	7.1	-	-
10		6.5	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in **bold** breach the relevant trigger.

Deterministic risk assessment demonstrated acceptable risk to non-target terrestrial plants provided that 10 m unsprayed buffer zone from non-agricultural land is respected or the spray drift is reduced by 90 % using appropriate nozzles at a standard buffer distance of 1 m.

## TER calculations including volatilisation

For semi-volatile compounds EVA 2.1 calculations were performed and presented in the following:

**Table 9.10-6: Percentage and amount of volatilisation and subsequent deposition**

Distance [m]	% volatilisation and subsequent deposition
	Clopyralid (VP = $1.36 \times 10^{-3}$ at 25 °C)
1	0.22
3	0.20
5	0.18
10	0.14
15	0.10
20	0.08

For the following risk assessment, as a worst case approach, the deposition values (%) are added to the spray drift values of the product.

**Table 9.10-7: Percentage and amount of volatilisation and subsequent deposition**

Distance [m]	[%]		
	Spray drift	Volatilisation Clopyralid	Sum of deposition
1	2.77	0.22	2.99
3	0.95	0.20	1.15
5	0.57	0.18	0.75
10	0.29	0.14	0.43
15	0.20	0.10	0.30
20	0.15	0.08	0.23

**Table 9.10-8: Risk assessment for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) and deposition – vegetative vigour**

Intended use		Grassland – Cereals (BBCH 21–39)			
Active substance/product		AG-CDF1-480 EC			
Application rate (mL/ha)		1 × 2000			
MAF		1.0			
Buffer-strip (m)	Drift rate (%)	PER <sub>off-field</sub> (mL/ha)	PER <sub>off-field</sub> 50 % drift red. (mL/ha)	PER <sub>off-field</sub> 75 % drift red. (mL/ha)	PER <sub>off-field</sub> 90 % drift red. (mL/ha)
1	2.99	60	30	15	6
3	1.15	23	12	6	–
5	0.75	15	8	–	–
10	0.43	9	5	–	–
Toxicity value ER <sub>50</sub> = 43 g/ha		TER criterion: TER ≥ 5			
1		0.7	1.4	2.9	7.2
3		1.9	3.6	7.2	–
5		2.9	5.4	–	–
10		4.8	8.6	–	–

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

**Table 9.10-9: Risk assessment for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) and deposition – seedling emergence**

Intended use		Grassland – Cereals (BBCH 21 – 39)			
Active substance/product		AG-CDF1-480 EC			
Application rate (mL/ha)		1 × 2000			
MAF		1.0			
Buffer strip (m)	Drift rate (%)	PER <sub>off-field</sub> (mL/ha)	PER <sub>off-field</sub> 50 % drift red. (mL/ha)	PER <sub>off-field</sub> 75 % drift red. (mL/ha)	PER <sub>off-field</sub> 90 % drift red. (mL/ha)
1	2.99	60	30	15	6
3	1.15	23	12	6	-
5	0.75	15	8	4	-
10	0.43	9	5	-	-
Toxicity value ER <sub>50</sub> = 39 g/ha		TER criterion: TER ≥ 5			
1		0.7	1.3	2.6	6.5
3		1.7	3.3	6.5	-
5		2.6	4.9	9.8	-
10		4.3	7.8	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

It has to be emphasized, that this approach should be considered as a worst case assumption because the whole product was considered to behave as completely semi-volatile and to deposit to a certain amount – which is a theoretical overestimation and will not occur in reality.

#### zRMS comments:

The risk assessment for seedling emergence was considered not necessary since acceptable risk for this parameter could be concluded for new variant of the formulation with no need for risk mitigation measures. Taking this into account, calculations presented in Tables 9.10-5 and 9.10-9 were struck through.

With regard to the risk assessment for vegetative vigour, the approach presented by the Applicant was correct, but the higher endpoint for the old version of the formulation has been considered, while lower endpoint is available for the new variant which will be placed on the market. For this reason recalculation of the TER values was deemed necessary, however in order to avoid intensive correction, Tables 9.10-4 and 9.10-8 were struck through and relevant calculations were performed by the zRMS below.

During the commenting period the Applicant proposed to change the approach in calculation of the off-field exposure. Initially, the potential deposition of clopyralid due to volatilisation was summed up with the standard drift rate and applied to the whole formulation. In reality, only clopyralid will be a subject of potential volatilisation, while for 2,4-D and fluroxypyr standard drift rates may be considered. In the Reporting Table the Applicant provided proposal for such a calculation with clopyralid fraction in the formulation (0.04) applied to the volatilisation rate (0.22%) and summed up with the standard drift rate, resulting with sum of deposition of 2.78%. However, assessment of reliability of this approach is difficult and it does not seem to be entirely appropriate to apply the fraction of clopyralid in formulation to its deposition rate. In opinion of the zRMS more appropriate and transparent way for calculation of the relevant off-field exposure is to calculate PER<sub>off-field</sub> for each substance with consideration of the standard drift rates assumed for 2,4-D EHE as well as fluroxypyr meptyl and drift rates considering deposition (as presented in Table 9.10-7 above) assumed for clopyralid, then sum up individual PER<sub>off-field</sub> values and compare them with formulation endpoint expressed in terms of the sum of active compounds (based on the analysed content of 2,4-D EHE, clopyralid and fluroxypyr meptyl in tested formulation, see Table 9.10-1 for details). Respective PER<sub>off-field</sub> for individual compounds depending on the assumed unsprayed buffer zone are presented in table below. All calculations were performed in Excel on unrounded values.

Use pattern	Distance	Substance <sup>1)</sup>	Application rate [g a.s./ha]	Drift rate [%]	Individual PER <sub>off-field</sub> [g a.s./ha]	PER <sub>off-field</sub> [g sum a.s./ha]
Grassland + cereals BBCH 21-39 1 x 2000 mL/ha	1 m	2,4-D EHE	1125	2.77	31.16	38.94
		Clopyralid	60	2.99	1.79	
		Fluroxypyr meptyl	216	2.77	5.98	
	3 m	2,4-D EHE	1125	0.95	10.69	13.43
		Clopyralid	60	1.15	0.69	
		Fluroxypyr meptyl	216	0.95	2.05	
	5 m	2,4-D EHE	1125	0.57	6.41	8.09
		Clopyralid	60	0.75	0.45	
		Fluroxypyr meptyl	216	0.57	1.23	
	10 m	2,4-D EHE	1125	0.29	3.29	4.15
		Clopyralid	60	0.43	0.26	
		Fluroxypyr meptyl	216	0.29	0.63	
	15 m	2,4-D EHE	1125	0.20	2.25	2.86
		Clopyralid	60	0.30	0.18	
		Fluroxypyr meptyl	216	0.20	0.43	

<sup>1)</sup> Based on intended maximum rate of 2,4-D ester, clopyralid and fluroxypyr meptyl

The risk assessment performed with consideration of the above PER<sub>off-field</sub> values is presented below and supersedes the initial risk assessment which was struck through as no longer relevant. Values in bold indicate unacceptable risk.

Intended use		Grassland – Cereals (BBCH 21 – 39)			
Active substance/product		ADM.3304.H.1.A			
Application rate (mL/ha)		1 × 2000			
MAF		1.0			
Buffer strip (m)	Drift rate <sup>1)</sup> (%)	PER <sub>off-field</sub> (g sum a.s./ha)	PER <sub>off-field</sub> 50 % drift red. (g sum a.s./ha)	PER <sub>off-field</sub> 75 % drift red. (g sum a.s./ha)	PER <sub>off-field</sub> 90 % drift red. (g sum a.s./ha)
1	2.77 + 2.99 + 2.77	38.94	19.47	9.73	3.89
3	0.95 + 1.15 + 0.95	13.43	6.71	3.36	1.34
5	0.57 + 0.75 + 0.57	8.09	4.05	2.02	0.81
10	0.29 + 0.43 + 0.29	4.15	2.07	1.04	0.41
15	0.20 + 0.30 + 0.20	2.86	1.43	0.72	0.29
Toxicity value		TER			
ER <sub>50</sub> = 17.69 g sum a.s./ha		criterion: TER ≥ 5			
1		0.45	0.91	1.82	4.54
3		1.32	2.63	5.27	13.2
5		2.19	4.37	—	—
10		4.27	8.53	—	—
15		6.18	—	—	—

<sup>1)</sup> Drift rates given for 2,4-D ester, clopyralid and fluroxypyr meptyl, respectively

Based on the performed above deterministic risk assessment, acceptable risk to non-target terrestrial plants from the intended uses of ADM.3304.H.1.A in the Central Zone may be concluded provided that following risk mitigation measures are respected:

- 15 m unsprayed buffer zone to non-agricultural land,
- 10 m unsprayed buffer zone to non-agricultural land is combined with 50% drift reduction,
- 3 m unsprayed buffer zone to non-agricultural land is combined with 75% drift reduction,

Please note that only off field exposure calculated with consideration of deposition of clopyralid due to volatilisation was taken into account as representing worst case.

Intended use	Grassland – Cereals (BBCH 21 – 39)
Active substance/product	AG-CDF1-480-EC
Application rate (mL/ha)	1 x 2000



MAF		1.0			
Buffer strip (m)	Drift rate (%)	PER <sub>off-field</sub> (mL/ha)	PER <sub>off-field</sub> 50 % drift red. (mL/ha)	PER <sub>off-field</sub> 75 % drift red. (mL/ha)	PER <sub>off-field</sub> 90 % drift red. (mL/ha)
1	2.99	60	30	15	6.0
3	1.15	23	12	6.0	2.3
5	0.75	15	8.0	3.8	-
10	0.43	9.0	5.0	-	-
15	0.30	6.0	-	-	-
20	0.23	4.6	-	-	-
Toxicity value ER <sub>50</sub> = 24.8 mL/ha		TER criterion: TER ≥ 5			
1		0.4	0.8	1.7	4.1
3		1.1	2.1	4.1	10.8
5		1.7	3.1	6.5	-
10		2.8	5.0	-	-
15		4.1	-	-	-
20		5.4	-	-	-

Based on the performed above deterministic risk assessment, acceptable risk to non-target terrestrial plants from the intended uses of ADM.3304.H.1.A in the Central Zone may be concluded provided that following risk mitigation measures are respected:

- 20 m unsprayed buffer zone to non-agricultural land;
- 10 m unsprayed buffer zone to non-agricultural land is combined with 50% drift reduction;
- 5 m unsprayed buffer zone to non-agricultural land is combined with 75% drift reduction;
- 3 m unsprayed buffer zone to non-agricultural land is combined with 90% drift reduction.

Taking into account that sufficient number of species that was tested in both tests, probabilistic risk assessment was carried out. Species sensitivity distribution (SSD) and HC<sub>5</sub> values were thus calculated in order to check if risk mitigation measures identified in deterministic risk assessment may be reduced.

#### Probabilistic approach

Taking into account that sufficient number of species that was tested in both tests, probabilistic risk assessment was carried out. According to the Terrestrial Guidance Document (2002), “if the ED<sub>50</sub> for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable” (page 34). Thus, species sensitivity distribution (SSD) and HC<sub>5</sub> values were calculated in order to check if risk mitigation measures identified in deterministic risk assessment may be reduced.

The HC<sub>5</sub> was calculated using the software package ETX 2.0<sup>13</sup>, developed by the RIVM, the Netherlands, based on Aldenberg and Jaworska (2000)<sup>14</sup> and Aldenberg and Luttik (2002)<sup>15</sup>. HC<sub>5</sub> evaluations are based on the results of the tests conducted with AG-CDF1-480 EC. This has to be done using all measured toxicity data as well as a single limit value according to the aquatic guidance document (EFSA Journal 2013; 11(7):3290). For graphical representations of the species distribution and the fit to those data please refer to **Appendix 6**.

Based on this methodology, for vegetative vigour the lowest HC<sub>5</sub> (median) is 14.43 mL/ha. For seedling emergence, the HC<sub>5</sub> is 39.06 mL/ha.

<sup>13</sup> Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T. 2004. ETX 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68 pp.

<sup>14</sup> Aldenberg T, Jaworska JS. 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicol Environ Saf 46: 1-18.

<sup>15</sup> Aldenberg T., Luttik R. (2002): Extrapolation factors for tiny toxicity data sets from species sensitivity distributions with known standard deviation. In: Posthuma L, Suter II GW, Traas TP, eds. Species Sensitivity Distributions in Ecotoxicology. Boca Raton, USA. Lewis Publishers. p. 103-118.

**Table 9.10-10: HC<sub>5</sub> values for vegetative vigour (ER<sub>50</sub> [mL/ha])**

Parameter tested	Test substance [mL/ha]		
	Lower	Median	Upper
Plants height	8.358	74.10	196.7
Plants weight	1.322	14.43	49.74

**Table 9.10-11: HC<sub>5</sub> values for seedling emergence (ER<sub>50</sub> [mL/ha] of all dicots)**

Parameter tested	Test substance [mL/ha]		
	Lower	Median	Upper
Seedling emergence	40.22	277.3	709.4
Plants height	8.358	74.10	196.7
Plants weight	5.748	39.06	105.5

According to the Guidance Document on Terrestrial Ecotoxicology SANCO/10329/2002 rev.2 (final), 17 October 2002, the risk for terrestrial plants is assumed to be acceptable if the ER/EC<sub>50</sub> for less than 5 % of the species is below the highest predicted exposure level. As this is the case for terrestrial non target plants, for the refined risk assessment the TER values considering the HC<sub>5</sub> are compared to a trigger of 1. The risk assessment was performed with the worst case HC<sub>5</sub> value of 14.43 mL/ha from the vegetative vigour test and the HC<sub>5</sub> of 39.06 mL/ha from the seedling emergence test.

Risk assessment based on HC<sub>5</sub> values is provided in Tables below. The trigger was reduced to 1.

**Table 9.10-12: Risk assessment for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) + deposit – vegetative vigour**

Intended use		Grassland – Cereals (BBCH 21 – 39)			
Active substance/product		AG-CDF1-480 EC			
Application rate (mL/ha)		1 × 2000			
MAF		1.0			
Buffer strip (m)	Drift rate (%)	PER <sub>off-field</sub> (mL/ha)	PER <sub>off-field</sub> 50 % drift red. (mL/ha)	PER <sub>off-field</sub> 75 % drift red. (mL/ha)	PER <sub>off-field</sub> 90 % drift red. (mL/ha)
1	2.99	60	30	15	6
3	1.15	23	12	6	-
5	0.75	15	-	-	-
Toxicity value ER <sub>50</sub> = 14.43 mL/ha		TER criterion: TER ≥ 1			
1		0.2	0.5	1*	2.4
3		0.6	1.2	2.4	-
5		1*	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

\* value with all digits is 0.962. Taking into consideration the worst case assumption of a deposit of 2.99% for the whole product instead of restricting this to the two volatile compounds Clopyralid and Fluroxypyr meptyl, the risk is deemed to be acceptable.

**Table 9.10-13: Risk assessment for non-target terrestrial plants due to the use of ADM.3304.H.1.A (old code AG-CDF1-480 EC) in Grassland/Cereals considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles) + deposit – seedling emergence**

Intended use		Grassland – Cereals (BBCH 21 – 39)			
Active substance/product		AG-CDF1-480 EC			
Application rate (mL/ha)		1 × 2000			
MAF		1.0			
Buffer strip (m)	Drift rate (%)	PER <sub>off-field</sub> (mL/ha)	PER <sub>off-field</sub> 50 % drift red. (mL/ha)	PER <sub>off-field</sub> 75 % drift red. (mL/ha)	PER <sub>off-field</sub> 90 % drift red. (mL/ha)
1	2.99	60	30	15	6
3	1.15	23	12	6	-
5	0.75	15	8	-	-
Toxicity value ER <sub>50</sub> = 39.06 mL/ha		TER criterion: TER ≥ 1			
1		0.7	1.3	2.0	6.5

3	1.7	3.3	6.5	-
5	2.6	4.9	9.8	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in **bold** breach the relevant trigger.

Probabilistic risk assessment demonstrated acceptable risk to non-target terrestrial plants provided that 5 m unsprayed buffer zone from non-agricultural land is respected or the spray drift is reduced by 75 % using appropriate nozzles at a standard buffer distance of 1 m.

#### zRMS comments:

The probabilistic risk assessment performed by the Applicant above is not agreed by the zRMS since for calculation of the HC<sub>5</sub> value results of the studies performed with the old variant of the formulation were used, while there are endpoints for the new variant available which in case of the vegetative vigour are lower for all tested species comparing to the old variant. The zRMS is of the opinion that the risk assessment should be based on endpoints derived from studies performed with variant that will be placed on the market, i.e. ADM.3304.H.1.A.

Taking this into account, the risk assessment performed by the Applicant has been struck through above and new calculations were performed by the zRMS using the endpoints derived for the most sensitive parameter (plant dry weight in the vegetative vigour study by Duffner, 2019a). Seedling emergence was not considered, since acceptable risk for this parameter could be concluded for ADM.3304.H.1.A with no need for risk mitigation measures.

The HC<sub>5</sub> value for vegetative vigour was calculated by the zRMS using ETX 2.3 by RIVM on the basis of the EC<sub>50</sub> values obtained for all species tested in Duffner (2019a). Since shoot dry weight was most sensitive parameter, other parameters were not considered. For two species EC<sub>50</sub> was >6000 mL/ha, so single value at 6000 mL/ha was included in calculations. Due to obvious difference in sensitivity of dicotyledonous and monocotyledonous species, not all tests for normality were passed (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) demonstrating normal distribution. Results obtained by the zRMS are presented below.

#### Input data

Species	EC <sub>50</sub> [mL/ha]
<i>Brassica napus</i>	31.3
<i>Brassica rapa</i>	41.4
<i>Daucus carota</i>	74.0
<i>Glycine max</i>	70.3
<i>Lactuca sativa</i>	24.8
<i>Vicia faba</i>	83.7
<i>Allium cepa</i>	1401
<i>Avena sativa</i> and <i>Lolium multiflorum</i>	6000
<i>Zea mays</i>	2905

#### Goodness of fit

##### Anderson-Darling test for normality

Sign. level	Critical	Normal?
0.1	0.631	Rejected
0.05	0.752	Rejected
0.025	0.873	Accepted
0.01	1.035	Accepted

AD Statistic: 0.848  
n = 9

##### Kolmogorov-Smirnov test for normality

Sign. level	Critical	Normal?
0.1	0.819	Rejected
0.05	0.895	Rejected
0.025	0.995	Rejected
0.01	1.035	Rejected

AD Statistic: 1.044  
n = 9

### Cramer von Mises test for normality

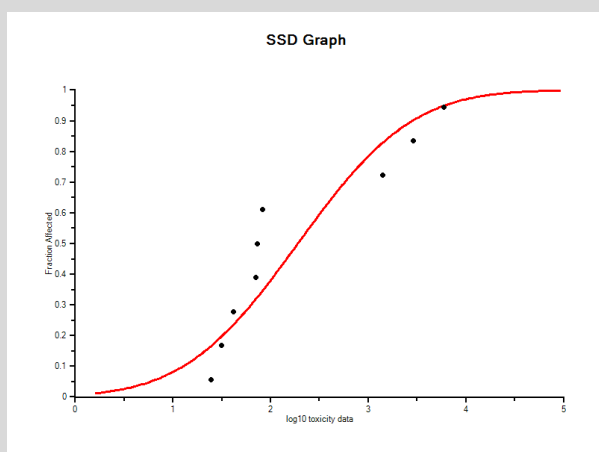
Sign. level	Critical	Normal?
0.1	0.104	Rejected
0.05	0.126	Rejected
0.025	0.148	Accepted
0.01	0.179	Accepted

AD Statistic: 0.143  
 n = 9

### HC<sub>5</sub> results

Name	Value	Description
LL HC <sub>5</sub>	0.318969	lower estimate of the HC <sub>5</sub>
HC <sub>5</sub>	<b>5.192384</b>	median estimate of the HC <sub>5</sub>
UL HC <sub>5</sub>	23.68177	upper estimate of the HC <sub>5</sub>
sprHC <sub>5</sub>	74.24464	spread of the HC <sub>5</sub> estimate

### SSD graph



It has to be noted that the zRMS had some concerns regarding obvious differences in sensitivity of monocotyledonous and dicotyledonous species and for this reason initially monocots were excluded from calculation of HC<sub>5</sub>. However, in order to check the differences between HC<sub>5</sub> derived with monocots excluded and included, additional HC<sub>5</sub> was calculated for all plants used for testing. It turned out that although all statistical analyses passed with monocots excluded, the median HC<sub>5</sub> (20.25 mL/ha) and lower limit HC<sub>5</sub> (7.52 mL/ha) were higher comparing to HC<sub>5</sub> calculated with consideration of toxicity data for all plants (5.19 mL/ha, as reported in the table above). Performed calculations were double checked and each time the same results were obtained using the ETX 2.3 calculator. To further confirm the differences in HC<sub>5</sub>, calculations were run using MOSAIC calculator, available at the Lyon University website and similar results were obtained. Taking this into account it was decided to consider HC<sub>5</sub> derived with all species included, as giving lower endpoint and representing thus worst case.

As indicated in the commenting box regarding deterministic risk assessment above, during the commenting period the Applicant proposed different approach in calculation of the off-field exposure, with volatilisation and deposition considered for clopyralid only. In order to make the resulting risk assessment transparent, respective calculations were performed by the zRMS with consideration of the exposure and endpoints expressed in terms of the sum of active compounds in the formulated product. Therefore the SSD endpoint was also recalculated and resulting HC<sub>5</sub> of 3.70 g sum a.s./ha was used in the risk assessment presented below. Please note that these calculations supersede the initial risk assessment which was struck through as no longer relevant. Values in bold indicate unacceptable risk.

Intended use	Grassland – Cereals (BBCH 21 – 39)
Active substance/product	ADM.3304.H.1.A
Application rate (mL/ha)	1 × 2000
MAF	1.0

Buffer strip (m)	Drift rate <sup>1)</sup> (%)	PER <sub>off-field</sub> (g sum a.s./ha)	PER <sub>off-field</sub> 50 % drift red. (g sum a.s./ha)	PER <sub>off-field</sub> 75 % drift red. (g sum a.s./ha)	PER <sub>off-field</sub> 90 % drift red. (g sum a.s./ha)
1	2.77 + 2.99 + 2.77	38.94	19.47	9.73	3.89
3	0.95 + 1.15 + 0.95	13.43	6.71	3.36	1.34
5	0.57 + 0.75 + 0.57	8.09	4.05	2.02	0.81
10	0.29 + 0.43 + 0.29	4.15	2.07	1.04	0.41
15	0.20 + 0.30 + 0.20	2.86	1.43	0.72	0.29
<b>Toxicity value</b> HC <sub>5</sub> = 3.70 g sum a.s./ha		<b>TER</b> criterion: TER ≥ 1			
1		0.10	0.19	0.38	0.95
3		0.28	0.55	1.10	2.76
5		0.46	0.92	-	-
10		0.89	1.79	-	-
15		1.29	-	-	-

<sup>1)</sup> Drift rates given for 2,4-D ester, clopyralid and fluroxypyr meptyl, respectively (see commenting box in area of deterministic risk assessment for details)

The outcome of the risk assessment was not improved in the probabilistic risk assessment comparing to the deterministic risk assessment and the same risk mitigation measures are required to protect non-target terrestrial plants following the intended uses of ADM.3304.H.1.A in the Central Zone, i.e.

- 15 m unsprayed buffer zone to non-agricultural land,
- 10 m unsprayed buffer zone to non-agricultural land is combined with 50% drift reduction,
- 3 m unsprayed buffer zone to non-agricultural land is combined with 75% drift reduction.

The probabilistic risk assessment based on the calculated HC<sub>5</sub> is presented below:

<b>Intended use</b>		Grassland – Cereals (BBCH 21 – 39)			
<b>Active substance/product</b>		AG-CDF1-480 EC			
<b>Application rate (mL/ha)</b>		1 × 2000			
<b>MAF</b>		1.0			
Buffer strip (m)	Drift rate (%)	PER <sub>off-field</sub> (mL/ha)	PER <sub>off-field</sub> 50 % drift red. (mL/ha)	PER <sub>off-field</sub> 75 % drift red. (mL/ha)	PER <sub>off-field</sub> 90 % drift red. (mL/ha)
1	2.99	60	30	15	6.0
3	1.15	23	12	6.0	2.3
5	0.75	15	8.0	3.8	-
10	0.43	9.0	5.0	-	-
15	0.30	6.0	-	-	-
20	0.23	4.6	-	-	-
<b>Toxicity value</b> HC <sub>5</sub> = 5.19 mL/ha		<b>TER</b> criterion: TER ≥ 1			
1		0.1	0.2	0.3	0.9
3		0.2	0.4	0.9	2.3
5		0.3	0.6	1.4	-
10		0.6	1.0	-	-
15		0.9	-	-	-
20		1.1	-	-	-

The outcome of the risk assessment was not improved in the probabilistic risk assessment comparing to the deterministic risk assessment and the same risk mitigation measures are required to protect non-target terrestrial plants following the intended uses of ADM.3304.H.1.A in the Central Zone, i.e.

- 20 m unsprayed buffer zone to non-agricultural land, or
- 10 m unsprayed buffer zone to non-agricultural land is combined with 50% drift reduction, or
- 5 m unsprayed buffer zone to non-agricultural land is combined with 75% drift reduction, or
- 3 m unsprayed buffer zone to non-agricultural land is combined with 90% drift reduction.

### 9.10.3 Overall conclusions

The risk assessment for non-target terrestrial plants from the intended Central Zone uses of ADM.3304.H.1.A has been performed using both, deterministic and probabilistic approach with consideration of the endpoints derived for the new variant of the formulation which will be placed in the market. The exposure was calculated using standard spray drift values and implementing potential deposition of clopyralid due to volatilisation.

On the basis of the deterministic risk assessment acceptable risk for seedling emergence could be concluded with no need for risk mitigation measures.

The deterministic risk assessment for vegetative vigour demonstrated that risk mitigation measures are necessary to demonstrate acceptable risk and for this reason probabilistic risk assessment has been performed, which, however, have not improved results of the deterministic risk assessment.

Overall, on the basis of the performed evaluation acceptable risk to non-target terrestrial plants may be concluded following the intended Central Zone uses of ADM.3304.H.1.A, provided that following risk mitigation measures are respected:

- 15 m unsprayed buffer zone to non-agricultural land, or
  - 10 m unsprayed buffer zone to non-agricultural land is combined with 50% drift reduction, or
  - 3 m unsprayed buffer zone to non-agricultural land is combined with 75% drift reduction.
- ~~• 3 m unsprayed buffer zone to non-agricultural land is combined with 90% drift reduction.~~

~~Probabilistic risk assessment demonstrated acceptable risk to non-target terrestrial plants provided that 5 m unsprayed buffer zone from non-agricultural land is respected or the spray drift is reduced by 75% using appropriate nozzles at a standard buffer distance of 1 m.~~

### 9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No other relevant data were identified in the EU review of the active substances and are considered necessary.

### 9.12 Monitoring data (KCP 10.8)

Not available.

## 9.13 Classification and Labelling

Classification and labelling of ADM.3304.H.1.A (old code AG-CDF1-480 EC) is proposed in accordance with Regulation 1272/2008/EC.

Taking into consideration of the toxicity endpoint of AG-CDF1-480 EC on the most sensitive aquatic organism (*Myriophyllum spicatum*):  $E_rC_{50} = 0.381$  mg/L (total shoot length) the following classification is proposed for the formulated product:

**Hazard** — **Class and Category:** Aquatic-Chronic 2      Chronic — (long term) — aquatic — hazard

GHS Pictogram, signal word, hazard statements and precautionary statements under Regulation 1272/2008:

GHS Pictograms:



Signal Word: ---

<b>Hazard statements:</b>	H411	Toxic to aquatic life with long lasting effects
<b>Precautionary Statement Prevention:</b>	P273	Avoid release to the environment
<b>Precautionary Statement Response:</b>	P391	Collect spillage
<b>Precautionary Statement Disposal:</b>	P501	Dispose of content/container in accordance with local regulations


### zRMS comments:

The CLP classification proposed by the Applicant above is not agreed by the zRMS, since the endpoint for the old variant of formulation (AG-CDF1-480 EC) was used, while considerably lower endpoint for the current variant of the formulation (ADM.3304.H.1.A) is available and should have been used for classification purposes since this variant will be placed on the market.

Taking into account the *Myriophyllum spicatum*  $E_rC_{50}$  of 0.054 mg/L, the formulation is classified for acute aquatic hazard in category 1 (H400).

Taking into account the *Myriophyllum spicatum*  $NOE_rC$  of 0.004 mg/L, the formulation is classified for chronic aquatic hazard in category 1 (H410).

Following labelling is considered relevant:

<b>Hazard pictograms:</b>	GHS09 
<b>Signal word:</b>	Warning
<b>Hazard statement(s):</b>	H410 - Very toxic to aquatic life with long lasting effects
<b>Precautionary statement(s):</b>	P391: Collect spillage P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

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



**List of data submitted by the applicant and relied on – Formulated product study summaries included in Appendix 2, studies on active substances / metabolite included in Appendix 3 to 5**

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 10.1.1/01	...	2015	Avian acute oral toxicity study of AG-CDF1-480 EC - Japanese quail - (limit test) ... Sponsor ID: ... Project ID: ... GLP, not published	Y	ADAMA Agan Ltd
KCP 10.2.1/01	....,	2014	Acute Toxicity of AG-CDF1-480 EC to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in a 96-hour Static Limit Test ... Sponsor ID: ... Project ID: ... GLP, not published	Y	ADAMA Agan Ltd
KCP 10.2.1/02	Hermes, H., Wydra, V.	2014	Acute Toxicity of AG-CDF1-480 EC to <i>Daphnia magna</i> in a Static 48-hour Immobilisation Limit Test Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90015326 Project ID: 90311220 GLP, not published	N	ADAMA Agan Ltd
KCP 10.2.1/03	Hermes, H., Wydra, V.	2015	Toxicity of AG-CDF1-480 EC to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90015333 Project ID: 90311210 GLP, not published	N	ADAMA Agan Ltd
KCP 10.2.1/04	Hermes, H., Wydra, V.	2015	Toxicity of AG-CDF1-480 EC to the Aquatic Plant <i>Lemna gibba</i> in a Static Growth Inhibition Test Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90015323 Project ID: 90311240 GLP, not published	N	ADAMA Agan Ltd
KCP 10.2.1/05	Falk, S.	2015	AG-CDF1-480 EC: Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: 90017682 Project ID: S15-00056 GLP, not published	N	ADAMA Agan Ltd

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 10.2.1/10	Gonsior, G.	2015	4-Chlorophenol: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agrosience Services EcoChem GmbH, Germany Sponsor ID: -- Project ID: S15-00666 GLP, not published	N	EU 2,4-D Annex III Taskforce
KCP 10.2.1/12	Gonsior, G.	2012	METHOXY: Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system Eurofins Agrosience Services EcoChem GmbH, Germany Sponsor ID: 90015185 Project ID: S12-00026 GLP, not published	N	ADAMA Agan Ltd
KCP 10.2.1/17	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, Germany Sponsor ID: 000102708 Project ID: S19-03357 GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.1.1/01	Franke, M.	2015	Acute toxicity of AG-CDF1-480 EC to the honeybee <i>Apis mellifera</i> L. under laboratory conditions BioChem agrar, Germany Sponsor ID: R-90015322 Project ID: 14 10 48 114 B GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.1.2/01	Noël, E.	2016	AG-CDF1-480 EC: A laboratory study to determine the chronic oral toxicity on the adult honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae). Sponsor ID: 90019009 Project ID: 307SRFR15C05 GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.1.3/01	Wilkins, S.	2018	AG-CDF1-480 EC1: In vitro 22-day toxicity test - repeated exposure to larval stage honeybee ( <i>Apis mellifera</i> L.). (report number) Sponsor ID: 90020554 Project ID: FR/000764 GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.2.2/01	Goßmann, A.	2014	Effects of AG-CDF1-480 EC on the Parasitoid <i>Aphidius rhopalosiphi</i> , Extended Laboratory Study - Dose Response Test Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90015324 Project ID: 90311002 GLP, not published	N	ADAMA Agan Ltd

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 10.3.2.2/02	Goßmann, A.	2014	Effects of AG-CDF1-480 EC on the Predatory Mite <i>Typhlodromus pyri</i> , Extended Laboratory Study - Dose Response Test Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90015330 Project ID: 90311062 GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.2.2/03	Goßmann, A.	2014	Effects of AG-CDF1-480 EC on the Lacewing <i>Chrysoperla carnea</i> , Extended Laboratory Study - Dose Response Test Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90017681 Project ID: 90311047 GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.2.2/04	Röhlig, U	2015	Effects of AG-CDF1-480 EC on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN under extended laboratory conditions (under semi-field conditions aged residues on maize plants). BioChem agrar, Germany Sponsor ID: 90017680 Project ID: 14 10 48 070 A GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.2.2/05	Walter, C.	2019a	ADM.3304.H.1.A: Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) after Exposure to Freshly Applied and Aged Spray Deposits on Maize Leaves. Eurofins Agrosience Services Ecotox GmbH, Germany Sponsor ID: 000102901 Project ID: S19-03574 GLP, not published	N	ADAMA Agan Ltd
KCP 10.3.2.2/06	Walter, C.	2019b	ADM.3304.H.1.A: Toxicity to the Ladybird <i>Coccinella septempunctata</i> (Coleoptera, Coccinellidae) under Extended Laboratory Conditions. Eurofins Agrosience Services Ecotox GmbH, Germany Sponsor ID: 000102899 Project ID: S19-01799 GLP, not published	N	ADAMA Agan Ltd
KCP 10.4.1.1/01	Friedrich, S.	2014	Sublethal toxicity of AG-CDF1-480 EC to the earthworm <i>Eisenia fetida</i> in artificial soil with 5 % peat BioChemagrar Labor für biologische und chemische Analytik GmbH, Germany Sponsor ID: R-90015329 Project ID: 14 10 48 131 S GLP, not published	N	ADAMA Agan Ltd

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 10.4.1/02	Wagenhoff, E.	2015	4-chlorophenol: Sublethal toxicity to the earthworm, <i>Eisenia fetida</i> (Annelida, Lumbricidae) in artificial soil with 10 % peat. Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: -- Project ID: S15-00154-L2 GLP, not published	N	EU 2,4-D Annex III Taskforce
KCP 10.4.2/01	Friedrich, S.	2014	Effects of AG-CDF1-480 EC on the reproduction of the collembolan <i>Folsomia candida</i> BioChemagrar Labor für biologische und chemische Analytik GmbH, Germany Sponsor ID: R-90015331 Project ID: 14 10 48 129 S GLP, not published	N	ADAMA Agan Ltd
KCP 10.4.2/02	Friedrich, S.	2015	Effects of AG-CDF1-480 EC on the reproduction of the collembolan <i>Folsomia candida</i> BioChemagrar Labor für biologische und chemische Analytik GmbH, Germany Sponsor ID: R-90017683 Project ID: 15 10 48 133 S GLP, not published	N	ADAMA Agan Ltd
KCP 10.4.2/03	Schulz, L.	2014	Effects of AG-CDF1-480 EC on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> BioChemagrar Labor für biologische und chemische Analytik GmbH, Germany Sponsor ID: R-90015325 Project ID: 14 10 48 130 S GLP, not published	N	ADAMA Agan Ltd
KCP 10.5/01	Schulz, L.	2015	Effects of AG-CDF1-480 EC on the activity of soil microflora (Nitrogen and carbon transformation tests) BioChem agrar, Germany Sponsor ID: 90017685 / 90017684 Project ID: 15 10 48 049 C/N GLP, not published	N	ADAMA Agan Ltd
KCP 10.6.2/01	Marquardt, J Braje, I.	2014	Effect of AG-CDF1-480 EC on vegetative vigour of terrestrial plants Agroscience GmbH, Germany Sponsor ID: R-90015328 Project ID: AS353 GLP, not published	N	ADAMA Agan Ltd
KCP 10.6.2/02	Marquardt, J Braje, I.	2014	Effect of AG-CDF1-480 EC on the seedling emergence and seedling growth of terrestrial plants Agroscience GmbH, Germany Sponsor ID: R-90015332 Project ID: AS352 GLP, not published	N	ADAMA Agan Ltd

<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 10.6.2/03	Duffner A.	2019a	ADM.3304.H.1.A: Effects on the Vegetative Vigour of Non-Target Terrestrial Plant Species under Greenhouse Conditions. Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: 000102903 Project ID: S19-03359 GLP, not published	N	ADAMA Agan Ltd
KCP 10.6.2/04	Duffner A.	2019b	ADM.3304.H.1.A: Effects on the Seedling Emergence and Seedling Growth of Non-Target Terrestrial Plant Species under Greenhouse Conditions. Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: 000102902 Project ID: S19-03358 GLP, not published	N	ADAMA Agan Ltd

**List of data 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>
As most of endpoints for 2,4-D, clopyralid and fluroxypyr as well as their relevant metabolites were taken from the EU review, for the list of respective studies please refer to Volume 2 of the RAR for particular active compounds.					

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.2.2/01	...	2012	Field study monitoring potential acute and long term effects of 2,4-D application in grassland on common vole populations in Germany and France ... GLP, unpublished	Y	ADAMA Agan Ltd
KCP 10.1.2.2/02	....	2015	2,4-D. Supportive information for the field study to monitor potential acute and long-term effects on common voles after application of 2,4-D in maize Project ID:... GLP no, unpublished	N	ADAMA Agan Ltd
KCP 10.2.1/06	Gonsior, G.	2014	LAF-74: Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system testing Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: -- Project ID: S14-03291 GLP, not published	N	EU 2,4-D Annex III Taskforce
KCP 10.2.1/07	...	2015	1,2,4-benzenetriol: Toxicity to the rainbow trout <i>Oncorhynchus mykiss</i> under laboratory conditions (acute toxicity test – static) ... Sponsor ID: -- Project ID: ... GLP, not published	Y	EU 2,4-D Annex III Taskforce
KCP 10.2.1/08	Zawadsky, C.	2015	1,2,4-Benzenetriol- Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test –Static) Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: -- Project ID: S15-00612 GLP, not published	N	EU 2,4-D Annex III Taskforce
KCP 10.2.1/9	Gonsior, G.	2015	1,2,4-Benzenetriol: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: -- Project ID: S15-00667 GLP, not published	N	EU 2,4-D Annex III Taskforce
KCP 10.2.1/11	Gonsior, G.	2012	Fluroxypyr acid - Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: 90015211 Project ID: S11-00188 GLP, not published	N	ADAMA Agan Ltd

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 10.2.1/13	Hodson, P.V.	1984	Measurement of median lethal dose as a rapid indication of contaminant toxicity to fish Environmental Toxicology and Chemistry, Vol. 3, pp. 243-254, 1984 GLP no, published yes	N	Publication
KCP 10.2.1/14	Kühn, R., Oattard, M., Pernak, K.-D., Winter, A.	1989	Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to <i>daphnia magna</i> Wat. Res. Vol. 23, No. 4, pp. 495-499, 1989 GLP no, published yes	N	Publication
KCP 10.2.1/ 15	Cowgill, U.M., Milazzo, D.P., Landenberger, B.D.	1989	Toxicity of nine benchmark chemicals to <i>Skeletonema costatum</i> , a marine diatom Environmental Toxicology and Chemistry, Vol. 8, pp. 451-455, 1989 GLP no, published yes	N	Publication
KCP 10.2.1/16	Kühn, R., Pattard, M.	1990	Results of the harmful effects of water pollutants to green algae ( <i>Scenedesmus subspicatus</i> ) in the cell multiplication inhibition test. Sponsor: - Project ID: Water Research, Vol 24 (1): 31 – 38 GLP no, Published yes	N	Publication
KCP 10.4.1/03	Witte, B.	2014	Effects of Methoxy (Fluroxypyr.) on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Sponsor ID: 90017404 Project ID: 92411022 GLP, not published	N	ADAMA Agan Ltd
KCP 10.4.2/04	Höhn, P.	2012	Methoxypyridine: Effects on the reproductive output of the springtail <i>Folsomia candida</i> Willem (Collembola, Isotomidae) using an artificial soil test with 5 % peat content including Report Amendment 1 and 2 Eurofins Agrosience Services EcoChem GmbH, Germany Sponsor ID: 90015180 Project ID: S 12-00021 GLP, not published	N	ADAMA Agan Ltd
KCP 10.4.2/06	Geary, N.	2016	FXP-211-6MeO – A laboratory test to determine the effects of fresh residues on the springtail <i>Folsomia candida</i> (Collembola, Isotomidae) Mambo-Tox Ltd., UK Sponsor ID: 90019202 Project ID: AGAN-16-24 GLP, not published	N	ADAMA Agan Ltd



<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</b> <b>Y/N</b>	<b>Owner</b>
KCP 10.5/02	Schöbinger, U.	2012	Effects of Pyridinol on the activity of the soil microflora - nitrogen transformation test – Eurofins Agroscience Services EcoChem GmbH, Germany Sponsor ID: 90015212 Project ID: S12-00189 GLP, not published	N	ADAMA Agan Ltd

**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</b> <b>Y/N</b>	<b>Owner</b>
There were no data relied on and not submitted by the Applicant.					

## Appendix 2 Detailed evaluation of the new studies – Formulated product

### A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

#### A 2.1.1 KCP 10.1.1 Effects on birds

#### A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

The following avian toxicity study on AG-CDF1-480 EC has not previously been reviewed and is provided in support of this assessment.

##### A 2.1.1.1.1 Study 1

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with minor deviations.</p> <p>It was noted that the age of birds was 7 weeks at the study initiation and the recommended age according to the guideline is at least 16 weeks. Also, the maximum temperature recorded during the study was 27.4°C which slightly exceeded the maximum range of 27°C, and the maximum relative humidity was 76.80% which exceeded the maximum range of 70%. However, the mean temperature during the study was 21.93°C (within the required range of 15-27°C) and the mean relative humidity was 53.08% (within the required range of 40-70%).</p> <p>Despite the deviations, all the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LD<sub>50</sub> &gt; 2000 mg product/kg b.w.</p>
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Reference:	<b>KCP 10.1.1.1/01</b>
Report	Avian acute oral toxicity study of AG-CDF1-480 EC - Japanese quail - (limit test). ...
Guideline(s):	OCSPP guideline 850.2100
Deviations:	Minor (see the commenting box above) -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No, representative product

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material**  
**Description**  
**Lot/batch**  
**Concentration/Purity**  
  
**Stability of test compound**
- Vehicle and/or control**
- Test animals**  
**Species**  
**Age at test start**

AG-CDF1-480 EC  
Brown-orange Liquid  
N6504  
Clopyralid: 30.6 g/L  
2,4-D-2-ethylhexyl: 583.0 g/L (equivalent to 2,4-D: 386.6 g/L)  
Fluroxypyr-meptyl: 113.5 g/L (equivalent to Fluroxypyr: 78.8 g/L)  
Expiry date: April 2017

Tap water

Japanese quail (*Coturnix coturnix japonica*)  
7 weeks

<b>Weight at test start</b>	Males: 177 – 215 g
<b>Source</b>	Females: 163 – 256 g Wachtelzucht Küberich GbR, 97353 Wiesentheid-Geesdorf, Germany
<b>Acclimation period</b>	14 days
<b>Diet</b>	Ssniff® V6120 served as standard diet. Feeding was discontinued approx. 19.5 hours before test item administration
<b>Water</b>	Drinking water was offered <i>ad libitum</i>
<b>Housing and cages</b>	During the adaptation period and the actual test, the Japanese quails were housed singly per pen with a surface area of 500 cm <sup>2</sup> per bird
<b>Dose</b>	2000 mg/kg bw
<b>Administration</b>	Oral, by gavage
<b>Number of birds per concentration and control</b>	10 birds per concentration and control (total 20 birds)
<b>Reference standard</b>	None
<b>4. Environmental conditions during testing</b>	
<b>Temperature</b>	15 – 27 °C
<b>Relative humidity</b>	45 – 70 %
<b>Ventilation</b>	-
<b>Photoperiod</b>	Light for a period of 10 h and dark for 14 h

## B. STUDY DESIGN AND METHODS

- In-life dates** 15.07.2015 – 05.08.2015
- Experimental design** A dose level of 2000 mg/kg body weight was tested in 10 birds. A control group of 10 animals was treated  
**Test concentrations** 2000 mg/kg bw  
Administration volume: 5 mL/kg bw  
**Chemical analysis and validation** HPLC  
**Test duration** 14 adaptation days  
1 test day with single dosing  
14 recovery days
- Observations**

Birds were observed individually during the first 2 hours after dosing, on at least 3 evenly spread additional occasions during the first 24 hours and thereafter at least twice daily for a total of 14 days.

Observations included regurgitation, signs of intoxication and remission, abnormal behaviour, deaths and time of death.

Body weight was determined within 24 hours of dosing and 3, 7 and 14 days after administration.

Food consumption was recorded for the periods 1 – 3 days, 4 – 7 days and 8 – 14 days (average daily food consumption per animal is reported).

Appropriate actions were taken to minimize loss of animals during the study.

All animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded

#### 4. Statistics

-

## II. RESULTS AND DISCUSSION

### A. Analytical results

The results of the analysis showed that the test item formulations were correctly prepared. The measured concentrations of clopyralid ranged from 113.1% to 114%, of 2,4-D-2-ethylhexyl from 103.2 % to 103.5% and of fluroxypyr-meptyl from 104.2% to 104.7% ~~114.0 %~~ of the nominal values and were well within the established limits of 85 % to 115 % for a suspension.

### B. Biological results

Under the present test conditions, a single oral administration of 2000 mg AG-CDF1-480 EC/kg b.w. revealed reduced mortality in all 5 of 5 male and 5 of 5 female animals, ruffled feathers in 1 male and 1 female, ataxia or abdominal position in respective 1 female animal up to 3 hours after administration. No animal died prematurely.

No signs of abnormalities were noted at necropsy.

**Table A 2.1.1.1-1: Acute oral LD<sub>50</sub> of AG-CDF1-480 EC in Japanese quail**

LD <sub>50</sub> [mg product/kg b.w.]	
Males	Females
> 2000	> 2000
Males and females combined	
> 2000	

### C. Validity criteria

- Birds were randomly assigned to treatment and control pens.
- The mortality in the control was 0 % at test end (required: ≤ 10 %).
- A minimum of ten birds were ~~not~~ used for dose level of the test substance and control.
- The test substance was orally administered, via gavage.
- A dose level of the test substance and a control were tested – limit test.

## III. CONCLUSION

Under the conditions of the test, the acute oral toxicity of AG-CDF1-480 EC by gavage to birds (Japanese quail) is higher than 2000 mg product/kg bw.

### A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional data submitted.

### A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

#### A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

For the study summary on the acute oral toxicity, please refer to Part B, Section 6.

## A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

The following supervised cage/field trials performed on 2,4-D were provided in support of the mammal assessment. The field trials include a study in grassland to evaluate the effects of 2,4-D on small herbivorous mammals (vole field populations).

A field trial was conducted at 750 g 2,4-D/ha in grassland to evaluate the effects of 2,4-D on small herbivorous mammals (vole field populations); this study found no visible treatment-related mortality or long-term effects on population development during the breeding season.

All studies have been summarised and evaluated in the RAR on 2,4-D (February 2013) and/or Addendum to the RAR on 2,4-D (February 2014).

Comments of zRMS:	Study was already evaluated during the EU renewal of 2,4-D in 2014 and rejected as not reliable. Furthermore, it was performed with formulations containing 2,4-D in a form of DMA salt, so results of this study are not relevant for authorisation of the product containing 2,4-D 2-EHE.
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Reference:	<b>KCP 10.1.2.2/01</b>
Report	Field study monitoring potential acute and long term effects of 2,4-D application in grassland on common vole populations in Germany and France. ...
Guideline(s):	No official test guideline(s) available at present
Deviations:	Minor (see the commenting box above) –
GLP:	Yes
Acceptability:	Rejected at the EU level as not reliable
Duplication (if vertebrate study)	Yes

~~Based on radio tracking, capture mark re-capture data, diet analysis and home range size determinations no evidence of any direct impact of 2,4-D application on voles, their abundance or population dynamics under field conditions in Southern France or Germany during the Field Phase of the study have been detected. No statistically significant differences between treatment and control plots were shown in the proportion of dicotyledons in diet samples and no differences in terms of survival or reproduction could be detected compared to the non-treated plots in France.~~

~~The following expert statement will focus on these concerns raised by EFSA and provide further supportive information for the validity of the results from the field study by ... (2012).~~

\*\*\*\*\*

Comments of zRMS:	Since study by ... (2012) was considered not reliable at the EU level and was performed with the different form of active substance (2,4-D DMA, while ADM.3304.H.1.A contains 2,4-D 2-EHE), additional information on the study was not considered by the zRMS.
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Reference:	<b>KCP 10.1.2.2/02</b>
Report	2,4-D. Supportive information for the field study to monitor potential acute and long-term effects on common voles after application of 2,4-D in maize. ... (2015) (2012). Project ID: ...
Guideline(s):	Not applicable
Deviations:	Minor (see the commenting box above) –
GLP:	Not applicable
Acceptability:	Not evaluated, since the referenced study was rejected at the EU level
Duplication	Yes

(if vertebrate study)	
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~~EFSA concluded that the field study by ... (2012) is “not considered suitable for the risk assessment because of a number of shortcomings...”~~

~~The conclusion of the expert statement is that the field study by ... (2012) was conducted according to recommendations of the current guidance document (EFSA 2009). The methods applied are widely accepted for the monitoring of potential acute (via radio-tracking) and long-term effects (via capture-mark-recapture approach), e.g. by EFSA (2009). Local common vole populations were investigated in two different geographic regions; in both areas no indication for acute effects and no contrasting population developments were recorded on treatment compared to control plots.~~

**A 2.1.3                      KCP 10.1.3                      Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)**

No additional data submitted.

## A 2.2 KCP 10.2 Effects on aquatic organisms

### A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

According to Commission Regulation (EU) No. 284/2013, tests for acute toxicity to fish, aquatic invertebrates or effects on algal growth/macrophytes are required when “the acute toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance which is especially the case if the formulation contains two or more active substances or co-formulants such as solvents, emulators, surfactants, dispersants, fertilisers which are able to increase the toxicity in comparison with the active substance.

#### A 2.2.1.1 Study 1: Acute toxicity to fish

The following fish acute toxicity study performed with AG-CDF1-480 EC were provided in support of the assessment.

Comments of zRMS:	<p>The study was conducted in line with OECD 203 (1992) guideline with minor deviations.</p> <p>It was noted that the current guideline OECD 203 (2019) recommends culture temperature in the range of 10-14°C for the rainbow trout while the test was conducted in line with OECD 203 (1992) when the recommended culture temperature for the rainbow trout was in the range of 13-17°C.</p> <p>Two deviations to the study plan were recorded. First, the oxygen concentration after 96 hours of test duration was 49% which is lower than the required minimum of 60%. The deviation was explained as a technical problem with the aeration system which probably was also the reason for the death of one of the test fish after 96 hours. It should be mentioned that the oxygen concentration was between 89 and 92% in the 24-72h test period.</p> <p>Second, in the analytical part of the study the fortification levels were prepared by dilution with the test water adjusted to pH 4 because the analytes are more stable at that pH. It was noted that the pH adjustment had no effect on the study since the biological samples were also adjusted to pH 4 immediately after sampling.</p> <p>The mean measured concentrations of all the active substances were maintained within 80-120% of nominal.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LC<sub>50</sub> &gt; 100 mg product/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/01
Report	Acute toxicity of AG-CDF1-480 EC to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in a 96-hour Static Limit Test. .... (2014).... (report number)
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C OECD Guideline for testing of chemicals, Section 2, No. 203 SANCO/3029/99 rev.4 11/07/00 Annex II (part A; Section 4) and Annex III (PART a; Section 5) of directive 91/414
Deviations:	Minor (see the commenting box above) -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No, representative product

## I. MATERIAL AND METHODS

### A. MATERIALS

<b>1. Test Material</b>	AG-CDF1-480 EC
<b>Description</b>	Yellowish liquid
<b>Lot/batch #</b>	D-N6401
<b>Concentration/Purity</b>	2,4-D: 367.7 g/L Fluroxypyr: 74.3 g/L Clopyralid: 30.1 g/L
<b>2. Vehicle and/or control</b>	Water
<b>3. Test animals (Species)</b>	Juvenile rainbow trout ( <i>Oncorhynchus mykiss</i> )
<b>Size (weight /length) at test start</b>	Mean length: 4.79 ± 0.26 cm
<b>Source</b>	Forellenzuchtbetrieb Störk, 88348 Bad Saulgau, Germany
<b>Acclimation period</b>	At least 12 days
<b>Diet</b>	Three times per week or daily until 24 hours before the test was started
<b>Water</b>	Reconstituted Water
<b>Holding</b>	12 L glass aquaria with 10 L test medium
<b>Number of animals per replicate</b>	2 treatment groups (one test item concentration at nominal 100 mg/L and one control); each containing 7 individuals
<b>Number of replicates</b>	1 per test concentration and control
<b>Untreated variant</b>	Reconstituted water
<b>Reference standard</b>	None
<b>4. Environmental conditions during testing</b>	
<b>Temperature</b>	14 – 15 °C water temperature
<b>pH</b>	7.3 – 7.9
<b>Hardness</b>	2.5 mmol/L (= 250 mg/L) as CaCO <sub>3</sub>
<b>Oxygen concentration</b>	49 – 101 % of the air saturation value
<b>Aeration</b>	The test media were slightly aerated during the test
<b>Photoperiod</b>	16 h light : 8 h dark
<b>Light intensity</b>	490 – 940 lux

### B. STUDY DESIGN AND METHODS:

<b>1. In-life dates</b>	16.05.2014 – 19.05.2014
<b>2. Experimental design</b>	The acute toxicity to unfed juvenile rainbow trout was determined in an aerated, static, 96-hour test. The test fish were observed after approximately 0, 2, 24, 48, 72 and 96 hours test duration for sublethal effects and mortality. The samples of the test medium were analysed via HPLC-UV method
<b>Test concentrations</b>	100 mg test item/L and a control
<b>Chemical analysis and validation</b>	HPLC-UV
<b>Test duration</b>	96 hours
<b>3. Observations</b>	The test fish were observed after approximately 0, 2, 24, 48, 72 and 96 hours test duration for sublethal effects and mortality. Dead fish were removed at least once daily and discarded
<b>4. Statistics</b>	The NOEC, the LOEC and the LC <sub>0</sub> were determined directly from the raw data. Due to the lack of mortality in the only test



concentration no LC<sub>50</sub> and LC<sub>100</sub> could be determined

## II. RESULTS AND DISCUSSION

### A. Analytical results

The quantification of the active ingredients Clopyralid, 2,4-D and 2,4-D 2-ethylhexyl ester (2,4-D EHE), Fluroxypyr, Fluroxypyr-1-meptylheptyl ester of the test item AG-CDF1-480 EC was performed using liquid chromatography (HPLC-UV). Results at the start of the test just before introduction of the fish (t = 0), after 96 hours test duration (t = 96 h) and the average nominal concentration are summarized in the following table.

**Table A 2.2.1-1: Summary of analytical results**

Substance	Concentration at t = 0 h [% of nominal]	Concentration at t = 96 h [% of nominal]	Mean concentration [% of nominal]
Clopyralid	105	103	104
2,4-D	< limit of quantification detection	14	--
2,4-D 2-ethylhexyl ester	106	77	91
Fluroxypyr	< limit of quantification detection	< limit of quantification detection	--
Fluroxypyr-1-meptylheptyl ester	103	75	89

### B. Biological results

In the control all fish survived until the end of the experiment and showed no sublethal effects during the exposure time. At the test concentration of 100 mg test item/L one fish died during the last 24 hours of the test and six fish showed strong ventilation. In parallel the oxygen content decreased from 89 – 92 % of the air saturation value during the first 72 hours to 49 % of the air saturation value at test end. This was caused by technical problems with the aeration system. Therefore the effects at the only test concentration of 100 mg test item/L are not considered to be substance related. Results of a pre-test support this conclusion. All biological results are listed in **Table A 2.2.1-2**.

**Table A 2.2.1-2: Acute mortality of AG- CDF1-480 EC in rainbow trout**

Nominal concentration [mg/L]	Mortality observed					
	Exposure time [h]					
	0	2	24	48	72	96
Control	0	0	0	0	0	0
100	0	0	0	0	0	1*
LC <sub>50</sub> [mg/L]	n.d.	>100	>100	>100	>100	>100
95% CI	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

\* One dead fish in the highest test concentration can be related to technical problems with oxygen supply.

n.d.: Could not be determined.

CI: Confidence interval.

Values refer to nominal test concentrations.

### C. Validity criteria

- In the control no fish died until the end of the test.
- Dissolved oxygen concentration: The dissolved oxygen concentration in the test media decreased to 49 % of the air saturation value during the last 24 hours of the test due to technical problems with the aeration system. Due to animal welfare reasons the test will not be repeated.

## III. CONCLUSION

Based on the test results the 96-hour LC<sub>50</sub> of AG-CDF1-480 EC for Rainbow Trout (*Oncorhynchus mykiss*) was determined to be higher than 100 mg test item/L based on nominal concentrations.

### A 2.2.1.2 Study 2: Acute toxicity to *Daphnia magna*

The following aquatic invertebrate toxicity study performed with AG-CDF1-480 EC were provided in support of the assessment.

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no major deviations.</p> <p>It was noted that in the analytical part of the test item concentrations the repeatability of injection should have been <math>\leq 2\%</math> but the relative standard deviation of repeated injection of one standard solution was 2.% for an unknown reason. Since the obtained value of 2.5% is only slightly above the required value of 2%, the deviation was considered to be acceptable.</p> <p>The mean measured concentrations of all the active substances were maintained within 80-120% of nominal.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>EC<sub>50</sub> &gt; 100 mg product/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/02
Report	Acute Toxicity of AG-CDF1-480 EC to <i>Daphnia magna</i> in a Static 48-hour Immobilisation Limit Test. Hermes, H. and Wydra, V., (2014). 90311220 (report number)
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C OECD Guideline for testing of chemicals, No. 202 SANCO/3029/99 rev.4 11/07/00 Annex II (Part A; Section 4) and Annex III (Part A; Section 5) of directive 91/414
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch :** D-N6401  
**Concentration/Purity:** 2,4-D: 367.7 g/L  
 Fluroxypyr: 74.3 g/L  
 Clopyralid: 30.1 g/L  
**Stability of test compound:** Expiry date: February 2016
- Vehicle and/or control:** Reconstituted water
- Test animals (Species)** Female *Daphnia magna*  
**Age at test start:** 6.5 – 22 hours  
**Source:** Breeding at test facility  
**Acclimation period:** Not necessary, since the test was performed in the same medium as the culturing  
**Feeding:** The daphnids in the stock culture were fed at least on all working days with green algae (*Desmodesmus subspicatus*)  
**Number of study organisms per** Two treatment groups (one test item concentration at

<b>concentration and control:</b>	nominal 100 mg/L and one control) each containing 20 individuals
<b>Number of animals per test vessel:</b>	5
<b>Number of replicates:</b>	4
<b>Test vessel:</b>	Glass beakers of 100 mL volume containing approximately 60 mL of test medium
<b>Untreated variant:</b>	Test medium without test substance
<b>Reference standard:</b>	The reference item potassium dichromate is tested at least twice a year to demonstrate satisfactory test conditions

#### 4. Environmental conditions during testing

<b>Temperature:</b>	20 °C water temperature (at test start and end)
<b>pH:</b>	8.1 to 8.4 at test start; 7.5 to 8.0 at test end <del>7.5–8.4</del>
<b>Hardness:</b>	2.5 mmol/L (= 250 mg/L) as CaCO <sub>3</sub>
<b>Oxygen-concentration:</b>	8.5 to 9.1 mg/L at test start; 7.9 to 8.3 <del>–9.1</del> mg/L at test end
<b>Aeration:</b>	Not stated
<b>Photoperiod:</b>	16 h light – 8 h dark
<b>Light intensity:</b>	560 – 760 lux

## B. STUDY DESIGN AND METHODS

- In-life dates:** Biological part: 04.06.2014 – 05.06.2014  
Analytical part: 27.06.2014
- Experimental design:** The mobility of the daphnids was determined in a static 48-hour test by visual observation after 24 and 48 hours.  
**Test concentration:** 100 mg test item/L (nominal) and a control  
**Chemical analysis and validation:** HPLC-UV  
**Test duration:** 48 hours
- Observations:** The mobility of the daphnids was determined by visual observation after 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile (even if they could still move their antennae)
- Statistics** No statistical analysis was performed. The NOEC and LOEC after 24 and 48 hours were determined directly from the raw data

## II. RESULTS AND DISCUSSION

### A. Analytical results

The quantification of the active ingredients Clopyralid, 2,4-D and 2,4-D 2-ethylhexyl ester (2,4-D EHE), Fluroxypyr, Fluroxypyr-1-meptylheptyl ester of the test item AG-CDF1-480 EC was performed using liquid chromatography (HPLC-UV). Results at the start of the test just before introduction of the fish (t = 0), after 96 hours test duration (t = 48 h) and the average nominal concentration are summarized in the following table.

**Table A 2.2.1-3: Summary of analytical results**

Substance	concentration at t = 0 h [% of nominal]	concentration at t = 48 h [% of nominal]	mean concentration [% of nominal]
Clopyralid	102	101	101
2,4-D	< limit of quantification detection	< limit of quantification detection	--
2,4-D 2-ethylhexyl ester	101	88	94
Fluroxypyr	< limit of quantification detection	< limit of quantification detection	--
Fluroxypyr-1-meptylheptyl ester	99	86	92

## B. Biological results

After 48 hours of exposure no immobilisation of the test animals was observed in the control and the test item concentration of 100 mg test item/L. For a summary of the results please refer to **Table A 2.2.1-4**.

**Table A 2.2.1-4: Summary of biological results (as nominal test concentrations)**

Nominal concentration [mg test item/L]	% of immobilised daphnids after	
	24 hours	48 hours
Control	0	0
100	0	0
EC <sub>50</sub> [mg/L]	>100	>100
95 % CI [mg/L]	-	-
EC <sub>20</sub> [mg/L]	>100	>100
95 % CI [mg/L]	-	-
EC <sub>10</sub> [mg/L]	>100	>100
95 % CI [mg/L]	-	-
NOEC [mg/L]	100	100
LOEC [mg/L]	>100	>100

CI: Confidence interval.

n.d.: Not determinable.

NOEC and LOEC were determined directly from the raw data.

## C. Validity criteria

- Control immobilisation rate: 0 %. Moreover, no daphnid showed signs of disease or stress; thus the validity criterion was met.
- Dissolved oxygen concentration:  $\geq 7.9$  mg O<sub>2</sub>/L in the control and test vessels at the end of the test; thus validity criterion was met.

## III. CONCLUSION

The toxic effect of the test item AG-CDF1-480 EC to *Daphnia magna* was assessed in a static limit test. The 48-hour NOEC was determined to be 100 mg test item/L. The 48-hour LOEC was determined to be > 100 mg test item/L and the 48-hour EC<sub>50</sub> value was determined to be >100 mg test item/L.

### A 2.2.1.3 Study 3: Toxicity to algae

The following algae toxicity study performed with AG-CDF1-480 EC were provided in support of the assessment.

Comments of zRMS:	<p>The study was conducted in line with OECD 201 with no deviations.</p> <p>The analytical measurements demonstrated that the mean measured concentrations of clopyralid and 2,4-D were maintained within 80-120% of nominal throughout the test period.</p> <p>Due to transformation of 2,4-D EHE and fluroxypyr-meptyl to acid forms, the measured concentrations of ester forms were within 80-120% at test initiation and declined on the next days while the measured concentrations of acid forms were increasing. Due to specific properties of the ester forms of these two compounds the zRMS is of the opinion that it is</p>
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	<p>justified to consider the measured concentrations of ester+acid throughout the study. Performed chemical analyses showed that the sum of ester+acid was within 80-120% during the test for both, 2,4-D and fluroxypyr.</p> <p>Overall, as concentrations of all active compounds were within 80-120% during the study it is justified to base the endpoints on nominal concentrations of the test item.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p><math>E_rC_{50} &gt; 100</math> mg product/L (based on nominal concentration)  <math>E_yC_{50} = 55.9</math> mg product/L (based on nominal concentration)  <math>NOE_rC = 1.0</math> mg product/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/03
Report	Toxicity of AG-CDF1-480 EC to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test. Hermes, H. and Wydra, V., (2015). 90311210 (report number)
Guideline(s):	OECD 201 (2011) Commission Regulation (EC) No 440/2008, Annex, Part C, C.3 OECD Guideline for testing of chemicals, Section 2, No. 202 SANCO/3029/99 rev.4 11/07/00; Annex II (Part A; Section 4) and Annex III (Part A; Section 5) of directive 91/414
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch, density:** D-N6401  
**Concentration/Purity:** 2,4-D: 367.7 g/L  
 Fluroxypyr: 74.3 g/L  
 Clopyralid: 30.1 g/L  
**Stability of test compound:** Expiry date: February 2016
- Vehicle and/or control:** Reconstituted water
- Test animals (Species):** *Pseudokirchneriella subcapitata*, Strain No. 61.81 SAG  
**Age or pre-culture:** The pre-culture was set up 4 days prior to the test start under the same conditions as in the test.  
**Source:** Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, 37073 Göttingen, Germany  
**Acclimation period:** 4 days  
**Feeding:** Not stated  
**Culture medium:** OECD medium  
**Test vessel:** Erlenmeyer flasks of 50 mL volume with 50 mL of test medium  
**Initial cell density:** 5000 algal cells per mL test medium  
**Number of replicates:** 6 treatment groups (5 dose rates of the test item, control)

<b>Untreated variant:</b>	with 3 replicates per test concentration and 6 replicates for the control
<b>Reference substance:</b>	Test medium without test substance The reference item potassium dichromate is tested at least twice a year to demonstrate satisfactory test conditions

#### 4. Environmental conditions during testing

<b>Temperature</b>	22 – 23 °C
<b>pH</b>	7.8 – 8.2 at the test start, 7.8 – 8.8 at the end of the test
<b>Hardness:</b>	0.24 mmol/L (= 24 mg/L) as CaCO <sub>3</sub>
<b>Oxygen-concentration [mg/L]</b>	Not stated
<b>Aeration</b>	Not stated
<b>Photoperiod</b>	continuous illumination
<b>Light intensity</b>	5520 lux ( 4850 – 6080 lux)

### B. STUDY DESIGN AND METHODS

- 1. In-life dates:** 10.06.2014 – 13.06.2014 (biological part)  
28.10.2014 (analytical part)
- 2. Experimental design:** At test start 50 mL of the test concentrations were inoculated with 5000 algal cells per mL test medium. Defined volumes of the algal suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours for determination of cell densities by spectrophotometrical measurement  
**Test concentrations:** 100, 32, 10, 3.2 and 1.0 mg test item/L, and a control.  
**Chemical analysis and validation:** HPLC-UV in samples taken at 0 and 72 h  
**Test duration:** 72 hours
- 3. Observations:** The cell densities in the samples were determined by spectrophotometrical measurement. Therefore defined volumes of the algal suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced.  
  
The algal cell densities were calculated by subtracting the absorption of the blanks, from each of the measured absorption of the test media (with algae).  
  
Based on the counted cell densities and the absorption from an algal suspension and its dilutions, a linear regression was performed for the calculation of the cell densities of the replicates during the test.
- 4. Statistics:** Based on the calculated cell densities, the 72-hour E<sub>r</sub>C<sub>50</sub> and the 72-hour E<sub>y</sub>C<sub>50</sub>, the corresponding EC<sub>20</sub> and EC<sub>10</sub> values and where possible their 95%-confidence limits were calculated by Probit analysis.  
  
For the determination of the 72-hour LOEC and the 72-hour NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams' t-test.

The software used to perform the statistical analysis was

ToxRat Professional, Version 2.10.05, ToxRat® Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. Analytical results

The quantification of the active ingredients Clopyralid, 2,4-D and 2,4-D 2-ethylhexyl ester (2,4-D EHE), Fluroxypyr, Fluroxypyr-1-meptylheptyl ester of the test item AG-CDF1-480 EC was performed using liquid chromatography (HPLC-UV). Results are summarised in the table below. Results at the start of the test just before introduction of the fish (t = 0), after 96 hours test duration (t = 72 h) and the average nominal concentration are summarized in the following table.

**Table A 2.2.1-5: Summary of analytical results**

Substance	concentration at t = 0 h [% of nominal]	concentration at t = 72 h [% of nominal]	mean concentration [% of nominal]
Clopyralid	96	102	99
2,4-D	insignificant	102	--
2,4-D 2-ethylhexyl ester	100	1 – 32 mg test item/L: insignificant 100 mg test item/L: 27 %	94
Fluroxypyr	<LOD or <LOQ 92	1, 3.2, 10 mg test item/L: 92% 32 mg test item/L: 59% 100 mg test item/L: 9%  32 mg test item/L: 9 % 100 mg test item/L: 59 %	--
Fluroxypyr-1-meptylheptyl ester	99	1 and 3.2 mg test item/L: insignificant 10 mg test item/L: 9 % 32 mg test item/L: 26 % 100 mg test item/L: 88 %	--

### B. Biological results

#### Yield y and Percentage Inhibition of y during the Test Period

Nominal concentration mg test item/L	Yields y [10000 cells/mL] and % inhibition of y					
	24 hours		48 hours		72 hours	
	y	%	y	%	y	%
Control	1.813	-	13.883	-	83.116	-
1.0	1.615	10.9	10.693	23.0 *	74.213	10.7
3.2	1.352	25.4	11.088	20.1 *	69.157	16.8 *
10	0.000	100.0	10.956	21.1 *	61.390	26.1 *
32	0.000	100.0	9.114	34.4 *	56.334	32.2 *
100	0.000	100.0	9.443	32.0	28.636	65.5 *

negative values in '% inhibition' indicate an increase in growth relative to that of the control

\* mean value significantly different from the control (tested with Bonferroni-Welch t-test (24h and 48h) and Williams t-test (72h),  $\alpha = 0.05$ , one-sided)

### Growth Rates $\mu$ and Percentage Inhibition of $\mu$ during the Test Period

Nominal concentration mg test item/L	Growth rates $\mu$ [1/day] and % inhibition of $\mu$					
	0 - 24 hours		0 - 48 hours		0 - 72 hours	
	$\mu$	%	$\mu$	%	$\mu$	%
Control	1.521	-	1.677	-	1.705	-
1.0	1.414	7.0	1.554	7.3	* 1.667	2.2
3.2	1.306	14.2	1.570	6.3	* 1.644	3.6
10	0.000	100.0	1.564	6.7	* 1.605	5.8
32	0.000	100.0	1.477	11.9	* 1.576	7.5
100	0.000	100.0	1.479	11.8	1.353	20.6

negative values in '% inhibition' indicate an increase in growth relative to that of the control  
\* mean value significantly different from the control (tested with Bonferroni-Welch t-test (24h and 48h) and Williams t-test (72h),  $\alpha = 0.05$ , one-sided)

The biological results are summarised in **Table A 2.2.1-6**.

**Table A 2.2.1-6: Summary of the biological results (as nominal test concentrations)**

Parameter	Yield [mg test item/L]	Growth rate [mg test item/L]
72-hour EC <sub>50</sub>	55.9	>100
95 % CI	33.8 – >100	n.d.
72-hour EC <sub>20</sub>	5.62	>100
95 % CI	1.92 – 10.1	77.7 – > 100
72-hour EC <sub>10</sub>	1.69	29.3
95 % CI	0.300 – 3.92	18.2 – 40.3
72-hour NOEC	1.0	1.0
72-hour LOEC	3.2	3.2

n.d.: Not determinable.  
CI: Confidence interval.

### C. Validity criteria (Cell Density)

- Increase in control cultures: 167-fold increase within 72 hours and thus, validity criterion was met.
- Coefficient of variation of sectional (daily) growth rates in control cultures: 11.4 % and thus, validity criterion was met.
- Coefficient of variation of average growth between control replicates: 2.2 % and thus, validity criterion was met.

## III. CONCLUSION

The influence of AG-CDF1-480 EC on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static dose-response test. The 72-hour E<sub>y</sub>C<sub>50</sub> was calculated to be 55.9 mg test item/L and the 72-hour E<sub>r</sub>C<sub>50</sub> value was calculated to be >100 mg test item/L. The 72-hour NOEC was determined to be 1.0 mg test item/L and the associated 72-hour LOEC was 3.2 mg test item/L for both, yield and growth rate.

### A 2.2.1.4 Study 4: Toxicity to macrophytes

The following aquatic plant toxicity study performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was conducted in line with OECD 221 with no deviations.</p> <p>The analytical measurements demonstrated that the mean measured concentrations of clopyralid and 2,4-D were maintained within 80-120% of nominal throughout the test period.</p> <p>Due to transformation of 2,4-D EHE and fluroxypyr-meptyl to acid forms, the measured concentrations of ester forms were within 80-120% at test initiation and declined on the</p>
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	<p>next days while the measured concentrations of acid forms were increasing. Due to specific properties of the ester forms of these two compounds the zRMS is of the opinion that it is justified to consider the measured concentrations of ester+acid throughout the study. Performed chemical analyses showed that the sum of ester+acid was within 80-120% during the test for both, 2,4-D and fluroxypyr.</p> <p>Overall, as concentrations of all active compounds were within 80-120% during the study it is justified to base the endpoints on nominal concentrations of the test item.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p><u>Fronnd number</u></p> <p><math>E_rC_{50}</math> = 82.1 mg product/L (based on nominal concentration)  <math>E_yC_{50}</math> = 2.92 mg product/L (based on nominal concentration)  <math>NOE_rC</math> = 0.1 mg product/L (based on nominal concentration)</p> <p><u>Dry weight</u></p> <p><math>E_rC_{50}</math> &gt;100 mg product/L (based on nominal concentration)  <math>E_yC_{50}</math> = 37.7 mg product/L (based on nominal concentration)  <math>NOE_rC</math> = 1.0 mg product/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/04
Report	Toxicity of AG-CDF1-480 EC to the aquatic plant <i>Lemna gibba</i> in a Static Growth Inhibition Test, Hermes. H. and Wydra, V., (2015). 90311240 (report number)
Guideline(s):	Commission Regulation (EC) No 761/2009, Annex, Part C, C.26. OECD Guideline 221: "Lemna sp. Growth Inhibition" SANCO/3029/99 rev.4 11/07/00; Annex II (Part A; Section 4) and Annex III (Part A; Section 5) of directive 91/414
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch, density:** D-N6401  
**Concentration/Purity:** 2,4-D: 367.7 g/L  
 Fluroxypyr: 74.3 g/L  
 Clopyralid: 30.1 g/L  
**Stability of test compound:** Expiry date: February 2016
- Vehicle and/or control:** Reconstituted water
- Test animals (Species):** *Lemna gibba* G 3  
**Age or pre-culture:** For at least 7 days under test conditions with weekly media exchange  
**Source:** Breeding at test facility  
**Culture medium:** Reconstituted water – (20× AAP-Growth Medium):  
 Analytical grade salts were added at the following nominal concentrations in deionised water (conductivity < 5 µS/cm<sup>-1</sup>):

**Macro-nutrients:**

- $\text{NaHCO}_3$ : 300 mg/L
- $\text{K}_2\text{HPO}_4 \times 3 \text{ H}_2\text{O}$ : 30 mg/L
- $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ : 290 mg/L
- $\text{NaNO}_3$ : 510 mg/L
- $\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ : 240 mg/L
- $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ : 90 mg/L

**Micro-nutrients:**

- $\text{H}_3\text{BO}_3$ : 3.7 mg/L
- $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ : 8.3 mg/L
- $\text{ZnCl}_2$ : 0.066 mg/L
- $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ : 0.029 mg/L
- $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ : 0.00024 mg/L
- $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ : 0.145 mg/L
- $\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$ : 3.2 mg/L
- $\text{Na}_2\text{EDTA} \times 2 \text{ H}_2\text{O}$ : 6.0 mg/L

**Test vessel:**

**Initial cell density:**

**Number of replicates:**

**Untreated variant:**

**Reference substance:**

The pH was adjusted with 2 M HCl to 7.5. The test water was prepared 3 days before test start to allow pH to stabilise.

Glass vessels of 250 mL volume containing approximately 200 mL of test medium, covered with glass dishes

12 fronds/test vessel

8 treatment groups (7 dose rates of the test item and a control) with 3 replicates

Test medium without test substance

The reference item 3,5-dichlorophenol is tested at least twice a year to demonstrate satisfactory test conditions.

**4. Environmental conditions during testing**

**Temperature**

**pH**

**Oxygen-concentration [mg/L]**

**Photoperiod**

**Light intensity**

Test solution temperature (range): 24 – 25 °C

7.6 (test start)

8.6 – 9.0 (at the end)

Not stated

Continuous

7608 lux (6950 to 8240 lux)

**B. STUDY DESIGN AND METHODS**

**1. In-life dates:**

Biological part: 04.06.2014 – 11.06.2014

Analytical part: 27.06.2014

**2. Experimental design:**

Growth of the aquatic plant *Lemna gibba* was determined in a static growth inhibition test:

At test start 12 fronds were introduced in each replicate and incubated for 7 days under static conditions. The frond numbers were determined on day 3, 5 and 7. The dry weight of each replicate from test end was determined.

**Test concentrations:**

100, 32, 10, 3.2, 1.0, 0.32 and 0.10 mg test item/L and a control.

**Chemical analysis and validation:**

**Test duration:**

7 days

**3. Observations:**

Number of Fronds: At test start frond and colony numbers were recorded. At days 3, 5 and 7 frond numbers and

appearance of colonies were observed.

Dry Weight of Fronds: At test start the dry weight of a sample of fronds identical to those used to inoculate the test vessels was determined. At the end of the test the dry weight of all plants from each vessel was determined. Accordingly, the plants were collected at the end of the test from the test vessels and dried at 60 °C to a constant weight.

#### 4. Statistics:

The  $E_rC_{50}$  and the  $E_yC_{50}$ , the corresponding  $EC_{20}$  and  $EC_{10}$  values and where possible their 95%-confidence limits were calculated by Probit analysis.

For the determination of the 7-day  $LOE_yC$  and  $NOE_yC$  values significant differences at the test concentrations compared to the control values were tested by the Welch t-test.

For the determination of the 7-day  $LOE_rC$  and  $NOE_rC$  values significant differences at the test concentrations compared to the control values were tested by the Williams t-test.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. Analytical results

The quantification of the active ingredients Clopyralid, 2,4-D and 2,4-D 2-ethylhexyl ester (2,4-D EHE), Fluroxypyr, Fluroxypyr-1-meptylheptyl ester of the test item AG-CDF1-480 EC was performed using liquid chromatography (HPLC-UV). Results at the start of the test (t = 0 days), after 7 days test duration (t = 7 days) and the average nominal concentration are summarized in the following table.

**Table A 2.2.1-7: Summary of analytical results**

Substance	concentration at t = 0 days [% of nominal]	concentration at t = 7days [% of nominal]	mean concentration [% of nominal]
Clopyralid*	99 <sup>1</sup>	108 <sup>1</sup>	103 <sup>1</sup>
2,4-D	Insignificant	99	--
2,4-D 2-ethylhexyl ester	94	Insignificant	--
Fluroxypyr	Insignificant	100	--
Fluroxypyr-1-meptylheptyl ester	96	Insignificant	--

<sup>1</sup> Average of the nominal test concentrations of 3.2, 10, 32 and 100 mg test item/L.

\*The measured concentrations of Clopyralid are below the valid fortification level

### B. Biological results

For a summary of the results please refer to **Table A 2.2.1-8**.

**Table A 2.2.1-8: Summary of biological results**

Nominal concentration [mg test item/L]	Yield (frond number) [mg test item/L]	Growth rate (frond number) [mg test item/L]	Yield (dry weight) [mg test item/L]	Growth rate (dry weight) [mg test item/L]
EC <sub>50</sub> (7-day)	2.92	82.1	37.4	> 100
95 % CI	1.91 – 4.44	47.5 – > 100	24.7 – 63.6	n.d.
EC <sub>20</sub> (7-day)	0.206	2.13	1.46	39.2
95 % CI	0.085 – 0.380	1.01 – 3.63	0.733 – 2.40	26.9 – 61.0
EC <sub>10</sub> (7-day)	0.052	0.316	0.266	4.13
95 % CI	0.015 – 0.117	0.089 – 0.716	0.091 – 0.555	2.12 – 6.63
NOEC [mg/L]	0.32	0.1	3.2	0.32
LOEC [mg/L]	1.0	0.32	10	1.0

n.d.: Not determinable.

Values refer to nominal test concentrations.

CI. Confidence interval.

At the higher test item concentrations the fronds showed deviations from the control replicates after 7 days; *i.e.* gibbous growth, shortened roots and chlorosis (100 mg test item/L), gibbous growth, shortened roots and separated fronds (32 mg test item/L) gibbous growth and slightly shortened roots (10 and 3.2 mg test item/L) and slight gibbous growth (1.0 mg test item/L).

### C. Validity criteria

- Doubling time of frond number in control: 1.6 days, validity criterion was met.

## III. CONCLUSION

The influence of AG-CDF1-480 EC on the growth of the freshwater plant *Lemna gibba* was assessed in a static dose-response test. The 7-day E<sub>y</sub>C<sub>50</sub> was calculated to be 2.92 and 37.4 mg test item/L for frond number and dry weight, respectively. The 7-day E<sub>r</sub>C<sub>50</sub> was calculated to be 82.1 and > 100 mg test item/L for frond number and dry weight, respectively. The 7-day NOE<sub>y</sub>C and the LOE<sub>y</sub>C were determined to be 0.32 and 1.0 mg test item/L for frond number and 3.2 and 10 mg test item/L for dry weight, respectively. The 7-day NOE<sub>r</sub>C and the LOE<sub>r</sub>C were determined to be 0.1 and 0.32 mg test item/L for frond number and 0.32 and 1.0 mg test item/L for dry weight, respectively.

### A 2.2.1.5 Study 5: Toxicity to macrophytes

The following study on growth inhibition of *Myriophyllum spicatum* in a water/sediment performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was conducted in line with OECD 239 with no deviations.</p> <p>The mean measured concentrations of the active substances clopyralid and 2,4-D were maintained within 80-120% of nominal but the mean measured concentrations of fluroxypyr were not maintained within 80-120% of nominal and for this reason results based on the measured concentrations are considered relevant (in tables below results based on both, nominal and measured concentrations, are presented).</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment (all based on mean measured concentrations related to fluroxypyr, being &lt;80%):</p> <p><u>Total shoot length</u></p> <p>E<sub>r</sub>C<sub>50</sub> = 0.306 mg product/L E<sub>y</sub>C<sub>50</sub> = 0.157 mg product/L NOE<sub>r</sub>C = 0.0214 mg product/L</p> <p><u>Fresh weight</u></p> <p>E<sub>r</sub>C<sub>50</sub> = 0.371 mg product/L</p>
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	$E_yC_{50} = 0.135 \text{ mg product/L}$ $NOE_rC = 0.0214 \text{ mg product/L}$  <u>Dry weight</u> $E_rC_{50} = 0.329 \text{ mg product/L}$ $E_yC_{50} = 0.121 \text{ mg product/L}$ $NOE_rC = 0.0214 \text{ mg product/L}$
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Reference:	KCP 10.2.1/05
Report	AG-CDF1-480 EC: Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system. Falk, S., (2015). S15-00056 (report number)
Guideline(s):	OECD Guideline 239: Water-Sediment <i>Myriophyllum spicatum</i> Toxicity Test (26 September 2014)
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Liquid / yellowish clear  
**Lot/batch, density:** D-N6405  
**Concentration/Purity:** Clopyralid 32.27 g/L and 2,4-D ethylhexyl ester 587.64 g/L (ester) or 389.66 g/L (acid) and Fluroxypyr-meptyl 113.17 g/L (ester) or 78.63 g/L (acid) (analysed)  
  
**Stability of test compound:** Expiry date: 30 June 2016
- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART AND BARKO medium
- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*  
**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agroscience Services EcoChem GmbH since November 2010. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.  
  
**Acclimation period:** Nine days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium under the same temperature, light, and water quality conditions as used during the exposure of the plants in the test.  
  
**Culture medium:** ANDREWS Medium  
**Test vessel:** Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with SMART AND BARKO medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of

added water was recorded, and the level marked on the outside of the test vessels.

Sediment used in the test (percentages based on dry weight):

- 4 % sphagnum peat (approximately pH 5.5 – 6.0; no visible plant remains, finely ground, air dried);
- 20 % kaolin clay (kaolinite content above 30 %);
- 75 – 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);
- approximately 0.2 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;
- organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;
- 100 mg of ammonium chloride and sodium phosphate per kg sediment (dry weight).

The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.

SMART AND BARKO medium:

- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L
- $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L
- $\text{NaHCO}_3$ : 58.4 mg/L
- $\text{KHCO}_3$ : 15.4 mg/L
- pH (air equilibrium) approximately 7.9

<b>Number of replicates:</b>	Five replicates per test item concentration and ten replicates for the control were used.
<b>Untreated variant:</b>	Test vessel/medium without test substance
<b>Reference substance:</b>	None

#### 4. Environmental conditions during testing

<b>Temperature</b>	The average temperature was measured to be $19.5 \pm 0.4$ °C
<b>pH</b>	The average pH-value was determined to be $7.97 \pm 0.45$
<b>Oxygen-concentration [mg/L]</b>	The oxygen saturation was determined to be $122 \pm 22\%$
<b>Photoperiod</b>	Photoperiod: 16 h day length
<b>Light intensity</b>	$120 - 160 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$

## B. STUDY DESIGN AND METHODS

- In-life dates:** 18.02.2015 – 05.05.2015
- Experimental design:** Test with *Myriophyllum spicatum* was conducted an in-house culture is maintained in a growth medium. Five replicates per test item concentration and ten replicates for the control were used. The duration of the test was 14 days. The test was performed under static test conditions. The nominal concentrations of the test item during the test were 0.00954, 0.0305, 0.0977, 0.313 and 1.00 mg/L and control. The test item was spiked to the water. Test item

concentrations in the definitive test were verified by analyses of the active ingredients at all concentration levels by analysing the overlying water at test start and test end and wet sediment at test termination on day 14. Further a 24-hour pulsed exposure test (parallel treatment group) with the same test item concentrations was run in parallel. After 24 h of exposure the test solution (water phase) was exchanged with untreated test medium. The objective of this parallel test was to quantify the effect of short term exposure to the test item on the growth of the rooted aquatic macrophyte, *Myriophyllum spicatum*.

**Test concentrations:** Nominal: 0.00954, 0.0305, 0.0977, 0.313, and 1 mg test item/L.

**Chemical analysis and validation:** HPLC-UV HPLC-MS/MS, samples taken at 0 and 14 days  
Samples taken: 0 and 14 days, in addition at the concentration level of 1.00 mg/L at day 7.

**Test duration:** 14 days

**3. Observations:** On day 14 plants were harvested from each treatment group for assessment of shoot length, total plant (i.e. shoots plus roots) fresh weight, total plant (i.e. shoots plus roots) dry weight and number and length of side shoots. Additionally the main shoot length was measured by use of a ruler on days 0, 7 and 14 during the test.

Temperature, pH and oxygen saturation (%) of the test solutions, measured after 0, 7 and 14 days, are reported.

**4. Statistics:** Endpoints reported are the  $EC_{50}$  for yield ( $E_yC_{50}$ ) and growth rate ( $E_rC_{50}$ ) based on the increase in total shoot length and total plant (i.e. shoots plus roots) biomass respectively after 14 days of exposure. The NOEC and LOEC for yield and growth rate were also determined.

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. If data were normally distributed and variance was homogeneous a Dunnett's t-test was performed. If Shapiro Wilk's test indicated a non-normal distribution of residuals a Bonferroni-U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3).

The  $EC_{50}$ ,  $EC_{20}$  and  $EC_{10}$  (yield and growth rate) was calculated where possible using Probit analysis. Only concentrations within a clear dose response were used for calculations.

## II. RESULTS AND DISCUSSION

### A. Analytical results

Measured concentrations of 2,4-D EHE in the overlying water immediately after treatment ranged between 86 and 122 % of nominal. The mean measured content for all concentrations at test start was 104 % of nominal. 2,4-D EHE was not detectable at test end. In the sediment 2,4-D EHE was not detectable at any test item concentration.

The measured concentration of the degradation product 2,4-D in the overlying water after treatment ranged between 0 (< LOQ) and 5 % of nominal. At test end the concentrations ranged between 79 and 87 % of nominal. The mean measured content for all concentrations at test end was 83 % of nominal. 2,4-D in the sediment was detectable above the LOQ for 0.0977, 0.313 and 1.00 mg test item/L test item with measured quantities of 14, 13 and 12 % of the amount applied, respectively.

Measured concentrations of clopyralid in the overlying water immediately after treatment ranged between 97 and 103 % of nominal. The mean measured content for all concentrations at test start was 100 % of nominal. The concentrations of clopyralid were between 90 – 119 % of nominal at test end. The mean measured content for all concentrations at test end was 99 % of nominal. In the sediment all values were below LOQ.

The measured concentration of the active ingredient Fluroxypyr-MHE in the overlying water immediately after treatment ranged between 71 – 84 % of nominal. The mean measured content for all concentrations at test start was 78 % of nominal. At test end, concentrations of Fluroxypyr-MHE ranged between 0 (< LOQ) and 2 % of nominal. Fluroxypyr-MHE in the sediment was not detectable for any test item concentration.

The measured concentration of the degradation product Fluroxypyr in the overlying water immediately after treatment ranged between 0 (< LOQ) and 1 % of nominal. At test end the concentrations ranged between 40 and 78 % of nominal. The mean measured content for all concentrations at test end was 62 % of nominal. In the sediment amounts above the LOQ were detected for Fluroxypyr at 0.313 and 1.00 mg test item/L test item with measured quantities being 12 % of the amount applied.

Since the measured concentrations were between 80 and 120 % as well as below 80 % of nominal (specially for the degradation products), all toxicological endpoints were evaluated using nominal and the actual concentrations based on the geometric mean of each test item concentration for 2,4-D EHE and the degradation product 2,4-D and Fluroxypyr – MHE/Fluroxypyr.

Results are presented in tables below.

Time	Nominal concentration		Overlying water (measured concentrations)	
	Test item	Clopyralid	Clopyralid	
[d]	[mg/L]	[mg/L]	[mg/L]	[% of nominal]
0	control	0.0	n.d.	-
14			< LOQ	-
0	0.00954	0.000284	0.000293	103
14			0.000337	119
0	0.0305	0.000909	0.000920	101
14			0.000930	102
0	0.0977	0.00291	0.00287	99
14			0.00275	95
0	0.313	0.00933	0.00938	101
14			0.00843	90
0	1.00	0.0298	0.0288	97
14			0.0267	90
Mean test start (0d)				100
Mean test end (14d)				99

LOQ = 0.000119 mg/L for water

n.d. = not detectable



Time	Nominal concentration			Overlying water (measured concentrations)				Amount of 2,4-D in sediment		Total % of nominal	Geometric mean [% of nominal]	Actual test concentration <sup>1)</sup>
	Test item	2,4-D EHE	2,4-D	2,4-D EHE		2,4-D						
[d]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[% of nominal l]	[mg/L]	[% of nominal]	[mg/kg]	[% of nominal]			[mg/L]
0	Control	0.0	0.0	n.d.	-	n.d.	-	-	-	-	-	0
14				n.d.	-	n.d.	-	n.d.	-	-		
0	0.00954	0.00518	0.00343	0.00633	122	< LOQ	0	-	-	122	99	0.00944
14				n.d.	0	0.00275	80	n.d.	0	80		
0	0.0305	0.0165	0.0110	0.0174	105	0.000583	5	-	-	110	96	0.0293
14				n.d.	0	0.00916	83	< LOQ	0	83		
0	0.0977	0.0530	0.0352	0.0560	106	0.00178	5	-	-	111	102	0.0997
14				n.d.	0	0.0279	79	0.0122	14	93		
0	0.313	0.170	0.113	0.147	86	0.00512	5	-	-	91	95	0.297
14				n.d.	0	0.0967	86	0.0370	13	99		
0	1.00	0.543	0.360	0.560	103	0.0182	5	-	-	108	103	1.03
14				n.d.	0	0.313	87	0.107	12	99		
Mean test start (0 d)				-	104	-	5	-	-	-	-	-
Mean test end (14d)				-	0	-	83	-	-	-	-	-

<sup>1)</sup> Based on the geometric mean (% of nominal) of each test item concentration

LOQ (2,4-D): = 0.0005 mg/L for water n.d. = not detectable

LOQ (2,4-D EHE): = 0.00217 mg/L for water

Time	Nominal concentration			Overlying water (measured concentrations)				Amount of Fluroxypyr in sediment		Total % of nominal	Geometric mean  [% of nominal]	Actual test concentration <sup>1</sup>
	Test item	Fluroxypyr- MHE	Fluroxypyr	Fluroxypyr-MHE		Fluroxypyr						
[d]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[% of nominal]	[mg/L]	[% of nominal]	[mg/kg]	[% of nominal]			[mg/L]
0	Control	0.0	0.0	n.d.	-	n.d.	-	-	-	-	-	0
14				n.d.	-	< LOQ	-	n.d.	0	-		
0	0.00954	0.00100	0.000693	0.000712	71	< LOD	0	-	-	71	53	0.00506
14				n.d.	0	0.000276	40	n.d.	0	40		
0	0.0305	0.00319	0.00221	0.00250	78	< LOQ	0	-	-	78	70	0.0214
14				0.0000628	2	0.00135	61	n.d.	0	63		
0	0.0977	0.0102	0.00709	0.00799	78	0.0000880	1	-	-	79	75	0.0733
14				0.000192	2	0.00493	70	n.d.	0	72		
0	0.313	0.0327	0.0227	0.0264	81	0.000238	1	-	-	82	77	0.241
14				< LOQ	0	0.0137	60	0.00677	12	72		
0	1.00	0.105	0.0726	0.0880	84	0.000684	1	-	-	85	87	0.870
14				0.0000964	0	0.0567	78	0.0223	12	90		
Mean test start (0 d)				-	78	-	1	-	-	-	-	-
Mean test end (14d)				-	1	-	62	-	-	-	-	-

<sup>1)</sup> Based on the geometric mean [% of nominal] of each test item concentration

LOQ (Fluroxypyr-MHE) = 0.0000523 mg/L for water mg/L for water

LOQ (Fluroxypyr) = 0.00007 mg/L for water mg/L for water n.d. = not detectable

## B. Biological results

The biological results are summarised in **Table A 2.2.1-9, A 2.2.1-10 and A 2.2.1-11**. The Applicant presented only results based on nominal concentrations, but due to measured concentrations for fluroxypyr being <80% the results based on measured concentrations should have been also presented and were added below by the zRMS. Since the results based on measured concentrations were available only in pdf file, they are added as images to tables below.

**Table A 2.2.1-9: Summary of biological results based on nominal concentrations of AG-CDF1-480 EC and total shoot length**

Parameter	Growth (total shoot length in cm) [mg test item/L]	rate	Yield (total shoot length in cm) [mg test item/L]
<b>Nominal</b>			
14-day EC <sub>50</sub>	0.381		0.203
95 % CI	0.314 – 0.471		0.170 – 0.242
14-day EC <sub>20</sub>	0.130		0.0772
95 % CI	0.0999 – 0.162		0.0593 – 0.0954
14-day EC <sub>10</sub>	0.0745		0.0466
95% CI	0.0520 – 0.0976		0.0331 – 0.0605
14-day NOEC	0.0305		0.0305
14-day LOEC	0.0977		0.0977

<b>Actual <sup>1)</sup></b>		
14-day EC <sub>50</sub>	0.378	0.201
95% Conf. Limits	0.312 – 0.469	0.168 – 0.239
14-day EC <sub>20</sub>	0.129	0.0765
95% Conf. Limits	0.0986 – 0.160	0.0587 – 0.0945
14-day EC <sub>10</sub>	0.0734	0.0462
95% Conf. Limits	0.0512 – 0.0963	0.0328 – 0.0600
14-day NOEC	0.0293	0.0293
14-day LOEC	0.0997	0.0997
<b>Actual <sup>2)</sup></b>		
14-day EC <sub>50</sub>	0.306	0.157
95% Conf. Limits	0.249 – 0.384	0.130 – 0.189
14-day EC <sub>20</sub>	0.0982	0.0566
95% Conf. Limits	0.0741 – 0.123	0.0429 – 0.0707
14-day EC <sub>10</sub>	0.0543	0.0332
95% Conf. Limits	0.0372 – 0.0721	0.0232 – 0.0438
14-day NOEC	0.0214	0.0214
14-day LOEC	0.0733	0.0733

<sup>1)</sup> Based on the geometric mean of each test item concentration of 2,4-D EHE and the degradation product 2,4-D

<sup>2)</sup> Based on the geometric mean of each test item concentration of Fluroxypyr-MHE and the degradation product Fluroxypyr

CI: Confidence limit.

**Table A 2.2.1-10: Summary of biological results based on nominal concentrations of AG-CDF1-480 EC and fresh weight**

Parameter	Growth (fresh weight in g) [mg test item/L]	rate	Yield (fresh weight in g) [mg test item/L]
<b>Nominal</b>			
14-day EC <sub>50</sub>	0.455		0.175
95 % CI	0.348 – 0.635		0.139 – 0.220
14-day EC <sub>20</sub>	0.0951		0.0434
95 % CI	0.0647 – 0.127		0.0287 – 0.0591
14-day EC <sub>10</sub>	0.0420		0.0209
95 % CI	0.0238 – 0.0621		0.0120 – 0.0313
14-day NOEC	0.0305		0.0305
14-day LOEC	0.0977		0.0977

<b>Actual <sup>1)</sup></b>		
14-day EC <sub>50</sub>	0.454	0.173
95% Conf. Limits	0.346 – 0.635	0.137 – 0.218
14-day EC <sub>20</sub>	0.0934	0.0425
95% Conf. Limits	0.0632 – 0.125	0.0280 – 0.0581
14-day EC <sub>10</sub>	0.0408	0.0204
95% Conf. Limits	0.0230 – 0.0607	0.0116 – 0.0306
14-day NOEC	0.0293	0.0293
14-day LOEC	0.0997	0.0997
<b>Actual <sup>2)</sup></b>		
14-day EC <sub>50</sub>	0.371	0.135
95% Conf. Limits	0.279 – 0.527	0.105 – 0.172
14-day EC <sub>20</sub>	0.0705	0.0308
95% Conf. Limits	0.0468 – 0.0961	0.0199 – 0.0427
14-day EC <sub>10</sub>	0.0296	0.0142
95% Conf. Limits	0.0162 – 0.0448	0.00786 – 0.0218
14-day NOEC	0.0214	0.0214
14-day LOEC	0.0733	0.0733

<sup>1)</sup> Based on the geometric mean of each test item concentration of 2,4-D EHE and the degradation product 2,4-D  
<sup>2)</sup> Based on the geometric mean of each test item concentration of Fluroxypyr-MHE and the degradation product Fluroxypyr

CI: Confidence limit.

**Table A 2.2.1-11: Summary of biological results based on nominal concentrations of AG-CDF1-480 EC and dry weight**

Parameter	Growth (dry weight in g) [mg test item/L]	rate	Yield (dry weight in g) [mg test item/L]
<b>Nominal</b>			
14-day EC <sub>50</sub>	0.404		0.161
95 % CI	0.297 – 0.590		0.124 – 0.212
14-day EC <sub>20</sub>	0.0615		0.0274
95 % CI	0.0418 – 0.0839		0.0181 – 0.0380
14-day EC <sub>10</sub>	0.0230		0.0109
95 % CI	0.0131 – 0.0346		0.00615 – 0.0167
14-day NOEC	0.0305		0.0305
14-day LOEC	0.0977		0.0977

<b>Actual <sup>1)</sup></b>		
14-day EC <sub>50</sub>	0.403	0.160
95% Conf. Limits	0.296 – 0.589	0.123 – 0.211
14-day EC <sub>20</sub>	0.0606	0.0270
95% Conf. Limits	0.0412 – 0.0829	0.0178 – 0.0374
14-day EC <sub>10</sub>	0.0225	0.0107
95% Conf. Limits	0.0128 – 0.0340	0.00601 – 0.0163
14-day NOEC	0.0293	0.0293
14-day LOEC	0.0997	0.0997
<b>Actual <sup>2)</sup></b>		
14-day EC <sub>50</sub>	0.329	0.121
95% Conf. Limits	0.236 – 0.496	0.0915 – 0.164
14-day EC <sub>20</sub>	0.0425	0.0176
95% Conf. Limits	0.0279 – 0.0596	0.0112 – 0.0251
14-day EC <sub>10</sub>	0.0146	0.00641
95% Conf. Limits	0.00789 – 0.0228	0.00342 – 0.0102
14-day NOEC	0.0214	0.0214
14-day LOEC	0.0733	0.0733

<sup>1)</sup> Based on the geometric mean of each test item concentration of 2,4-D EHE and the degradation product 2,4-D

<sup>2)</sup> Based on the geometric mean of each test item concentration of Fluroxypyr-MHE and the degradation product Fluroxypyr

CI: Confidence limit.

### C. Validity criteria

The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots. Over 14 days, the mean total shoot length increased more than 6.5-fold, fresh weight biomass increased more than 6.5-fold, and mean dry weight biomass increased more than 4.5-fold.

The mean control growth rate based on shoot length, fresh weight and dry weight was 0.1353, 0.1368 and 0.1072/day respectively, which is equivalent to a mean doubling time of 5.1, 5.1 and 6.5 days respectively. The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight was 6.4 %, 6.3 % and 9.2 % respectively.

The mean control yield (and C.V.) based on shoot length was 46.1 cm (C.V. = 13.2 %), for fresh weight yield was 1.8708 g (C.V. = 14.7 %), and for dry weight yield was 0.1696 g (C.V. = 18.5 %).

Since the CV for fresh weight and shoot length yield was below 35 % and a doubling of shoot biomass and length was reached within the test duration the mean control growth rates and variability were considered acceptable.

### III. CONCLUSION

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to AG-CDF1-480 EC for 14 days, the  $E_rC_{50}$  (growth rate) and  $E_yC_{50}$  (yield) values based on total shoot length were 0.381 mg test item/L and 0.203 mg test item/L respectively. The NOEC for growth rate and yield based on total shoot length was 0.0305 mg test item/L.

The  $E_rC_{50}$  and  $E_yC_{50}$  based on 2,4-D EHE and 2,4-D were 0.378 and 0.201 mg test item/L, respectively. The NOEC for growth rate and yield were 0.0293 mg test item/L. Based on Fluroxypyr-MHE and Fluroxypyr the actual values of the  $E_rC_{50}$  and  $E_yC_{50}$  were 0.306 and 0.157 mg test item/L, respectively. The NOEC for growth rate and yield were 0.0214 mg test item/L, respectively.

The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (fresh weight) were 0.455 mg test item/L and 0.175 mg test item/L, respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 0.0305 mg test item/L.

The  $E_rC_{50}$  and  $E_yC_{50}$  based on 2,4-D EHE and 2,4-D were 0.454 and 0.173 mg test item/L, respectively. The NOEC for growth rate and yield were 0.0293 mg test item/L. Based on Fluroxypyr-MHE and Fluroxypyr the actual values of the  $E_rC_{50}$  and  $E_yC_{50}$  were 0.371 and 0.135 mg test item/L, respectively. The NOEC for growth rate and yield were 0.0214 mg test item/L, respectively.

The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (dry weight) were 0.404 mg test item/L and 0.161 mg test item/L respectively. The NOEC for growth rate and yield were 0.0305 mg test item/L.

The  $E_rC_{50}$  and  $E_yC_{50}$  based on 2,4-D EHE and 2,4-D were 0.403 and 0.160 mg test item/L, respectively. The actual values of NOEC for growth rate and yield were 0.0293 mg test item/L. Based on Fluroxypyr-MHE and Fluroxypyr the actual values of the  $E_rC_{50}$  and  $E_yC_{50}$  were 0.329 and 0.121 mg test item/L, respectively. The NOEC for growth rate and yield were 0.0214 mg test item/L, respectively.

#### A 2.2.1.6 Study 6: Toxicity to macrophytes

The following study on growth inhibition of *Myriophyllum spicatum* in a water/sediment performed with ADM.3304.H.1.A was provided in support of the assessment.

Comments of zRMS:	<p>The study was conducted in line with OECD 239 with no deviations.</p> <p>The mean measured concentrations of clopyralid were maintained within 80-120% of nominal but the mean measured concentrations of 2,4-D and fluroxypyr were not maintained within 80-120% of nominal and for this reason results based on the measured concentrations are considered relevant (in tables below results based on both, nominal and measured concentrations, are presented).</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment (all based on mean measured concentrations related to fluroxypyr, giving the lowest measured concentrations):</p> <p><u>Total shoot length</u>  <math>E_rC_{50}</math> = 0.119 mg product/L  <math>E_yC_{50}</math> = 0.050 mg product/L  <math>NOE_rC</math> = 0.022 mg product/L</p> <p><u>Fresh weight</u>  <math>E_rC_{50}</math> = 0.054 mg product/L</p>
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	$E_yC_{50} = 0.023 \text{ mg product/L}$ $NOE_rC = 0.004 \text{ mg product/L}$  <u>Dry weight</u> $E_rC_{50} = 0.278 \text{ mg product/L}$ $E_yC_{50} = 0.068 \text{ mg product/L}$ $NOE_rC = 0.022 \text{ mg product/L}$
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Reference:	KCP 10.2.1/17*
Report	ADM.3304.H.1.A: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. Eser, S., (2019). S19-03357 (report number)
Guideline(s):	OECD Guideline 239: Water-Sediment <i>Myriophyllum spicatum</i> Toxicity Test (26 September 2014)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

\*It is important to note that this study KCP reference does not follow the numeration sequence due to the late inclusion of its summary.

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** ADM.3304.H.1.A  
**Description:** Liquid / orange  
**Lot/batch, density:** N6903-A  
**Concentration/Purity:** 2,4-D ester; content of a.i. (analysed): 380 g/L (acid equivalent); clopyralid; content of a.i. (analysed): 30.6 g/L; fluroxypyr-meptyl; content of a.i. (analysed): 75.7 g/L (acid equivalent).  
  
**Stability of test compound:** sufficient for the test purpose (at least 1 h)  
**Expiry date:** 30/03/2021
- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART AND BARKO (1985) medium
- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*  
**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at the test facility since February 2015. The cultures obtained from Federal Environment Agency Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.  
  
**Acclimation period:** Nine days prior to test start, submerged apical shoots of the same size (5 cm in length and without side shoots) were planted in a tub of stainless steel (52 cm length x 20 cm width x 35 cm height) in an artificial sterilised sediment overlaid with SMART AND BARKO medium under the same temperature, light, and water quality conditions as used during the exposure of the plants in the test.  
  
**Culture medium:** ANDREWS Medium  
**Test vessel:** Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with SMART AND BARKO medium under the same conditions as used in the

pre-culture. The study was conducted in 2 L glass-beakers measuring approximately 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.

Artificial soil (percentages based on dry weight):

- 4 % sphagnum peat;
- 20 % kaolin clay (kaolinite content above 30 %);
- 75 – 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);
- approximately 0.2 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;
- organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;
- 200 mg ammonium chloride and sodium phosphate per kg dry sediment.

The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.

SMART AND BARKO medium:

- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L
- $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L
- $\text{NaHCO}_3$ : 58.4 mg/L
- $\text{KHCO}_3$ : 15.4 mg/L
- pH (air equilibrium) approximately 7.9

**Number of replicates:**

Five replicates per test item concentration and ten replicates for the control were used.

**Untreated variant:**

Test vessel/medium without test substance

**Reference substance:**

3,5-Dichlorophenol

#### 4. Environmental conditions during testing

**Temperature**

$20.8 \pm 0.3$  °C

**pH**

$8.18 \pm 0.74$

**Oxygen-concentration [%]**

$119 \pm 16$  %

**Photoperiod**

Photoperiod: 16 h day length

**Light intensity**

$120 - 160 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$

## B. STUDY DESIGN AND METHODS

### 1. In-life dates:

29.08.2019 – 03.12.2019

### 2. Experimental design:

Five replicates per test item concentration and ten replicates for the control were used. The duration of the test was 14 days. The test was performed under static test conditions. On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight and number and length of side shoots.

**Test concentrations:**

The nominal concentrations of the test item during the test

were 2.98, 9.54, 30.5, 97.7, 313 and 1000 µg/L and control. The test item was spiked to the water.

**Chemical analysis and validation:** Test item concentrations in the definitive test were verified by analyses of 2,4-D ester, 2,4-D acid, fluroxypyr-MHE, fluroxypyr acid and clopyralid at all concentration levels by analysing the overlying water from samples taken at test start and after 14 days at test end and wet sediment from samples at test termination on day 14 and pore water at test end in the highest test item concentration.

**Test duration:** 14 days

### 3. Observations:

On day 14 plants were harvested from each treatment group for assessment of shoot length, shoot fresh weight, shoot dry weight and number and length of side shoots. Additionally, assessments of plant growth were made on days 0, 7 and 14 during the test.

Temperature, pH and oxygen saturation in fresh and aged solutions of the test solutions, measured after 0, 7 and 14 days, are reported.

### 4. Statistics:

Endpoints reported are the  $EC_{10, 20, 50}$  for yield ( $E_yC_{10, 20, 50}$ ) and growth rate ( $E_rC_{10, 20, 50}$ ) based on the increase in total shoot length and biomass (fresh and dry weight of shoot) respectively after 14 days of exposure. The NOEC and LOEC for yield and growth rate were also determined.

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. As all data were normally distributed and variance was homogeneous a Dunnett's t-test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3).

The  $EC_{50}$ ,  $EC_{20}$  and  $EC_{10}$  (yield and growth rate) were determined by probit analysis following the normal, logistic or Gompertz distribution. The specific procedure was chosen on the basis of the best ratio of confidence intervals at the  $EC_{50}$ . Only values within a clear dose response were used. Values below zero were set to zero. Values were considered not to be reliable if control C.V. exceeded the effect level (i.e.  $EC_x$  value).

## II. RESULTS AND DISCUSSION

### A. Analytical results

The measured concentration of the test item in the test vessels based on the 2,4-D ester content in the freshly prepared test solutions ranged between 78 and 96 % of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 84 % of nominal. 2,4-D ester was not detectable at test end. In the sediment 2,4-D ester was not detectable at any test item concentration. In pore water no concentrations of 2,4-D ester were detectable after 14 days at the highest nominal concentration level of 1000 µg test item/L.

The measured concentration of the degradation product 2,4-D acid in the overlying water after treatment ranged between 0 (< LOD) and 2 % of nominal. In the 14-day aged test solutions the measured



concentration of the test item based on the 2,4-D acid content in the test vessels ranged between 71 and 83% of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the 2,4-D acid content was 78 % of nominal in the overlaying water. In the sediment, concentrations of 2,4-D acid above the LOD were detectable at the concentration levels of 97.7, 313 and 1000 µg/L with recoveries of 6, 4 and 1 % at test end after 14 days. In pore water < 1% of the applied amount was measured after 14 days at the highest nominal concentration level of 1000 µg test item/L.

The measured concentration of the test item in the test vessels based on the content of fluroxypyr-MHE in the freshly prepared test solutions ranged between 67 and 80 % of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 74 % of nominal. fluroxypyr-MHE was not detectable at test end. In the sediment fluroxypyr-MHE was not detectable at any test item concentration. In pore water no concentrations of fluroxypyr-MHE was detectable after 14 days at the highest nominal concentration level of 1000 µg test item/L.

The measured concentrations of the test item in the test vessels based on the content of the degradation product fluroxypyr acid in the freshly prepared test solutions were < 1% of nominal or below the LOQ in the overlaying water. In the 14-day aged test solutions the measured concentration of the test item based on the fluroxypyr acid content in the test vessels ranged between 20 and 83 % of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the fluroxypyr acid content was 54 % of nominal in the overlaying water. In the sediment, no concentrations of fluroxypyr acid above the LOQ were detectable at test end after 14 days. In pore water < 1 % of the applied amount was measured after 14 days at the highest nominal concentration level of 1000 µg test item/L.

The measured concentration of the test item in the test vessels based on the content of clopyralid in the freshly prepared test solutions ranged between 80 and 95 % of nominal in the overlaying water. The mean measured content for all concentrations in the freshly prepared test solutions was 88 % of nominal. In the 14-day aged test solutions the measured concentration of the test item based on the clopyralid content in the test vessels ranged between 81 and 95 % of nominal in the overlaying water. The mean measured concentration of the test item in aged test solutions based on the clopyralid content was 87 % of nominal in the overlaying water. In the sediment, no concentrations of clopyralid above the LOQ were detectable at test end after 14 days. In pore water 2 % of the applied amount was measured after 14 days at the highest nominal concentration level of 1000 µg test item/L.

Since the initial measured concentrations of clopyralid were between 80 und 120 % of nominal, all toxicological endpoints were evaluated using nominal concentrations. As 2,4-D ester as well as the measured concentrations of the degradation product at test termination were below 80 % of nominal, all toxicological endpoints were evaluated using the actual concentrations based on the geometric mean of each test item concentration for 2,4-D ester/2,4-D acid. Since Fluroxypyr-MHE as well as the measured concentrations of the degradation product at test termination were below 80 % of nominal, all toxicological endpoints were evaluated using the actual concentrations based on the geometric mean of each test item concentration for Fluroxypyr-MHE/Fluroxypyr acid.

## B. Biological results

Following exposure to ADM.3304.H.1.A, total shoot length and shoot fresh weight were found to be more sensitive than shoot dry weight for the EC<sub>50</sub> as indicated in the tables below.

**Table A 2.2.1-12: Summary of Biological Results based on Nominal and Actual concentrations of ADM.3304.H.1.A and Total Shoot Length**

	Growth rate (total shoot length in cm) [µg/L]	Yield (total shoot length in cm) [µg/L]
<b>Nominal concentrations</b>		
<b>14-day EC<sub>50</sub></b>	165 <sup>1)</sup>	68.8 <sup>2)</sup>
<b>95% Conf. Limits</b>	135 - 203	57.9 - 81.7
<b>14-day EC<sub>20</sub></b>	45.8 <sup>1)</sup>	28.6 <sup>2)</sup>
<b>95% Conf. Limits</b>	33.9 - 58.6	22.2 - 35.1
<b>14-day EC<sub>10</sub></b>	23.5 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	15.9 - 31.9	-
<b>14-day NOEC</b>	30.5 <sup>3)</sup>	30.5 <sup>3)</sup>
<b>14-day LOEC</b>	97.7 <sup>3)</sup>	97.7 <sup>3)</sup>
<b>Actual concentrations<sup>4)</sup></b>		
<b>14-day EC<sub>50</sub></b>	135 <sup>1)</sup>	54.4 <sup>2)</sup>
<b>95% Conf. Limits</b>	110 - 168	45.6 - 64.9
<b>14-day EC<sub>20</sub></b>	36.0 <sup>1)</sup>	22.1 <sup>2)</sup>
<b>95% Conf. Limits</b>	26.5 - 46.3	17.1 - 27.2
<b>14-day EC<sub>10</sub></b>	18.0 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	12.1 - 24.7	-
<b>14-day NOEC</b>	22.9 <sup>3)</sup>	22.9 <sup>3)</sup>
<b>14-day LOEC</b>	77.2 <sup>3)</sup>	77.2 <sup>1)</sup>
<b>Actual concentrations<sup>5)</sup></b>		
<b>14-day EC<sub>50</sub></b>	119 <sup>1)</sup>	49.6 <sup>2)</sup>
<b>95% Conf. Limits</b>	96.0 - 147	41.0 - 59.5
<b>14-day EC<sub>20</sub></b>	32.3 <sup>1)</sup>	19.6 <sup>2)</sup>
<b>95% Conf. Limits</b>	23.2 - 42.2	14.6 - 24.7
<b>14-day EC<sub>10</sub></b>	16.4 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	10.6 - 22.9	-
<b>14-day NOEC</b>	22.3 <sup>3)</sup>	22.3 <sup>3)</sup>
<b>14-day LOEC</b>	74.3 <sup>3)</sup>	74.3 <sup>3)</sup>

<sup>1)</sup> Probit analysis following normal distribution

<sup>2)</sup> Probit analysis following logistic distribution

<sup>3)</sup> Following Dunnett's-t-test (left-sided, p ≤0.05)

<sup>4)</sup> Based on the geometric mean of each test item concentration of 2,4-D ester and the degradation product 2,4-D acid

<sup>5)</sup> Based on the geometric mean of each test item concentration of Fluroxypyr-MHE and the degradation product Fluroxypyr acid

( - ) Values not reliable, control CV exceeded the effect level

**Table A 2.2.1-13: Summary of Biological Results based on Nominal and Actual concentrations of ADM.3304.H.1.A and Shoot Fresh Weight**

	Growth rate (shoot fresh weight in g) [µg/L]	Yield (shoot fresh weight in g) [µg/L]
<b>Nominal concentrations</b>		
<b>14-day EC<sub>50</sub></b>	81.2 <sup>1)</sup>	36.7 <sup>2)</sup>
<b>95% Conf. Limits</b>	66.2 - 99.8	30.3 - 44.5
<b>14-day EC<sub>20</sub></b>	21.3 <sup>1)</sup>	12.4 <sup>2)</sup>
<b>95% Conf. Limits</b>	16.0 - 27.3	9.31 - 15.6
<b>14-day EC<sub>10</sub></b>	10.6 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	7.36 - 14.3	-
<b>14-day NOEC</b>	9.54 <sup>3)</sup>	9.54 <sup>3)</sup>
<b>14-day LOEC</b>	30.5 <sup>3)</sup>	30.5 <sup>3)</sup>
<b>Actual concentrations<sup>4)</sup></b>		
<b>14-day EC<sub>50</sub></b>	65.8 <sup>1)</sup>	29.2 <sup>2)</sup>
<b>95% Conf. Limits</b>	53.5 - 81.2	24.1 - 35.4
<b>14-day EC<sub>20</sub></b>	16.9 <sup>1)</sup>	9.76 <sup>2)</sup>
<b>95% Conf. Limits</b>	12.6 - 21.6	7.38 - 12.3
<b>14-day EC<sub>10</sub></b>	8.30 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	5.74 - 11.2	-
<b>14-day NOEC</b>	8.11 <sup>3)</sup>	8.11 <sup>3)</sup>
<b>14-day LOEC</b>	22.9 <sup>3)</sup>	22.9 <sup>3)</sup>
<b>Actual concentrations<sup>5)</sup></b>		
<b>14-day EC<sub>50</sub></b>	53.9 <sup>1)</sup>	22.7 <sup>1)</sup>
<b>95% Conf. Limits</b>	42.9 - 67.6	18.1 - 28.4
<b>14-day EC<sub>20</sub></b>	12.3 <sup>1)</sup>	5.65 <sup>1)</sup>
<b>95% Conf. Limits</b>	8.81 - 16.3	4.07 - 7.45
<b>14-day EC<sub>10</sub></b>	5.69 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	3.72 - 8.03	-
<b>14-day NOEC</b>	3.53 <sup>3)</sup>	3.53 <sup>3)</sup>
<b>14-day LOEC</b>	22.3 <sup>3)</sup>	22.3 <sup>3)</sup>

<sup>1)</sup> Probit analysis following normal distribution

<sup>2)</sup> Probit analysis following logistic distribution

<sup>3)</sup> Following Dunnett's-t-test (left-sided,  $p \leq 0.05$ )

<sup>4)</sup> Based on the geometric mean of each test item concentration of 2,4-D ester and the degradation product 2,4-D acid

<sup>5)</sup> Based on the geometric mean of each test item concentration of Fluroxypyr-MHE and the degradation product Fluroxypyr acid

( - ) Values not reliable, control CV exceeded the effect level

**Table A 2.2.1-14: Summary of biological results based on nominal and actual concentrations of ADM.3304.H.1.A and Shoot Dry Weight**

	Growth rate (shoot fresh weight in g) [µg/L]	Yield (shoot fresh weight in g) [µg/L]
<b>Nominal concentrations</b>		
<b>14-day EC<sub>50</sub></b>	403 <sup>1)</sup>	95.4 <sup>1)</sup>
<b>95% Conf. Limits</b>	297 - 570	76.5 - 118
<b>14-day EC<sub>20</sub></b>	41.6 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	25.2 - 61.2	-
<b>14-day EC<sub>10</sub></b>	-	-
<b>95% Conf. Limits</b>	-	-
<b>14-day NOEC</b>	30.5 <sup>2)</sup>	9.54 <sup>2)</sup>
<b>14-day LOEC</b>	97.7 <sup>2)</sup>	30.5 <sup>2)</sup>
<b>Actual concentrations<sup>4)</sup></b>		
<b>14-day EC<sub>50</sub></b>	344 <sup>1)</sup>	77.4 <sup>1)</sup>
<b>95% Conf. Limits</b>	251 - 492	61.8 - 96.5
<b>14-day EC<sub>20</sub></b>	33.1 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	19.8 - 49.2	-
<b>14-day EC<sub>10</sub></b>	-	-
<b>95% Conf. Limits</b>	-	-
<b>14-day NOEC</b>	22.9 <sup>2)</sup>	8.11 <sup>2)</sup>
<b>14-day LOEC</b>	77.2 <sup>2)</sup>	22.9 <sup>2)</sup>
<b>Actual concentrations<sup>5)</sup></b>		
<b>14-day EC<sub>50</sub></b>	278 <sup>1)</sup>	67.6 <sup>1)</sup>
<b>95% Conf. Limits</b>	204 - 395	52.7 - 86.2
<b>14-day EC<sub>20</sub></b>	27.3 <sup>1)</sup>	-
<b>95% Conf. Limits</b>	16.0 - 40.8	-
<b>14-day EC<sub>10</sub></b>	-	-
<b>95% Conf. Limits</b>	-	-
<b>14-day NOEC</b>	22.3 <sup>2)</sup>	3.53 <sup>2)</sup>
<b>14-day LOEC</b>	74.3 <sup>2)</sup>	22.3 <sup>2)</sup>

<sup>1)</sup> Probit analysis following normal distribution

<sup>2)</sup> Probit analysis following logistic distribution

<sup>3)</sup> Following Dunnett's-t-test (left-sided,  $p \leq 0.05$ )

<sup>4)</sup> Based on the geometric mean of each test item concentration of 2,4-D ester and the degradation product 2,4-D acid

<sup>5)</sup> Based on the geometric mean of each test item concentration of Fluroxypyr-MHE and the degradation product Fluroxypyr acid

(-) Values not reliable, control CV exceeded the effect level

The average pH-value was determined to be  $8.18 \pm 0.74$ , the average temperature was measured to be  $20.8 \pm 0.3$  °C and the oxygen saturation was determined to be  $119 \pm 16$  %. The test item had no influence on the pH-value of the test solutions.

### C. Validity criteria

Since the C.V. for shoot fresh weight yield was below 35 % (19.2 %, respectively) and a doubling of shoot biomass and length was reached within the test duration, the mean control growth rates and variability were considered acceptable. Furthermore, control plants did not show any visual symptoms of chlorosis and have been be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the aqueous growth medium.

### III. CONCLUSION

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to ADM.3304.H.1.A for 14 days, the  $E_rC_{50}$  and  $E_yC_{50}$  values based on total shoot length were 165 µg/L (nominal) and 68.8 µg/L (nominal) respectively. The NOEC for growth rate and yield was 30.5 µg/L (nominal).

The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (fresh weight) were 81.2 µg/L (nominal) and 36.7 µg/L (nominal) respectively. The NOEC for growth rate and yield was 9.54 µg/L (nominal).

The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (dry weight) were 403 µg/L (nominal) and 95.4 µg/L (nominal). The NOEC for growth rate and yield was 30.5 µg/L (nominal) and 9.54 µg/L (nominal), respectively.

**A 2.2.2                      KCP 10.2.2                      Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms**

Since the formulation AG-CDF1-480 EC will not persevere as formulation in water, the long-term toxicity to aquatic organisms can be extrapolated from active substances data. Accordingly, chronic toxicity tests to aquatic organisms are not deemed necessary.

**A 2.2.3                      KCP 10.2.3                      Further testing on aquatic organisms**

No additional data submitted.

## A 2.3 KCP 10.3 Effects on arthropods

### A 2.3.1 KCP 10.3.1 Effects on bees

#### A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

#### A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

#### A 2.3.1.1.1.1 Study 1: Acute oral toxicity to honey bees

The following bee toxicity study performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in line with OECD 213 and 214 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48 h LD<sub>50</sub> (oral) &gt; 684.0 µg product/bee (corresponding to &gt; 300.1 µg sum of a.s./bee)</p> <p>48 h LD<sub>50</sub> (contact) &gt; 911.6 µg product/bee (corresponding to &gt; 400.0 µg sum of a.s./bee)</p>
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Reference:	KCP 10.3.1.1/01
Report	Acute toxicity of AG-CDF1-480 EC to the honeybee <i>Apis mellifera</i> L. under laboratory conditions. Franke, M., (2015). 14 10 48 114 B (report number)
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	None -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Liquid  
**Lot/batch #:** D-N6401  
**Concentration/Purity:** 30.34 g/L Clopyralid, 365.34 g/L 2,4-D, 74.72 g/L Fluroxypyr (analysed)  
**Stability of test compound:** Expiry date: June 2016
- Vehicle and/or positive control:** Contact test: Deionised water with wetting agent (Tween®80)  
Oral test: 50 % (w/v) sucrose solution
- Test animals (Species)** *Apis mellifera* L. subspecies *iberica* G. (honeybee), young adult worker bees  
**Age at test start:** Young bees  
**Source:** Beekeeper Joaquin Cordero, Paseo de Colón No. 19, 41370 Cazalla (Seville), Spain  
**Acclimation period:** 1 h  
**Feeding:** Food (50 % (w/v) sucrose solution) was provided *ad libitum* after application.  
**Test cages:** For the observation of the bees disposable cages of

	cardboard with holes in the bottom for ventilation and a glass plate in front are used (95 mm × 50 mm × 65 mm, length × width × height)
<b>Number of study organisms:</b>	10 per test unit
<b>Number of animals per test vessel:</b>	10 bees per cage per treatment group
<b>Number of replicates:</b>	3 replicates
<b>Control (untreated variant):</b>	<u>Contact test:</u> Deionised water with wetting agent (Tween®80)
	<u>Oral test:</u> 50 % (w/v) sucrose solution
<b>Reference standard:</b>	Dimethoate EC 400 (analysed content of dimethoate: 400.9 g/L)

#### 4. Environmental conditions during testing

<b>Temperature:</b>	24.2 – 26.9 °C
<b>Relative humidity:</b>	50.1 – 69.3%
<b>Photoperiod:</b>	Constant darkness throughout the test

### B. STUDY DESIGN AND METHODS:

- In-life dates:** 04.11.2014 – 06.11.2014
- Experimental design**

Contact (LD<sub>50</sub> test):  
 48 hours; 5 dose rates of test item, 3 replicates each consisting of 10 bees.

Oral (LD<sub>50</sub> test):  
 48 hours; 5 dose rates of test item; 3 replicates each consisting of 10 bees.  
 The mortality and the behaviour were assessed 4, 24 and 48 h after application in the contact and oral toxicity tests.

**Test concentrations:** Contact test: 911.6, 638.1, 446.7, 312.7, 218.9 µg product/bee (nominal).

Oral test: 648.0, 410.4, 246.2, 147.7, 88.6 µg product/bee (nominal).

**Test duration:** 48 h
- Observations:** Mortality: Number of dead bees after 4, 24 and 48 hours.  
  
Behaviour: The number of bees showing normal behaviour and the number of bees showing any behavioural abnormalities after 4, 24 and 48 hours e.g. health, effectiveness (paralysis, lateral position, lying on the back, abnormal movements), any differences in activity, in position within the cage (e.g. all on the bottom) or any abnormality in amount and colour of excretion were documented. All observations were made in comparison with the control bees.
- Statistics:** Statistical program used: ToxRat Professional 3.0. beta (2014).  
  
LD<sub>50</sub>:
  - Contact / oral test item: no calculation
  - Oral and contact reference item: Probit (linear maximum likelihood regression)

Statistical significance of mortality values:

- Contact and oral test item: Fisher's Exact Binominal Test with Bonferroni Correction ( $p \leq 0.05$ )
- Contact and oral reference item: Fisher's Exact Binomial Test with Bonferroni Correction ( $p \leq 0.05$ )

## II. RESULTS AND DISCUSSION

### A. Contact toxicity test

In the contact toxicity test, no mortality occurred in the control groups either treated with deionised water or tween solution after 48 hours exposure.

In the test item group, no mortality on treated bees occurred at all tested dose rates up to 911.6 µg AG-CDF1-480 EC/bee.

Thus, the LD<sub>50</sub> (48 h) was determined to be > 911.6 µg AG-CDF1-480 EC/bee, which is corresponding to > 400.0 µg a.s./bee.

Behavioural abnormalities were observed at a low level and thereof, only at the higher dose rates.

The respective LD<sub>50</sub> values of the contact toxicity test are presented in **Table A 2.3.1.1-1**.

### B. Oral toxicity test

In the oral toxicity test, no mortality occurred in the control fed with pure sucrose solution.

In the test item treatment group, no statistically significant mortality occurred at all tested dose rates, but nevertheless, slight mortality of 10.0 and 3.3 % was observed at the higher dose rate of 684.0 and 410.4 µg consumed AG-CDF1-480 EC/bee, respectively.

Thus, the LD<sub>50</sub> (48 h) was > 684.0 µg consumed AG-CDF1-480 EC/bee, which is corresponding to > 300.1 µg consumed a.s./bee.

Behavioural abnormalities were only observed at a very low level and thereof, at the higher dose rates. The respective LD<sub>50</sub> values of the oral toxicity test are presented in **Table A 2.3.1.1-1**.

**Table A 2.3.1.1-1: Contact and oral toxicity of AG-CDF1-480 EC to honeybees (*Apis mellifera* L.)**

Assessment	Contact toxicity test		Oral toxicity test*	
	24 h	48 h	24 h	48 h
LD <sub>50</sub> value				
LD <sub>50</sub> [µg product/bee]	> 911.6	> 911.6	> 684.0	> 684.0
LD <sub>50</sub> [µg a.i./bee]	> 400.0	> 400.0	> 300.1	> 300.1

\* Values refer to consumed dosages

### C. Validity of the tests

The validity criteria of the acute honeybee study (contact and oral test) with AG-CDF1-480 EC are given in **Table A 2.3.1.1-2**. The contact and oral LD<sub>50</sub> (24 h) of the reference item was calculated to be 0.208 µg dimethoate/bee and 0.170 µg dimethoate/bee, respectively. All validity criteria have been met in this study.



**Table A 2.3.1.1-2: Validity criteria of the acute honeybee study**

Validity criterion		Occurred / calculated	Recommended
<b>Control mortality (24 h)</b>	Contact test: - Deionised water - Tween solution	0.0 % 0.0 %	≤ 10 %
	Oral test: - Sucrose solution	0.0 %	≤ 10 %
<b>LD<sub>50</sub> – value of the reference (24 h)</b>	Contact toxicity test	0.208 µg a.s./bee	0.10 – 0.30 µg a.s./bee
	Oral toxicity test	0.170 µg a.s./bee	0.10 – 0.35 µg a.s./bee

### III. CONCLUSION

The acute contact and oral toxicity of AG-CDF1-480 EC was tested on honeybees under laboratory conditions.

In the contact toxicity test, no mortality occurred after thoracic application of 911.6, 638.1, 446.7, 312.7 and 218.9 µg AG-CDF1-480 EC/bee, after 48 hours.

The LD<sub>50</sub> (48 h) was > 911.6 µg AG-CDF1-480 EC/bee, which is corresponding to > 400.0 µg a.s./bee in the contact toxicity test.

In the oral toxicity test, no statistically significant mortality occurred at the tested dose rates of 684.0, 410.4, 246.2, 147.7 and 88.6 µg consumed AG-CDF1-480 EC/bee after 48 hours.

The LD<sub>50</sub> (48 h) was > 684.0 µg consumed AG-CDF1-480 EC/bee, which is corresponding to > 300.1 µg consumed a.s./bee in the oral toxicity test.

#### A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

##### A 2.3.1.1.2.1 Study 1: Acute contact toxicity to honey bees

Please refer to Study 1 included in Point A 2.3.1.1.1.1.

#### A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

The following bee toxicity study performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in 2015/2016 in line with modified Decourtye et al. (2005) and EFSA (2013) and was evaluated against the current OECD 245 guideline published in 2017.</p> <p>The test design adhered to indications of OECD 245 and all validity criteria were met, however no analytical measurements of the test item was conducted as they were not required in the previous version of the guideline. It is noted that in the larvae toxicity test summarised below under 10.3.1.3/01 the test item (measured in terms of particular active compounds) was stable in the prepared stock solutions. In addition to that it is noted that in aquatic toxicity studies ester forms of 2,4-D and fluroxypyr declined, but they were transformed into acid forms and on balance the concentrations of 2,4-D and fluroxypyr were maintained at 80-120% of nominal over period of 2-4 days. Clopyralid was stable in all aquatic studies. Overall, based on available data it is not expected that active substances present in ADM.3304.H.1.A would decline in the feeding solutions prepared on a daily basis and offered to bees. Taking this into account, the zRMS is of the opinion that lack of analytical measurements should not invalidate the test results.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p>
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	10-d LD <sub>50</sub> = 85.86 µg consumed product/bee/day 10-d LC <sub>50</sub> = 2720 mg product/L NOED = 29.91 µg consumed product/bee/day NOEC = 1000 mg product/L
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Reference:	<b>KCP 10.3.1.2/01</b>
Report	AG-CDF1-480 EC: A laboratory study to determine the chronic oral toxicity on the adult honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae). Noël, E., (2016). 307SRFR15C05 (report number)
Guideline(s):	Decourtye et al. (2005) modified and EFSA (2013)
Deviations:	Minor (see commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish clear liquid  
**Lot/batch #:** N6504  
**Concentration/Purity:** 29.2 g/L Clopyralid, 566.8 g/L 2,4-D 2-EHE, 110.7 g/L Fluroxypyr (analysed)  
**Stability of test compound:** Expiry date: July 2017
- Vehicle and/or positive control:** Negative control: untreated sucrose solution  
Reference toxic item: Rogor Plus (Dimethoate 400 g/L)
- Test animals (Species)** Newly emerged adult worker bees of the same race (*Apis mellifera mellifera* L.), similar age and same health status. No chemical substance had been used during at least one month prior to this study. Brood frames with capped cells were taken out from the colonies, brought to the laboratory and put inside an incubator at the study climatic conditions.  
Bees were collected as they emerged, and none of the captured bees exceeded 3 days old when the first application started.  
They were separated in groups of 20 adults with the help of mouth vacuum and put in test cages. Each test unit were labelled with the study number and a unique test unit number.  
**Age at test start:** 1 – 3 days old  
**Source:** SynTech Research  
**Acclimation period:** Before the start of the exposure period, the bees were fed *ad libitum* with sucrose solution in distilled water with a final concentration of 500 g/L (50 % w/v) and with pollen during the first 24 hours  
**Feeding:** During the 10-day exposure period, treatments were added to the sucrose solution and provided to the bees *ad libitum* using the sucrose feeder (replaced every day).  
**Test cages:** The test units consisted of an untreated and well ventilated 350 cm<sup>3</sup> stainless steel cage with a removable glass sheet

- as front side. A hole on the top of the cage allow the introduction of a syringe (sucrose feeder) containing the test solutions.
- Number of animals per test vessel:** 20 bees per cage  
**Number of replicates per treatment group:** 3 replicates  
**Control (untreated variant):** Control item: 500 g/L sucrose solution in distilled water  
**Reference standard:** Rogor Plus (1 mg a.s./L sucrose solution)
- 4. Environmental conditions during testing**  
**Temperature:** 31.0 – 35.0 °C  
**Relative humidity:** 51.0 – 71.0 %  
**Photoperiod:** Constant darkness throughout the test

## B. STUDY DESIGN AND METHODS:

- 1. In-life dates:** 28.09.2015 – 10.10.2015
- 2. Experimental design**  
The potential adverse effects of the test item AG-CDF1-480 EC on the survival of young adults of honey bees *Apis mellifera* L. (Hymenoptera: Apidae) was investigated under laboratory conditions, following a 10-day oral exposure at the concentrations of 37.03, 111.1, 333.3, 1000 and 3000 mg f.p./L sucrose solution. A reference item (400 g/L dimethoate) was also applied at the dose of 1 mg a.s./L sucrose solution to demonstrate the relative susceptibility of the test organisms and the sensitivity of the test system. Untreated sucrose solution control was included to assess the natural mortality of the test organisms and for comparison with the test item treatment.
- Test concentrations:** Test item: AG-CDF1-480 EC (37.03, 111.1, 333.3, 1000 and 3000 mg f.p./L sucrose solution)  
**Test duration:** 10 days
- 3. Observations:** Direct treatment effects (mortality and other observed biological effects) at daily intervals during 10-day exposure period.  
Temperature and humidity were recorded continuously
- 4. Statistics:** Mortality data (except toxic reference results) were analysed with the statistical software Minitab® Release14 (ANOVA plus Dunnett's (p ≤ 0.05) after log transformation) to determine any significant differences between untreated sucrose solution control and test item treatments and with the EPA Probit analysis program V1.5 to determine a rate-response relationship

## II. RESULTS AND DISCUSSION

### A. Biological findings

The results of the test are shown in the table below.

**Table A 2.3.1.2-1: Summary of *A. mellifera* chronic oral toxicity data**

Item		AG-CDF1-480 EC
Test organism / Exposure		Honey bees Adult / oral chronic
Assessment		Mortality after 10 days [%]*
Target concentrations [mg f.p./L sucrose solution]	Consumed doses [ $\mu$ g f.p./bee/day]**	
Untreated sucrose solution control		13.33
37.03	0.976	3.846 <sup>[1]</sup> (ns)
111.1	2.644	3.846 <sup>[1]</sup> (ns)
333.3	8.674	7.692 <sup>[1]</sup> (ns)
1000	29.91	9.615 <sup>[1]</sup> (ns)
3000	95.18	57.69 <sup>[1]</sup> (s)
NOEC [mg f.p./L]		1000
Corresponding NOED [ $\mu$ g f.p./bee/day]		29.91
LOEC [mg f.p./L]		3000
Corresponding LOED [ $\mu$ g f.p./bee/day]		95.18
LC <sub>50</sub> [mg f.p./L]		2720 (95% confidence limits: 1957 - 3746)
Corresponding LD <sub>50</sub> [ $\mu$ g f.p./bee/day]		85.86 (95% confidence limits: 60.66 - 120.2)

\* Based on the number of dead organisms. \*\* Based on actual consumption of the test item solution. NA = Not Applicable.

[1] Value corrected from untreated sucrose solution control, according to Abbott (1925). f.p.= formulated product. Treatment groups significantly (s) or not significantly (ns) different from the untreated sucrose solution control (ANOVA plus Dunnett's after Log transformation).

## B. Validity criteria

The study is valid since cumulative mean mortality did not exceed 15 % in the untreated sucrose solution control (actual value: 13.33 %) and cumulative mean mortality in the toxic reference item resulted in a 10-day mortality higher than 50% (actual value: 100 %).

## III. CONCLUSION

There was no significant difference between mean mortalities in the untreated sucrose solution control and the four lowest concentrations of the test item test of 37.03, 111.1, 333.3, 1000 mg f.p./L sucrose solution.

Thus the NOEC was 1000 mg f.p./L sucrose solution, the LOEC was 3000 mg f.p./L sucrose solution and the LC<sub>50</sub> was calculated to be 2720 mg f.p./L sucrose solution (95 % confidence limits: 1957 – 3746 mg f.p./L sucrose solution).

This corresponds to a NOED of 29.91  $\mu$ g f.p./bee/day, a LOED of 95.18  $\mu$ g f.p./bee/day and a LD<sub>50</sub> calculated to be 85.86  $\mu$ g f.p./bee/day (95 % confidence limits: 60.66 – 120.2  $\mu$ g f.p./bee/day), based on actual consumption of the test solution.

This chronic bee study with AG-CDF1-480 EC was conducted in 2015. As the current test guideline (OECD 245) was published in 2017, the study was conducted in accordance to Decourtye et al. (2005) modified and EFSA Guidance Document (2013). Therefore, no analytical measurements of the test solutions are available. However, the test shows a clear dose-response relationship and the results are in line with the current available toxicity data for bees. Acute oral and contact studies with the formulation AG-CDF1-480 EC showed low toxicity to bees (oral LD<sub>50</sub> > 684  $\mu$ g product/bee and contact LD<sub>50</sub> > 911.6  $\mu$ g product/bee). The results of the larvae study with the product showed a NOEL of 77.0  $\mu$ g product/larva. The LD<sub>50</sub> of the chronic study of 85.86  $\mu$ g product/bee/d can therefore be considered reliable.

### A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

The following bee toxicity study conducted with AG-CDF1-480 EC1 was provided in support of the assessment. From an ecotoxicological point of view, the formulation EC1 is equivalent to EC as no change in the percentage of active substances and/or inclusion of classified co-formulant(s) is involved in the composition of the formulation EC1. A comparison of composition of both formulations is given in the in the confidential dossier of this submission (Registration Report – Part C).

Comments of zRMS:	<p>The study was performed in line with OECD 239 with minor deviations.</p> <p>It was noted in the study report that the Study Plan stated that the larvae would be assigned to treatment and accepted onto the study on Day 2, however, the larvae were assigned to treatment and accepted onto the study on Day 3. Day 3 is when they were first fed the dosed feed and so it made sense to assign the bees to treatment immediately prior to feeding on Day 3. As nothing study specific occurred on Day 2, this had no impact on the integrity or outcome of the study. On Day 1 and Day 2 (prior to acceptance of larvae onto the study) the larvae were fed 10 µL of untreated diet A per day. The feeding schedule used on Day 1 and Day 2 is different to the OECD guidelines, which state that the larvae should be grafted on to 20 µL of untreated diet A and not fed anything on the following day. Based on previous work, the testing facility feels that the initial feeding quantity of 20 µL is considered too large and increases the chance of the larvae being drowned. The risk of the larvae drowning on subsequent days with larger quantities of diet is reduced due to the fact that as the study progresses the larvae grow. By grafting the larvae onto 10 µL diet and feeding the remaining 10 µL the following day there is no change to the total amount of food received by each larva. On Day 3 they were fed 20 µL of diet B treated with the test solutions. On Day 4, Day 5, and Day 6 the larvae were fed 30, 40 and 50 µL diet C treated with the test solutions respectively.</p> <p>The data logger used to monitor temperature and humidity for this study records high humidity readings as 0. This is due to a fault with the humidity sensor such that when it becomes wet it records 100% humidity as 0. However, the fact that this fault occurred suggests that the humidity in the chamber was high (~95%). Apart from this the humidity was as required, except during observations, for D3-8 and D15-22. Between D8 and D15 the humidity was higher than required (~95% rather than ~80%). This may have been due to the NaCl solution not being sufficiently saturated. There was no record effect on control emergence and so this is not thought to have affected the integrity or outcome of the study. The temperature was within tolerance (except during observations) except for the final week (Days 15-22) where the temperature recorded was within 0.5oC of required. There was no recorded effect on control mortality or emergence. These deviations did not have any impact on the integrity or outcome of the study.</p> <p>In zRMS opinion deviations described above had no impact on the outcome of the study since all the validity criteria were met and no effects on development of larvae in the untreated control was observed.</p> <p>The endpoints are expressed as nominal concentrations since the measured concentrations of the active substances were maintained at 80-120% of nominal.</p> <p>It is noted that at the dose set as NOED (77.0 µg product/larva) the pupal mortality exceeded 10% and adult emergence was &gt;10% lower comparing to controls. Although these effects were statistically not significant, they could be of biological relevance. Since no ED<sub>10</sub> values were calculated, which could be used instead of uncertain NOED, the zRMS is of the opinion that the NOED should be set to the next lower dose, which produced effect &lt;10%, i.e. 250 ppm (38.5 µg product/larva).</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>NOED = 38.5 µg product/larva (corresponding to NOEC of 250 ppm)</p>
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	<p>During the commenting period the Applicant disagreed with lowering the NOED value by the zRMS indicating that the endpoint based on valid statistical methods should always be considered as the relevant endpoint to be used in a risk assessment. It was further pointed out that there is neither a scientific justification to use the 10% for ecological relevance nor is this a requirement according to the test guideline OECD 239 and that the NOED of 77.0 µg product/larvae was determined based on the sound scientific statistical analysis and is in line with the endpoint determination as outlined in the OECD test guideline 239. The Applicant indicated also that the ED<sub>10</sub> values were actually calculated and substantiated this with table copied from the study report.</p> <p>First of all the zRMS would like to point out that the Applicant copied table presenting actually LCx/LDx values (Table A 2.3.1.3-4), which are based on mortality and are thus not equivalent to ECx/EDx values, which should be based on adult emergence. Taking into account that no effect values based on adult emergence were calculated in the study report, the zRMS comment in this area still stands.</p> <p>The zRMS would like to further point out that although there are no formal rules regarding the biological relevance of effects at &gt;10%, it should be noted that the NOEC/NOED value should not be lower than EC<sub>10</sub>/ED<sub>10</sub>. This approach is commonly agreed within the EU and applied to the active substance evaluations. Moreover, at the Central Zone level there were multiple comments in the past when &gt;10% effects were observed at the NOEC/NOED value with requests to invalidate this endpoint. Therefore, the approach taken by the zRMS is in line with the approach taken at the EU level, as well as requested by MS in the course of the zonal assessments.</p> <p>The zRMS would like also to emphasise that actually, in line with indications of OECD 239, ECx/EDx values should be calculated, provided that it is possible based on the study results:</p> <p><i>If data allows an EC<sub>50</sub>/ED<sub>50</sub> and any ECx/EDx, including the associated lower and upper confidence limits, is/are calculated for emerged adult bees on D22</i></p> <p>Furthermore, in line with Commission Regulation (EU) No 283/2013 and 284/2013, calculation of EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values from the bee larvae study is mandatory. Therefore presentation of these endpoints is actually a data requirement, which was not fulfilled in this case and resulted with lowering the endpoint.</p> <p>As the test design was sufficient to cover ECx values and a dose response was observed, the ECx/EDx values should have been calculated and in case the ED<sub>10</sub> value was lower than the NOED, it would be considered to be most relevant endpoint for the risk assessment purposes, which is in line with indications of EFSA bee guidance (2013):</p> <p><i>In line with the latest version of the data requirements under 1107/2009, when assessing the long-term/chronic/reproductive toxicity of an active substance the toxicological NOEC endpoint may be used; however, it is accepted that assessment using a toxicological effective concentration (ECx) endpoint is from a scientifically and statistically perspective more appropriate. Therefore, if possible an EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub>, when required, along with corresponding 95 % confidence intervals. If an ECx approach is used, a NOEC should still be determined</i></p> <p>Taking this into account, in case the Applicant wishes the zRMS to recommend the relevant endpoint for the risk assessment and avoid lowering of the NOED value, respective ECx/EDx values must be calculated based on the study results. Until that time, the NOED remains reduced as based on the available data it may be higher than ED<sub>10</sub> value.</p>
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Reference:	KCP 10.3.1.3/01
Report	AG-CDF1-480 EC1: In vitro 22-day toxicity test - repeated exposure to larval stage honeybee ( <i>Apis mellifera</i> L.). Wilkins, S., (2018). FR/000764 (report number)
Guideline(s):	OECD Guidance Document 239 (2016): Honey Bee ( <i>Apis mellifera</i> ) Larval Toxicity Test Following Repeated Exposure
Deviations:	Minor (see the commenting box above) -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC1  
**Description:** Yellowish liquid  
**Lot/batch #:** N6702-A  
**Concentration/Purity:** Clopyralid – 28.9 g/L  
2,4-D – 560.8 g/L (as ester)  
Fluroxypyr – 108.6 g/L (as ester)  
**Stability of test compound:** Expiry date: 12<sup>th</sup> February 2019
- Vehicle and/or positive control:** Negative control: untreated sucrose solution  
Reference toxic item: Technical Dimethoate (99.8 % w/w)
- Test animals (Species)** Larval honey bees (*Apis mellifera* L.) were obtained from colonies belonging to the Fera National Bee Unit. The colonies used to provide the bees are not managed according to GLP and no claim for GLP compliance is made for them. However, the colonies are maintained and managed according to NBU Standard Operating Procedures by Fera staff. The frames from the colonies were collected from the hives and brought into the CCSS bee facilities laboratory in preparation for grafting (GLP compliant facility).  
  
The colonies used to provide the larvae were queen-right and showed no signs of American foul brood (AFB) or European foul brood (EFB). The colonies were not treated with a varroacide or antibiotics within the 4 weeks prior to collection.  
  
**Age at test start:** Larvae were assigned to treatment on Day 3 and it was at this point that the larvae were accepted onto the study.  
**Source:** Fera National Bee Unit  
**Acclimation period:** Before the start of the exposure period, the bees were fed *ad libitum* with sucrose solution in distilled water with a final concentration of 500 g/L (50 % w/v) and with pollen during the first 24 hours  
**Feeding:** The larvae were fed different diet solutions on each day of the study with the dosing commencing on Day 3 and continuing for 4 days.  
  
The larval diet consists of two components i) diet solution (either A, B or C dependent on developmental stage of the

larvae), which was prepared in advance, and ii) royal jelly. To accomplish the diet (A, B or C) the diet solution (A, B or C) was mixed with royal jelly in a ratio of 1:1. For each diet, the diet solutions were filtered through a 0.22 µm syringe filter before being mixed with the royal jelly. The compositions of the diet solutions used was as follows:

*Diet Solution A* is an aqueous solution containing 2 % yeast extract, 12 % glucose and 12 % fructose

*Diet Solution B* is an aqueous solution containing 3 % yeast extract, 15 % glucose and 15 % fructose

*Diet Solution C* is an aqueous solution containing 4 % yeast extract, 18 % glucose and 18 % fructose.

(All figures expressed in w/w).

Quantities and weights used to make up each diet solution are recorded in the raw data.

On Day 1 and Day 2 (prior to acceptance of larvae onto the study) the larvae were fed 10 µL of untreated diet A per day. The feeding schedule used on Day 1 and Day 2 is different to the OECD guidelines, which state that the larvae should be grafted on to 20 µL of untreated diet A and not fed anything on the following day. Based on previous work, the testing facility feels that the initial feeding quantity of 20 µL is considered too large and increases the chance of the larvae being drowned. The risk of the larvae drowning on subsequent days with larger quantities of diet is reduced due to the fact that as the study progresses the larvae grow. By grafting the larvae onto 10 µL diet and feeding the remaining 10 µL the following day there is no change to the total amount of food received by each larva. On Day 3 they were fed 20 µL of diet B treated with the test solutions. On Day 4, Day 5, and Day 6 the larvae were fed 30, 40 and 50 µL diet C treated with the test solutions respectively. The diet was warmed in the incubator prior to each feed.

#### **Test system:**

Each queen was confined to a brood frame containing empty cells and emerging worker brood in a queen frame cage within their own colony. In order to ensure the production of enough larvae queens from a number of colonies were caged at one time. The combs were left in the colony for four days to ensure that larvae of the correct age would be present. On test Day 1, the combs were taken to the GLP compliant laboratory to allow grafting of the young larvae into the test cells.

Larvae were grafted and reared using grafting cells with a 9 mm internal diameter (Thornes, UK) housed in a 48 well tissue culture plate (CELLSTAR®, Greiner Bio One, Germany). Each cell was placed into one well in the tissue culture plate. The wells had been previously half-filled with a piece of dental roll wetted with sterilant (12ml/litre of Milton's sterilising solution with 15.5% glycerol). The plates were then sterilised by UV exposure for a minimum of 20 minutes. The plates were warmed in the incubator prior to the grafting of the larvae.



The larvae were grafted onto 10 µL of pre-warmed untreated Diet A (50 % fresh royal jelly, 50 % aqueous solution containing 2 % yeast extract, 12 % glucose and 12 % fructose (all w/w)) and placed in the bottom of each cell by delicately transferring from the comb to the cell on the surface of the diet substance using a grafting tool. When the plates were complete with 48 larvae, they were placed into a Plexiglas container in the incubator. The following two days (Days 2 and 3) the larvae were examined under a dissection microscope and any dead, damaged or duplicate (2 or more in 1 cell) larvae were removed.

**Number of animals per test vessel:** 48  
**Number of replicates per treatment group:** Larvae from 3 hives with 1 plate per treatment rate containing 48 larvae (16 from each hive)  
**Control (untreated variant):** Untreated diet  
**Reference standard:** Technical Dimethoate at 1 concentration of 48 mg a.s./kg diet

#### 4. Environmental conditions during testing (except during observations and feeding)

**Temperature:** 34.0 – 35.0 °C

**Relative humidity:** Days 1 – 8:  
 ~ 95 %

(This was maintained chemically with the use of a dish of saturated K<sub>2</sub>SO<sub>4</sub> solution contained within the Plexiglas container)

Days 8 – 15:  
 ~ 80 %

(This was maintained chemically with the use of a dish of saturated NaCl solutionj contained within the Plexiglas container).

Days 15 – 22:  
 65 ± 10 %

**Photoperiod:**

(This was maintained by the incubator).  
 Constant darkness throughout the test

### B. STUDY DESIGN AND METHODS:

1. **In-life dates:** 12.07.2017 – 09.01.2018

2. **Experimental design** Three colonies were used to supply the larvae for each of 5 test item dose rates. A single plate was used per dose rate, using 16 larvae from each hive.

A record of the position of the larvae on the plates and the source colonies were maintained to allow any differences between colonies to be identified if necessary. A single plate was also set up for the controls and toxic reference item using larvae from the 3 colonies in the same manner as for the test item

**Test concentrations:** The test was performed at 5 nominal concentrations: 62.5, 125, 250, 500, and 1000 mg formulation/kg diet. These

doses are equivalent to a total dose of 9.63, 19.3, 38.5, 77, and 154 mg formulation/larva over the test period. This conversion is based on an assumed uptake of the complete offered dose within 140 µL of diet over the exposure period and an assumed weight of 1 µL of larval diet of 1.1 mg (as specified in the OECD Guidance document 239). The equivalent concentrations and doses in terms of each a.s and are given in Tables below. These conversions are based on the analysed content values given in the CoA (Certificate of the Analysis).

#### Treatment concentrations

mg formulation/kg	mg clopyralid /kg	mg 2,4-D/kg	mg fluroxypyr/kg
62.5	1.66	21.4	4.34
125	3.33	42.9	8.69
250	6.65	85.7	17.4
500	13.3	171.5	34.8
1000	26.6	342.9	69.5

#### Treatment dose rates

µg formulation/larva	µg clopyralid/larva	µg 2,4- D /larva	µg fluroxypyr/larva
9.63	0.26	3.30	0.67
19.3	0.51	6.60	1.34
38.5	1.02	13.2	2.68
77.0	2.05	26.4	5.35
154	4.10	52.8	10.7

**Test duration:**

22 days

### 3. Observations:

Treatment-related mortality checks were made on Days 3-8 before feeding. An immobile larva or one which did not respond when touched by a paintbrush, was recorded as dead and removed.

Between Days 8 and 15 the plates were examined daily and any larvae/pupae that appeared to be dead (black in colour or decomposing) or that showed signs of fungal growth were removed without record.

On the Day 15 mortality check any developing bees that were still larvae were removed along with any dead or fungal infected pupae that had not previously been removed. The number alive was recorded along with any observations of discolouration, abnormal positioning or incorrect growth stage (e.g. white eyed pupae).

On the Day 22 emergence assessments each plate was checked for the number of emerged adult bees. A bee was recorded as emerged if it had developed wings even if the bee had subsequently died. It was also recorded if the bees (whether alive or dead) had any morphological problems such as deformed wings. Of those bees that did not successfully emerge it was recorded whether the pupae had reached the final development stage (almost a fully

developed bee but with wings not expanded). The presence of mould on the plates would also have been recorded but was not present in the successful test of this study.

Samples of the stocks were taken on Day 3 and Day 6 for analysis by LC-DAD.

#### 4. Statistics:

The LOEC/D (lowest observable effect concentration/dose) and NOEC/D (no observable effect concentration/dose) were estimated in R v3.2.2. The LOEC/D and NOEC/D were tested using a step-down test for a trend in proportions. The test was undertaken as follows: The number tested and number responding was put into groups by quantity of treatment and ordered by quantity of treatment. A chi-square test for a significant linear trend in log odds of the number responding with quantity of treatment was applied to all of the data, and then sequentially after removing each highest quantity remaining in the data set. Because the interval between quantities of treatment was a fixed proportion this test has optimal detection power where the log odds of a response increases linearly with the log of the quantity of treatment. The LOEC/D was determined to be the maximum quantity of treatment in the data set with the lowest maximum quantity of treatment for which a significant ( $p < 0.05$ ) trend was detected. The NOEC/D was determined to be the next treatment quantity below the LOEC/D.

The LD/LC<sub>10, 20 and 50</sub> for Day 22 were generated in Genstat version 16.1.

## II. RESULTS AND DISCUSSION

### A. Analytical verification

The analysed content of clopyralid, 2,4 D and fluroxypyr in the stock, highest and lowest dosing solutions collected on study Days 3 and 6 showed an average deviation from expected nominal concentrations of less than 20 %.

### B. Biological findings

A summary of the mortality results for all treatment groups is presented in the tables below.

**Table A 2.3.1.3-1: Number of surviving larvae, at each treatment dose, at each observation time point**

Nominal concentration [mg formulation/kg larval diet]	Nominal Dose <sup>1</sup> [µg formulation /larva/test period]	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 15	Day 22
Control	0	48	48	48	48	48	47	45	44
62.5	9.63	48	48	48	48	48	47	44	44
125	19.3	48	48	48	48	48	47	46	46
250	38.5	48	48	48	48	48	47	46	45
500	77.0	48	48	47	47	47	46	41	39
1000	154	48	48	45	41	37	32	14	12
Toxic Reference	7.39	48	39	18	13	9	4	0	0

<sup>1</sup> These values were calculated using the nominal concentrations given and assuming total consumption of offered diet over the course of the exposure period.

**Table A 2.3.1.3-2: Percent cumulative mortality of larvae, at each test item treatment dose, at each observation time point (corrected using Abbott 1925 with control group mortality data)**

Nominal concentration [mg formulation/kg larval diet]	Nominal Dose <sup>1</sup> [µg formulation/ larva/ test period]	Day 4	Day 5	Day 6	Day 7	Day 8	Day 15	Day 22
62.5	9.63	0.0	0.0	0.0	0.0	0.0	2.2	0.0
125	19.3	0.0	0.0	0.0	0.0	0.0	-2.2	-4.5
250	38.5	0.0	0.0	0.0	0.0	0.0	-2.2	-2.3
500	77.0	0.0	2.1	2.1	2.1	2.1	8.9	11.4
1000	154	0.0	6.3	14.6	22.9	31.9	68.9	72.7

<sup>1</sup> These values were calculated using the nominal concentrations given and assuming total consumption of offered diet over the course of the exposure period.

**Table A 2.3.1.3-3: Larval mortality rate, pupal mortality rate and adult emergence rate at 22 days**

Nominal concentration [mg formulation/kg larval diet]	Larval mortality rate (%)	Pupal mortality rate (%)	Adult emergence rate (%)
Control	2.08	6.38	91.67
62.5	2.08	6.38	91.67
125	2.08	2.13	95.83
250	2.08	4.26	93.75
500	4.17	15.22	81.25
1000	33.33	62.50	25.00
Toxic Reference	91.67	100.00	0.00

A summary of the LC/D<sub>x</sub> values, NOEC/D & LOEC/D values is given in tables below.

**Table A 2.3.1.3-4: The Day 8 and 22 LC/D<sub>x</sub> values (95 % CI, confidence intervals) for the formulation (AG-CDF1-480 EC)**

LC <sub>x</sub> [mg formulation/kg]			LD <sub>x</sub> [µg formulation/larva]	
	Day 8*	Day 22	Day 8*	Day 22
LC/D <sub>10</sub>	> 500	472.2 (330.6 – 566.0)	> 77.0 <del>500</del>	72.73 (50.98 – 87.10)
LC/D <sub>20</sub>	> 500	561.8 (430.7 – 650.7)	> 77.0 <del>500</del>	86.51 (66.39 – 100.2)
LC/D <sub>50</sub>	> 1000	783.0 (682.1 – 889.6)	> 154	120.6 (105.1 – 137.0)

\* It was not possible to fit a suitable model to the Day 8 data or estimate 95% Confidence Intervals therefore these figures are by observation

**Table A 2.3.1.3-5: The Day 8 and 22 NOEC/D and LOEC/D values for the formulation (AG-CDF1-480 EC)**

	NOEC [mg/kg]	LOEC [mg/kg]	NOED [µg/bee]	LOED [µg/bee]
Formulation	500	1000	77.0	154
Clopyralid	13.3	26.6	2.05	4.10
2,4-D	171.5	343	26.4	52.8
Fluroxypyr	34.8	69.5	5.35	10.7

### C. Validity criteria

The acceptance criteria for this study were:

- ≤ 15% or less pre-pupal mortality (Day 3 – Day 8) in the controls (observed 2.08%).
- An adult emergence rate ≥ 70 % on Day 22 in the control plates (across all replicates) (observed 91.67%).
- A dimethoate pre-pupal mortality rate of ≥ 50 % (observed 91.67%).

### **III. CONCLUSION**

The overall NOEC for larvae and pupae was determined to be 500 mg formulation/kg diet. This corresponds to a NOED of 77.0 µg formulation/bee.

#### **A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects**

No additional data submitted.

#### **A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests**

No additional data submitted.

#### **A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees**

No additional data submitted.

## A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

### A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing

No additional data submitted.

### A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing and aged residue studies

#### A 2.3.2.2.1 Study 1: Toxicity to *Aphidius rhopalosiphi*

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 2000 mL product/ha</p>
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Reference:	KCP 10.3.2.2/01
Report	Effects of AG-CDF1-480 EC on the Parasitoid <i>Aphidius rhopalosiphi</i> , Extended Laboratory Study - Dose Response Test. Goßmann, A., (2014). 90311002 (report number)
Guideline(s):	Mead-Briggs M.A. <i>et al.</i> (2000)
Deviations:	None -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch #:** D-N6401  
**Concentration/Purity:** 2,4-D: 367.7 g/L  
 Fluroxypyr: 74.3 g/L  
 Clopyralid: 30.1 g/L  
**Stability of test compound:** Expiry date: 25.02.2014
- Vehicle and/or control:** 400 L deionised water/ha
- Test animals (Species)** Parasitoid (*Aphidius rhopalosiphi*)  
**Source:** Katz Biotech AG, Baruth, Germany  
**Age:** Adults, not older than 48 hours old  
**Number of test organisms:** 7 treatment groups (5 dose rates of the test item, control, reference item) with 6 replicates each containing 5 female parasitoids  
**Number of replicates:** Exposure period: 6 replicates per treatment group  
 Post-exposure period: 20 replicates per treatment group  
**Food:** *Ad libitum*: 10 % fructose solution  
**Acclimation** Approx. 1 – 2 days under test conditions  
**Test unit:** Hatching chambers:  
 Glass tubes with a length of approximately 15 cm and a diameter of 1.5 cm at the large and 0.5 cm at the small opening.  
  
 Exposure units:  
 Treated pots (13 cm in diameter) with 9 – 10 barley seedlings

(*Hordeum vulgare* ‘Xanadu’, BBCH 12) per pot. The plants were enclosed within a clear polyacrylic cylinder.

Post-exposure units

Untreated pots (13 cm in diameter) with barley seedlings (*Hordeum vulgare* ‘Xanadu’; 10 – 15 seedlings, 10 days old) infested with 100 – 150 host aphids of all developmental stages (*Rhopalosiphum padi*; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder.

**Untreated variant:**  
**Reference standard:**

Deionised water  
 Perfekthion (Dimethoate, 400.9 g/L, nominal: 400 g/L)

#### 4. Environmental conditions

**Temperature:** 20 – 22 °C

**Relative humidity:** 69 – 79 % (acclimatisation and exposure period)  
 86 – 87 % (post-exposure period, within the test units)

**Photoperiod** 16 h light/8h dark

**Light intensity:** 570 – 740 lux (acclimatisation and exposure period)  
 1090 – 1180 lux (parasitisation period)

11620 – 16040 lux (post-parasitisation period)  
**Ventilation** yes

### B. STUDY DESIGN AND METHODS:

1. **In-life dates** 22.04.2014 – 07.05.2014 (experimental phase)

2. **Experimental design** The spraying dilutions were sprayed onto leaves via laboratory spraying equipment. The parasitoids were exposed to dried residues on treated plant surfaces (barley plants). Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups with < 50 % corrected mortality survived females were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult parasitoids were removed after 24 hours and the aphid-infested plants left for further 11 – 12 days before the numbers of aphid mummies that had developed were assessed.

**Test concentrations:** Control, 24.7, 74.1, 222, 667 and 2000 mL product/ha (in 400 L water/ha) and reference item (10.0 mL Perfekthion/ha).

**Test duration:** Exposure time: 48 h

#### 3. Observations:

Mortality and behavior:

Observations of mortality were recorded approx. 2, 24 and 48 hours after test initiation. The number of parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead.

- Live: alive and apparently unaffected
- Affected: upright and attempting to walk, but with reduced coordination
- Moribund: on their back or side, twitching slightly
- Dead: not moving

Settling of the parasitoids:

To determine whether residues of the test item were repellent to the wasps, observations on the position of the individual insects were

made during the initial 3 h after their release. Five separate observations were made at approx. 30-minute intervals starting approx. 30 minutes after the introduction of all wasps. Each wasp was recorded as being on the:

- Plants: on the treated plants
- Cylinder: on the walls of the test arena
- Sand: on the sand below the plants

Fecundity:

Number of aphid mummies was counted 11 – 12 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hour parasitisation period ( $n = 17 - 20$ ). The number of live, dead or not found females after the 24 hours parasitisation period was documented in the raw data and reported in the final report. The fecundity assessment was performed where the corrected mortality ( $M_{\text{corr}}$ ) was  $\leq 50\%$ .

No fecundity assessment was performed for the reference item.

#### 4. Statistics:

Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis.

Settling data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ( $\alpha = 0.05$ ) and the Levene's test ( $\alpha = 0.05$ ).

Test item: Because settling data were normally distributed and homogenous the Dunnett's t-test (multiple comparison, one-sided,  $\alpha = 0.05$ ) was used.

Reference item: Because settling data were normally distributed and homogenous the Student t-test (pair wise comparison, one-sided,  $\alpha = 0.05$ ) was used.

Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ( $\alpha = 0.05$ ) and the Levene's test ( $\alpha = 0.05$ ). Because reproduction data were normally distributed and homogenous the Dunnett's t-test (multiple comparison, one-sided,  $\alpha = 0.05$ ) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. Mortality

For the data on mortality please refer to **Table A 2.3.2.2-1**.



**Table A 2.3.2.2-1: Mortality and parasitisation efficiency of *Aphidius rhopalosiphi***

Treatment group	Mortality <sup>a</sup> [%]	Mortality corr. [%]
Control	0.0 ± 0.0	--
24.7 mL product/ha	0.0 ± 0.0 n.s.	0.0 <del>n.s.</del>
74.1 mL product/ha	3.3 ± 8.2 n.s.	3.3 <del>n.s.</del>
222 mL product/ha	0.0 ± 0.0 n.s.	0.0 <del>n.s.</del>
667 mL product/ha	0.0 ± 0.0 n.s.	0.0 <del>n.s.</del>
2000 mL product/ha	0.0 ± 0.0 n.s.	0.0 <del>n.s.</del>
Reference item	90.0 ± 16.7 *	90.0 <del>n.s.</del>

[Fisher's Exact Test,  $\alpha = 0.05$ ; n.s. = not significant, \* = significant]

<sup>a</sup> After 48 hours exposure to the test item residues on plant surfaces; percentage values represent means and standard deviation from 6 replicates each with 5 females note: the tabulated results represent rounded values calculated on the exact raw data.

The reference item applied at a rate of 10.0 mL Perfekthion/ha produced a statistically significant corrected mortality of 90 % after 48 hours.

## B. Reproductive capacity

The reproductive capacity of *A. rhopalosiphi* was tested at all dose rates. There was no effect on reproduction up to and including 2000 ml product/ha compared to the control (**Table A 2.3.2.2-2**).

**Table A 2.3.2.2-2: Parasitisation efficiency of *A. rhopalosiphi***

Treatment group	Parasitisation rate <sup>a</sup> [mummies/female]	Reduction of parasitisation efficiency <sup>b</sup> [%]
Control	26.9 ± 18.1	-
24.7 mL product/ha	32.9 ± 15.2 n.s.	-21.9
74.1 mL product/ha	27.1 ± 17.3 n.s.	-0.6
222 mL product/ha	28.1 ± 15.2 n.s.	-4.1
667 mL product/ha	34.1 ± 16.8 n.s.	-26.5
2000 mL product/ha	28.8 ± 17.9 n.s.	-6.7

[Dunnett's t-test,  $\alpha = 0.05$ ; n.s. = not significant]

<sup>a</sup> Parasitoids previously exposed to the test item residues on plant surfaces; values represent means and standard deviation from maximum 20 replicates each with 1 female.

<sup>b</sup> Negative values indicate better performance compared to the control note: the tabulated results represent rounded values calculated on the exact raw data.

## C. Validity criteria

- Control mortality: 0.0 %, validity criterion was met.
- Reference item mortality: 90.0 % corrected mortality, validity criterion was met.
- Control reproduction rate: 26.9 mummies per female, validity criterion was met. Two parasitoids produced zero values, validity criterion was met.

## III. CONCLUSION

Under extended laboratory conditions the LR<sub>50</sub> of AG-CDF1-480 EC is estimated to be greater than 2000 mL product/ha. No repellent effect of the test item was observed compared to the control.

The reproductive capacity of *A. rhopalosiphi* was tested at all dose rates. There was no effect on reproduction up to and including 2000 mL product/ha compared to the control.

### A 2.3.2.2.2 Study 2: Toxicity to *Typhlodromus pyri*

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with a minor deviation.</p> <p>It was noted that there were six replicates with 10 protonymphs each while the guideline recommends 5 replicates with 20 protonymphs. However, since all the validity criteria were met, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p>
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	LR <sub>50</sub> = 279.7 mL product/ha
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Reference:	KCP 10.3.2.2/02
Report	Effects of AG-CDF1-480 EC on the Predatory Mite <i>Typhlodromus pyri</i> , Extended Laboratory Study - Dose Response Test. Goßmann, A., (2014). 90311062 (report number)
Guideline(s):	IOBC (Blümel et al. 2000), Oomen (1998)
Deviations:	Minor (see the commenting box above) -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch #:** D-N6401  
**Concentration/Purity:** 2,4-D: 367.7 g/L  
Fluroxypyr: 74.3 g/L  
Clopyralid: 30.1 g/L  
**Stability of test compound:** Expiry date: February 2016
- Vehicle and/or control:** 200 L deionised water/ha
- Test animals (Species)** Predatory mite (*Typhlodromus pyri*)  
**Source:** Katz Biotech AG, Baruth, Germany  
**Age:** Protonymphs less than 24 hours old  
**Number of test organisms:** 7 treatment groups (5 dose rates of the test item, control, reference item) with 6 replicates each contain 10 mites  
**Number of replicates:** 6  
**Food:** A mixture of pine (*Pinus nigra*) and birch (*Betula sp.*) pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one *resp.* at least every four days  
Tap water, available from a thin needle which was pierced through the leaf surface  
**Test unit:** Detached maize leaves (*Zea mays* ‘Tatonky, F1’) were cut to slices of about 10 – 20 cm<sup>2</sup>. These leaf cuts were treated on their upper surface  
Test container: The test unit was placed with its treated side upward on a wet cotton wool pad in a petri dish. The petri dish was constantly filled with tap water during the trial. A glue barrier was added to prevent the escaping of the mites. Small needles were stuck into the leaf to guarantee the water supply of the mites.  
**Untreated variant:** Deionised water  
**Reference standard:** Perfekthion (Dimethoate, 400.9 g/L, nominal: 400 g/L)
- Environmental conditions**  
**Temperature:** 24 – 27 °C  
**Relative humidity:** 41 – 97 %  
**Photoperiod** 16 h light / 8h dark  
**Light intensity:** 290 – 640 lux  
**Ventilation** Not stated

## B. STUDY DESIGN AND METHODS:

1. **In-life dates** 05.05.2012 – 19.05.2012 (experimental phase)
2. **Experimental design**

The spraying dilutions were sprayed onto leaves via laboratory spraying equipment, which were then air dried. 30 to 50 minutes after application the mites were exposed to dried residues on treated leaf surfaces (maize leaves). Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where the corrected mortality was < 50 % were sexed and the number of eggs per females was recorded on 3 assessment days within one week

**Test concentrations:** Control, 51.2, 128, 320, 800 and 2000 mL product/ha (in 200 L water/ha) and reference (40.0 mL Perfekthion/ha)

**Test duration:** 14 days (for those treatments for which a reproduction test was performed)
3. **Observations:**

Mortality:  
The number of living, dead and escaped mites was counted twice in the first week (on day 3 and at day 7) after test initiation. Dead mites were removed, escaped mites were considered as dead.

Sex-ratio:  
The sex-ratio for reproduction testing at day 7 was 1 male : 5 females at a minimum. If at day 7 the sex-ratio was less than 1 male : 5 females, males originating from another replicate from the same treatment were added until an appropriate sex-ratio was reached.

Reproduction:  
Number of eggs laid and number of live and dead juvenile stages per female were counted and removed afterwards on 3 assessment days from day 7 on with a maximum interval of 3 days up to day 14 (inclusive). Eggs laid until day 7 inclusive were removed from the test arena and were not counted. The reproduction assessment was performed where the corrected mortality ( $M_{\text{corr}}$ ) was  $\leq 50$  %.

No reproduction assessment was performed for the reference item.
4. **Statistics:**

The  $LR_{50}$  of the mortality was calculated by applying the Probit-Analysis. Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis.

Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ( $\alpha = 0.05$ ) and the Levene's test ( $\alpha = 0.05$ ). Because reproduction data were normally distributed and homogenous the Dunnett's t-test (multiple comparison, one-sided,  $\alpha = 0.05$ ) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. Biological findings

For the data on mortality please refer to **Table A 2.3.2.2-3.**

**Table A 2.3.2.2-3: Percent mortalities of *Typhlodromus pyri***

Treatment group	Mortality after 7 d exposure [%] <sup>b</sup>		Corrected mortality [%]	Escapers [%] <sup>b</sup>
Control	10.0 ± 11.0		--	5.0 ± 8.4
51.2 mL product/ha	26.7 ± 12.1	*	18.5	13.3 ± 8.2
128 mL product/ha	38.3 ± 21.4	*	31.5	15.0 ± 10.5
320 mL product/ha	46.7 ± 34.4	*	40.7	20.0 ± 17.9
800 mL product/ha	80.0 ± 19.0	*	77.8	13.3 ± 8.2
2000 mL product/ha	95.0 ± 8.4	*	94.4	23.3 ± 16.3
Reference item	100.0 ± 0.0	*	100.0	25.0 ± 12.2

[Fisher's Exact Test,  $\alpha = 0.05$ ; \* = significant]

<sup>b</sup> Percentage values represent means and standard deviation from 6 replicates each with 10 mites.

The reference item applied at a rate of 40.0 mL Perfekthion/ha produced a statistically significant mortality of 100.0 % after 7 days.

## B. Reproductive capacity

The reproductive capacity of *T. pyri* was tested at 51.2, 128 and 320 mL product/ha. There was no effect on reproduction up to and including 128 mL product/ha compared to the control. At 320 mL product/ha a statistically significant (Dunnett's t-test,  $\alpha = 0.05$ ) effect on reproduction of 77.4 % occurred (Table A 2.3.2.2-4).

**Table A 2.3.2.2-4: Reproduction of adult *T. pyri***

Treatment group	Reproduction <sup>a</sup> [eggs/female]		Effect on reproduction [%]
Control	6.6 ± 1.3		--
51.2 mL product/ha	5.6 ± 3.0	n.s.	15.0
128 mL product/ha	4.7 ± 1.8	n.s.	28.4
320 mL product/ha	1.5 ± 2.4	*	77.4

[Dunnett's t-test,  $\alpha = 0.05$ ; n.s. = not significant, \* = significant]

<sup>a</sup> From day 7 to day 14 after test start; values represent means and standard deviation from 6 replicates.

Note: the tabulated results represent rounded values calculated on the exact raw data.

## C. Validity criteria

- Control mortality: 10.0 % at day 7, validity criterion was met.
- Reference item mortality: 100.0 %, validity criterion was met.
- Control reproduction rate: 6.6 eggs per female for the second week, validity criterion was met.

## III. CONCLUSION

Under extended laboratory conditions the LR<sub>50</sub> of AG-CDF1-480 EC is 279.7 mL product/ha with 95% confidence limits of 104.7 – 577.4 mL product/ha.

The reproductive capacity of *T. pyri* was tested at 51.2, 128 and 320 mL product/ha. There was no effect on reproduction up to and including 128 mL product/ha compared to the control. At 320 mL product/ha a statistically significant (Dunnett's t-test,  $\alpha = 0.05$ ) effect on reproduction of 77.4 % occurred.

### A 2.3.2.2.3 Study 3: Toxicity to *Chrysoperla carnea*

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 2.0 L product/ha</p>
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Reference:	KCP 10.3.2.2/03
Report	Effects of AG-CDF1-480 EC on the Lacewing <i>Chrysoperla carnea</i> , Extended Laboratory Study - Dose Response Test. Goßmann, A., (2014). 90311047 (report number)
Guideline(s):	Vogt et al. 2000; this guideline was modified for exposure of <i>C. carnea</i> on natural substrate.
Deviations:	None -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch #:** D-N6401  
**Concentration/Purity:** 2,4-D: 367.7 g/L  
Fluroxypyr: 74.3 g/L  
Clopyralide: 30.1 g/L  
**Stability of test compound:** Expiry date: February 2016
- Vehicle and/or control:** 200 L deionised water/ha
- Test animals (Species)** Lacewing (*Chrysoperla carnea*)  
**Source:** Katz Biotech AG, Baruth, Germany  
**Acclimation** 2 – 3 days  
**Age:** Larvae (2 to 3 days old)  
**Number of test organisms:** 7 treatment groups (5 dose rates of the test item, control, reference item) with 40 replicates each containing 1 larvae  
**Number of replicates:** Exposure period: 40  
Post-exposure period: maximum 38 adults per replicate (all survivors, adults were divided among two test units)  
**Food:** Larvae: UV-sterilised *Sitotroga cerealella* Oliv. eggs, *ad libitum*  
Adults: artificial diet out of: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheat germ and deionised water (approximately 45 mL) mixed homogeneously, *ad libitum*  
**Test unit:** petri dish (60 mm in diameter)  
**Untreated variant:** Deionised water  
**Reference standard:** Perfekthion (nominal: 400 g dimethoate/L)
- Environmental conditions**  
**Temperature:** 24 – 27 °C  
**Relative humidity:** 77 – 90 %  
**Photoperiod** 16 h light / 8h dark  
**Light intensity:** 1030 – 1780 lux

**Ventilation**

Not stated

**B. STUDY DESIGN AND METHODS:**

**1. In-life dates**

27.08.2014 (main test: dosing, application)  
25.09.2014 (main test: observation)

**2. Experimental design**

Detached leaves of vine plants (*Vitis vinifera* 'Phoenix') were cut to discs (55 mm in diameter), treated (single application) on their upper surface and placed on a wet cotton wool pad in a petri dish. After 45 to 55 min larvae were exposed to dried residues on treated leaf surfaces. Exposure time lasted until pupae were transferred to the reproduction units for development of adults.

**Test concentrations:**

Control, 24.7, 74.1, 222, 667 and 2000 mL product/ha and reference item (140 mL Perfekthion/ha).

**Test duration:**

Exposure time: 12 – 19 days (until cocoons were transferred to oviposition cages)  
Pre-oviposition period: 6 – 11 days (time interval from adult hatch to start of oviposition)  
Oviposition period: 1 week

**3. Observations:**

Mortality:

Number of living and dead larvae and number of pupae developed was determined at least 3 times a week after test start and number of adults hatched was checked regularly.

Reproduction:

The number of eggs was counted after 24 hours egg-laying periods (checks) and 2 checks were done within one week. The number of larvae was determined after hatching of all larvae and the hatching rate was calculated.

Reproduction was performed where the corrected mortality ( $M_{\text{corr.}}$ ) was  $\leq 50\%$ .

No reproduction testing was performed with the reference item.

Fertility and Fecundity:

Number of eggs was counted by two egg samples (checks) within one week. Each check covered an egg laying period of 24 h.

Eggs of each sample were incubated in separate plastic boxes. For assessment of fertility hatched larvae were removed daily; *Sitotroga* eggs were added to avoid cannibalism. When no further hatching of larvae was observed (after 6 days), the remaining eggs where no alive larva had hatched were determined as unhatched. All eggs were counted and the percentage of hatched eggs was determined.

**4. Statistics:**

Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. Mortality

For the data on mortality, reproduction and hatching please refer to **Table A 2.3.2.2-5**. Under extended laboratory conditions the LR<sub>50</sub> of AG-CDF1-480 EC is > 2000 mL product/ha.

### B. Reproductive capacity

The reproductive capacity of *Chrysoperla carnea* was tested at all dose rates. Reproduction was > 15 eggs per female per day at all dose rates except 677 mL product/ha where 12.6 eggs per female were evaluated. In the next higher treatment group of 2000 mL product /ha the number of eggs per female per day was 25.2. It can be concluded that the reduced fecundity at 667 mL product/ha was not test item related. The mean hatching rate was > 70 % at all tested dose rates.

**Table A 2.3.2.2-5: Pre-imaginal mortality and reproduction of *Chrysoperla carnea***

	Rate <sup>1)</sup> [mL/ha]	Mortality <sup>2)</sup> [%]	Mortality <sub>corr.</sub> <sup>3)</sup> [%]	Reproduction [eggs/female/day]	Larval hatching rate [%]
Control	0	5.0	--	28.3	84.3
AG-CDF1- 480 EC	24.7	5.0 n.s.	0.0	29.8	87.4
	74.1	5.0 n.s.	0.0	23.4	89.0
	222	5.0 n.s.	0.0	28.3	89.5
	667	7.5 n.s.	2.6	12.6	88.1
	2000	5.0 n.s.	0.0	25.2	88.3
Endpoint					
LR <sub>50</sub> (95% CL): > 2000 mL product/ha					

1) Application rate in 200 L deionised water/ha.

2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces (Fisher's Exact Test,  $\alpha = 0.05$ ; n.s. = not significant).

3) Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli.

### C. Validity criteria

- Control Mortality: 5.0 %, validity criterion was met
- Reference Item Mortality: 97.4 % corrected mortality, validity criterion was met
- Fecundity in the Control Group: 28.3 eggs per female per day (mean number), validity criterion was met
- Fertility in the Control Group: 84.3 % larval hatching rate (mean value), validity criterion was met

## III. CONCLUSION

Under extended laboratory conditions the LR<sub>50</sub> of AG-CDF1-480 EC is > 2000 mL product/ha. The reproductive capacity of *C. carnea* was tested at all rates. Reproduction was > 15 eggs per female per day at all dose rates except 677 mL product/ha where 12.6 eggs per female were evaluated. As in the next higher treatment group of 2000 mL product/ha the number of eggs per female per day was 25.2 it can be concluded that the reduced fecundity at 667 mL product/ha was not test item related. The mean hatching rate was > 70 % at all tested dose rates. This indicates that there was no negative effect of the test item on reproductive performance of *C. carnea* up to and including 2000 mL product/ha.

#### A 2.3.2.2.4 Study 4: Toxicity to *Typhlodromus pyri*

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 2.0 L product/ha  ER<sub>50</sub> &gt; 2.0 L product/ha</p>
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Reference:	KCP 10.3.2.2/04
Report	Effects of AG-CDF1-480 EC on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN under extended laboratory conditions (under semi-field conditions aged residues on maize plants). Röhlig, U., (2015). 14 10 48 070 A (report number)
Guideline(s):	Blümel <i>et al.</i> (2000)
Deviations:	None -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

### I. MATERIAL AND METHODS

#### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Yellowish liquid  
**Lot/batch #:** D-N6405  
**Concentration/Purity:** 2,4-D: 386.6 g/L  
Fluroxypyr: 79.7 g/L  
Clopyralid: 32.5 g/L  
**Stability of test compound:** Expiry date: May 2016
- Vehicle and/or control:** 400 L deionised water/ha
- Test animals (Species):** Predatory mite (*Typhlodromus pyri*)  
**Source:** Katz Biotech AG, Baruth, Germany  
**Age:** Protonymphs less than 24 hours old  
**Number of test organisms:** 3 treatment groups (1 dose rate of the test item, control, reference item) with 6 replicates each contain 20 mites  
**Number of replicates:** 5  
**Food:** A mixture of pine (*Pinus nigra*) and birch (*Betula pendula*) pollen (1:1)  
**Feeding** At the start of each exposure and each assessment day  
**Test unit:** Maize (*Zea mays*), which had not been treated with any plant production products; variety “Suleyka”. Supplier: Nordssat Saatzucht GmbH, Saatzucht Langenstein, Böhnshauser Str. 1, 38895 Langenstein, Germany. Grown in soil (cultivated in pots with a diameter of 30 cm with Dehner® Blumenerde near the test facility under semi-field conditions), which had not been treated with any plant protection products. BBCH at application: 32. Height of the potted plants: approx. 0.60 – 0.70 cm leaves were cut shortly before each bioassay.  
  
Exposure units:  
Maize leaf disc (4 cm diameter) surrounded with insect glue



	(TEM MEN Insektenleim), on cotton wool moistened with tap water in a Petri dish (9 cm diameter).
<b>Untreated variant:</b>	Deionised water
<b>Reference standard:</b>	Dimethoate EC 400
<b>4. Environmental conditions</b>	
<b>Controlled-environment test room:</b>	
<b>Temperature:</b>	23 – 27 °C
<b>Relative humidity:</b>	65 – 74 % (DAT 0) and 67 – 74% (DAT 7) <del>86 – 87 %</del>
<b>Photoperiod:</b>	16 h light/8h dark
<b>Light intensity:</b>	2190 lux
<b>Outdoor weather conditions (non-GLP) – valid for the full time of ageing:</b>	
<b>Temperature (mean per day):</b>	21.2 – 27.9 °C
<b>Temperature (min/max)</b>	13.9/36.1 °C
<b>Relative humidity (mean per day)</b>	47.0 – 74.1 %
<b>Rainfall</b>	0
	2.7 mm (on DAT 6 after application) are not relevant, since the treated plants were placed under a rain-protected carpot

## B. STUDY DESIGN AND METHODS:

<b>1. In-life dates</b>	15.07.2014 – 05.08.2014 (experimental phase)
<b>2. Experimental design</b>	Protonymphs were exposed via freshly dried or aged residues of the test item on maize leaves. The test comprised 3 treatment groups (1 test item rates, water treated control, reference item). Treatments were applied to potted maize plants using a plot-sprayer. Following treatment and between DAT 4 and DAT 7, the plants were kept protected against rain in a UV-permeable carpot. Extended laboratory bioassays were initiated within 1 h of treatment application, i.e. 0 days after treatment (DAT 0) and 7 days after treatment (DAT 7), set up with 5 replicates for the test item, control and reference item treatment consisting of 20 mites each. For each bioassay the replicate leaves were gently cut to leaf discs, which were placed with the treated side upward, on moistened cotton wool in Petri dishes.
<b>Test concentrations:</b>	Control, 2 L product/ha in 400 L/ha deionized water, and reference item (30 mL product/ha in 400 L/ha of deionized water on DAT 0, 15 mL product in 200 L/ha of deionized water on DAT 7)
<b>Test duration:</b>	In each bioassay mortality assessments were carried out 3 and 7 days after exposure and reproduction assessments were carried out on days 9, 11 and 14 after exposure.
<b>3. Observations:</b>	Mortality. Number of surviving, dead, trapped and escaped mites. Reproduction: number of eggs laid and number of juveniles per evaluation period per female
<b>4. Statistics:</b>	FISHER's Exact Binomial test ( $\alpha = 0.05$ ) for mortality. STUDENT-t-test ( $\alpha = 0.05$ ) for reproduction

## II. RESULTS AND DISCUSSION

### A. Biological findings

The results of bioassays are summarized in **Table A 2.3.2.2-6**.

**Table A 2.3.2.2-6: Effects on *T. pyri* exposed to freshly dried or aged residues of AG-CDF1-480 EC in an extended laboratory trial**

Treatment group	Rate <sup>1</sup>	Corrected mortality <sup>2</sup> [%]	Corrected mortality <sup>3</sup> [%]	Reproduction <sup>4</sup> [eggs/female]	Effect on reproduction <sup>5</sup> [%]
<b>Bioassay initiated on DAT 0</b>					
Control	-	3.0	-	7.06	-
AG-CDF1-480 EC	2 L product/ha	3.0 (n.s.)	0	7.62 (n.s.)	-7.9
Reference item	30 mL product/ha	95.0*	94.8	n.d.	-
<b>Bioassay initiated on DAT 7</b>					
Control	-	3.0	-	71.6	-
AG-CDF1-480 EC	2 L product/ha	3.0 (n.s.)	0	7.14	0.3
Reference item	30 mL product/ha	84.0*	83.5	n.d.	-

<sup>1</sup> Application rate in 400 L water/ha (control, test item and reference item on DAT 0) or in 200 L water/ha (reference item on DAT 7).

<sup>2</sup> Mortality: percentage of individuals, which died, trapped or escaped, results compared to control by FISHER's Exact Binomial Test ( $\alpha = 0.05$ ).

<sup>3</sup> Corrected mortality according to Abott (1925).

<sup>4</sup> Results for reproduction compared to control by Student-t-test ( $\alpha = 0.05$ ).

<sup>5</sup> Change in mean number of eggs per female, relative to control. A positive value indicates a decrease and a negative an increase, relative to the control.

\* Statistically significantly different compared to control.

n.d. not determined.

### B. Validity criteria

- Control mortality:  $\leq 20$  % (dead, trapped and escaped mites) on day 7.
- Reference item mortality: 50 – 100 % on day 7.
- Control reproduction rate:  $\geq 4$  eggs per female (only, when a fecundity test was performed with surviving mites on the test item group).

All validity criteria were met.

## III. CONCLUSION

To assess the duration and extent of possible effect of AG-CDF1-480 EC on survival and reproduction of the predatory mite, *T. pyri*, one application rate of the test item, a control and a toxic reference item were applied to maize plants. After defined time periods, protonymphs of *T. pyri* were exposed to the residues on maize leaves taken from treated plants, in a series of extended laboratory plants.

No adverse effects on survival and reproduction of *T. pyri* were observed when the mites were exposed to 0-day-old residues (bioassay started on DAT 0) and 7-day-old residues (bioassay started on DAT 7) of AG-CDF1-480 EC, applied at a rate 2 L product/ha in 400 L water/ha.

### A 2.3.2.2.5 Study 5: Toxicity to *Typhlodromus pyri*

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with a minor deviation.</p> <p>It was noted that there were 10 replicates with 10 protonymphs each while the guideline recommends 5 replicates with 20 protonymphs. However, since all the validity criteria were met, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 2.0 L product/ha              ER<sub>50</sub> &gt; 2.0 L product/ha</p>
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Reference:	KCP 10.3.2.2/05*
Report	ADM.3304.H.1.A: Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) after Exposure to Freshly Applied and Aged Spray Deposits on Maize Leaves. Walter, C. (2019a). S19-03574 (report number)
Guideline(s):	Blümel <i>et al.</i> (2000)
Deviations:	Minor (see the commenting box above) -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

\*It is important to note that this study KCP reference does not follow the numeration sequence due to the late inclusion of its summary.

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** ADM.3304.H.1.A  
**Description:** Liquid/orange  
**Lot/batch #:** N6903-A  
**Concentration/Purity:** 2,4-D ester: 380.0 g/L (as acid equivalent)  
 Fluroxypyr-methyl: 75.7 g/L (as acid equivalent)  
 Clopyralid: 30.6 g/L (as acid equivalent)  
**Expiry date:** 30 March 2021  
**Stability of test compound:** Sufficient for the test purpose (at least 1 h)
- Vehicle and/or control:** 400 L water/ha
- Test animals (Species):** Predatory mite (*Typhlodromus pyri*)  
**Source:** Laboratory culture at the test facility: Eurofins Agrosience Services Ecotox GmbH Eutinger Straße 24 D – 75223 Niefern-Öschelbronn Germany  
**Age:** Protonymphs (less than 24h old)  
**Number of test organisms:** 3 treatment groups (control, test and reference item group) with 10 replicates each. Ten mites were exposed per replicate unit.  
**Number of replicates:** 10  
**Food:** Pollen of bean (*Vicia faba*) and birch (*Betula pendula*)  
**Feeding:** Each type of pollen was supplied separately at test initiation and replenished at each assessment date (except day 14).  
**Test unit:** A leaf disc (4 cm diameter) was positioned treated side facing upwards on top of the wet cotton wool pad in a Petri dish (diameter approx. 9 cm) after drying of the treatment deposits. In order to prevent mites from escaping a glue barrier was set up around the leaf.  
**Untreated variant:** Tap water

**Reference standard:** Dimethoate EC 400

#### 4. Environmental conditions

##### **Controlled-environment test room:**

**Temperature:** Trial L1: 24.8 – 26.1 °C  
 Trial L2: 24.7 – 26.2 °C  
 Trial L3: 24.6 – 26.2 °C

**Relative humidity:** Trial L1: 60.0 – 80.4 %  
 Trial L2: 60.0 – 80.4 %  
 Trial L3: 64.3 – 80.1 %

**Photoperiod:** 16 h light/8h dark (all trials)

**Light intensity:** Trial L1: 3500 - 4400 lux  
 Trial L2: 3200 - 4000 lux  
 Trial L3: 3400 - 4530 lux

**Outdoor weather conditions (non-GLP) – valid for the full time of ageing:** During the ageing period, the maize plants were kept rain protected under an UV-permeable roof. An electronically controlled system opened and closed the roof depending on prevailing weather conditions (based on cloud cover). Temperature and humidity data during the ageing period were recorded continuously with a calibrated monitoring system. The daily sum of sunshine hours during the ageing period was recorded by an official weather station (Bauschlott) at a distance of approx. 3 km from the field station (non-GLP data).

**Temperature (mean per day):** 16.33 – 25.09 °C

**Temperature (min/max)** 11.9/33.9 °C

**Relative humidity (mean per day)** 49.1 – 78.38 %

**Rainfall** maize plants were kept rain protected under an UV-permeable roof

#### **B. STUDY DESIGN AND METHODS:**

1. **In-life dates** 07/06/2019 – 05/07/2019

2. **Experimental design** The test item was applied at a single rate to potted maize plants with a spray volume of 400 L/ha. The application was performed with a motorized knapsack sprayer under semi-field conditions (i.e. rain protected). The application also included a control (tap water) and a reference item group (a.i. dimethoate). A total of 3 trials were carried out in the laboratory. Protonymphs of *T. pyri* were exposed to the treated leaves for 14 days. Each trial comprised of 3 treatment groups (control, test and reference item group) with 10 replicates each. Ten mites were exposed per replicate unit. Direct treatment effects (dead and escaped mites) and any change in behaviour with respect to the control were assessed 3 (4 in trial L1) and 7 days after the start of exposure. Reproduction (offspring/female) was assessed on day 10, 12 and 14 in each test item group with a corrected mortality  $\leq 50$  % and the control group. The first trial was conducted with freshly treated foliage (trial L1), the following trials with foliage aged for 7 days (L2) and 14 days (L3), respectively. The interval between each trial was 7 days. During ageing plants were kept rain protected, under an UV-permeable roof. For trials L2 and L3, the reference item was freshly applied with a spray volume of 200 L/ha on detached maize leaves with a track sprayer in the laboratory.

- Test concentrations:** Test item: 2.0 L product/ha  
Reference item: 0.2 L product/ha for semi-field application, 0.03 L product/ha for laboratory applications
- Test duration:** Protonymphs of *T. pyri* were exposed to the treated leaves for 14 days.
- 3. Observations:** Cumulative juvenile mortality and cumulative mean number of eggs per female, for each trial.
- 4. Statistics:** Chi<sup>2</sup>-test (one-sided greater) was used to detect significant differences between mortality data of the test item groups and the control. Student's t-test (one-sided smaller) was used to compare reproduction data between the test item treatment groups and the control groups. The significance level was set to  $\alpha = 0.05$  for all hypothesis tests.

## II. RESULTS AND DISCUSSION

### A. Biological findings

The results of bioassays are summarized in **Table A 2.3.2.2-7**.

**Table A 2.3.2.2-7: Effects on *T. pyri* exposed to ADM.3304.H.1.A in an aged residue laboratory trial**

Trial	Treatment group	Mean mortality [%]	Corrected mortality [%] <sup>3)</sup>	Reproduction [eggs/female ± SD]	Reduction in reproduction rate [%]
<b>L1 (0 DAA1)</b>	Control	10.0	-	10.4 ± 1.3	-
	ADM.3304.H.1.A 2.0 L/ha	25.0 <sup>a</sup>	16.7	11.2 ± 2.4	-7.7
	Reference item <sup>1)</sup>	100.0	100.0	n.a.	n.a.
<b>L2 (7 DAA1)</b>	Control	9.0	-	10.4 ± 1.4	-
	ADM.3304.H.1.A 2.0 L/ha (aged residues)	10.0	1.1	10.0 ± 2.2	3.8
	Reference item <sup>2)</sup>	99.0	98.9	n.a.	n.a.
<b>L3 (14 DAA1)</b>	Control	17.8	-	9.8 ± 2.4	-
	ADM.3304.H.1.A 2.0 L/ha (aged residues)	20.0	2.7	7.5 <sup>b</sup> ± 2.4	23.5
	Reference item <sup>2)</sup>	96.0	95.1	n.a.	n.a.

n.a.: not assessed

SD: Standard Deviation

<sup>1)</sup> BAS 152 11 I at 0.2 L/ha (semi-field application)

<sup>2)</sup> BAS 152 11 I at 0.03 L/ha (laboratory application)

<sup>3)</sup> According to ABBOTT (1925), corrected by SCHNEIDER-ORELLI (1947)

\* Statistically significantly different compared to the control (<sup>a</sup> Chi<sup>2</sup>-test, one-sided greater; <sup>b</sup> Student's t-test, one-sided smaller; all tests with  $\alpha = 0.05$ )

### B. Validity criteria

- Control mortality: The mean mortality (dead and escaped individuals) in the control should be  $\leq 20$  % on day 7 of exposure (actual: 10.0 % mortality in Trial L1, 9.0 % mortality in Trial L2 and 17.8 % mortality in Trial L3).
- Reference item mortality: The corrected cumulative mean mortality in the reference item group should range between 50 % and 100 % on day 7 after application (actual: 100.0 % mortality in Trial L1, 98.9 % mortality in Trial L2 and 95.1 % mortality in Trial L3).
- Control reproduction rate: The cumulative mean number of eggs per female in the control (from day 7 to day 14) should be  $\geq 4.0$  eggs/female (actual: 10.4 eggs/female in Trial L1, 10.4 eggs/female in Trial L2 and 9.8 eggs/female in Trial L3).

All validity criteria were met.

### III. CONCLUSION

ADM.3304.H.1.A, applied at 2.0 L/ha to potted maize plants under semi-field conditions caused 25.0 % mortality in trial L1 (freshly applied), 10.0 % mortality in trial L2 (7 day-aged spray residues) and 20.0 % mortality in trial L3 (14 day-aged spray residues). Mortality rates of *Typhlodromus pyri* were therefore clearly below the trigger value of 50 %.

The effect on reproduction of *Typhlodromus pyri* was -7.7 % in trial L1 (freshly applied), 3.8 % in trial L2 (7 day-aged spray residues) and 23.5 % in trial L3 (14 day-aged residues) and therefore clearly below the trigger value of 50 %.

#### A 2.3.2.2.6 Study 2: Toxicity to *Coccinella septempunctata*

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 2000 mL product/ha                      ER<sub>50</sub> &gt; 2000 mL product/ha</p>
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Reference:	KCP 10.3.2.2/06*
Report	ADM.3304.H.1.A: Toxicity to the Ladybird <i>Coccinella septempunctata</i> (Coleoptera, Coccinellidae) under Extended Laboratory Conditions. Walter, C. (2019b). S19-01799 (report number)
Guideline(s):	IOBC (SCHMUCK et al., 2000) modified for the exposure on natural substrate
Deviations:	None with impact on the outcome of the study
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

\*It is important to note that this study KCP reference does not follow the numeration sequence due to the late inclusion of its summary.

### I. MATERIAL AND METHODS

#### A. MATERIALS

- Test Material:** ADM.3304.H.1.A  
**Description:** Liquid/orange  
**Lot/batch #:** N6903-A  
**Concentration/Purity:** 2,4-D: 380.0 g/L (as acid equivalent)  
 Fluroxypyr-methyl: 75.7 g/L  
 Clopyralid: 30.6 g/L (as acid equivalent)  
**Expiry date:** 30 March 2021  
**Stability of test compound:** Sufficient for the test purpose (at least 1 h)
- Vehicle and/or control:** Deionized water
- Test animals (Species):** *Coccinella septempunctata*  
**Source:** Katz Biotech AG<sup>1</sup>, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany  
**Age:** 3–4 days old larvae  
**Number of test organisms:** Each treatment group included 40 replicates containing one larva each  
**Number of replicates:** 40 replicates  
**Food:** Aphids of the species *Acyrtosiphon pisum ad libitum* during larval development plus honey-water solution (2:1) and a mixture of

<b>Test unit:</b>	different unspecified pollen types during the reproduction phase After spray residues had dried, the leaves were placed with the treated surface upwards on the top of a wet cotton pad in a Petri dish which was moistened regularly with tap water throughout the test. Plastic vessels (size: 17 cm x 12.5 cm x 6 cm) with gauze covered lids were used as maintenance units for emerged beetles.
<b>Untreated variant:</b>	Deionised water
<b>Reference standard:</b>	BAS 152 11 I

#### 4. Environmental conditions

<b>Temperature:</b>	24.5 – 26.1 °C
<b>Relative humidity:</b>	61.5 – 82.0 %
<b>Photoperiod:</b>	16 h light/8h dark
<b>Light intensity:</b>	1100 - 2900 lux

### B. STUDY DESIGN AND METHODS:

- In-life dates** 06/05/2019 – 18/06/2019
- Experimental design**

Test and reference item were diluted in water and applied with a laboratory track sprayer to detached bean leaves. A control group treated with deionised water was included in the study. All applications were performed with a spray volume of 200 L/ha. After drying of the treated leaves the test units were assembled. Each treatment group included 40 replicates containing one larva each. The larvae were exposed to the dried residues on the bean leaves. The larvae were fed with aphids of the species *Acyrtosiphon pisum ad libitum*. During the reproduction phase adults were provided with aphids (same species as used for the larvae), honey-water solution (2:1) and a mixture of unspecified pollen types. The mortality was determined from the larval stage until pupation and emergence of the adults at intervals of 1–3 days. Reproductive performance (fecundity and fertility) was assessed taking eight egg samples (each covering a 24-hour egg laying period) within two weeks. Eggs from this period were counted and the hatching success of the larvae was recorded.

**Test concentrations:** Test item: 24.7, 74.1, 222, 667 and 2000 mL ADM.3304.H.1.A/ha  
Reference item: 70.0 mL BAS 152 11 I/ha

**Test duration:** The percentage mortality was assessed after 16 days of exposure. After additional 12 days the reproduction test started. During a period of two weeks the mean number of eggs/female/day and the percentage of fertile eggs/female/day was assessed.
- Observations:** Percentage mortality, mean number of eggs/female/day and percentage of fertile eggs/female/day as well as the LR<sub>50</sub> (median lethal rate), if possible
- Statistics:**

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater,  $\alpha=0.05$ ) was used to detect significant differences between mortality data of the test item groups and the control.

The LR<sub>50</sub> could not be determined since mortality was clearly below 50 % in all test item treatment groups.

The reproduction test was evaluated only qualitatively due to the very high species-inherent variability in egg laying performance (SCHMUCK et al., 2000). Statistical evaluations were therefore not

conducted.

## II. RESULTS AND DISCUSSION

### A. Biological findings

The results of bioassays are summarized in **Table A 2.3.2.2-8**.

**Table A 2.3.2.2-8: Effects on *C. septempunctata* exposed to ADM.3304.H.1.A**

Treatment group	Application rates [mL product/ha]	Mortality [%]	Corrected mortality [%]	Fecundity [Mean no. of eggs/female/day]	Hatching rate [%]	Fertility [Mean no. of fertile eggs/female/day]
Control	0	5.0	-	18.5	95.6	17.7
Test item ADM.3304.H.1.A	24.7	10.0	5.3	26.3	95.6	25.4
	74.1	12.8	8.2	19.8	96.1	19.1
	222	7.5	2.6	21.8	91.6	19.8
	667	10.0	5.3	12.3	94.5	11.7
	2000	7.5	2.6	13.0	97.1	12.6
LR <sub>50</sub>	n.d. but assumed > 2000 mL product/ha					

n.d.: not determined, as corrected mortality was clearly below 50 % in all test item treatment groups

The reference item caused a mortality of 100.0 % (corrected: 100.0 %).

ADM.3304.H.1.A applied to detached bean leaves caused no statistically significant increase in the mortality of *Coccinella septempunctata* compared to the control group at any test item rate up to and including 2000 mL product/ha, the highest rate tested.

The mean fecundity was between 12.3 and 26.3 eggs per female per day compared to 18.5 eggs per female per day in the control group. The hatching rate was between 91.6 and 97.1 % in the test item treatment groups compared to 95.6 % in the control group. The mean fertility in the test item groups was between 11.7 and 25.4 fertile eggs per female per day compared to 17.7 fertile eggs per female per day in the control group.

### B. Validity criteria

- Control mortality: The maximum cumulative mortality in the control group was  $\leq 30$  % (actual: 5.0 %).
- Reference item mortality: The cumulative mortality in the toxic reference group was  $\geq 40$  % (actual: 100.0 %).
- Control reproduction rate: The mean number of fertile eggs per female per day in the control was  $\geq 2$  (actual: 17.7).

All validity criteria were met.

## III. CONCLUSION

ADM.3304.H.1.A applied to bean leaves caused no statistically significant increase in the mortality of *Coccinella septempunctata* at test item rates up to and including 2000 mL product/ha, compared to the control. The LR<sub>50</sub> was not determined, since the highest effects on reproduction were clearly below 50 % up to a rate of 2000 mL product/ha. Thus, the LR<sub>50</sub> for ADM.3304.H.1.A is assumed to be higher than 2000 mL product/ha. It can be assumed that there are no adverse effects on the reproductive performance of the test organism up to 2000 mL product/ha, because the reproductive output was within the historical data base (SCHMUCK et al., 2000) for control beetles ( $\geq 2$  fertile eggs per female per day).



**A 2.3.2.3      KCP 10.3.2.3 Semi-field studies**

No additional data submitted.

**A 2.3.2.4      KCP 10.3.2.4 Field studies**

No additional data submitted.

## A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

### A 2.4.1 KCP 10.4.1 Earthworms

#### A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

##### A 2.4.1.1.1 Study 1: Sub-lethal toxicity to Earthworms

The following study on sublethal effects on *Eisenia fetida* performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>It was noted that the test design was not relevant to derive both NOEC and EC<sub>x</sub> values as there should have been 8 concentrations tested with 4 replicates per treatment, 8 replicates for control, and the spacing factor should have not exceeded 1.8. In the study there were 5 concentrations tested with 4 replicates per treatment, 8 replicates for control, and the spacing factor was 2. Therefore, the test design was relevant to derive only the NOEC value. However, since EC<sub>10</sub> value for reproduction derived in the study was lower than the NOEC value, the reliability of EC<sub>10</sub> was checked by the zRMS in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> <li>NW (normalised width) of 1.05 was calculated, which results in rating “poor” in line with Table E9 in EFSA Supporting publication 2019:EN-1673,</li> <li>median EC<sub>10</sub> is lower than EC<sub>20,low</sub>, indicating high certainty of the protection level,</li> <li>the dose-response curve is shallow with steepness of 0.24 (i.e. &lt;0.33).</li> </ul> <p>Overall, although the confidence intervals for the EC<sub>10</sub> are too wide, the zRMS is of the opinion that the EC<sub>10</sub> may be considered to be sufficiently reliable due to shallow dose-response and high certainty of the protection level.</p> <p>As all the validity criteria were met, overall the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>56 d NOEC (mortality, biomass) ≥ 250 mg product/kg dw soil 56 d NOEC (reproduction) = 62.5 mg product/kg dw soil 56 d EC<sub>10</sub> (reproduction) = 60.2 mg product/kg dw soil</p>
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Reference:	KCP 10.4.1.1/01
Report	Sublethal toxicity of AG-CDF1-480 EC to the earthworm <i>Eisenia fetida</i> in artificial soil with 5% peat. Friedrich, S., (2014). 14 10 48 131 S (report number)
Guideline(s):	OECD 222 (2004)
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test Material:	AG-CDF1-480 EC
Description:	Not stated
Lot/batch:	D-N6401
Concentration/Purity:	30.34 g/L Clopyralid, 365.34 g/L 2,4 D, 74.72 g/L Fluroxypyr, 551.00 g/L 2,4-D Ethylhexyl ester, 107.63 g/L

<b>Stability of test compound:</b>	Fluroxypyr meptyl Expiry date: June 2016
<b>2. Vehicle and/or control:</b>	Deionised water
<b>3. Test animals (Species):</b>	Earthworm <i>Eisenia fetida</i> (SAVIGNY, 1826) Subspecies <i>Eisenia andrei</i> (BOUCHÉ, 1972)
<b>Age:</b>	Adult worms (approximately 3 months old with clitellum)
<b>Mean weight:</b>	250 – 448 mg/worm
<b>Source:</b>	Reared under ambient laboratory conditions in the test facility (original breeding of animals was purchased from “W. Neudorff GmbH KG”, An der Mühle 3, 31860 Emmerthal, Germany)
<b>Feeding:</b>	Mixture of horse manure, straw, peat (1:1:1) origin: horse manure and straw were purchased from farmers, peat was purchased from the company “Torfwerk Moorkultur Ramsloh”
<b>Acclimation period:</b>	Approximately 24 hours in the artificial substrate (with food)
<b>Animals per test concentration:</b>	4 per treated group and 8 per control group
<b>Number of replicates:</b>	10 replicates = 40 animals (80 for control group)
<b>Artificial soil components:</b>	<ul style="list-style-type: none"> <li>• 5 % sphagnum peat; classified according to DIN 11540 (as close to pH 5.5 – 6.0 as possible, no visible plant remained, finely ground, dried to measured moisture content)</li> <li>• 20 % kaolin clay (kaolinite content &gt; 30 %); type: Kaolin W</li> <li>• 0.3 % calcium carbonate;</li> <li>• 74.7 % industrial quartz sand; type: Millisil W3 (fine sand is dominant with more than 50 % of the particles between 50 and 200 Nm)</li> <li>• Deionised water</li> </ul>
	Max. water holding capacity: $WHC_{max}$ (g/100 g dry soil): 42.9 %
<b>Test unit:</b>	Plastic vessel of Bellaplast (inside dimensions: about 16.5 cm × 12 cm × 6 cm) with a lid pervious to air and light
<b>Untreated variant:</b>	The control substrate was left untreated
<b>Reference standard:</b>	Nutdazim 50 FLOW (Carbendazim, SC 500)
<b>4. Environmental conditions</b>	
<b>Temperature:</b>	18.1 – 21.5°C
<b>pH:</b>	Guideline requirement: $6.0 \pm 0.5$ Test start: 5.93 – 6.09 Test end: 5.67 – 5.78
<b>Humidity (Moisture content of the soil):</b>	Guideline requirement: 40 – 60 % of $WHC_{max}$ . Test start: 24.9 – 25.0 (equivalent to 58.0 – 58.3 % of $WHC_{max}$ ) Test end: 24.5 – 24.9 (equivalent to 57.1 – 58.0 % of $WHC_{max}$ )
<b>Photoperiod:</b>	16 h light / 8 h dark
<b>Light intensity:</b>	480 lux

## B. STUDY DESIGN AND METHODS

- 1. In-life dates:** 07.10. 2014 – 02.12.2014 (experimental phase)

## 2. Experimental design:

Each test vessel was then filled with the treated soil. The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food. After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The final assessment included counting of juveniles per test vessel, determination of the water content and pH measurements of the artificial soil. Juveniles were counted by manual inspection of the substrate.

### Test concentrations:

15.63, 31.25, 62.5, 125, 250 mg test item/kg soil dry weight (spacing factor: 2)

### Test duration:

8 weeks (4 weeks adult mortality; 4 weeks juvenile development)

## 3. Observations:

At test start: individual fresh weight (mg/worm) behaviour of earthworms determination of physico-chemical parameters (water content, pH) of the artificial soil

Weekly: observation of behavioural and pathological symptoms (including the feeding activity)

4 weeks after start of exposure: number of surviving adult earthworms per replicate observation of behavioural and pathological symptoms (including morphological alterations) fresh weight of surviving adult earthworms per replicate

8 weeks after start of exposure: number of juveniles per replicate observation of behavioural and pathological symptoms (including morphological alterations) determination of physico-chemical parameters (water content, pH) of the artificial soil

## 4. Statistics:

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 2.10.06 (Ratte 2010). The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (number of juveniles) were estimated by Probit analysis using the maximum likelihood method (Finney 1971). Confidence limits (95 %) of the EC<sub>x</sub> values were computed by normal approximation. For identifying the NOEC value the Williams-t-test was used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was

used.

## II. RESULTS AND DISCUSSION

### A. Biological results

At test start, the fresh weight of the earthworms used was in a range of 250 – 448 mg/worm. The physico-chemical parameters measured at the start and at the end of the tests met the guideline requirements. Mortality rates of 0% were recorded in the test item treatment groups. 0 % mortality was observed in the control group.

The weight change of adult worms ranged between 25.3 and 27.5 % in the treated variants and 26.6 % in the control group. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control treatment at any concentration tested (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller).

Statistically significant effects (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller) on number of juveniles compared to the control group were recorded at concentrations of 125 and 250 mg test item/kg d.w.

The NOEC for biomass and reproduction was determined to be 250 and 62.5 mg test item/kg soil dry weight, respectively. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values for reproduction were estimated to be 60.2, 112 and > 250 mg test item/kg soil dry weight, respectively.

**Table A 2.4.1.1-1: Sublethal effects of AG-CDF1-480 EC on *Eisenia fetida* in a 56-day reproduction study**

Endpoint	Treatment group [mg test item/kg soil dry weight]					
	control	15.63	31.25	62.5	125	250
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean biomass change (%)	26.6	27.5	25.3	27.1	26.0	25.5
Mean number of juveniles after 8 weeks	109.4	115.8	110.0	96.5	82.5*	68.3*
Change of reproduction compared to control (%)	-	-5.8	-0.6	11.8	24.6	37.6
NOEC (mortality)	≥ 250					
NOEC (biomass)	≥ 250					
NOEC (reproduction)	62.5					
LC <sub>50</sub> (mortality)	> 250					
EC <sub>10</sub> (reproduction)	60.2 (95 % confidence limits 36.4 to 99.7)					
EC <sub>20</sub> (reproduction)	112 (95 % confidence limits 83.3 to 151)					
EC <sub>50</sub> (reproduction)	> 250					

No statistically significant differences between control and test item were calculated for biomass (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller).

\* Statistically significantly different compared to control for reproduction (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller).

Negative values = increase, relative to control.

### B. Validity criteria

All criteria were met in the test:

- Adult mortality after 4 weeks in the control group: ≤ 10 %
- Number of juveniles per replicate: ≥ 30
- Coefficient of variation of reproduction: ≤ 30 %

### III. CONCLUSION

In a 56-day earthworm reproduction study with AG-CDF1-480 EC no effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to and including 250 mg test item/kg soil dry weight, i.e. the highest concentration tested.

The NOEC for biomass and reproduction was determined to be 250 and 62.5 mg test item/kg soil dry weight, respectively. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values for reproduction were estimated to be 60.2, 112 and > 250 mg test item/kg soil dry weight, respectively.

#### A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not required since the risk assessment demonstrates that safe use is possible.

#### A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

##### A 2.4.2.1 KCP 10.4.2.1 Species level testing

##### A 2.4.2.1.1 Study 1: Sub-lethal toxicity to Collembolan

The following study on sublethal effects on *Folsomia candida* performed on the product AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>It was noted that the test design was relevant to derive both NOEC and EC<sub>x</sub> values as there were 8 concentrations tested with 4 replicates per treatment, 8 replicates for control, and the spacing factor was 2 but should have not exceeded 1.8. However, this is considered to have no effect on the derived EC<sub>10</sub>.</p> <p>The reliability of EC<sub>10</sub> was checked by the zRMS in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> <li>NW (normalised width) of 1.09 was calculated, which results in rating “poor” in line with Table E9 in EFSA Supporting publication 2019:EN-1673,</li> <li>median EC<sub>10</sub> is higher than EC<sub>20,low</sub>, but lower than the EC<sub>50,low</sub> indicating medium certainty of the protection level,</li> <li>the dose-response curve is medium with steepness of 0.41 (i.e. &lt;0.33).</li> </ul> <p>Overall, due to too wide confidence intervals for the EC<sub>10</sub> and only medium certainty of the protection level, the zRMS is of the opinion that the EC<sub>10</sub> is not fully reliable.</p> <p>All the validity criteria were met and overall the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>28d NOEC (mortality) = 31.25 mg product/kg soil dw 28d NOEC (reproduction) = 62.5 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/01
Report	Effects of AG-CDF1-480 EC on the reproduction of the collembolan <i>Folsomia candida</i> . Friedrich, S. (2014). 14 10 48 129 S (report number)
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

- 1 Test Material:**  
**Active ingredients / purity:** AG-CDF1-480 EC  
30.34 g/L Clopyralid, 365.34 g/L 2,4-D, 74.72 g/L Fluroxypyr, 551.00 g/L 2,4-D Ethylhexyl ester, 107.63 g/L Fluroxypyr meptyl  
**Description:** Not stated  
**Lot/Batch no.:** D-N6401  
**Density:** 1.072 g/mL
- 2 Reference toxicant:**  
**Purity / Active ingredient** The reference item boric acid (100 % analysed)
- 3 Test Animals:**  
**Species:** Collembolan *Folsomia candida* (Willem)  
**Age/growth stage:** 9 – 12 days (juvenile collembolans)  
**Source:** Originally purchased from “Biologische Bundesanstalt (BBA)”, Berlin-Dahlem in May 2000  
**Acclimation:** Not stated  
**Food:** Granulated dry yeast  
**Number of animals / test vessel** 10  
**Number of collembolans** 80 (control group), 40 (treated group)
- 4 Environmental Conditions**  
**Temperature** 18.0 – 20.1 °C  
**Photoperiod** Light : dark = 16 h : 8 h  
**Light intensity** 490 lux  
**Composition of artificial soil**
  - 5 % sphagnum peat; classified according to DIN 11540 (as close to pH 5.5 – 6.0 as possible, no visible plant remained, finely ground, dried to measured moisture content)
  - 20 % kaolin clay (kaolinite content > 30 %); type: Kaolin W,
  - 0.3 % calcium carbonate
  - 74.7 % industrial quartz sand; type: Millisil W3, (fine sand is dominant with more than 50 % of the particles between 50 and 200 µm)
  - deionised water

## B. STUDY DESIGN AND METHODS

- 1. In-life dates:** 22.10.2014 – 19.11.2014
- 2. Experimental design:**

On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40 – 60 % of its WHC. The control substrate contained the corresponding amount of deionised water only. After thorough mixing, 30 g (wet weight) of the test substrate was placed into each vessel, avoiding compression.

The test was started using juvenile collembolans, *Folsomia candida*, well-fed and 9 – 12 days old. Test organisms of a uniform age were obtained by transferring egg clusters from the breeding containers to fresh containers of fresh substrate 12 days before starting the experiment. After 72

hours these egg clusters were removed from the containers and the juveniles that had hatched during the preceding 72 hours were fed with granulated dry yeast. After a further 9 days the test organisms were collected and used for the test. Ten test organisms were introduced to each vessel, using an exhaustor. After addition of the test organisms, the test vessels were positioned randomly in a controlled-environment test room, and these positions were re-randomized weekly. The test containers were tightly covered with a lid and briefly opened twice a week for aeration. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 mg test item/kg soil d.w.)

**Test concentrations:**

**Test duration:**

28 days

**3. Observations:**

The pH and water content of the test substrate were determined at the start and at the end of the test. The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2 % of the initial water content.

Four weeks after introducing the test organisms the parental and juvenile collembolans in the test and control vessels were counted. The test substrate of each replicate was poured into an individual 150 – 200 mL container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring the numbers of parental and juvenile collembolans floating on the surface were determined. Missing parental collembolans are assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to a digital image storage and analysis system. This technique fulfils the requirement of the ISO guideline regarding precision of the counting method (average error < 10 %). The extraction efficiency of the extraction method was determined to be 98 % in a separate extraction run using vessels containing a known number of juveniles kept in untreated test substrate.

**4. Statistics:**

The statistical analysis was performed with the software ToxRat Professional 2.10.06 (RATTE 2010). Fisher's Exact Binomial Test with Bonferroni Correction and the Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment were used to compare the control with the independent test item groups. The  $LC_x$  and  $EC_x$  values were calculated by Probit analysis using the maximum likelihood regression. Confidence limits (95 %) of the  $LC_x/EC_x$  values were computed by normal approximation



## II. RESULTS AND DISCUSSION

### A. Biological results

Mortality rates of 0 – 100.0% were recorded in the test item treatment groups. 3.8% parental mortality was observed in the control. Statistically significant effects (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , one-sided greater) on parental mortality were recorded at 62.5, 125, 250 and 500 mg test item/kg soil d.w. The NOEC for mortality of the parental collembolans was determined to be 31.25 mg test item/kg soil dry weight. The LC<sub>50</sub> was determined to be 76.8 mg test item/kg soil dry weight.

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 502 in the control and 520, 508, 492, 520, 461, 174, 122 and 32 at concentrations of 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 mg test item/kg soil d.w., respectively. Statistically significant effects (Welch-t-test,  $\alpha = 0.05$ , one-sided smaller) on the number of juveniles compared to the control group were recorded at 125, 250 and 500 mg test item/kg soil d.w.

The NOEC for reproduction was determined to be 62.5 mg test item/kg soil dry weight.

The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (based on reproduction) were calculated to be 49.2, 66.7 and 119 mg test item/kg soil dry weight, respectively.

**Table A 2.4.2.1-1: Chronic effects of AG-CDF1-480 EC on *Folsomia candida* in a 28-day reproduction study**

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	3.91	7.81	15.63	31.25	62.5	125	250	500
Parental mortality [%]	3.8	2.5	2.5	0.0	7.5	45.0 <sup>*a</sup>	87.5 <sup>*a</sup>	90.0 <sup>*a</sup>	100.0 <sup>*a</sup>
No. of juveniles	502	520	508	492	520	461	174 <sup>*b</sup>	122 <sup>*b</sup>	32 <sup>*b</sup>
Reduction of reproduction [%] compared to control	-	-4	-1	2	-4	8	65	76	94
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	31.25								
NOEC (reproduction)	62.5								
LC <sub>50</sub>	76.8 (95 % confidence limits 53.9 to 109)								
EC <sub>10</sub>	49.2 (95 % confidence limits 29.2 to 82.9)								
EC <sub>20</sub>	66.7 (95 % confidence limits 45.0 to 98.7)								
EC <sub>50</sub>	119 (95 % confidence limits 92.7 to 153)								

\* Statistically significantly different compared to the control (<sup>a</sup> Fisher-exact test with Bonferroni Correction for mortality;  $\alpha = 0.05$ , one-sided greater; <sup>b</sup> Welch-t-test for reproduction;  $\alpha = 0.05$ , one-sided smaller).

Negative values = increase, relative to control.

### B. Validity criteria

The validity criteria for the control group were accomplished:

- Mean adult mortality:  $\leq 20$  % (observed: 3.8 %).
- Mean number of juveniles per test vessel:  $\leq 100$  (observed: average of 502/vessel).
- Coefficient of variation for the mean number of juveniles:  $< 30$  % (observed: 7.1 %).

The requirement of the ISO guideline concerning the precision of the counting method (average error  $< 10$  %) was fulfilled, the determined overall error of counting amounted to 2.6 %.

## III. CONCLUSION

The NOEC for mortality of the parental collembolans and for reproduction was determined to be 31.25 and 62.5 mg test item/kg soil dry weight, respectively. The LC<sub>50</sub> value for mortality was calculated to be

76.8 mg test item/kg soil dry weight. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (based on reproduction) were calculated to be 49.2, 66.7 and 119 mg test item/kg soil dry weight, respectively.

#### A 2.4.2.1.2 Study 2: Sub-lethal toxicity to Collembolan

The following study on sublethal effects on *Folsomia candida* performed on the product AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>The test design was relevant to derive both NOEC and EC<sub>x</sub> values as there were 8 concentrations tested with 4 replicates per treatment, 8 replicates for control, and the spacing factor was 1.3.</p> <p>The reliability of EC<sub>10</sub> was checked by the zRMS in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> <li>NW (normalised width) of 0.22 was calculated, which results in rating “good” in line with Table E9 in EFSA Supporting publication 2019:EN-1673,</li> <li>median EC<sub>10</sub> is lower than EC<sub>20,low</sub>, indicating high certainty of the protection level,</li> <li>the dose-response curve is steep with steepness of 0.71 (i.e. &lt;0.33).</li> </ul> <p>Overall, based on the above indications, the calculated EC<sub>10</sub> is considered to be reliable.</p> <p>All the validity criteria were met and overall the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>28d NOEC (mortality, reproduction) = 88.8 mg product/kg soil dw  28d EC<sub>10</sub> (reproduction) = 103.0 mg product/kg soil dw  28d EC<sub>20</sub> (reproduction) = 115.8 mg product/kg soil dw  28d EC<sub>50</sub> (reproduction) = 144.8 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/02
Report	Effects of AG-CDF1-480 EC on the reproduction of the collembolan <i>Folsomia candida</i> . Friedrich, S. (2015). 15 10 48 133 S (report number)
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

### I. MATERIALS AND METHODS

- Test Material:** AG-CDF1-480 EC  
**Active ingredients / purity:** 30.34 g/L Clopyralid, 365.34 g/L 2,4-D, 74.72 g/L Fluroxypyr, 551.00 g/L 2,4-D Ethylhexyl ester, 107.63 g/L Fluroxypyr meptyl  
**Description:** Not stated  
**Lot/Batch no.:** D-N6401  
**Density:** 1.072 g/mL
- Reference toxicant:**  
**Purity / Active ingredient** The reference item boric acid (100 % analysed)

### 3 Test Animals:

<b>Species:</b>	Collembolan <i>Folsomia candida</i> (Willem)
<b>Age/growth stage:</b>	9 – 12 days (juvenile collembolans)
<b>Source:</b>	Originally purchased from “Biologische Bundesanstalt (BBA)”, Berlin-Dahlem in May 2000
<b>Acclimation:</b>	Not stated
<b>Food:</b>	Granulated dry yeast
<b>Number of animals / test vessel</b>	10
<b>Number of collembolans</b>	80 (control group), 40 (treated group)

### 4 Environmental Conditions

<b>Temperature</b>	20.2 – 22.0°C
<b>Photoperiod</b>	Light : dark = 16 h : 8 h
<b>Light intensity</b>	540 lux
<b>Composition of soil</b>	<p>Natural soil: LUFA 2.2</p> <ul style="list-style-type: none"><li>• C<sub>org</sub> (%): 1.59</li><li>• pH (CaCl<sub>2</sub>): 5.4</li><li>• CEC (meq/100 g): 9.7</li></ul> <p>Particles sizes (mm) distribution according to German DIN classification (%):</p> <ul style="list-style-type: none"><li>• &lt; 0.002: 8.3</li><li>• 0.002 – 0.006: 3.7</li><li>• 0.006 – 0.02: 5.2</li><li>• 0.02 – 0.063: 8.0</li><li>• 0.063 – 0.2: 33.3</li><li>• 0.2 – 0.63: 40.9</li><li>• 0.63 – 2.0: 0.5</li></ul> <p>Soil type: loamy sand</p> <p>Particle sizes (mm) distribution according to USDA classification (%):</p> <ul style="list-style-type: none"><li>• &lt; 0.002 mm: 7.7</li><li>• 0.002 – 0.05 mm: 16.2</li><li>• 0.05 – 2.0 mm: 76.1</li></ul> <p>Soil type: loamy sand</p> <ul style="list-style-type: none"><li>• Max. water holding capacity WHC<sub>max.</sub> (g/100 g soil d.w.): 43.5. Water content guideline requirement: 40 – 60 % of WHC<sub>max.</sub> (g/100 g soil d.w.):</li><li>• Test initiation: 19.6 – 19.8 (equivalent to 45.1 – 45.5 % of WHC)</li><li>• Test completion: 18.8 – 19.0 (equivalent to 43.2 – 43.7 % of WHC)</li></ul> <p>pH-value: guideline requirement: 6.0 ± 0.5</p> <ul style="list-style-type: none"><li>• Test initiation: 5.78 – 5.81</li><li>• Test completion : 5.68 – 5.81</li></ul>

## B. STUDY DESIGN AND METHODS

- In-life dates:** 18.08.2015 – 15.09.2015
- Experimental design:** On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40 – 60 % of its WHC. The control substrate contained the corresponding amount of deionised

water only. After thorough mixing, 30 g (wet weight) of the test substrate was placed into each vessel, avoiding compression.

The test was started using juvenile collembolans, *Folsomia candida*, well-fed and 9 – 12 days old. Test organisms of a uniform age were obtained by transferring egg clusters from the breeding containers to fresh containers of fresh substrate 12 days before starting the experiment. After 72 hours these egg clusters were removed from the containers and the juveniles that had hatched during the preceding 72 hours were fed with granulated dry yeast. After a further 9 days the test organisms were collected and used for the test. Ten test organisms were introduced to each vessel, using an exhaustor. After addition of the test organisms, the test vessels were positioned randomly in a controlled-environment test room, and these positions were re-randomized weekly. The test containers were tightly covered with a lid and briefly opened twice a week for aeration. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. 23.9, 31.1, 40.4, 52.5, 68.3, 88.8, 115.4, 150 mg test item/kg soil d.w.

**Test concentrations:**

**Test duration:**

28 days

**3. Observations:**

The pH and water content of the test substrate were determined at the start and at the end of the test. The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2 % of the initial water content.

Four weeks after introducing the test organisms the parental and juvenile collembolans in the test and control vessels were counted. The test substrate of each replicate was poured into an individual 150 – 200 mL container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring the numbers of parental and juvenile collembolans floating on the surface were determined. Missing parental collembolans were assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to a digital image storage and analysis system. This technique fulfils the requirement of the ISO guideline regarding precision of the counting method (average error < 10 %). The extraction efficiency of the extraction method was determined to be 99 % in a separate extraction run using vessels containing a known number of juveniles kept in untreated test substrate.

**4. Statistics:**

Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, Williams-t-test ( $\alpha = 0.05$ , one-sided),

Moving average computation after Thompson (1947),  
Probit analysis.  
Statistical program: ToxRat Professional 3.1.0 (2015).

## II. RESULTS AND DISCUSSION

### A. Biological results

Mortality rates of 0 – 62.5 % were recorded in the test item treatment groups. 3.8 % parental mortality was observed in the control. Statistically significant effects (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm,  $\alpha = 0.05$ , one-sided greater) on parental mortality were recorded at 115.4 and 150 mg test item/kg soil d.w.

The NOEC for mortality of the parental collembolans was determined to be 88.8 mg test item/kg soil dry weight. The LC<sub>50</sub> was determined to be 139.9 mg test item/kg soil dry weight.

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 513 in the control and 512, 521, 513, 524, 516, 523, 416 and 227 at concentrations of 23.9, 31.1, 40.4, 52.5, 68.3, 88.8, 115.4 and 150 mg test item/kg soil d.w., respectively. Statistically significant effects (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller) on the number of juveniles compared to the control group were recorded at 115.4 and 150 mg test item/kg soil d.w.

The NOEC for reproduction was determined to be 88.8 mg test item/kg soil dry weight.

The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (based on reproduction) were calculated to be 103.0, 115.8 and 144.8 mg test item/kg soil dry weight, respectively.

**Table A 2.4.2.1-2: Chronic effects of AG-CDF1-480 EC on *Folsomia candida* in a 28-day reproduction study**

Endpoint	Treatment group [mg test item/kg soil dry weight]								
	Control	23.9	31.1	40.4	52.5	68.3	88.8	115.4	150
Parental mortality [%]	3.8	2.5	2.5	0.0	5.0	2.5	2.5	22.5 <sup>*a</sup>	62.5 <sup>*a</sup>
No. of juveniles	513	512	521	513	524	516	523	416 <sup>*b</sup>	227 <sup>*b</sup>
Reduction of reproduction [%] compared to control	-	0	-2	0	-2	-1	-2	19	56
<b>Endpoints [mg test item/kg soil dry weight]</b>									
NOEC (mortality)	<b>88.8</b>								
NOEC (reproduction)	<b>88.8</b>								
LC <sub>50</sub> (mortality) <sup>1</sup>	<b>139.9</b> (95 % confidence limits 128.0 to 153.0)								
EC <sub>10</sub> (reproduction) <sup>2</sup>	<b>103.0</b> (95 % confidence limits 92.4 to 114.9)								
EC <sub>20</sub> (reproduction) <sup>2</sup>	<b>115.8</b> (95 % confidence limits 107.4 to 124.8)								
EC <sub>50</sub> (reproduction) <sup>2</sup>	<b>144.8</b> (95 % confidence limits 137.3 to 152.6)								

<sup>\*a</sup> Statistically significantly different compared to the control for mortality.

(Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm,  $\alpha = 0.05$ , one-sided greater).

<sup>\*b</sup> Statistically significantly different compared to the control for reproduction (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller).

<sup>1</sup> Based on Moving average computation

<sup>2</sup> Based on Probit analysis

### B. Validity criteria

The validity criteria for the control group were accomplished:

- Mean adult mortality:  $\leq 20$  % (observed: 3.8 %).
- Mean number of juveniles per test vessel:  $\leq 100$  (observed: average of 513/vessel).
- Coefficient of variation for the mean number of juveniles:  $< 30$  % (observed: 14.0 %).

### III. CONCLUSION

The NOEC for mortality of the parental collembolans and for reproduction was determined to be 88.8 mg test item/kg soil dry weight. The LC<sub>50</sub> value for mortality was calculated to be 139.9 mg test item/kg soil dry weight. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (based on reproduction) were calculated to be 103.0, 115.8 and 144.8 mg test item/kg soil dry weight, respectively.

#### A 2.4.2.1.3 Study 3: Sub-lethal toxicity to soil mites

The following study on sublethal effects on *Hypoaspis aculeifer* performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in line with OECD 226 with no deviations.</p> <p>The study design (5 concentrations, 8 replicates for control, 4 replicates per treatment group) was relevant to derive only the NOEC values and not the EC<sub>x</sub> values. Effects ≥ 10% were not observed at any of the concentrations tested.</p> <p>All the validity criteria were met and overall the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>14d NOEC (reproduction) &gt; 1000 mg product/kg soil dw</p>
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Reference:	KCP 10.4.2.1/03
Report	Effects of AG-CDF1-480 EC on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> . Schulz, L. (2014). 14 10 48 130 S (report number)
Guideline(s):	OECD 226 (2008)
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

### I. MATERIALS AND METHODS

#### A. MATERIALS

<b>1. Test Material</b>	AG-CDF1-480 EC
<b>Lot/batch #</b>	D-N6401
<b>Concentration/Purity</b>	<p>Clopyralid: 30 g/L (nominal)</p> <p>2,4 D: 375 g/L (nominal)</p> <p>Fluroxypyr: 75 g/L (nominal)</p>
<b>2. Vehicle and/or control</b>	Untreated substrate
<b>3. Test animals (Species)</b>	<i>Hypoaspis aculeifer</i> (CANESTRINI), adult females
<b>Source</b>	In-house culture of BioChem agrar
<b>Feeding</b>	At the beginning and every 2-3 days during the whole test period with <i>Tyrophagus putrescentiae</i> (SCHRANK)
<b>Holding</b>	100 mL SCHOTT-bottle with 20 g soil dry weight
<b>Number of animals per replicate</b>	4 replicates were performed for the test item group and 8 replicates for the control group; each replicate consisted of ten female soil mites
<b>Reference item</b>	Dimethoate (analysed purity: 99.8 %, tolerance ± 1.0 %). The effects of the reference item were investigated in a separate study.

#### 4. Environmental conditions during testing

Soil	Artificial soil according to OECD 226
Temperature	19.7 – 20.8 °C
pH	5.7 – 6.1
Water content	At test initiation: 51.00 – 51.82 %, At test termination: 47.95 – 51.44 %, of maximum water holding capacity (WHC <sub>max</sub> )
Photoperiod	16 h light: 8 h dark
Light intensity	510 lux

### B. STUDY DESIGN AND METHODS:

- 1. In-life dates** 29.09.2014 – 21.10.2014
- 2. Experimental design** A chronic laboratory experiment over a time period of 14 days according to OECD 226 was conducted. Each of the five different test item concentrations were homogeneously mixed into artificial soil and filled into glass vessels.  
**Test concentrations** 198, 296, 444, 667, 1000 mg test item/kg soil dry weight (spacing factor: 1.5)  
**Test duration** 14 days
- 3. Observations** Number of juveniles per test vessel and mortality of the adult female mites were determined. The reproductive output of the mites exposed to the test substance was compared to that of the control in order to determine the no observed effect concentration (NOEC).
- 4. Statistics** Mortality: Fisher's Exact Binomial Test with Bonferroni Correction ( $\alpha = 0.05$ , one-sided greater)  
Reproduction: Dunnett-t-test ( $\alpha = 0.05$ , one-sided smaller)  
Statistical program: ToxRat Professional

## II. RESULTS AND DISCUSSION

### A. Mortality

In the control group the mortality rate was 1.3 %. Mortality rates of 0.0 – 2.5 % were recorded in the test item treatment groups. The observed mortality rates for adults in the test item treatment groups compared to control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction,  $\alpha = 0.05$ , one-sided greater) at all tested concentrations. The results are summarised in the table below.

### B. Fecundity

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 274.0, 290.5, 280.8, 261.8 and 235.8 at concentrations of 198, 296, 444, 667 and 1000 mg/kg soil d.w., respectively. The mean reproduction in the control reached 265.6 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations (Dunnett-t-test,  $\alpha = 0.05$ , one-sided smaller). The results are summarised in the table below.

**Table A 2.4.2.1-3: Effect of the test item on *Hypoaspis aculeifer* mortality and reproduction**

AG-CDF1-480 EC [mg test item/kg soil d.w.]						
Endpoint	Control	198	296	444	667	1000
Mortality of soil mites after 14 days (%)	1.3	2.5	2.5	0.0	0.0	2.5
Mean number of juveniles after 14 days	265.6	274.0	290.5	280.8	261.8	235.8
CV (%)	12.1	6.4	5.2	5.6	9.1	20.5
Reproduction (% to control)	100	103	109	106	99	89

No statistically significant differences compared to control were calculated (Fisher's Exact Binomial Test for mortality,  $\alpha = 0.05$ ; Dunnett-t-test for reproduction;  $\alpha = 0.05$ ).

Calculations were done using non-rounded values.

Percent reproduction:  $(R_t / R_c) * 100 \%$

$R_t$  = mean number of juvenile mites in the treated group(s).

$R_c$  = mean number of juvenile mites in the control group.

### C. Validity criteria

The validity criteria for the control group were accomplished:

- Mean mortality of adult females:  $\leq 20 \%$  (observed: 1.3 %)
- Mean number of juveniles per replicate:  $\geq 50$  (observed: 265.6)
- Coefficient of variation (mean number of juveniles per replicate):  $\leq 30 \%$  (calculated: 12.1 %)

### III. CONCLUSION

In a 14-day *Hypoaspis aculeifer* reproduction study with AG-CDF1-480 EC, the  $LC_{50}$  for mortality and the  $EC_{50}$  for reproduction could not be calculated, but it can be concluded that the  $LC_{50}$  and the  $EC_{50}$  are greater than 1000 mg test item/kg soil dry weight.

Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be  $\geq 1000$  mg test item/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be  $> 1000$  mg test item/kg soil dry weight.

#### A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No additional data submitted.



## A 2.5 KCP 10.5 Effects on soil nitrogen transformation

### A 2.5.1 Study 1: Toxicity to the soil microflora

The following laboratory study on effects on soil microbial activity performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed fully in line with OECD 216 with no deviations.</p> <p>Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason the part of the study pertaining to carbon mineralisation was not validated by the zRMS and was struck through.</p> <p>All the validity criteria were met and the study is considered acceptable.</p> <p>It may be concluded that the effects of the test item on soil nitrogen formation rates were &lt; 25 % at the end of the study period (28 days) up to 14.47 mg product/kg soil dw</p>
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Reference:	KCP 10.5/01
Report	Effects of AG-CDF1-480 EC on the activity of soil microflora (nitrogen and carbon transformation tests). Schulz, L., (2015). 15 10 48 049 C/N (report number)
Guideline(s):	OECD 216 (2000), OECD 217 (2000)
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC

**Description:** Yellowish liquid

**Lot/batch:** D-N6406

**Concentration/Purity:** Clopyralid: 30 g/L + 2,4-D: 375 g/L + Fluroxypyr: 75 g/L

**Stability of test compound:** Not stated
- Vehicle and/or control:** Untreated soil
- Test animals (Species):** Soil microorganisms

**Replicates:** 3 per control (water control) and test item

**Test vessel:** N-transformation test: The incubation of the soil samples was performed as a series of individual and equally sized sub-samples of each treatment group.

200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1).

One additional soil sample (without lucerne meal) was used for determination of the initial  $\text{NH}_4^+$ -N- and  $\text{NO}_3^-$ -N content. The test item was mixed with deionised water and the test solution was subsequently mixed with the soil by means of a hand

stirrer. Water was added to the soil to achieve a water content of approximately 45 % of WHC.

The incubation of the prepared soil was carried out in wide-mouth glass flasks (500 mL) under the conditions mentioned above. The screw caps of the flasks used permit air exchange.

~~Carbon transformation test: The incubation of the soil samples was performed as a series of individual and equally sized sub-samples of each treatment group. 1000 g soil dry weight (= one sub-sample) per vessel was weighed in the mixing vessel of a laboratory mixer ("KitchenAid").~~

~~The test item was mixed with deionised water and the test solution was subsequently mixed with the soil in the laboratory mixer. Water was added to the soil to achieve a water content of approximately 45 % of WHC.~~

~~The incubation of the prepared soil was carried out in steel vessels (4 L) under the conditions mentioned above. The lids on the vessels used permit air exchange.~~

**Test soil:**

A common agricultural soil type was used for the study (see below)

**Soil parameter:**

Biologically active agricultural soil: loamy sand (DIN 4220).  
Physico-chemical data of the soil:

- pH (H<sub>2</sub>O): 6.4
- C<sub>org</sub> [%]: 1.44
- Humus content [%]: 2.48
- Carbon content of microbial biomass [mg C/100 g soil d.w.]: 42.94 = 2.98 % of C<sub>org</sub>.
- N<sub>min</sub> [mg/100 g soil d.w.]: 0.56
- Total-N [%]: 0.15

Particle size distribution [%]:

According to USDA:

- clay (< 0.002 mm): 8.9
- silt (0.002 – 0.050 mm): 28.8
- sand (0.05 – 2.0 mm): 62.3
- soil class: sandy loam

According to ISO 11277:

- clay (< 0.002 mm): 8.9
- silt (0.002 – 0.063 mm): 31.3
- sand (0.063 – 2.0 mm): 59.9
- soil class (DIN 4220): loamy sand

Water holding capacity:

- WHC [g/100 g soil d.w.]: 35.59
- Water content [g/100 g soil d.w.]: 10.45

Cation exchange capacity [cmol+/kg soil]: 8.0

soil d.w. = soil dry weight

**Untreated variant:**

Untreated soil with lucerne meal

**Reference standard:**

Dinoterb (purity: 98.0 ± 0.5 % analysed). The reference item was tested in a separate study at concentrations of 6.80, 16.00 and 27.00 mg/kg.

#### 4. Environmental conditions

<b>Temperature:</b>	18.9 – 21.0 °C
<b>Photoperiod:</b>	Incubation in the dark
<b>pH</b>	5.8 – 6.1 (N-transformation test) <del>5.7 – 5.9 (Respiration test)</del>
<b>Soil moisture:</b>	Water content: approx. 45% of its maximum water holding capacity. Nitrogen transformation test: water content: 15.01 – 16.04 g/100 g dry soil. <del>Carbon transformation test: water content: 15.46 – 16.01 g/100 g dry soil</del>

### B. STUDY DESIGN AND METHODS

- In-life dates:** 19.08.2015 – 16.09.2015
- Experimental design:**

Nitrogen transformation test  
The test was performed in accordance with OECD guideline 216 (2000). Determination of the nitrogen transformation (NO<sub>3</sub>-nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH<sup>4+</sup>-nitrogen, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>-nitrogen were determined by using the Autoanalyzer. Sampling scheme: 0, 7, 14 and 28 days after treatment.

~~Carbon transformation test  
The test was performed in accordance with OECD guideline 217 (2000). Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non treated soil. Three replicates per treatment and concentration. A respirometer system (BSB digi, SELUTECH) was used to determine the O<sub>2</sub> consumption over a period of 12 hours at different sampling intervals.~~

~~Sampling scheme: 0, 7, 14 and 28 days after treatment.~~

**Test concentrations:** Control, 2.89 mg test item/kg dry soil (corresponding to an application rate of 2 L test item/ha) and 14.47 mg test item/kg dry soil (corresponding to an application rate of 10 L test item/ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm<sup>3</sup>.

**Test duration:** 28 days
- Observations:** During the nitrogen ~~and carbon~~ transformation test samples of soil (from each replicate of treatments and control) were collected after 3 h, 7, 14, 28 days of incubation. pH-value, water content, soil nitrification ~~and respiration~~ was determined.

For the quantitative determination of the mineralized part of nitrogen an autoanalyzer was used. The autoanalyzer is a continuous flow analysis system. Ammonium reacts with salicylate and dichloroisocyanuric acid to form an indophenoleblue compound. The intensity of the formed compound is colorimetrically measured at a wavelength of 625 nm. Nitrate is reduced to nitrite by hydrazinesulphate. The nitrite reacts with sulphanilamide in an acidic solution to form a

diazocompound. The diazotized product is then coupled with naphthylamine. The intensity of the formed azodye, which is proportional to the sum of the nitrate and nitrite originally present in the sample, is colorimetrically measured at a wavelength of 525 nm. The differences between the nitrate/nitrite sum and the nitrite contents are the nitrate contents. The nitrite contents are determined without nitrate reduction.

~~The respiration of microorganisms was measured with a respirometer. The respiration of micro-organisms leads to O<sub>2</sub>-consumption and formation of CO<sub>2</sub> that is absorbed in NaOH solution. The absorption of CO<sub>2</sub> decreases the pressure in the reaction flask, which is compensated with O<sub>2</sub> delivered by the respirometer.~~

#### 4. Statistics:

Calculation of treatment means and deviation from solvent control in %.

## II. RESULTS AND DISCUSSION

### A. Nitrogen Transformation

#### *Evaluation method I (day based evaluation)*

No adverse effects of the test item on nitrogen transformation in soil were observed at both test concentrations (2.89 mg/kg dry soil and 14.47 mg/kg dry soil) after 28 days.

The results are summarised in the table below.

**Table A 2.5.1-1: Effects on nitrogen transformation in soil after treatment with the test item – evaluation method I**

Days after application	Control	2.89 mg test item/kg soil dry weight equivalent to 2 L test item/ha		14.47 mg test item/kg soil dry weight equivalent to 10 L test item/ha	
	NO <sub>3</sub> -N [mg/kg soil d.w.]	NO <sub>3</sub> -N [mg/kg soil d.w.]	Deviation from control [%] <sup>1)</sup>	NO <sub>3</sub> -N [mg/kg soil d.w.]	Deviation from control [%] <sup>1)</sup>
0	20.97	20.87	-0.5	20.60	-1.7
7	50.80	50.30	-1.0	52.67	+3.7
14	63.37	62.93	-0.7	64.17	+1.3
28	82.23	81.63	-0.7	83.80	+1.9

The calculations were performed with unrounded values.

<sup>1)</sup> Based on NO<sub>3</sub>-nitrogen-production; - = inhibition; + = stimulation.

#### *Evaluation method II (interval based evaluation)*

No adverse effects of the test item on nitrogen transformation in soil were observed at both test concentrations (2.89 mg/kg dry soil and 14.47 mg/kg dry soil) after 28 days (time interval 0 – 28).

The results are summarised in the table below.

**Table A 2.5.1-2: Effects on nitrogen transformation in soil after treatment with the test item – evaluation method II**

Time Interval (days)	Control	2.89 mg test item/kg dry weight soil equivalent to 2 L test item/ha		14.47 mg test item/kg dry weight soil equivalent to 10 L test item/ha	
	NO <sub>3</sub> -N/day [mg/kg soil d.w.]	NO <sub>3</sub> -N/day [mg/kg soil d.w.]	Deviation from control [%] <sup>1)</sup>	NO <sub>3</sub> -N/day [mg/kg soil d.w.]	Deviation from control [%] <sup>1)</sup>
0 - 7	29.83	29.43	-1.3	32.07	+7.5
0 - 14	42.40	42.07	-0.8	43.57	+2.8
0 - 28	61.27	60.77	-0.8	63.20	+3.2

The calculations were performed with unrounded values.

<sup>1)</sup> Based on NO<sub>3</sub>-nitrogen-production; - = inhibition; + = stimulation.

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +33.2 % and +46.9 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

### **B. Carbon Transformation**

No adverse effects of the test item on carbon transformation in soil were observed at both test concentrations (2.89 mg/kg dry soil and 14.47 mg/kg dry soil) after 28 days.

The results are summarised in the table below.

**Table A 2.5.1-3: Effects on carbon transformation in soil after treatment with the test item**

Days after application	Control	2.89 mg test item/kg soil dry weight equivalent to 2 L item/ha		14.47 mg test item/kg soil dry weight equivalent to 10 L item/ha	
	O <sub>2</sub> -consumption [mg/kg soil d.w./h]	O <sub>2</sub> -consumption [mg/kg soil d.w./h]	Deviation from control [%] <sup>1)</sup>	O <sub>2</sub> -consumption [mg/kg soil d.w./h]	Deviation from control [%] <sup>1)</sup>
0	16.25	16.33	+0.5	16.16	-0.5
7	15.58	15.47	-0.7	15.13	-2.9
14	14.35	14.21	-1.0	14.20	-1.1
28	13.09	13.16	+0.5	13.10	+0.0

The calculations were performed with non rounded values.

<sup>1)</sup> Based on O<sub>2</sub>-consumption; - = inhibition; + = stimulation.

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of -30.1 % and -39.6 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

### **C. Validity criteria**

The coefficients of variation in the control group of the nitrogen and carbon transformation tests were maximum 3.1 % and 2.5 %, respectively (demanded range ≤ 15 %).

#### Nitrogen transformation test:

In the most recent test, dated 06.01.2015 to 03.02.2015 the toxic standard Dinoterb caused effects of +33.2 % and +46.9 % (required ≥ 25 %) on the nitrogen transformation at the tested concentrations of 16.00 mg and 27.00 mg/kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

#### Carbon transformation test:

In the most recent test, dated 06.01.2015 to 03.02.2015, the toxic standard dinoterb caused effects of -30.1 % and -39.6 % (required ≥ 25 %) on the carbon transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

### III. CONCLUSION

The test item caused no adverse effects (deviation from control < 25 %, OECD 217) on soil nitrogen transformation (expressed as NO<sub>3</sub>-N production) ~~and on soil carbon transformation (measured as O<sub>2</sub>-consumption)~~ at the end of the 28-day incubation period.

The study was performed in a field soil at concentrations equivalent up to an application rate of 10 L test item/ha (five-fold of a single application rate).

## **A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants**

### **A 2.6.1 KCP 10.6.1 Summary of screening data**

No additional data submitted.

### **A 2.6.2 KCP 10.6.2 Testing on non-target plants**

#### **A 2.6.2.1 Study 1: Toxicity to non-target plants (vegetative vigour)**

Comments of zRMS:	<p>The study was performed in line with OECD 227 with minor deviations.</p> <p>It was noted that the number of plants per pot was 5 for all plant species while the guideline recommends e.g. 3 seeds for oilseed rape, 2 for bean and corn, and 5-10 for onion. The guideline states that the number of plants per pot depends on the species, pot size and test duration, and should provide adequate and uniform growth conditions and avoid overcrowding and shading of plants by each other. For the 15 cm container (used in the study and indicated in the guideline), 1-2 or 3 seeds should be sown for bigger plants and for smaller plants 5-10 seeds should be used. In principle, after the seeds have emerged, thinning should be completed so that there is only one plant per pot for larger-growing species, while for smaller growing species more than one plant per pot is allowed. Although for some species the number of plants per pot could have been slightly too high, all plants survived and no phytotoxic symptoms were observed for all tested plant species in the control group and the validity criteria of the test were met. Therefore, in the zRMS opinion these deviations had no significant impact on the outcome of the study.</p> <p>It was noted that the study plan stated that the daily mean air humidity should be 70% ± 25%. But in several hours measuring errors occurred for soya bean, turnip, field bean and oilseed rape (values of 100%) and in several days measuring errors occurred for soya bean, turnip, field bean and oilseed rape (values of 36% - 40%). Therefore the error values of 100% were excluded from the calculation of the mean value. The study report states that the reason for the deviation was dripping condensates or rain drops (air exchange via gable ventilation) that caused the measuring errors. This error occurs, when the sensor detects air humidity &gt;100%. As mentioned above, the validity criteria of the test were met and in zRMS opinion these deviations also had no significant impact on the outcome of the study.</p> <p>It is noted that the product contains three active substances and in line with the requirements of OECD 227 the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of fluroxypyr-meptyl was measured and the analyses of 2,4-D and clopyralid were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. From the data on fate and behaviour in water it may be concluded that of all three compounds, 2,4-D in an ester form is the least stable compound. However, transformation of this compound to the acid form starts immediately after addition to water, so chemical analyses performed immediately after preparation of the test solutions would be not reliable. The second least stable substance is fluroxypyr in the ester form, which is also rapidly transformed into the acid form, but slower than 2,4-D EHE. Clopyralid and acid forms of 2,4-D and fluroxypyr are stable in the aqueous solutions. Taking all this into account, the zRMS is of the opinion that selection of fluroxypyr-meptyl for chemical verification in the test solutions is justified. It should be also noted that under practical conditions of use the formulated product will be used and behaviour of particular active compounds will be the same as in the performed study. Taking this into account, confirmation of measured concentration of fluroxypyr-meptyl is deemed sufficient, even if not ideal, since ideally concentration of all three compounds should be measured.</p> <p>The study is considered acceptable with following endpoint relevant for the risk assessment:</p> <p>lowest ER<sub>50</sub> = 43 mL product/ha (<i>Lactuca sativa</i>)</p>
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Reference:	KCP 10.6.2/01
Report	Effect of AG-CDF1-480 EC on vegetative vigour of terrestrial plants. Marquardt, J & Braje, I., (2014). AS353 (report number)
Guideline(s):	OECD Guideline for the Testing of Chemicals, Guideline 227 Terrestrial Plant Test: Vegetative Vigour Test (adopted July 2006)
Deviations:	Minor (see the commenting box above) -
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** AG-CDF1-480 EC  
**Description:** Not stated  
**Lot/batch:** D-N6401  
**Concentration/Purity:** Clopyralid: nominal content 30 g/L  
2,4-D-2EHE: nominal content 375 g/L  
Fluroxypyr-meptyl: nominal content 75 g/L  
**Stability of test compound:** Expiry date: June 2016
- Vehicle and/or control:** Deionised water
- Test species:** 6 dicotyledonous and 4 monocotyledonous plant species were tested. The tested plant species were Field bean (*Vicia faba*), Oilseed rape (*Brassica napus*), Carrot (*Daucus carota*), Soya bean (*Glycine max*), Lettuce (*Lactuca sativa*), Turnip (*Brassica rapa* var *rapa*), Oats (*Avena sativa*), Onion (*Allium cepa*), Rye grass (*Lolium multiflorum*), Corn (*Zea mays*)  
**Source of seeds:**
  - KWS Lochow GmbH: Oats
  - Hild Samen GmbH: Carrot, onion, lettuce, field bean
  - Power-Soja: Soya bean
  - Meiners Saaten: Rye grass
  - KWS Saat AG: Oilseed rape, corn
  - Biogartenversand OHG: Turnip**Replicates:** 6 replicates per variant  
**Test vessel:** Not stated  
**Soil:** The soil medium PS1-2014 was used for the tested plant species.  
**Soil parameter:**

Soil type: medium loamy sand  
Particle size distribution:  
Clay [ $< 2 \mu\text{m}$ ]: 4.7 %  
Silt [ $2 - 63 \mu\text{m}$ ]: 24.0 %  
Sand [ $> 63 \mu\text{m}$ ]: 71.3 %  
Organic Carbon [% C]: 0.72  
Salt content [g KCl/L]: 1.22  
pH ( $\text{CaCl}_2$ ): 6.93

**Untreated variant:** Deionised water-treated control  
**Reference standard:** Fluroxypyr-1-meptylheptylester      PESTANAL<sup>®</sup>      (Short: Fluroxypyr-meptyl)
- Environmental conditions**  
**Temperature:** Daily mean 25 – 27 °C



<b>Soil pH</b>	6.93
<b>Carbon dioxide concentration:</b>	Not stated
<b>Rel. humidity:</b>	Daily mean 45 – 59 %
<b>Photoperiod:</b>	16 h light per day
<b>Light intensity:</b>	< 10 lux

## B. STUDY DESIGN AND METHODS

- In-life dates:** 12.06.2014 – 03.09.2014
- Experimental design:** For all plant species 6 variants were tested (5 test item rates plus a deionised water-treated control); 6 replicates per variant; 1 pot per replicate; 5 plants per pot.

This test was performed under worst case conditions, as it was conducted in the greenhouse with good and permanent wetting of the soil in combination with a low content of clay and organic carbon of the soil medium. This ensured a high availability of AG-CDF1-480 EC for the plants. Furthermore, the pots were irrigated from the bottom, i.e. the test item could not be removed by dissipation.

### Test concentrations:

AG-CDF1-480 EC was applied post-emergence at BBCH-scale 12-14 in a volume of 200 L/ha using a laboratory spray cabin. Following the application, all plants were grown for 21 days ( $\pm 1$  day). Assessments for phytotoxicity were carried out at weekly intervals for all plants. At test termination 21 days ( $\pm 1$  day) after application (DAA), the plant height per plant and the plant fresh weight of the plant biomass above ground per replicate was determined.

The test rates ranged from 8.0 mL to 6000 mL AG-CDF1-480 EC/ha (8.0, 25, 74, 222, 667, 2000, 6000 mL/ha). The factor of the geometric range of the test rates was 3. To verify the concentrations of the test item in the application solutions, a concentration control analysis of the active substance Fluroxypyr-meptyl in aqueous solution was performed using HPLC method. The analysis of the test solution AS353-TG7 yielded an analytical recovery of 104.1 % (for carrot, lettuce, onion, oats, rye grass, corn), 103.6 % (for field bean, oilseed rape, turnip) and of 115.7 % (for soya bean) of the theoretically expected concentration. No false positive value for the active substance in the control specimens was observed.

### Test duration:

21 days ( $\pm 1$  day)

### Chemical analysis:

Reverse phase High Performance Liquid Chromatography (HPLC) with UV-detection

- Observations:** On all three assessment dates, symptoms of phytotoxicity were observed in all tested rates. The observed symptoms were chlorosis, necrosis, growth reductions, deformations and dead plants.

At test termination (21 DAA  $\pm 1$  day), the average plant height and plant fresh weight was recorded.

- Statistics:** Calculation of mean values, standard deviations, Analysis of

variance (ANOVA) followed by Williams or Bonferroni-Welch t-test ( $\alpha = 5\%$ ). If no normal distribution could be detected, Holm's Bonferroni U-test was performed. Linear Maximum Likelihood Regression, based on a Probit or Logit-Model.

## II. RESULTS AND DISCUSSION

### A. Verification of application rate

Analysis of the active substance Fluroxypyr-meptyl in the application solution of the highest application rate resulted in a range of the recovery of 103.6 – 115.7 % of nominal. Therefore, the correct dosing was confirmed.

### B. Biological results

For some of the plants it was not possible to reach a 25% or 50% effect level in plant height or plant fresh weight. In case that the effects were statistically significant but < 25%, the ER<sub>x</sub> values were extrapolated. Because of that the ER<sub>x</sub> values exceeded the highest test rate.

For the species Field bean and Turnip symptoms of phytotoxicity of  $\geq 10\%$  or in every replicate were assessed in the lowest test rate. Therefore the NOER is given as < lowest test rate. Further for the species Field bean and Turnip the plant height was more than 25% reduced and/or statistically significant different in the lowest test rate. Therefore the NOER is given as < lowest test rate. For the species Turnip the plant fresh weight was more than 25% reduced and/or statistically significant different in the lowest test rate. Therefore the NOER is given as < lowest test rate.

NOER, ER<sub>25</sub> and ER<sub>50</sub> values for phytotoxicity, plant height and plant fresh weight of all tested plant species at test termination are summarised in the following table.

**Table A 2.6.2.1-1: No Observed Effect Rates NOER, ER<sub>25</sub> and ER<sub>50</sub> values for the ten tested plant species at test termination after post-emergence application of AG-CDF1-480 EC**

Effect rate [mL test item/ha]	Field bean	Oilseed rape	Carrot	Soy bean	Lettuce	Turnip	Oat	Onion	Rye grass	Corn
<b>Phytotoxicity</b>										
<b>NOER</b>	< 25	25	25	< 25	8	< 25	667	74	222	2000
<b>Plant height</b>										
<b>NOER</b>	< 25	25	74	< 25	25	< 25	222	$\geq 2000$	$\geq 6000$	$\geq 6000$
<b>ER<sub>25</sub></b>	85	43	463	n.d.	66	73	> 6000	> 2000	> 6000	> 6000
<b>ER<sub>50</sub></b> (extrapolated)	> 2000 (2877)	517	> 2000 (2639)	84	320	> 2000 (21880)	> 6000	> 2000	> 6000	> 6000
<b>Plant fresh weight (shoots above ground)</b>										
<b>NOER</b>	25	25	25	< 25	8	< 25	667	74	$\geq 6000$	$\geq 6000$
<b>ER<sub>25</sub></b> (extrapolated)	94	36	107	39	24	< 25 (18)	> 6000	212	> 6000	> 6000
<b>ER<sub>50</sub></b>	297	55	342	190	43	59	> 6000	605	> 6000	> 6000

n.d.: not determined

### C. Validity criteria

There was no control mortality > 10% observed and all control plants remained healthy throughout the complete test period. Thus, any adverse influences on the study results can be excluded and the study can be considered as valid.

## III. CONCLUSION

At test termination clearly treatment-related symptoms of phytotoxicity were observed for all tested plant species. Adverse effects on plant height were observed for all plant species except of rye grass and corn.

The most sensitive plant species in terms of plant height was soya bean with an ER<sub>50</sub> of 84 mL test item/ha. Adverse effects on plant fresh weight were observed for all plant species except of Rye grass and Corn. The most sensitive plant species in terms of plant fresh weight was lettuce with an ER<sub>50</sub> of 43 mL test item/ha. NOER, ER<sub>25</sub> and ER<sub>50</sub> values for phytotoxicity, plant height and plant fresh weight of all tested plant species at test termination are summarised in the following table.

#### A 2.6.2.2 Study 2: Toxicity to non-target plants (seedling emergence)

The following seedling emergence and seedling growth study performed with AG-CDF1-480 EC was provided in support of the assessment.

Comments of zRMS:	<p>The study was performed in line with OECD 208 with minor deviations.</p> <p>It was noted that the number of plants per pot was 6-9 for all plant species while the guideline recommends e.g. 3 seeds for oilseed rape, 1-2 for bean and corn, and 5-10 for onion. The guideline states that the number of plants per pot depends on the species, pot size and test duration, and should provide adequate and uniform growth conditions and avoid overcrowding and shading of plants by each other. For the 15 cm container (used in the study and indicated in the guideline), 1-2 or 3 seeds should be sown for bigger plants and for smaller plants 5-10 seeds should be used. Although for some species the number of plants per pot could have been slightly too high, all plants survived and no phytotoxic symptoms were observed for all tested plant species in the control group and the validity criteria of the test were met. Therefore, in the zRMS opinion these deviations had no significant impact on the outcome of the study.</p> <p>It is noted that the product contains three active substances and in line with the requirements of OECD 208 the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of fluroxypyr-meptyl was measured and the analyses of 2,4-D and clopyralid were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. From the data on fate and behaviour in water it may be concluded that of all three compounds, 2,4-D in an ester form is the least stable compound. However, transformation of this compound to the acid form starts immediately after addition to water, so chemical analyses performed immediately after preparation of the test solutions would be not reliable. The second least stable substance is fluroxypyr in the ester form, which is also rapidly transformed into the acid form, but slower than 2,4-D EHE. Clopyralid and acid forms of 2,4-D and fluroxypyr are stable in the aqueous solutions. Taking all this into account, the zRMS is of the opinion that selection of fluroxypyr-meptyl for chemical verification in the test solutions is justified. It should be also noted that under practical conditions of use the formulated product will be used and behaviour of particular active compounds will be the same as in the performed study. Taking this into account, confirmation of measured concentration of fluroxypyr-meptyl is deemed sufficient, even if not ideal, since ideally concentration of all three compounds should be measured.</p> <p>The study is considered acceptable with following endpoint relevant for the risk assessment:</p> <p>lowest ER<sub>50</sub> = 39 mL product/ha (<i>Lactuca sativa</i>)</p>
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Reference:	KCP 10.6.2/02
Report	Effect of AG-CDF1-480 EC on the seedling emergence and seedling growth of terrestrial plants. Marquardt, J & Braje, I., (2014). AS352 (report number)
Guideline(s):	OECD Guideline for the Testing of Chemicals, Guideline 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth (adopted July 2006)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable

Duplication (if vertebrate study)	-
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## I. MATERIAL AND METHODS

### A. MATERIALS

1. **Test Material:** AG-CDF1-480 EC  
**Description:** Not stated  
**Lot/batch:** D-N6401  
**Concentration/Purity:** Clopyralid: nominal content 30 g/L  
2,4-D-2EHE: nominal content 375 g/L  
Fluroxypyr-meptyl: nominal content 108 g/L  
**Stability of test compound:** Expiry date: June 2016
2. **Vehicle and/or control:** Deionised water
3. **Test species:** The tested plant species were Field bean (*Vicia faba*), Oilseed rape (*Brassica napus*), Carrot (*Daucus carota*), Soya bean (*Glycine max*), Lettuce (*Lactuca sativa*), Turnip (*Brassica rapa* var *rapa*), Oats (*Avena sativa*), Onion (*Allium cepa*), Rye grass (*Lolium multiflorum*), Corn (*Zea mays*).  
**Source of seeds:**
  - KWS Lochow GmbH: Oats
  - Hild Samen GmbH: Carrot, onion, lettuce, field bean
  - Power-Soja: Soya bean
  - Meiners Saaten: Rye grass
  - KWS Saat AG: Oilseed rape, corn
  - Biogartenversand OHG: Turnip**Replicates:** 5 replicates per variant; 1 pot per replicate; 6 to 9 seeds per pot (species dependent)  
**Test vessel:** Pots with 6 to 9 seeds  
**Soil:** The soil medium PS1-2014 was used for the tested plant species.  
**Soil parameter:** Soil type: medium loamy sand  
Particle size distribution:  
Clay [ $< 2 \mu\text{m}$ ]: 4.7 %  
Silt [ $2 - 63 \mu\text{m}$ ]: 24.0 %  
Sand [ $> 63 \mu\text{m}$ ]: 71.3 %  
Organic Carbon [% C]: 0.72  
Salt content [g KCl/L]: 1.22  
pH ( $\text{CaCl}_2$ ): 6.93  
**Untreated variant:** Deionised water-treated control  
**Reference standard:** Fluroxypyr-1-meptylheptylester    PESTANAL<sup>®</sup>    (Short: Fluroxypyr-meptyl)
4. **Environmental conditions**  
**Temperature:** 25 – 28 °C; daily mean 27°C  
For Field bean and Onion: 25-29°C; daily mean 28°C  
**Soil pH** 6.93  
**Carbon dioxide concentration:** Not stated  
**Rel. humidity:** 45 – 73 %; daily mean 54%  
For Field bean and Onion: 52-73%; daily mean 58%  
**Photoperiod:** 16 h light per day  
**Light intensity:** < 10 lux

## B. STUDY DESIGN AND METHODS

1. **In-life dates:** 18.06.2014 – 25.07.2014
2. **Experimental design:** For all plant species 6 variants were tested (5 test item rates plus a deionised water-treated control); 5 replicates per variant; 1 pot per replicate; 6 to 9 seeds per pot (species dependent); This test was performed under worst case conditions, as it was conducted in the greenhouse with good and permanent wetting of the soil in combination with a low content of clay and organic carbon of the soil medium. This ensured a high availability of AG-CDF1-480 EC for the plants. Furthermore, the pots were irrigated from the bottom, i.e. the test item could not be removed by dissipation.  
  
AG-CDF1-480 EC was applied pre-emergence in a water volume of 200 L/ha using a laboratory spray cabin. Following the application, the plants were grown for 21 ( $\pm$ 1) days.  
  
**Test concentrations:** The test rates ranged from 25 mL to 6000 mL AG-CDF1-480 EC/ha. The factor of the geometric range of the test rates was 3. To verify the concentrations of the test item in the application solutions, a concentration control analysis of the active substance Fluroxypyr-meptyl in aqueous solution was performed using HPLC method. The analysis of the test solution AS352-TG6 yielded an analytical recovery of 107.7 % (for all plant species except of field bean and onion) and 103.6 % for field bean and onion of the theoretically expected concentration. No false positive value for the active substance in the control specimens was observed.  
  
**Test duration:** 21 days ( $\pm$  1 day)  
**Chemical analysis:** Reverse phase High Performance Liquid Chromatography (HPLC) with UV-detection
3. **Observations:** Assessments of seedling emergence and phytotoxicity were done 7, 14 (for Onion 9, 16) and 21 ( $\pm$ 1) DAA for all replicates. At test termination, the plant height per plant and the plant fresh weight of the plant biomass above ground per replicate or plant were determined.
4. **Statistics:** Calculation of mean values, standard deviations, Analysis of variance (ANOVA) followed by Williams or Bonferroni-Welch t-test ( $\alpha = 5 \%$ ). If no normal distribution could be detected, Holm's Bonferroni U-test was performed. Linear Maximum Likelihood Regression, based on a Probit or Logit-Model.

## II. RESULTS AND DISCUSSION

### A. Verification of application rate

Analysis of the active substance Fluroxypyr-meptyl in the application solution of the highest application rate resulted in a range of the recovery of 103.6 – 107.7 % of nominal. Therefore, the correct dosing was confirmed.

## B. Biological results

NOER, ER<sub>10</sub>, ER<sub>25</sub> and ER<sub>50</sub> values in terms of seedling emergence, plant height and plant fresh weight of all tested plant species at test termination are summarised in the following table.

**Table A 2.6.2.2-1: No Observed Effect Rates NOER, ER<sub>25</sub> and ER<sub>50</sub> values for the ten tested plant species at test termination after pre-emergence application of AG-CDF1-480 EC**

Effect rate [mg/ha]	Field bean	Oilseed rape	Carrot	Soy bean	Lettuce	Turnip	Oats	Onion	Rye grass	Corn
<b>Phytotoxicity</b>										
<b>NOER</b>	74	25	< 25	74	25	25	222	222	222	667
<b>Seedling emergence</b>										
<b>NOER</b> <b>ER<sub>10</sub></b> (extrapolated)	667	667	74* < 74# (16)	< 74	25	667	≥ 6000	222	≥ 6000	≥ 6000
<b>ER<sub>25</sub></b> (extrapolated)	2244	755	280	< 74 (16)	92	< 667 (640)	> 6000	481	> 6000	> 6000
<b>ER<sub>50</sub></b> (extrapolated)	5337	1064	>2000# (6653)	4885	254	> 2000 (2449)	> 6000	1367	> 6000	> 6000
<b>Plant height</b>										
NOER ER <sub>10</sub> (extrapolated)	< 74 < 74 (19)	222	74	222	25	74	222	222	222	2000
<b>ER<sub>25</sub></b>	84	392	93	284	46	1242	2212	524	965	3519
<b>ER<sub>50</sub></b> (extrapolated)	456	698	375	575	96	3059	4785	1867	4150	5503
<b>Plant fresh weight (shoots above ground)</b>										
<b>NOER</b>	< 74	222	< 25	222	< 25	74	222	222	222	667
<b>ER<sub>25</sub></b> (extrapolated)	109	301	< 25 (5)	266	< 25 (22)	100	805	< 222 (150)	517	2754
<b>ER<sub>50</sub></b>	368	450	92	533	39	155	2109	605	1625	4352

\* The NOER for seedling emergence has been set to 74 mL test item/ha.

# Not reliable, as the dose-response-relationship was not continuous.

At test termination, the estimated degree of phytotoxicity in some of the tested rates of Field bean, Soya bean, Turnip, Onion, Rye grass and Corn was on the average less than 10% compared to the control and/or the symptoms did not appear in every replicate. At test termination, the estimated degree of phytotoxicity in some of the tested rates of Onion was on the average more than 10% compared to the control, but the symptoms did not appear in every replicate and the following test rate showed no effects. Therefore, these minor symptoms are considered as experimental artefacts and cannot be clearly attributed to the treatment.

At test termination, the data of seedling emergence for Carrot allowed the calculation or extrapolation of ER<sub>x</sub> values with rate-response curves by Linear Maximum Likelihood Regression, but the results are not reliable, as the dose-response-relationship was not continuous and the Goodness of fit- Parameter (r<sup>2</sup>) was 0.347. In the tested rate of 74 mL TI/ha the seedling emergence for Carrot was less reduced compared to the control (-8%) than in the former tested rate of 25 mL TI/ha (-26%). The seedling emergence in the tested rate of 667 mL TI/ha could be observed as treatment related effect because of a general tendency of attributed effects in the tested rates of 222 mL TI/ha and 2000 mL TI/ha. To be on the side of caution, the NOER for seedling emergence has been set to 74 mL TI/ha. The observation indicates that the ER<sub>25</sub> value of 280 mL TI/ha was accepted as reasonable. In case of seedling emergence (Oilseed rape, Turnip, Oats), plant height (Oilseed rape, Soya bean, Turnip) and plant fresh weight (Oilseed rape, Lettuce) no variance homogeneity could be detected and therefore a Bonferroni-Welch t-test was performed for statistical evaluation.

### C. Validity criteria

There was no control mortality > 10 % observed and all control plants remained healthy throughout the complete test period and the rate of seedling emergence in the control was 70% for all tested plant species. Thus, any adverse influences on the study results can be excluded and the study can be considered as valid.

### III. CONCLUSION

At test termination clearly treatment-related symptoms of phytotoxicity were observed for all tested plant species. Adverse effects on seedling emergence, plant height and plant weight were observed for the species Field bean, Oilseed rape, Carrot, Soya bean, Lettuce, Turnip and Onion. The most sensitive plant species in terms of seedling emergence, plant height and plant fresh weight was lettuce with an ER<sub>50</sub> of 254 mL item/ha, 96 mL test item/ha and 39 mL test item/ha, respectively.

#### A 2.6.2.3 Study 3: Toxicity to non-target plants (vegetative vigour)

Comments of zRMS:	<p>The study was performed in line with OECD 227 with no major deviations.</p> <p>All the validity criteria were met.</p> <p>It is noted that the product contains three active substances and in line with the requirements of OECD 227 the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of fluroxypyr-meptyl and clopyralid was measured and the analyses of 2,4-D were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. From the data on fate and behaviour in water it may be concluded that of all three compounds, 2,4-D in an ester form is the least stable compound. However, transformation of this compound to the acid form starts immediately after addition to water, so chemical analyses performed immediately after preparation of the test solutions would be not reliable. The second least stable substance is fluroxypyr in the ester form, which is also rapidly transformed into the acid form, but slower than 2,4-D EHE. Clopyralid and acid forms of 2,4-D and fluroxypyr are stable in the aqueous solutions. Taking all this into account, the zRMS is of the opinion that selection of fluroxypyr-meptyl for chemical verification in the test solutions is justified. It should be also noted that under practical conditions of use the formulated product will be used and behaviour of particular active compounds will be the same as in the performed study. Taking this into account, confirmation of measured concentration of fluroxypyr-meptyl and clopyralid is deemed sufficient, even if not ideal, since ideally concentration of all three compounds should be measured.</p> <p>The study is considered acceptable with following endpoint relevant for the risk assessment:</p> <p>lowest ER<sub>50</sub> = 24.8 mL product/ha (<i>Lactuca sativa</i>)</p>
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Reference:	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. Duffner A., (2019a). S19-03359 (report number)
Guideline(s):	OECD Guideline for the Testing of Chemicals, Guideline 227 Terrestrial Plant Test: Vegetative Vigour Test (adopted July 2006)
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

\*It is important to note that this study KCP reference does not follow the numeration sequence due to the late inclusion of its summary.

## I. MATERIAL AND METHODS

### A. MATERIALS

1. **Test Material:** ADM.3304.H.1.A  
**Description:** Liquid / orange  
**Lot/batch:** N6903-A  
**Concentration/Purity:** Clopyralid: 30.6 g/L  
 2,4-D ester: 380 g/L  
 Fluroxypyr-meptyl: 75.7 g/L  
**Stability of test compound:** Expiry date: 30/03/2021
2. **Vehicle and/or control:** Tap water
3. **Test species:** Dicotyledonous species: *Brassica napus* (oilseed rape), *Brassica rapa* (turnip), *Daucus carota* (carrot), *Glycine max* (soybean), *Lactuca sativa* (lettuce), *Vicia faba* (faba bean)  
 Monocotyledonous species: *Allium cepa* (onion), *Avena sativa* (oat), *Lolium multiflorum* (ryegrass), *Zea mays* (maize)  
**Source of seeds:**
  - KWS: Oilseed rape
  - Bingenheimer: Carrot, turnip, onion
  - Saatbau Linz: Soybean
  - Hild: Lettuce, maize
  - Hof Jeebel: Faba bean
  - Partnerbio: Oat
  - Samenshop24: Ryegrass**Replicates:** 5-10 replicates (2-4 seeds per replicate) per variant/treatment group  
**Test vessel:** Plant pots (Ø 15 cm) were filled with the test soil substrate (approximately 1.5 kg)  
**Soil:** A substrate composed of sand, loam or clay was used for cultivation of the plant species (natural soil mixed with sand).  
**Soil parameter:** Soil type: Loamy Sand  
 Particle size distribution:  
 Clay [ $< 2 \mu\text{m}$ ]: 3.1 %  
 Silt [ $2 - 63 \mu\text{m}$ ]: 20.2 %  
 Sand [ $> 63 \mu\text{m}$ ]: 76.7 %  
 TOC [%]: 0.17  
 Organic matter content: 0.29%  
 Electrical conductivity: 77.6  $\mu\text{S/cm}$   
 pH: 7.97  
**Untreated variant:** Tap water-treated control  
**Reference standard:** -
4. **Environmental conditions**  
**Temperature:** 19.96 – 30.86 °C; mean 25.02°C  
**Soil pH:** 7.97  
**TOC:** 0.17 %  
**Rel. humidity:** 40.11\* – 88.09 %; mean 64.41  
 \*Short-term deviation ( $< 2$  hours) from the recommended humidity range was not considered as deviation as it does not affect the integrity and outcome of the study.



**Photoperiod:** 16 h light/ 8 h dark per day  
**Light intensity:** 310 – 395  $\mu\text{mol}/\text{m}^2/\text{s}$

## B. STUDY DESIGN AND METHODS

1. **In-life dates:** 22.07.2019 – 19.08.2019
2. **Experimental design:** Six dicotyledonous and four monocotyledonous species were cultivated in soil. ADM.3304.H.1.A was applied at five application rates per species. In each treatment group a total number of 20 plants at BBCH growth stage 12-13 were applied. The test duration was 21 days following application. During this period, plants were assessed for mortality and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant shoot dry weight and on plant growth stage were determined for day 21. Results of a species were compared to the respective water treated control.  
**Test concentrations:** 0 (control), 8.23, 24.7, 74.1, 222 and 667 mL product/ha (*Lactuca sativa*)  
 0, 24.7, 74.1, 222, 667 and 2000 mL product/ha (*Brassica napus*, *Brassica rapa*, *Daucus carota*, *Glycine max*, *Vicia faba* and *Allium cepa*)  
 0, 74.1, 222, 667, 2000 and 6000 mL product/ha (*Avena sativa*, *Lolium multiflorum* and *Zea mays*)  
**Test duration:** 21 days  
**Chemical analysis:** Analysis of the test item solution from the highest application rate (6000 mL product/ha) and the control solution (C) by HPLC-MS/MS.
3. **Observations:** NOER (no observed effect rate), LOER (lowest observed effect rate) and  $\text{ER}_{50}$  (effect rate for 50 % effect) for mortality and shoot dry weight on day 21, where possible.
4. **Statistics:** A statistical evaluation was performed for the data of mortality and shoot dry weight. If no mortality occurred, no statistical evaluation was conducted for this parameter. For determination of significant difference to the control the significance level was set to  $\alpha = 0.05$  for all hypothesis tests. For the pre-tests (monotony and homogeneity of variance) the significance level was set to  $\alpha = 0.01$ . The data of mortality were tested for monotony and homogeneity of variance followed by a Cochran-Armitage test with Rao-Scott adjustment since the homogeneity of variance was not given. If no mortality occurred, no further computations were performed. The data of shoot dry weight were tested for normality and homoscedasticity using Shapiro-Wilk's Test and Levene-Test followed by a William's test in case that both requirements were fulfilled and the trend analysis by contrast was significant, in case the trend analysis by contrast was not significant Dunnett's t-test was conducted. The multiple Welch's t-test with Bonferroni-Holm adjustment was carried out in case that the data were non-homogenous. If the data were non-homogenous and not normal distributed the  $\text{Chi}^2$ -test was used. Statistical analyses of mortality and shoot dry weight also included the determination of effect rates ( $\text{ER}_{50}$ ) and their

95 % confidence limits. For mortality the determination was conducted by Probit or Weibull analysis using linear max. likelihood regression, where possible. For shoot dry weight the determination was conducted by a non-linear 3-parameter logistic cumulative distribution function without weighting (optimization method: Levenberg-Marquardt). For the latter approach, confidence limits were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs), where possible. Statistical analysis was performed using the program ToxRatPro Version 3.3.0.

## II. RESULTS AND DISCUSSION

### A. Verification of application rate

The analysed concentration of clopyralid and fluroxypyr-meptyl in the test item solution of the highest application rate corresponded to 108 % and 115 % of the target concentration, respectively.

### B. Biological results

#### Mortality:

Mortality occurred in all dicotyledonous species tested. 100 % mortality down to 667 mL product/ha occurred in *Brassica rapa*, *Daucus carota*, *Glycine max*, and *Vicia faba*. No mortality occurred in any of the monocotyledonous species tested.

**Table A 2.6.2.3-1: LOER, NOER and ER<sub>50</sub> of ADM.3304.H.1.A for mortality 21 days after application**

Species	ADM.3304.H.1.A [mL product/ha]		
	LOER	NOER	ER <sub>50</sub> (95% confidence limits)
<b>Dicotyledonous species</b>			
<i>Brassica napus</i>	74.1 <sup>a</sup>	24.7	286 (205 / 400)
<i>Brassica rapa</i>	74.1 <sup>a</sup>	24.7	128 (93.2 / 176)
<i>Daucus carota</i>	222 <sup>a</sup>	74.1	229 (n.d.)
<i>Glycine max</i>	667 <sup>a</sup>	222	375 (315 / 448)
<i>Lactuca sativa</i>	74.1 <sup>a</sup>	24.7	537 (317 / 1471)
<i>Vicia faba</i>	667 <sup>a</sup>	222	355 (295 / 428)
<b>Monocotyledonous species</b>			
<i>Allium cepa</i>	-	≥ 2000	> 2000
<i>Avena sativa</i>	-	≥ 6000	> 6000
<i>Lolium multiflorum</i>	-	≥ 6000	> 6000
<i>Zea mays</i>	-	≥ 6000	> 6000

n.d.: not determined

LOER determined with: <sup>a</sup> Cochran-Armitage test with Rao-Scott adjustment; one-sided greater,  $\alpha = 0.05$

#### Phytotoxicity:

Symptoms of phytotoxicity occurred in all species tested except *Avena sativa*. The monocotyledonous species in general were less affected after the treatment than the dicotyledonous species.

The observed symptoms were rolled leaves, stem deformations, chlorosis, necrosis and stunted growth. Symptoms on nearly the total plant (up to 80 %) or even moribund plants (up to but not including 100 %) occurred in all dicotyledonous species down to 74.1 mL product/ha except for *Daucus carota* with

plants affected in this severity only down to 222 mL product/ha. The highest mean phytotoxicity in relation to the application rate occurred in *Lactuca sativa* with 78 % at 74.1 mL product/ha.

#### Growth Stage:

Differences in the growth stage of the plants (=BBCH stage) were observed between the test item groups and the respective control group of all dicotyledonous species on the last assessment day (21 DAA). No differences occurred in the monocotyledonous species.

The difference in all dicotyledonous species affected the number of true leaves (e.g. BBCH growth stage of 16 in all treatment groups of *Brassica napus* except at 222 mL product/ha with 12) except for *Glycine max* and *Vicia faba*.

In the control and at 24.7 mL product/ha the plants of *Glycine max* already initiated flowers (BBCH growth stage of 62) in comparison to the further higher test item rates (BBCH growth stage of 14 at 74.1 mL product/ha and 13 at 222 mL product/ha).

In the control and at 24.7 mL product/ha the plants of *Vicia faba* initiated sprouts (BBCH growth stage of 21) in comparison to the further higher test item rates (BBCH growth stage of 14 at 74.1 and 222 mL product/ha).

#### Shoot Dry Weight:

Statistically significant differences in shoot dry weight were detected between the test item groups and the control group of all species tested except *Avena sativa* on the last assessment day (21 DAA).

Inhibitions of shoot dry weight above 60 % down to 222 mL product/ha occurred in all dicotyledonous species. No inhibitions above 50 % occurred in the monocotyledonous species.

The highest inhibition compared to the control in relation to the test item rate occurred in *Lactuca sativa* with 97.4 % at 74.1 mL product/ha.

**Table A 2.6.2.3-2: LOER, NOER and ER<sub>50</sub> of ADM.3304.H.1.A for shoot dry weight 21 days after application**

Species	ADM.3304.H.1.A [mL product/ha]		
	LOER	NOER	ER <sub>50</sub> (95% confidence limits)
<b>Dicotyledonous species</b>			
<i>Brassica napus</i>	24.7 <sup>a</sup>	< 24.7	31.3 (24.9 / 37.9)
<i>Brassica rapa</i>	24.7 <sup>b</sup>	< 24.7	41.4 (26.5 / 56.8)
<i>Daucus carota</i>	74.1 <sup>b</sup>	24.7	74.0 (49.8 / 98.7)
<i>Glycine max</i>	24.7 <sup>b</sup>	< 24.7	70.3 (52.8 / 88.2)
<i>Lactuca sativa</i>	8.23 <sup>c</sup>	< 8.23	24.8 (22.6 / 27.1)
<i>Vicia faba</i>	24.7 <sup>b</sup>	< 24.7	83.7 (53.8 / 114.3)
<b>Monocotyledonous species</b>			
<i>Allium cepa</i>	222 <sup>b</sup>	74.1	1401 (698 / 2119)
<i>Avena sativa</i>	-	≥ 6000	> 6000
<i>Lolium multiflorum</i>	74.1 <sup>b</sup>	< 74.1	> 6000
<i>Zea mays</i>	667 <sup>b</sup>	222	2905 (1728 / 4109)

LOER determined with: <sup>a</sup> Multiple Welch's t-test with Bonferroni-Holm adjustment, <sup>b</sup> Williams' test, <sup>c</sup> Chi<sup>2</sup>-test; all tests one-sided smaller,  $\alpha = 0.05$

### C. Validity criteria

- Seedling emergence: The emergence rate of the seeds used in this study was  $\geq 70$  % (actual 84 – 94%).
- Phytotoxicity: The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibited only normal variation in growth and morphology for the particular species.
- Mean survival: The mean survival of emerged control seedlings was  $\geq 90$  % (actual: 100 %) 21 days after the application.
- Cultivation conditions: The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

### III. CONCLUSION

The target concentration of the active ingredients clopyralid and fluroxypyr-meptyl in the test item solution from the highest application rate was confirmed by the analytical dose verification.

The study was conducted to determine the effects of ADM.3304.H.1.A on the vegetative vigour of six dicotyledonous and four monocotyledonous species. Since the validity criteria were met for all species tested on day 21, the study can be regarded as valid.

Mortality occurred in all dicotyledonous species tested in contrary to the monocotyledonous species where no plant died in the course of this study. The most sensitive species with regard to the  $ER_{50}$  was *Brassica rapa*. The  $ER_{50}$  with 95 % confidence limits was calculated to be 128 (93.2 / 176) mL product/ha.

Statistically significant differences in shoot dry weight of day 21 were detected for all species tested except *Avena sativa*. The most sensitive species with regard to the  $ER_{50}$  was *Lactuca sativa*. The  $ER_{50}$  with 95 % confidence limits was calculated to be 24.8 (22.6 / 27.1) mL product/ha.

#### A 2.6.2.4 Study 4: Toxicity to non-target plants (seedling emergence)

Comments of zRMS:	<p>The study was performed in line with OECD 208 with minor deviations.</p> <p>It was noted in the study report that due to technical reasons on one day during the exposure in the greenhouse the air temperature was above the recommended maximum of 32 °C for a period longer than two hours (maximum temperature reached 33.71°C for 3.5 hours) and on five days during the exposure in the greenhouse the relative air humidity was below the recommended minimum of 45 % for a period longer than two hours, but less than 4 hours (minimum relative air humidity reached 33.2 %). However, as all the validity criteria were met for all species and no effects were observed in any of the control groups, these deviations are considered to have no impact on the outcome of the study.</p> <p>It is noted that the product contains three active substances and in line with the requirements of OECD 208 the test concentrations of all active substances should be verified in the respective chemical analyses or at a minimum the least stable active substance should be analysed. However, in the present study only the concentration of fluroxypyr-meptyl and clopyralid was measured and the analyses of 2,4-D were not carried out. No explanation or justification for the active substance selected for the chemical analysis was provided in the study report. From the data on fate and behaviour in water it may be concluded that of all three compounds, 2,4-D in an ester form is the least stable compound. However, transformation of this compound to the acid form starts immediately after addition to water, so chemical analyses performed immediately after preparation of the test solutions would be not reliable. The second least stable substance is fluroxypyr in the ester form, which is also rapidly transformed into the acid form, but slower than 2,4-D EHE. Clopyralid and acid forms of 2,4-D and fluroxypyr are stable in the aqueous solutions. Taking all this into</p>
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	<p>account, the zRMS is of the opinion that selection of fluroxypyr-meptyl for chemical verification in the test solutions is justified. It should be also noted that under practical conditions of use the formulated product will be used and behaviour of particular active compounds will be the same as in the performed study. Taking this into account, confirmation of measured concentration of fluroxypyr-meptyl and clopyralid is deemed sufficient, even if not ideal, since ideally concentration of all three compounds should be measured.</p> <p>The study is considered acceptable with following endpoint relevant for the risk assessment:</p> <p>lowest ER<sub>50</sub> = 341 mL product/ha (<i>Lactuca sativa</i>)</p>
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Reference:	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. Duffner A., (2019b). S19-03358 (report number)
Guideline(s):	OECD Guideline for the Testing of Chemicals, Guideline 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth (adopted July 2006)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

\*It is important to note that this study KCP reference does not follow the numeration sequence due to the late inclusion of its summary.

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** ADM.3304.H.1.A  
**Description:** Liquid / orange  
**Lot/batch:** N6903-A  
**Concentration/Purity:** Clopyralid: 30.6 g/L  
 2,4-D ester: 380 g/L (as acid equivalent), 573.9 g/L in ester form  
 Fluroxypyr-meptyl: 75.7 g/L (as acid equivalent), 109 g/L in ester form  
**Stability of test compound:** sufficient for the test purpose (at least 1 h)  
**Expiry date:** 30/03/2021
- Vehicle and/or control:** Tap water
- Test species:** Dicotyledonous species: *Brassica napus* (oilseed rape), *Brassica rapa* (turnip), *Daucus carota* (carrot), *Glycine max* (soybean), *Lactuca sativa* (lettuce), *Vicia faba* (faba bean).  
 Monocotyledonous species: *Allium cepa* (onion), *Avena sativa* (oat), *Lolium multiflorum* (Italian ryegrass), *Zea mays* (maize)  
**Source of seeds:**
  - KWS: Oilseed rape
  - Bingenheimer: Turnip, carrot
  - Saatbau Linz: Soybean
  - Hild: Lettuce, onion, maize
  - Raiffeisen: Faba bean
  - Partnerbio: Oat

<b>Replicates:</b>	<ul style="list-style-type: none"> <li>Samenshop24: Italian ryegrass</li> </ul> 6 treatment group (5 test item treatment groups and 1 control group). 5 to 10 replicates per variant; 2 to 4 seeds per replicate
<b>Test vessel:</b>	Pots with 2 to 4 seeds. Non-porous plastic pots (Ø 15 cm) were filled with the test soil substrate (approximately 1.5 kg).
<b>Soil:</b>	natural soil mixed with sand
<b>Soil parameter:</b>	Soil type: loamy sand (Batch: Göbrichen_2019_1) Particle size distribution: Clay [ $< 2 \mu\text{m}$ ]: 3.1 % Silt [ $2 - 63 \mu\text{m}$ ]: 20.2 % Sand [ $> 63 \mu\text{m}$ ]: 76.7 % TOC [%]: 0.17 Electrical conductivity [ $\mu\text{S}/\text{cm}$ ]: 77.6 pH: 7.97
<b>Untreated variant:</b>	Tap water-treated control
<b>Reference standard:</b>	-
<b>4. Environmental conditions</b>	
<b>Temperature:</b>	18.67 – 34.86 °C; daily mean 24.26°C
<b>Soil pH</b>	7.97
<b>TOC:</b>	0.17%
<b>Organic matter content:</b>	0.29%
<b>Rel. humidity:</b>	33.2 – 81.66 %; daily mean 60.66%
<b>Photoperiod:</b>	16 h light / 8 h dark
<b>Light intensity:</b>	320 – 400 $\mu\text{mol}/\text{m}^2/\text{s}$
<b>B. STUDY DESIGN AND METHODS</b>	
<b>1. In-life dates:</b>	24.06.2019 – 19.08.2019
<b>2. Experimental design:</b>	<p>Six dicotyledonous and four monocotyledonous species were sown in pots and the pots were sprayed with ADM.3304.H.1.A at five application rates per plant species. In each treatment group a total of 20 seeds were sown. The test duration was from application until 21 days after at least 50 % of the seeds in the control had emerged (21 DA50E) in each species. During this period, plants were assessed for seedling emergence, post-emergence mortality and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant growth stage and shoot dry weight were determined for day 21. Results were compared to the tap water treated control.</p>
<b>Test concentrations:</b>	0 (control), 24.7, 74.1, 222, 667, 2000 mL product/ha for <i>Brassica napus</i> , <i>Brassica rapa</i> , <i>Daucus carota</i> , <i>Lactuca sativa</i> , <i>Allium cepa</i> , <i>Glycine max</i> . 0 (control), 74.1, 222, 667, 2000 and 6000 mL product/ha for <i>Vicia faba</i> , <i>Avena sativa</i> , <i>Lolium multiflorum</i> , <i>Zea mays</i> .
<b>Test duration:</b>	21 days
<b>Chemical analysis:</b>	Analysis of the test item solution from the highest application rate (6000 mL product/ha) and the control solution (C) by HPLC-MS/MS.
<b>3. Observations:</b>	NOER (no observed effect rate), LOER (lowest observed effect rate) and ER <sub>50</sub> (effect rate for 50 % effect) for seedling

emergence, post-emergence mortality and shoot dry weight 21 DA50E, where possible.

#### 4. Statistics:

The data of seedling emergence and post-emergence mortality were analysed with the Multiple Fisher's exact test with Bonferroni-Holm adjustment in case of no monotonicity of the data. If monotonicity as well as extrabinominal variance was given the Cochran-Armitage test was conducted. If the test on monotonicity failed, the Cochran-Armitage test with Rao-Scott adjustment was conducted.

For determination of significant difference to the control the significance level was set to  $\alpha = 0.05$  for all hypothesis tests. For the pre-tests (monotony and homogeneity of variance) the significance level was set to  $\alpha = 0.01$ .

The data of shoot dry weight were tested for normality and homoscedasticity using Shapiro-Wilk's Test and Levene-Test followed by a William's test in case that both requirements were fulfilled and the trend analysis by contrast was significant. If the trend analysis by contrast was not significant the Dunnett's t-test was conducted. In case the data were normal distributed but non-homogenous, the Welch's t-test was applied. In case the data were not normal distributed but homogenous and trend analysis by contrast were significant the Jonckheere-Terpstra test was used. In case the data were not normal distributed and non-homogenous the Multiple Median Chi2-test with Bonferroni-Holm adjustment was used.

Statistical analyses of seedling emergence, post-emergence mortality and shoot dry weight also included the determination of effect rates (ER50) and their 95 % confidence limits. For seedling emergence and post-emergence mortality the determination was conducted by Probit or Weibull analysis using linear max. likelihood regression, where possible. For shoot dry weight the determination was conducted by Probit analysis using linear max. likelihood regression or a non-linear 3-parameter normal cumulative distribution function without weighting (optimization method: Levenberg-Marquardt). Confidence limits were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs), where possible.

Statistical analysis was performed using the program ToxRatPro Version 3.3.0.

## II. RESULTS AND DISCUSSION

### A. Verification of application rate

The analysed concentration of fluroxypyr-meptyl and clopyralid in the test item solution of the highest application rate corresponded to 117 % and 113 % of the target concentration, respectively.

### B. Biological results

#### Seedling emergency:

Statistically significant differences in seedling emergence were determined for *Brassica rapa* and *Daucus carota* (Cochran-Armitage test with Rao-Scott adjustment, one-sided greater,  $\alpha = 0.05$ ).

The highest inhibition of seedling emergence was observed in *Brassica rapa* with 38.9 % at 2000 mL product/ha.

**Table A 2.6.2.4-1: LOER, NOER and ER<sub>50</sub> of ADM.3304.H.1.A for seedling emergence 21 days after at least 50 % of the seedlings in the control group had emerged**

Species	ADM.3304.H.1.A [mL product/ha]		
	LOER	NOER	ER <sub>50</sub> (95% confidence limits)
<b>Dicotyledonous species</b>			
<i>Brassica napus</i>	-	≥ 2000	> 2000
<i>Brassica rapa</i>	667 <sup>a</sup>	222	> 2000
<i>Daucus carota</i>	74.1 <sup>a</sup>	24.7	> 2000
<i>Glycine max</i>	-	≥ 2000	> 2000
<i>Lactuca sativa</i>	-	≥ 2000	> 2000
<i>Vicia faba</i>	-	≥ 6000	> 6000
<b>Monocotyledonous species</b>			
<i>Allium cepa</i>	-	≥ 2000	> 2000
<i>Avena sativa</i>	-	≥ 6000	> 6000
<i>Lolium multiflorum</i>	-	≥ 6000	> 6000
<i>Zea mays</i>	-	≥ 6000	> 6000

LOER determined with: <sup>a</sup> Cochran-Armitage test with Rao-Scott adjustment; one-sided greater,  $\alpha = 0.05$

#### Post-Emergence Mortality:

Statistically significant post-emergence mortality occurred in *Daucus carota*, *Glycine max*, *Lactuca sativa* and *Vicia faba* (Cochran-Armitage test with Rao-Scott adjustment, one-sided greater,  $\alpha = 0.05$ ).

The highest mortality occurred in *Daucus carota* with 78.6 % at 2000 mL product/ha and in *Vicia faba* with 100 % at 6000 mL product/ha.

**Table A 2.6.2.4-2: LOER, NOER and ER<sub>50</sub> of ADM.3304.H.1.A for post-emergence mortality 21 days after at least 50 % of the seedlings in the control group had emerged**

Species	ADM.3304.H.1.A [mL product/ha]		
	LOER	NOER	ER <sub>50</sub> (95% confidence limits)
<b>Dicotyledonous species</b>			
<i>Brassica napus</i>	-	≥ 2000	> 2000
<i>Brassica rapa</i>	-	≥ 2000	> 2000
<i>Daucus carota</i>	222 <sup>a</sup>	74.1	923 (601 / 1676)
<i>Glycine max</i>	2000 <sup>a</sup>	667	n.d.*
<i>Lactuca sativa</i>	74.1 <sup>a</sup>	24.7	1549 (699 / 10239)
<i>Vicia faba</i>	2000 <sup>a</sup>	667	2176 (n.d.)
<b>Monocotyledonous species</b>			
<i>Allium cepa</i>	-	≥ 2000	> 2000
<i>Avena sativa</i>	-	≥ 6000	> 6000
<i>Lolium multiflorum</i>	-	≥ 6000	> 6000
<i>Zea mays</i>	-	≥ 6000	> 6000

\* No reliable ER<sub>50</sub> could be calculated, even though the mortality was 53.3 % at 2000 mL product/ha.

LOER determined with: <sup>a</sup> Cochran-Armitage test with Rao-Scott adjustment; one-sided greater,  $\alpha = 0.05$

n.d. not determined



### Phytotoxicity:

Symptoms of phytotoxicity were more pronounced in dicotyledonous species in comparison to monocotyledonous species. The observed symptoms were chlorosis, leaf deformation, necrosis, rolled leaves, stem deformation and stunted growth. Phytotoxic effects >50 % as a mean value did not occur in the tested plant species. The highest mean phytotoxicity in relation to the application rate occurred in *Vicia faba* with 49 % at 2000 mL product/ha.

### Growth Stage:

No differences in the growth stage of the plants (=BBCH stage) were observed in all treatments groups when compared with the control group.

### Shoot Dry Weight:

Statistically significant differences in shoot dry weight were detected between the test item groups and the control group for *Brassica napus*<sup>a</sup>, *Daucus carota*<sup>a</sup>, *Glycine max*<sup>a</sup>, *Lactuca sativa*<sup>b</sup>, *Allium cepa*<sup>a</sup>, *Avena sativa*<sup>a</sup>, *Lolium multiflorum*<sup>a</sup> and *Zea mays*<sup>c</sup> (<sup>a</sup> Williams' test, <sup>b</sup> Jonckheere-Terpstra test, <sup>c</sup> Multiple Median Chi<sup>2</sup>-test with Bonferroni- Holm adjustment; all tests one-sided smaller,  $\alpha = 0.05$ ). The highest inhibition compared to the control occurred in *Lactuca sativa* with 75.1 % at 2000 mL product/ha.

**Table A 2.6.2.4-3: LOER, NOER and ER<sub>50</sub> of ADM.3304.H.1.A for shoot dry weight 21 days after at least 50 % of the seedlings in the control group had emerged**

Species	ADM.3304.H.1.A [mL product/ha]		
	LOER	NOER	ER <sub>50</sub> (95% confidence limits)
<b>Dicotyledonous species</b>			
<i>Brassica napus</i>	2000	667	> 2000
<i>Brassica rapa</i>	-	≥ 2000	> 2000
<i>Daucus carota</i>	222 <sup>a</sup>	74.1	574 (242 / 1363)
<i>Glycine max</i>	222 <sup>a</sup>	74.1	> 2000
<i>Lactuca sativa</i>	74.1 <sup>b</sup>	24.7	341 (84.3 / 1383)
<i>Vicia faba</i>	> 2000 <sup>*</sup>	≥ 2000	> 2000
<b>Monocotyledonous species</b>			
<i>Allium cepa</i>	667 <sup>a</sup>	222	> 2000
<i>Avena sativa</i>	2000 <sup>a</sup>	667	> 6000
<i>Lolium multiflorum</i>	667 <sup>a</sup>	222	> 6000
<i>Zea mays</i>	6000 <sup>c</sup>	2000	> 6000

\* At 6000 mL product/ha all plants died and therefore no assessments were conducted  
LOER determined with: <sup>a</sup> Williams' test, <sup>b</sup> Jonckheere-Terpstra test; both one-sided smaller,  $\alpha = 0.05$

### **C. Validity criteria**

- Seedling emergence: The control seedling emergence was ≥ 70 % (actual: 80 % to 100 %) 21 days after at least 50 % emergence in the control.

- Phytotoxicity: The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibited only normal variation in growth and morphology for the particular species.

- Mean survival: The mean survival of emerged control seedlings was ≥ 90 % (actual: 100 %) 21 days after the application.

- Cultivation conditions: The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

### III. CONCLUSION

The target concentration of the active ingredients fluroxypyr-meptyl and clopyralid in the test item solution from the highest application rate was confirmed by the analytical dose verification.

The study was conducted to determine the effects of ADM.3304.H.1.A on the seedling emergence and early growth of six dicotyledonous and four monocotyledonous species. Since the validity criteria were met for all species tested on the last assessment day (21 DA50E), the study can be regarded as valid.

Statistically significant differences in seedling emergence were determined for *Brassica rapa* and *Daucus carota* (Cochran-Armitage test with Rao-Scott adjustment, one-sided greater,  $\alpha = 0.05$ ). The ER<sub>50</sub> values could not be calculated since the inhibitions compared to control were below 50 % for all species and can therefore be assumed to be greater than the highest rate tested. The highest inhibition of seedling emergence was observed in *Brassica rapa* with 38.9 % at 2000 mL product/ha.

Statistically significant post-emergence mortality occurred in *Daucus carota*, *Glycine max*, *Lactuca sativa* and *Vicia faba*. The most sensitive species with regard to the LOER was *Lactuca sativa* with 74.1 mL product/ha. The most sensitive species with regard to ER<sub>50</sub> (with 95 % confidence limits) was *Daucus carota* with 923 (601 / 1676) mL product/ha.

Statistically significant differences in shoot dry weight were detected between the test item groups and the control group for *Brassica napus*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Allium cepa*, *Avena sativa*, *Lolium multiflorum* and *Zea mays*. The most sensitive species with regard to LOER and ER<sub>50</sub> was *Lactuca sativa*. The LOER was 74.1 mL product/ha and the ER<sub>50</sub> (with 95 % confidence limits) was calculated to be 341 (84.3 / 1383) mL product/ha.

#### A 2.6.3                      KCP 10.6.3                      Extended laboratory studies on non-target plants

No additional data submitted.

#### A 2.7                              KCP 10.7      Effects on other terrestrial organisms (flora and fauna)

No additional data submitted.

#### A 2.8                              KCP 10.8      Monitoring data

No additional data submitted.

## Appendix 3 Studies performed on active substances/metabolites in support of the evaluation – Aquatic organisms

### Studies with 2,4-D and metabolites

#### A 3.1 KCP 10.2 Effects on aquatic organisms

##### A 3.1.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Comments of zRMS:	<p>The study was performed with 2,4-D in a form of dimethylamine salt and for this reason is not relevant for evaluation of ADM.3304.H.1.A which contains 2,4-D 2-EHE.</p> <p>EU agreed endpoints for 2,4-D in a form of acid and ester are already available in the Bridging Report (2018) so no additional study is deemed necessary, especially in a form not present in the formulation for which authorisation is sought. The study below was not evaluated and its summary is thus struck through.</p>
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Reference:	KCP 10.2.1/06
Report	LAF-74: Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system testing. Gonsior, G., (2014). S14-03291 (report number)
Guideline(s):	OECD Guideline 239: Water-Sediment <i>Myriophyllum spicatum</i> Toxicity Test (26 September 2014)
Deviations:	-
GLP:	Yes
Acceptability:	Not evaluated, not relevant for evaluation of ADM.3304.A.1.H.
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** LAF-74

**Description:** Amber liquid

**Lot/batch, density:** 2A14150103

**Concentration/Purity:** 724 g/L 2,4 D DMA (60 % w/w), equivalent to 600 g/L 2,4-D (50 % w/w)

**Stability of test compound:** Expiry date: 22 April 2016
- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART AND BARKO medium
- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*

**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agroscience Services EcoChem GmbH since November 2010. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.

*M. spicatum* is cultivated under sterile conditions submersed in a modified, aqueous ANDREWS medium containing sucrose. This laboratory stock culture is used to provide

**Acclimation period:**

**Culture medium:**

**Test vessel:**

uniform plants throughout the year, eliminating seasonal variation in plant quality and contamination by other species (e.g. algae). The stock culture plants were held under the same

environmental conditions as used in the test

Seven days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium under the same temperature, light, and water quality conditions as used during the exposure of the plants in the test.

ANDREWS Medium

Plants were grown in a static water sediment system using artificial sterilized sediment overlaid with SMART AND BARKO medium under the same conditions as used in the pre culture. The study was conducted in 2 L glass beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.

Sediment used in the test (percentages based on dry weight):

- 4 % sphagnum peat (approximately pH 5.5–6.0; no visible plant remains, finely ground, air dried);
- 20 % kaolin clay (kaolinite content above 30 %);
- 75–76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);
- approximately 0.2 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;
- organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;
- 100 mg ammonium chloride and sodium phosphate per kg sediment (dry weight).

The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.

SMART AND BARKO medium:

- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L
- $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L
- $\text{NaHCO}_3$ : 58.4 mg/L
- $\text{KHCO}_3$ : 15.4 mg/L
- pH (air equilibrium) approximately 7.9

**Number of replicates:**

**Untreated variant:**

**Reference substance:**

Five replicates per test item concentration and ten replicates for the control were used

Test vessel/medium without test substance

None

#### 4. Environmental conditions during testing

Temperature	Test solution temperature (range): $20.9 \pm 0.5$ °C
pH	Test solution pH (range): $8.05 \pm 0.52$
Oxygen concentration [mg/L]	The oxygen saturation was determined to be $120 \pm 39$ %
Photoperiod	Photoperiod: 16 h day length
Light intensity	$120\text{--}160 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

### B. STUDY DESIGN AND METHODS

1. In-life dates: 16.09.2014 – 16.10.2014

2. Experimental design: Test with *Myriophyllum spicatum* was conducted in an in-house culture maintained in a growth medium. Five replicates per test item concentration and ten replicates for the control were used. The duration of the test was 14 days. The test was performed under static test conditions. The nominal concentrations of the test item during the test were 0.00954, 0.0305, 0.0977, 0.313 and 1.00 mg/L and control. The test item was spiked to the water. Test item concentrations in the definitive test were verified by analyses of 2,4-D at all concentration levels by analysing the overlying water at test start and test end and wet sediment at test termination on day 14

Test concentrations: Nominal: 0.00954, 0.0305, 0.0977, 0.313, and 1 mg test item/L

Chemical analysis and validation: HPLC MS/MS  
 Samples taken: 0 and 14 days, in addition at the concentration level of 1.00 mg/L at day 7  
 Limit of Detection: 30% of the LOQ (= 0.000222 mg 2,4-D/L in test medium resp. 0.00120 mg 2,4-D/kg in sediment)  
 Limit of Quantitation: 0.0015 mg test item (0.000740 mg 2,4-D/L) in test medium and 0.004 mg 2,4-D/kg in sediment

Test duration: 14 days

3. Observations: On day 14 plants were harvested from each treatment group for assessment of shoot length, total plant (i.e. shoots plus roots) fresh weight, total plant (i.e. shoots plus roots) dry weight and number and length of side shoots. Additionally, the main shoot length was measured by use of a ruler on days 0, 7 and 14 during the test.

Temperature, pH and oxygen saturation (%) of the test solutions, measured after 0, 7 and 14 days, are reported.

4. Statistics: Endpoints reported are the  $EC_{50}$  for yield ( $E_yC_{50}$ ) and growth rate ( $E_rC_{50}$ ) based on the increase in total shoot length and total plant (i.e. shoots plus roots) biomass respectively after 14 days of exposure. The NOEC and LOEC for yield and growth rate were also determined.

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. If data were normally distributed and variance was homogeneous a Dunnett's t-test was performed. If Shapiro-Wilk's test

indicated a non-normal distribution of residuals a Bonferroni U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3).

The EC<sub>50</sub> (yield and growth rate) was calculated where possible using Probit analysis. Only concentrations within a clear dose response were used for calculations.

## H. RESULTS AND DISCUSSION

### A. Analytical results

The measured concentration of the test item based on the 2,4 D content in the test vessels at test start ranged between 86 and 123 % of nominal in the overlaying water. A mean measured content of 106 % was observed. As the content of 2,4 D was > 80 % of nominal at test start all toxicological endpoints were evaluated using nominal concentrations of the test item.

After 14 days 2,4 D concentrations in the water ranged between 82 – 90 % of nominal. In the sediment, concentrations of 2,4 D were detectable with recoveries ranged between 10 – 14 % of the amount applied.

### B. Biological results

The biological results are summarised in Table 4 to 6.

**Table 4: Summary of Biological Results based on Nominal Concentrations of LAF-74 and Total Shoot Length**

Parameter	Growth rate (total shoot length in cm) [mg/L]	Yield (total shoot length in cm) [mg/L]
14 day EC <sub>50</sub>	0.715	0.376
95 % CI	0.590 – 0.910	0.323 – 0.437
14 day NOEC	0.0977	0.0977
14 day LOEC	0.313	0.313

CI: Confidence interval.

**Table 5: Summary of Biological Results based on Nominal Concentrations of LAF-74 and Fresh Weight**

Parameter	Growth rate (total shoot length in cm) [mg/L]	Yield (total shoot length in cm) [mg/L]
14 day EC <sub>50</sub>	0.783	0.373
95 % CI	0.619 – 1.06	0.314 – 0.447
14 day NOEC	0.0977	0.0977
14 day LOEC	0.313	0.313

CI: Confidence interval.

**Table 6: Summary of Biological Results based on Nominal Concentrations of LAF-74 and Dry Weight**

Parameter	Growth rate (total shoot length in cm) [mg/L]	Yield (total shoot length in cm) [mg/L]
14 day EC <sub>50</sub>	>1.00 <sup>†</sup>	0.560
95 % CI	–	0.466 – 0.692
14 day NOEC	0.0977	0.0977
14 day LOEC	0.313	0.313

<sup>†</sup> No effect > 50% could be observed, therefore the EC<sub>50</sub> was estimated to be > 1.00 mg/L.

– Not calculable.

CI: Confidence interval.

### C. Validity criteria

The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots. Over 14 days, the mean total shoot length increased more than 9.5 fold, fresh weight biomass increased more than 7.5 fold, and mean dry weight biomass increased more than 4 fold.

The mean control growth rate based on shoot length, fresh weight and dry weight was 0.1623, 0.1437 and 0.1026 /day respectively, which is equivalent to a mean doubling time of 4.2, 4.8 and 6.8 days respectively. The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight was 8.3 %, 12.2 % and 19.6 % respectively.

The mean control yield (and C.V.) based on shoot length was 54.0 cm (C.V. = 20.5 %), for fresh weight yield was 1.7354 g (C.V. = 27.5 %), and for dry weight yield was 0.1409 g (C.V. = 33.4 %).

Since the CV for fresh weight and shoot length yield was below 35 % and a doubling of shoot biomass and length was reached within the test duration the mean control growth rates and variability were considered acceptable.

### III. CONCLUSION

The measured concentration of the test item based on the 2,4 D content in the test vessels at test start ranged between 86 and 123 % of nominal in the overlaying water. A mean measured content of 106 % was observed. As the content of 2,4 D was > 80 % of nominal at test start all toxicological endpoints were evaluated using nominal concentrations of the test item.

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to LAF 74 for 14 days, the  $E_rC_{50}$  and  $E_yC_{50}$  values based on total shoot length were 0.715 mg/L and 0.376 mg/L respectively (equivalent to 0.357 mg 2,4 D/L and 0.188 mg 2,4 D/L respectively). The NOEC for growth rate and yield based on total shoot length was 0.0977 mg/L (equivalent to 0.049 mg 2,4 D/L).

The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (fresh weight) were 0.783 mg/L and 0.373 mg/L respectively (equivalent to 0.391 mg 2,4 D/L and 0.186 mg 2,4 D/L respectively). The NOEC for growth rate and yield based on biomass (fresh weight) was 0.0977 mg/L.

The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (dry weight) were > 1.00 mg/L and 0.560 mg/L, respectively (equivalent to > 0.499 mg 2,4 D/L and 0.280 mg 2,4 D/L respectively). The NOEC for growth rate and yield based on biomass (dry weight) was 0.0977 mg/L. Overall, *Myriophyllum spicatum* is much less sensitive to 2,4 D after a 24 hour pulsed exposure to the test item.

The following study on growth inhibition of *Myriophyllum spicatum* in a water/sediment performed with Fluroxypyr acid was provided in support of the assessment.

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

### **1,2,4-Benzenetriol**

The following fish acute toxicity study performed with 1,2,4-Benzenetriol were provided in support of the assessment.

#### **Study 1: Acute toxicity to fish**

Comments of zRMS:	<p>The study was performed in line with OECD 203 with no major deviation regarding the test design and conditions with exception of the temperature which was in range of 15.8-16.2°C, while in the most recent version of the guideline (June 2019) temperature of 10-14°C. However, as all validity criteria were met, this deviation is considered to have no impact on the study results.</p> <p>It is, however, noted that metabolite 1,2,4-benzenetriol degraded rapidly in the test system with measured concentration &lt;LOD already after 1 hour after test initiation. Since no measurable residues of the test item were present in the test solutions by the end of the study, the endpoints were expressed in terms of the nominal and initial measured concentrations. However, the endpoints may be expressed in terms of nominal concentrations only when the test item measured concentrations are maintained at 80-120% of nominal throughout the study period. It is also possible to base the endpoints on initial measured concentrations, provided that the measured concentrations over the study period were maintained within 80-120% of the initially measured concentrations. However, this procedure is not described for the acute fish study (OECD 203), but for <i>Daphnia</i> acute study (OECD 202), algae study (OECD 201), <i>Lemna</i> study (OECD 221) and <i>Myriophyllum</i> studies (OECD TG 238 and 239).</p> <p>Since in the study performed with 1,2,4-benzenetriol the measured concentrations of the test item were not maintained within 80-120% of nominal or mean measured concentrations, the endpoints should be expressed in terms of the mean measured concentrations. However, as the concentration of 1,2,4-benzenetriol dropped rapidly &lt;LOD, this was not possible and new test with adjusted exposure regime (semi-static or flow-through) should have been performed.</p> <p>Since according to the current standards it is not possible to derive reliable endpoints from the study, the study is considered not valid.</p> <p>The summary below has been struck through.</p>
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Reference:	KCP 10.2.1/07
Report	1,2,4-benzenetriol: Toxicity to the rainbow trout <i>Oncorhynchus mykiss</i> under laboratory conditions (acute toxicity test – static). ... (2015). S15-00611 (report number)
Guideline(s):	OECD Guideline for testing of chemicals, Section 2, No. 203 SANCO/3029/99 rev.4 11/07/00 Annex II (part A; Section 4) and Annex III (PART a; Section 5) of directive 91/414
Deviations:	-
GLP:	Yes
Acceptability:	Not valid, test item concentrations <LOD already 1 hour after test initiation and due to the static test design the mean measured concentrations could not be determined
Duplication (if vertebrate study)	-

## **I. MATERIAL AND METHODS**

### **A. MATERIALS**

#### **1. Test Material**

#### **1,2,4-benzenetriol**



<b>Description</b>	Solid / brown
<b>Lot/batch #</b>	DE3-150845-38 TSN309430
<b>Concentration/Purity</b>	96 % w/w
<b>2. Vehicle and/or control</b>	Water
<b>3. Test animals (Species)</b>	Juvenile rainbow trout ( <i>Oncorhynchus mykiss</i> Walbaum; Salmoniformes: Salmonidae)
<b>Size (weight /length) at test start</b>	4–6 cm
<b>Source</b>	Forellenzuchtbetrieb Störk, 88348 Bad Saulgau, Germany
<b>Acclimation period</b>	> 12 days
<b>Diet</b>	The fish were fed each day with granular rearing food with approx. 2 % of their body weight. The food was obtained from the fish supplier
<b>Water</b>	Water composed of dechlorinated drinking water and deionised water.
<b>Holding</b>	25 L glass aquaria, filled with 15 L
<b>Number of animals per replicate</b>	7 individuals per concentrate
<b>Number of replicates</b>	1 per test concentration and control
<b>Untreated variant</b>	Reconstituted water
<b>Reference standard</b>	None
<b>4. Environmental conditions during testing</b>	
<b>Temperature</b>	15–17 °C water temperature
<b>pH</b>	6.0–8.5
<b>Hardness</b>	140–250 mg/L (as CaCO <sub>3</sub> ), corresponding to 7.8–14 °dH
<b>Oxygen concentration</b>	> 60 % of the saturation
<b>Aeration</b>	Continuous aeration of the test tanks with a membrane pump using a Pasteur pipette
<b>Photoperiod</b>	12 to 16 hours daily.
<b>Light intensity</b>	Not stated

## **B. STUDY DESIGN AND METHODS:**

<b>1. In-life dates</b>	18.05.–30.06.2015
<b>2. Experimental design</b>	The acute toxicity to rainbow trout was determined in an aerated, static, 96-hour test. The test fish were observed after approximately 0, 2, 24, 48, 72 and 96 hours test duration for sublethal effects and mortality.
<b>Test concentrations</b>	2.13, 4.70, 10.3, 22.7 and 50.0 mg/L and control
<b>Chemical analysis and validation</b>	GC/MS method
<b>Test duration</b>	96 hours
<b>3. Observations</b>	The test fish were observed after 0, 4, 24, 48, 72 and 96 hours test duration for sublethal effects and mortality. Dead fish were removed if observed and mortality, length and weight were recorded.
<b>4. Statistics</b>	The NOEC was determined directly from the raw data. Since no mortality above the allowed control mortality was observed the LC <sub>50</sub> -value (96 h) was not calculated.

## H. RESULTS AND DISCUSSION

### A. Analytical results

The quantification of the active ingredient 1,2,4 benzenetriol in the test solution samples was performed using a liquid/liquid extraction, derivatization with MSTFA and final determination by GC-MS. Analytical samples from control, 2.13, 4.70, 10.3, 22.7 and 50.0 mg/L were analyzed at  $t = 0$  h fresh. Additionally, samples from control and 50.0 mg/L taken at  $t = 1$  h aged,  $t = 2$  h aged,  $t = 4$  h aged and  $t = 24$  h aged were analyzed.

The measured content of 1,2,4 benzenetriol in fresh test solutions ranged from 58 % to 71 % of nominal. The measured content of 1,2,4 benzenetriol in aged test solutions was below the limit of detection (LOD = 0.075 mg/L) within 1 hour of dosing. Thus, more than 99 % degradation of the test material occurred within 1 hour of test initiation. Since the initial measured concentrations of 1,2,4 benzenetriol were below 80 % of nominal concentrations, the biological endpoints were evaluated using nominal and initial measured test item concentrations.

The concentration course of the test item is presented in the following table.

**Table 7: Determined test item concentrations and 1,2,4 benzenetriol concentrations (corrected for 96 % purity) during the test**

Test item nominal [mg/L]	1,2,4-benzenetriol [mg/L]	Sampling [h]	1,2,4 benzenetriol found		Test item actual* [mg/L]
			[mg/L]	[%]	
0	0	0 h fresh	n.d.	-	-
		1 h aged	n.d.	-	-
		2 h aged	n.d.	-	-
		4 h aged	n.d.	-	-
		24 h aged	n.d.	-	-
2.13	2.04	0 h fresh	1.44	71	1.51
4.70	4.51	0 h fresh	2.93	65	3.06
10.3	9.89	0 h fresh	5.74	58	5.97
22.7	21.8	0 h fresh	13.0	60	13.6
50.0	48.0	0 h fresh	28.4	59	29.5
		1 h aged	n.d.	-	-
		2 h aged	n.d.	-	-
		4 h aged	n.d.	-	-
		24 h aged	n.d.	-	-

LOQ = 0.250 mg/L 1,2,4 benzenetriol

\*: Calculated from measured 0 h concentrations

-: Not calculable

n.d.: not detectable (LOD = 0.075 mg/L)

### B. Biological results

In the control all fish survived until the end of the experiment and showed no sublethal effects during the exposure time. At the test item concentrations up to and including 50.0 mg/L no mortality above the allowed control mortality was observed within the period of the test. At 50.0 mg/L one fish was accidentally killed during inspection after 72 h; the death was not caused by the test item.

A dark coloration of the test solutions (10.3, 22.7 and 50.0 mg/L), which decreased in intensity with decreasing test concentration, could be observed throughout the period of the test. It was therefore difficult to identify sublethal effects with absolute certainty.

All fish were weighed and measured. The average weight of the test organisms was  $1.26 \pm 0.30$  g; the average length was  $51 \pm 4$  mm.

**Table 8: Mortality of fish in the test [%]**

Test item concentration [mg/L]	Control	2.13	4.70	10.3	22.7	50.0
Time [h]	Mortality [%]					
4	0	0	0	0	0	0
24	0	0	0	0	0	0
48	0	0	0	0	0	0
72	0	0	0	0	0	14 <sup>†)</sup>
96	0	0	0	0	0	14 <sup>†)</sup>

<sup>†)</sup> Fish accidentally killed.

### C. Validity criteria

- In the control no fish died until the end of the test.
- Dissolved oxygen concentration: > 60 % of the air saturation.

The criteria of the OECD 203 were met, so the study can be considered as valid.

### III. CONCLUSION

According to the results of the test, the  $LC_{50}$  (96 h) of the test item was determined to be > 50 mg/L test item (nominal). The corresponding NOEC (mortality) (96 h) was 50 mg/L test item (nominal).

Based on initial (t = 0 h) measured concentrations, the  $LC_{50}$  (96 h) of the test item was determined to be > 29.5 mg/L test item (actual). The corresponding NOEC (mortality) (96 h) was 29.5 mg/L test item (actual).

No sublethal effects were observed in the control and at all test item concentrations after 96 h.

### Study 1: Acute toxicity to aquatic invertebrates

Comments of zRMS:	<p>The study was performed in line with OECD 202 with no deviation regarding the test design and conditions.</p> <p>It is, however, noted that metabolite 1,2,4-benzenetriol degraded rapidly in the test system with measured concentration &lt;LOD already after 1 hour after test initiation. Since no measurable residues of the test item were present in the test solutions by the end of the study, the endpoints were expressed in terms of the nominal and initial measured concentrations. However, the endpoints may be expressed in terms of nominal concentrations only when the test item measured concentrations are maintained at 80-120% of nominal throughout the study period. It is also possible to base the endpoints on initial measured concentrations, provided that the measured concentrations over the study period were maintained within 80-120% of the initially measured concentrations.</p> <p>Since in the study performed with 1,2,4-benzenetriol the measured concentrations of the test item were not maintained within 80-120% of nominal or initial mean measured concentrations, the endpoints should be expressed in terms of the mean measured concentrations. However, as the concentration of 1,2,4-benzenetriol dropped rapidly &lt;LOD, this was not possible and new test with adjusted exposure regime (semi-static or flow-through) should have been performed.</p> <p>Since according to the current standards it is not possible to derive reliable endpoints from the study, the study is considered not valid.</p> <p>The summary below has been struck through.</p>
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Reference:	KCP 10.2.1/08
Report	1,2,4-Benzenetriol- Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test –Static). Zawadsky, C. (2015). S15-00612 (report number)
Guideline(s):	OECD Guideline 202
Deviations:	-
GLP:	Yes
Acceptability:	Not valid, test item concentrations <LOD already 1 hour after test initiation and due to the static test design the mean measured concentrations could not be determined
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** 1,2,4 Benzenetriol  
**Description:** Solid / brown  
**Lot/batch :** DE3-150845-38, TSN309430  
**Concentration/Purity:** 96 % w/w  
**Stability of test compound:** Expiry date: February 2016
- Vehicle and/or control:** Elendt M4 test medium
- Test animals**  
**Species:** *Daphnia magna* Straus, Clone V  
**Age at test start:** Not exceeding 24 hours old.  
**Source:** The animals are continuously bred in the laboratory and were originally purchased in a healthy condition from the Federal Environmental Agency in Berlin/Germany.  
**Acclimation period:** Not necessary, since the test was performed in the same medium as the culturing  
**Feeding:** The animals were fed with single cell green algae (*Desmodesmus subspicatus*, former *Scenedesmus subspicatus*) at least three times a week.  
**Number of study organisms per concentration and control:** Nominal: 6.25, 12.5, 25.0, 50.0, and 100 mg/L  
**Number of animals per test vessel:** 5  
**Number of replicates:** 4  
**Test vessel:** Four 100 mL glass beakers per concentration, each filled with 50 mL, one additional replicate for physico-chemical measurements without organisms  
**Untreated variant:** Test medium without test substance  
**Reference standard:** The reference item: Potassium dichromate
- Environmental conditions during testing**  
**Temperature:** 20.3 ± 0.2 °C  
**pH:** 7.79 ± 0.03  
**Hardness:** 232 mg/L as CaCO<sub>3</sub>  
**Oxygen concentration:** 7.5 ± 2.4 mg/L  
**Aeration:** Not stated  
**Photoperiod:** 16 h light – 8 h dark

### B. STUDY DESIGN AND METHODS

- In-life dates:** 20.05.2015 – 03.07.2015

## 2. Experimental design:

~~Following a static non-GLP range-finding test with concentrations of 0, 1.00, 10.0 and 100 mg/L a static main test with concentrations of 0, 6.25, 12.5, 25.0, 50.0 and 100 mg/L was performed. Twenty daphnids per test concentration (4 replicates of 5 animals each) were used. The duration of the test was 48 hours. Test solutions were prepared by dilution of the test item in test medium and application of defined volumes of the test solutions to the test vessels~~

**Test concentration:**

~~0, 6.25, 12.5, 25.0, 50.0 and 100 mg/L~~

**Chemical analysis and validation:**

~~Liquid/liquid extraction of acidified test medium samples with ethyl acetate, derivatisation with MSTFA and final determination by GC-MS~~

**Test duration:**

~~48 hours~~

## 3. Observations:

~~Freshly hatched daphnids max. 24 hours old were exposed to increasing concentrations of the test item for 48 hours. After 24 h and 48 h the immobilised daphnids were counted. All daphnids not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. If present, behavioural changes of daphnids were recorded at 24 and 48 hours after starting the test.~~

~~Temperature, pH value and oxygen concentration of the test solutions measured after 0, 24 and 48 hours are reported.~~

~~Hardness of the test water was measured on the day of application.~~

~~The necessary amount of 1,2,4 Benzenetriol for preparing the highest test solution was weighed on a weighing scoop and transferred to a volumetric flask. Test medium was added up to the bench mark and the solution was homogenised by shaking. The solution was slightly red coloured after shaking. The lower test solutions were prepared by serial dilution with test medium. Defined volumes of the prepared solution were transferred to each test vessels. The test solution volume was 50 mL per test vessel.~~

~~Analytical samples were taken from all test concentrations up to 100 mg/L and control at 0 hours (initial value) from fresh test solution, from the highest test item concentration and control after 1, 2, 4 and 24 hours from aged test solution and from all test concentrations up to 100 mg/L and control after 48 hours from aged test solution. The control and all test item concentrations were analysed at 0 hours (initial value) and the control and highest test item concentration of 100 mg/L were analysed at t = 1 h, 2 h, 4 h and 24 h.~~

## 4. Statistics

~~The 24 h and 48 h EC<sub>50</sub> are the estimated concentrations~~

where 50 % of the daphnids were immobilised after 24 and 48 hours, respectively.

For the evaluation of the 24 h and 48 h EC<sub>50</sub>, probit analysis using linear max. likelihood regression was used. The evaluation of data was performed by using ToxRat Professional 3.0.0.

The NOEC was established based on the highest concentration at which the immobilisation is not higher than the allowed control immobilisation ( $\leq 10$  % immobilisation).

## II. RESULTS AND DISCUSSION

### A. — Analytical results

The test item is rapidly degraded in aqueous solution. Consequently, the initial measured concentrations of 1,2,4 benzenetriol in the test item concentrations ranged from 13 % to 22 % of nominal. In the 100 mg/L aged test solution the content of 1,2,4 benzenetriol was below the detection limit (LOD = 0.075 mg/L) within 1 hour of dosing. Thus, it can be inferred that more than 99 % degradation of the test material occurred in all test solutions within 1 hour of test initiation. Since the initial measured concentrations of 1,2,4 benzenetriol were below 80 % nominal, and all concentrations were below the limit of detection within 1 hour of exposure, the toxicological endpoints were evaluated using nominal and initial measured concentrations of the test item.

### B. — Biological results

After 24 hours of exposure no immobilisation higher than the allowed control immobilisation was observed in the control and up to and including 12.5 mg/L (nominal) / 2.13 mg/L (initial). At 25.0 mg/L (nominal) / 4.50 mg/L (initial) 30 % immobilisation was observed. At 50.0 mg/L (nominal) / 7.50 mg/L (initial) and the highest test item concentration of 100 mg/L nominal) / 13.0 mg/L (initial) all daphnids were found immobile. After 48 hours of exposure no immobilisation higher than the allowed control immobilisation was observed at the control and up to and including 12.5 mg/L (nominal) / 2.13 mg/L (initial). At 25.0 mg/L (nominal) / 4.50 mg/L (initial) 55 % immobilisation was observed. At 50.0 mg/L (nominal) / 7.50 mg/L (initial) and the highest test item concentration of 100 mg/L (nominal) / 13.0 mg/L (initial) all daphnids were found immobile.

At test start, a decrease in oxygen concentration could be observed which was dependent on the test item concentration. The highest test item concentrations resulted in oxygen levels of 6.0, 2.1 and 0.7 mg/L for the 25.0, 50.0 and 100 mg/L test concentrations respectively at test start. Oxygen levels increased with time at these test item concentrations but only recovered to control levels after 48 h in the highest test item concentration. This reduction in oxygen levels may have contributed to the mortality of the test organisms.

At test start, a slight pH reduction could be observed which was dependent on the test item concentration, particularly for the 25.0, 50.0 and 100 mg/L test concentrations. pH levels returned to control levels in the 25.0 and 50.0 mg/L concentrations after 24 h. This reduction in pH may have contributed to the mortality of the test organisms.

The 24 h and 48 h EC<sub>50</sub> are the estimated concentrations where 50 % of the daphnids were immobilised after 24 and 48 hours, respectively. For the evaluation of the 24 h and 48 h EC<sub>50</sub> Probit analysis using linear max. likelihood regression was used. The evaluation of data was performed by using ToxRat Professional 3.0. The NOEC was established based on the highest concentration at which the immobilisation was not higher than the allowed control immobilisation ( $\leq 10$  % immobilisation).

**Table 9: Effect of 1,2,4 Benzenetriol on immobilisation**

Treatment [mg test item/L]	24-hr		48-hr	
	No. immobile	% Immobility	No. immobile	% Immobility
Negative control	0	0	0	0
6.25	0	0	2	10
12.5	1	5	1	5
25.0	6	30	11	55
50.0	20	100	20	100
100	20	100	20	100
NOEC	12.5 mg/L (nominal) 2.13 mg/L (initial measured)		12.5 mg/L (nominal) 2.13 mg/L (initial measured)	
EC <sub>50</sub>	27.0 mg/L (nominal) 4.64 mg/L (initial measured)		21.0 mg/L (nominal) 3.66 g/L (initial measured)	

### C. Validity criteria

All the criteria were met in the test:

- In the control no daphnid died until the end of the test.
- The dissolved oxygen concentration at the end of the test was 3 mg/L in the control and the test vessels.

### III. CONCLUSION

According to the results of the test, the EC<sub>50</sub> (48 h) for immobilisation is determined to be 21.0 mg/L (nominal) and 3.66 mg/L (initial measured). The corresponding NOEC (48 h) was 12.5 mg/L (nominal) and 2.13 mg/L (initial measured).

### Study 1: Toxicity to macrophytes

Comments of zRMS:	<p>The study was performed in line with OECD 239, but the replication and the number of plants was modified so there were 10 and 5 replicates per control and test item groups, respectively with one plant each, while the test guideline for water/sediment test indicates that there should be 6 and 4 replicates for control and test item groups, respectively, with 3 shoots per replicate. However, modified replication of the study performed in line with OECD 239 was considered acceptable during the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (see EFSA Supporting publication 2019:EN-1673), so it is not challenged here despite the reduced number of plants per test concentration.</p> <p>It is, however, noted that metabolite 1,2,4-benzenetriol degraded rapidly in the test system with measured concentration &lt;LOD already after 1 hour after test initiation in all tested concentrations and &lt;LOD at 0 h in two lowest test concentrations. Since no measurable residues of the test item were present in the test solutions by the end of the study, the endpoints were expressed in terms of the nominal and initial measured concentrations. However, the endpoints may be expressed in terms of nominal concentrations only when the test item measured concentrations are maintained at 80-120% of nominal throughout the study period. It is also possible to base the endpoints on initial measured concentrations, provided that the measured concentrations over the study period were maintained within 80-120% of the initially measured concentrations.</p> <p>Since in the study performed with 1,2,4-benzenetriol the measured concentrations of the test item were not maintained within 80-120% of nominal or initial mean measured concentrations, the endpoints should be expressed in terms of the mean measured concentrations. However, as the concentration of 1,2,4-benzenetriol dropped rapidly &lt;LOD, this was not possible and new test with adjusted exposure regime (semi-static) should have been performed.</p> <p>The residues of the test item in the sediment were not measured, however partitioning is not expected due to rapid degradation of 1,2,4-benzenetriol observed in fish and <i>Daphnia</i> study, performed without sediment.</p>
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	<p>Since according to the current standards it is not possible to derive reliable endpoints from the study, the study is considered not valid.</p> <p>The summary below has been struck through.</p>
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Reference:	KCP 10.2.1/9
Report	1,2,4-Benzenetriol: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. Gonsior, G., (2015). S15-00667 (report number)
Guideline(s):	OECD Guideline 239
Deviations:	-
GLP:	Yes
Acceptability:	Not valid, test item concentrations <LOD already 1 hour after test initiation and due to the static test design the mean measured concentrations could not be determined
Duplication (if vertebrate study)	-

## ~~I. MATERIAL AND METHODS~~

### ~~A. MATERIALS~~

- Test Material:** 1,2,4 Benzenetriol

**Description:** Solid brown

**Lot/batch, density:** DE3-150845-38, TSN309430

**Concentration/Purity:** 96 % w/w

**Stability of test compound:** Re-certification date: 05-October-2017
- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART ANBARKO medium
- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*

**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agroscience Services EcoChem GmbH since November 2015. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.

*M. spicatum* is cultivated under sterile conditions submersed in a modified, aqueous ANDREWS medium containing sucrose. This laboratory stock culture is used to provide uniform plants throughout the year, eliminating seasonal variation in plant quality and contamination by other species (e.g. algae). The stock culture plants were held under the same environmental conditions as used in the test.

**Acclimation period:** Nine days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium under the same temperature, light, and water quality conditions as used during the exposure of the plants in the test. Shoot were anchored in an upright position using glass rings and were maintained under controlled environment conditions.

**Culture medium:** ANDREWS Medium

**Test vessel:** Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with SMART AND



~~BARKO medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.~~

~~Sediment used in the test (percentages based on dry weight):~~

- ~~▪ 4 % sphagnum peat (approximately pH 5.5–6.0; no visible plant remains, finely ground, air dried);~~
- ~~▪ 20 % kaolin clay (kaolinite content above 30 %);~~
- ~~▪ 75–76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);~~
- ~~▪ approximately 0.2 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;~~
- ~~▪ organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;~~
- ~~▪ 100 mg ammonium chloride and sodium phosphate per kg sediment (dry weight).~~

~~The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.~~

~~SMART AND BARKO medium:~~

- ~~▪  $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L~~
- ~~▪  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L~~
- ~~▪  $\text{NaHCO}_3$ : 58.4 mg/L~~
- ~~▪  $\text{KHCO}_3$ : 15.4 mg/L~~
- ~~▪ pH (air equilibrium) approximately 7.9~~

<del>Number of replicates:</del>	<del>Five replicates per test item concentration and ten replicates for the control were used</del>
<del>Untreated variant:</del>	<del>Test vessel/medium without test substance</del>
<del>Reference substance:</del>	<del>None</del>

#### ~~4. Environmental conditions during testing~~

<del>Temperature</del>	<del>Test solution temperature (range): <math>19.8 \pm 0.3</math> °C</del>
<del>pH</del>	<del>Test solution pH (range): <math>8.20 \pm 0.30</math></del>
<del>Oxygen concentration [mg/L]</del>	<del>The oxygen saturation was determined to be <math>107 \pm 16</math> %</del>
<del>Photoperiod</del>	<del>Photoperiod: 16 h day length</del>
<del>Light intensity</del>	<del><math>120\text{--}160 \mu\text{Em}^{-2}\text{s}^{-1}</math></del>

## ~~B. STUDY DESIGN AND METHODS~~

- ~~1. In-life dates:~~ ~~16.04.2015–03.07.2015~~
- ~~2. Experimental design:~~ ~~Plants were grown in a static water sediment system using artificial sterilised sediment overlaid with Smart and Barko medium under the same conditions as used in the pre-~~

culture. The study was conducted in 2 L glass beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels. Each vessel contained approx. 350 g of moist sediment containing growth nutrients (ammonium chloride and sodium phosphate), with the sediment surface overlaid with moist sediment without nutrients, and a thin layer of washed quartz sand, to minimise displacement of the sediment when the growth medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L). Two days after preparation of the test vessels and before application of the test item, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring. The test item was spiked to the water at nominal concentrations of 0.477, 1.53, 4.88, 15.6 and 50.0 mg 1,2,4 Benzenetriol /L. Ten replicates were used for the control and five for each test item group. On day 0 fifteen additional plants, representative of those used in the test, were selected from the available plant material. The plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours.

**Test concentrations:**

Nominal: 50.0, 15.6, 4.88, 1.53 and 0.477 mg test item/L and control

**Chemical analysis and validation:** HPLC MS/MS

**Test duration:**

Samples taken: 0 hours, 1 hour, 2 hours, 4 hours, 1 day, 7 days and 14 days

Limit of Detection: The limit of detection (LOD) was defined as 30 % of the limit of quantification (= 0.0300 mg/L of 1,2,4 benzenetriol). Limit of Quantitation: The limit of quantification was 0.100 mg/L of 1,2,4 benzenetriol. Recoveries from QC fortifications: (70 ± 110 % mean recovery, ≤ 20 % RSD)

**3. Observations:**

Test item concentrations in the definitive test were verified by analyses of 1,2,4 Benzenetriol at all concentration levels and control by analysing the overlaying water at test start. Only the highest test item concentration in the overlaying water and control was analysed from samples taken at t=1 h, t=2 h, t=4 h, t=1 d and t=7 d.

The weight of the dry plant samples was recorded. On day 14 plants were harvested from each treatment group for assessment of total plant fresh weight, total plant dry weight, shoot length and number and length of side shoots. In addition, observations on shoot and root development (e.g. necrosis, deformation) were documented

Temperature (°C), pH and oxygen saturation (%) of the test solutions, measured after 0, 7 and 14 days, are reported.

#### 4. Statistics:

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. As data were normally distributed and variance was homogeneous a Dunnett's t test was performed (SAS® Proprietary Software 9.3).

The  $EC_{50}$  (yield and growth rate) was calculated where possible using Probit analysis.

The  $EC_{10}$  and  $EC_{20}$  (yield and growth rate) were calculated where the C.V. in the control cultures was low enough to allow for reliable estimates to be determined.

For example, estimates of  $EC_{10}$  and  $EC_{20}$  values are only reliable if the C.V. in control plants is below the effect level being estimated, i.e. C.V. should be < 20 % for robust estimation of an  $EC_{20}$  and C.V. should be < 10 % for robust estimation of an  $EC_{10}$ .

Only concentrations within a clear dose response were used for calculations. Negative values were set to zero.

## H. RESULTS AND DISCUSSION

### A. Analytical results

The measured concentration of 1,2,4 Benzenetriol was below the limit of detection (LOD = 0.03 mg/L) at test start in the 0.477 and 1.53 mg/L treatment levels. At the higher concentrations, 4.88, 15.6 and 50 mg/L, measured Day 0 concentrations were 80, 72 and 61 % of nominal respectively. Because initial concentrations were quantified for treatment levels representing the NOEC level and above, the low recoveries (< LOD) for the 0.477 and 1.53 mg/L treatment levels did not prevent determination of the relevant ecotoxicological endpoints for this study. Following dosing of the test system, 1,2,4 Benzenetriol showed a very fast oxidation. No 1,2,4 Benzenetriol could be detected (LOD = 0.03 mg/L) in the water phase of the highest test concentration after 1 hour. This illustrates that degradation of the test item was extremely rapid, with greater than 99.9% degradation within 1 hour of dosing the overlaying water. This was also indicated by an observed change in the colour of the test medium to dark brown within 1 day. Due to the fast oxidation, 1,2,4 Benzenetriol was not measured in the sediment. As the content of 1,2,4 Benzenetriol was less than 80 % of nominal at test start, all toxicological endpoints were determined and reported using both nominal and initial measured concentrations of the test item (1,2,4 Benzenetriol).

### C. Biological results

Summary of the biological results are shown in the following tables.

Table 10: Mean total shoot length including side shoots (cm)

Nominal concentration [µg/L]	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 <sup>1)</sup>	14				
Control	8.1	51.3	43.2	-	0.1313	-
0.477	8.1	44.3	36.2	16.2	0.1208	8.0
1.53	8.1	52.2	44.1	-2.1	0.1328	-1.1
4.88	8.1	62.2	54.1	-25.2	0.1450	-10.4
15.6	8.1	48.1	40.0	7.4	0.1264	3.7
50.0	8.1	28.3	20.2*	53.2*	0.0889*	32.3*

\* Significantly different reduction compared to the pooled control

<sup>1)</sup> Based on 15 additional plants, representative of those used in the test

**Table 11: Mean total plant fresh weight (g)**

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 <sup>1)</sup>	14				
Control	0.2605	1.8521	1.5916	-	0.1392	-
0.477	0.2605	1.5976	1.3371	16.0	0.1286	7.6
1.53	0.2605	1.7548	1.4943	6.1	0.1359	2.4
4.88	0.2605	2.0940	1.8335	15.2	0.1482	-6.5
15.6	0.2605	1.4882	1.2277*	22.9*	0.1239	11.0
50.0	0.2605	0.8330	0.5725*	64.0*	0.0814*	41.5*

\* Significantly different reduction compared to the pooled control

<sup>1)</sup> Based on 15 additional plants, representative of those used in the test

**Table 12: Mean total plant dry weight (g)**

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 <sup>1)</sup>	14				
Control	0.0347	0.1755	0.1408	-	0.1145	-
0.477	0.0347	0.1417	0.1070	24.0	0.0986	13.9
1.53	0.0347	0.1529	0.1182	16.1	0.1055	7.9
4.88	0.0347	0.1574	0.1227	12.9	0.1069	6.6
15.6	0.0347	0.1036	0.0689*	51.1*	0.0774*	32.4*
50.0	0.0347	0.0614	0.0267*	81.0*	0.0384*	66.5*

\* Significantly different reduction compared to the pooled control

<sup>1)</sup> Based on 15 additional plants, representative of those used in the test

The calculated EC<sub>50</sub> values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below. Where the C.V. in the control cultures allowed for reliable estimates of EC<sub>10</sub> and/or EC<sub>20</sub> to be determined, these were also reported below.

**Table 13: Summary of biological results (based on nominal or measured concentrations) of 1,2,4-Benzenetriol**

Parameter (mg/L)	Total shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14 day EC <sub>50</sub>	≥ 50.0	46.9	≥ 50.0	34.4	28.9	16.8
95% Conf. Limits	n.a.	40.0–57.5	n.a.	29.2–41.7	23.6–36.8	13.9–20.3
14 day EC <sub>20</sub>	35.9	25.0	25.7	16.2	10.3	-
95% Conf. Limits	29.1–43.8	20.4–29.3	20.4–31.3	13.0–19.3	7.68–12.9	-
14 day EC <sub>10</sub>	24.6	-	16.4	-	-	-
95% Conf. Limits	17.8–30.2	-	11.6–20.7	-	-	-
14 day NOEC	15.6	15.6	15.6	4.88	4.88	4.88
14 day LOEC	50.0	50.0	50.0	15.6	15.6	15.6

(n.a.) not applicable, EC<sub>50</sub> above maximum test concentration; (–) Values not reliable, control CV exceeded the effect level

**Table 14: Summary of biological results based on initial measured concentrations of 1,2,4-Benzenetriol**

Parameter (mg/L)	Total shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14 day EC <sub>50</sub>	≥ 30.5	28.9	≥ 30.5	22.1	19.0	11.7
95% Conf. Limits	n.a.	25.2–34.5	n.a.	19.2–26.1	15.9–23.4	9.95–13.9
14 day EC <sub>20</sub>	22.9	16.8	17.1	11.5	7.67	-
95% Conf. Limits	19.1–27.2	14.1–19.2	14.0–20.4	9.44–13.4	5.90–9.34	-
14 day EC <sub>10</sub>	16.6	-	11.6	-	-	-
95% Conf. Limits	12.4–19.8	-	8.58–14.2	-	-	-
14 day NOEC	11.2	11.2	11.2	3.90	3.90	3.90
14 day LOEC	30.5	30.5	30.5	11.2	11.2	11.2

(n.a.) not applicable, EC<sub>50</sub> above maximum test concentration; (–) Values not reliable, control CV exceeded the effect level.

### C. ~~Validity criteria (from OECD 239)~~

- ~~• The control plants showed uniform growth over the test period and the mean total shoot length increased more than 6 fold, fresh weight biomass increased more than 7 fold, and mean dry weight biomass more than 5 fold.~~
- ~~• The coefficient of variation (C.V.) for control growth rates based on shoot length, fresh weight and dry weight was 6.9 %, 8.5 % and 12.7 % respectively. The mean C.V. for control yield based on shoot length, fresh weight and for dry weight was 14.9%, 19.1% and 25.4% respectively.~~

~~Since shoot biomass and length more than doubled within the test duration, and the C.V. for fresh weight and shoot length yield was below 35 %, the performance of the plants used for this test was considered acceptable.~~

### III. ~~CONCLUSION~~

~~Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to 1,2,4 Benzenetriol for 14 days, the  $E_rC_{50}$  and  $E_yC_{50}$  values based on total shoot length were  $> 30.5$  mg test item/L and 28.9 mg test item/L respectively based on initial measured concentrations. The NOEC for growth rate and yield based on total shoot length was 11.2 mg test item/L (measured). The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (fresh weight) were  $> 30.5$  mg test item/L and 22.1 mg test item/L respectively based on initial measured concentrations. The NOEC for growth rate and yield based on biomass (fresh weight) were 11.2 mg test item/L and 3.90 mg test item/L respectively based on initial measured concentrations. The  $E_rC_{50}$  and  $E_yC_{50}$  values based on biomass (dry weight) were 19.0 mg test item/L and 11.7 mg test item/L respectively based on initial measured concentrations. The NOEC for growth rate and yield based on biomass (dry weight) was 3.90 mg test item/L based on initial measured concentrations.~~

## 4-Chlorophenol

### Study 1: Toxicity to macrophytes

Comments of zRMS:	<p>The study was performed in line with OECD 239, with no major deviations. All the validity criteria were met.</p> <p>The replication and the number of plants were modified so there were 10 and 5 replicates per control and test item groups, respectively with one plant each, while the test guideline for water/sediment test indicates that there should be 6 and 4 replicates for control and test item groups, respectively, with 3 shoots per replicate. However, modified replication of the study performed in line with OECD 239 was considered acceptable during the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (see EFSA Supporting publication 2019:EN-1673), so it is not challenged here despite the reduced number of plants per test concentration.</p> <p>Since the test item concentrations were not maintained at 80-120% of nominal, the endpoints from the study were expressed in terms of geometric mean measured concentrations, in line with indications of OECD 239. It is noted that at the lowest test concentration the measured concentration of 4-Chlorophenol on day 14 were &lt;LOD, however there was sufficient information from other sampling days in this test item group and from all sampling days in other test item groups to determine the overall geometric mean measured concentrations.</p> <p>Overall, the study is considered acceptable with following endpoints relevant for the risk assessment (all based on geometric mean measured concentrations):</p> <p><u>Total shoot length</u></p> <p><math>E_rC_{50} = 13.1</math> mg pm/L  <math>E_yC_{50} = 10.4</math> mg pm/L</p>
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	<u>Fresh weight</u> $E_rC_{50} = 48.0 \text{ mg pm/L}$ $E_yC_{50} = 18.2 \text{ mg pm/L}$  <u>Dry weight</u> $E_rC_{50} = 56.7 \text{ mg pm/L}$ $E_yC_{50} = 15.4 \text{ mg pm/L}$
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Reference:	KCP 10.2.1./10
Report	4-Chlorophenol: Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System. Gonsior, G., (2015). S15-00666 (report number)
Guideline(s):	OECD Guideline 239
Deviations:	Minor (see commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** 4-Chlorophenol  
**Description:** Solid white  
**Lot/batch, density:** MKBJ7452V, TSN304318  
**Concentration/Purity:** 100 % w/w  
**Stability of test compound:** Re-certification date: 26 January 2015
- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART ANBARKO medium
- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*  
**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agrosience Services EcoChem GmbH since November 2010. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerökologie Koblenz, Germany.  
  

*M. spicatum* is cultivated under sterile conditions submersed in a modified, aqueous ANDREWS medium containing sucrose. This laboratory stock culture is used to provide uniform plants throughout the year, eliminating seasonal variation in plant quality and contamination by other species (e.g. algae). The stock culture plants were held under the same environmental conditions as used in the test.

**Acclimation period:** Nine days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium under the same temperature, light, and water quality conditions as used during the exposure of the plants in the test. Shoot were anchored in an upright position using glass rings and were maintained under controlled environment conditions.

**Culture medium:**

**Test vessel:**

**ANDREWS Medium**

Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with SMART AND BARKO medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.

Sediment used in the test (percentages based on dry weight):

- 4 % sphagnum peat (approximately pH 5.5 – 6.0; no visible plant remains, finely ground, air dried);
- 20 % kaolin clay (kaolinite content above 30 %);
- 75 – 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);
- approximately 0.2 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;
- organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;
- 100 mg ammonium chloride and sodium phosphate per kg sediment (dry weight).

The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.

**SMART AND BARKO medium:**

- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L
- $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L
- $\text{NaHCO}_3$ : 58.4 mg/L
- $\text{KHCO}_3$ : 15.4 mg/L
- pH (air equilibrium) approximately 7.9

**Number of replicates:**

Five replicates per test item concentration and ten replicates for the control were used

**Untreated variant:**

Test vessel/medium without test substance

**Reference substance:**

None

**4. Environmental conditions during testing**

**Temperature**

Test solution temperature (range):  $19.9 \pm 0.3$  °C

**pH**

Test solution pH (range):  $7.93 \pm 0.23$

**Oxygen-concentration [mg/L]**

The oxygen saturation was determined to be  $92 \pm 10$  %

**Photoperiod**

Photoperiod: 16 h day length

**Light intensity**

120 – 160  $\mu\text{Em}^{-2}\text{s}^{-1}$

**B. STUDY DESIGN AND METHODS**

**1. In-life dates:**

16.04.2015 – 03.07.2015

## 2. Experimental design:

Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with Smart and Barko medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels. Each vessel contained approx. 350 g of moist sediment containing growth nutrients (ammonium chloride and sodium phosphate), with the sediment surface overlaid with moist sediment without nutrients, and a thin layer of washed quartz sand, to minimise displacement of the sediment when the growth medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L). Two days after preparation of the test vessels and before application of the test item, one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Shortly afterwards, application of the test item was performed and mixed in with gentle stirring. The test item was spiked to the water at nominal concentrations of 0.477, 1.53, 4.88, 15.6 and 50.0 mg 4-Chlorophenol /L. Ten replicates were used for the control and five for each test item group. On day 0 fifteen additional plants, representative of those used in the test, were selected from the available plant material. The plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours.

**Test concentrations:**

**Chemical analysis and validation:** HPLC-MS/MS

**Test duration:**

Nominal: 0.477, 1.53, 4.88, 15.6 and 50.0 mg test item /L

Samples taken: 0 hours, 1 day, 3 day, 7 days and 14days

Limit of Detection: The limit of detection (LOD) was defined as 30 % of the limit of quantification (= 0.0300 mg/L of 4-Chlorophenol). Limit of Quantitation: The limit of quantification was 0.0700 mg/L of 4-Chlorophenol in water and 0.500 mg/kg 4-Chlorophenol in sediment. Recoveries from QC fortifications: (70 ± 110 % mean recovery, ≤ 20 % RSD)

## 3. Observations:

Test item concentrations in the definitive test were verified by analyses of 4-Chlorophenol at all concentration levels by analyzing the overlaying water at test start, 1, 3 and 7 days after test start and at test end and wet sediment at termination on day 14.

The weight of the dry plant samples was recorded. On day 14 plants were harvested from each treatment group for assessment of total plant fresh weight, total plant dry weight, shoot length and number and length of side shoots. In addition, observations on shoot and root development (e.g. necrosis, deformation) were documented

Temperature (°C), pH and oxygen saturation (%) of the test solutions, measured after 0, 7 and 14 days, are reported.



#### 4. Statistics:

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's or Levene's test was performed. If data were normally distributed and variance was homogeneous a Dunnett's t-test was performed. If Shapiro Wilk's test indicated a non-normal distribution of residuals a Bonferroni-U Exact Test was performed to determine significant differences from controls (SAS® Proprietary Software 9.3).

The  $EC_x$  (yield and growth rate) was calculated where possible using Probit analysis.

The  $EC_{10}$  and  $EC_{20}$  (yield and growth rate) were calculated when the C.V. in the control cultures was low enough to allow for reliable estimates to be determined.

For example, estimates of  $EC_{10}$  and  $EC_{20}$  values are only reliable if the C.V. in control plants is below the effect level being estimated, i.e. C.V. should be < 20 % for robust estimation of an  $EC_{20}$  and C.V. should be < 10 % for robust estimation of an  $EC_{10}$ .

Only concentrations within a clear dose response were used for calculations.

## II. RESULTS AND DISCUSSION

### A. Analytical results

The measured concentration of the test item based on the 4-Chlorophenol content in the overlaying water in the test vessels at test start ranged between 117 and 132 % of nominal. At test end, concentrations of 4-Chlorophenol ranged between < LOD and 90 % of nominal. At test end, concentrations of 4-Chlorophenol in the sediment were detectable at between 10 – 15 % of the amount applied in the three highest test concentrations 4.88, 15.6 and 50.0 mg/L. Since the initial mean measured concentrations of 4-Chlorophenol were > 80 % of nominal, and the mean measured concentrations at test termination were below 80 % of nominal, all toxicological endpoints were evaluated using nominal and the geometric mean measured concentrations based on 4-Chlorophenol concentration. Results of analytical measurements are presented in tables below.

Time	Nominal concentration		Overlaying water (measured concentrations)	
	Test item	4-Chlorophenol	4-Chlorophenol	
[d]	[mg/L]	[mg/L]	[mg/L]	[% of nominal]
0	Control	0.00	n.d.	-
1			n.d.	-
3			n.d.	-
7			n.d.	-
14			n.d.	-
0	0.477	0.477	0.558	117
1			0.480	101
3			0.472	99
7			0.279	58
14			n.d.	-
0	1.53	1.53	1.81	118
1			1.54	101
3			1.45	95
7			1.04	68
14			0.397	26
0	4.88	4.88	6.34	130
1			5.40	111
3			5.23	107
7			4.08	84
14			2.54	52
0	15.6	15.6	19.5	125
1			18.8	121
3			17.9	115
7			16.1	103
14			12.4	79
0	50.0	50.0	66.1	132
1			65.9	132
3			60.8	122
7			49.5	99
14			44.9	90

LOQ = 0.0700 mg/L 4-Chlorophenol in water

n.d. = not detectable

Time	Sample		Sediment
	Test item	4-Chlorophenol	4-Chlorophenol
[d]	[mg/L]	[mg/L]	[mg/kg] <sup>1)</sup>
14	Control	0.00	n.d.
	0.477	0.477	n.d.
	1.53	1.53	<LOQ
	4.88	4.88	1.24
	15.6	15.6	4.49
	50.0	50.0	18.7

LOQ = 0.500 mg/kg 4-Chlorophenol in sediment

n.d. = not detectable

<sup>1)</sup> based on wet weight

	Test item	4-Chlorophenol	Amount of sediment	4-Chlorophenol
	[d]	[mg/L]	[mg/test vessel]	[g]
14	Control	0.00	613.6	-
	0.477	0.716	614.1	-
	1.53	2.30	625.4	-
	4.88	7.32	602.0	10
	15.6	23.4	612.5	12
	50.0	75.0	619.4	15

## B. Biological results

Summary of the biological results are shown in the following tables.

**Table 15: Mean total shoot length including side shoots (cm)**

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 <sup>1)</sup>	14				
Control	8.2	24.4	16.2	-	0.0769	-
0.477	8.2	24.4	16.2	0.0	0.0778	-1.2
1.53	8.2	23.4	15.2	6.2	0.0716	6.9
4.88	8.2	21.9	13.7	15.4	0.0694	9.8
15.6	8.2	14.9	6.7*	58.6*	0.0420*	45.4*
50.0	8.2	7.6	-0.6*	103.7*	-0.0058*	107.5*

\* Significantly different reduction compared to the control.

<sup>1)</sup> Based on 15 additional plants, representative of those used in the test.

**Table 16: Mean total plant fresh weight (g)**

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 <sup>1)</sup>	14				
Control	0.2271	0.9237	0.6966	-	0.0988	-
0.477	0.2271	0.8922	0.6651	4.5	0.0975	1.3
1.53	0.2271	0.8166	0.5895	15.4	0.0880	10.9
4.88	0.2271	0.7649	0.5378	22.8	0.0847	14.3
15.6	0.2271	0.6149	0.3878*	44.3*	0.0699*	29.3*
50.0	0.2271	0.4111	0.1840*	73.6*	0.0412*	58.3*

\* Significantly different reduction compared to the control.

<sup>1)</sup> Based on 15 additional plants, representative of those used in the test.

**Table 17: Mean total plant dry weight (g)**

Nominal concentration (µg/L)	Days after application		Yield (cm)	Reduction in yield (%)	Growth rate (1/day)	Reduction in growth rate (%)
	0 <sup>1)</sup>	14				
Control	0.0265	0.1306	0.1041	-	0.1132	-
0.477	0.0265	0.1250	0.0985	5.4	0.1104	2.5
1.53	0.0265	0.1078	0.0813	21.9	0.0983	13.2
4.88	0.0265	0.0996	0.0731*	29.8*	0.0931*	17.8*
15.6	0.0265	0.0868	0.0603*	42.1*	0.0839*	25.9*
50.0	0.0265	0.0524	0.0259*	75.1*	0.0481*	57.5*

\* Significantly different reduction compared to the control.

<sup>1)</sup> Based on 15 additional plants, representative of those used in the test.

The calculated EC<sub>50</sub> values, NOEC and LOEC based on growth rate and yield for each of the measured parameters (total shoot length, fresh weight and dry weight) are presented below. Where the C.V. in the control cultures allowed for reliable estimates of EC<sub>10</sub> and/or EC<sub>20</sub> to be determined, these were also reported below.

**Table 18: Summary of biological results based on nominal concentrations of 4-Chlorophenol**

Parameter (mg/L)	Total shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC <sub>50</sub>	12.9	10.6	38.7	17.2	43.8	15.1
95% Conf. Limits	11.0 – 15.3	9.07 – 12.4	27.1 – 62.7	13.0 – 24.1	28.8 – 78.6	11.2 – 21.6
14-day EC <sub>20</sub>	5.69	-	6.23	-	5.40	2.14
95% Conf. Limits	4.62 – 6.78	-	4.42 – 8.39	-	3.67 – 7.52	1.40 – 2.97
14-day EC <sub>10</sub>	-	-	-	-	1.81	-
95% Conf. Limits	-	-	-	-	0.999 – 2.76	-
14-day NOEC	4.88	4.88	4.88	4.88	1.53	1.53
14-day LOEC	15.6	15.6	15.6	15.6	4.88	4.88

(-) Values not reliable, control CV exceeded the effect level.

**Table 19: Summary of biological results based on geometric mean measured concentrations of 4-Chlorophenol**

Parameter (mg/L)	Total shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
14-day EC <sub>50</sub>	13.1	10.4	48.0	18.2	56.7	15.4
95% Conf. Limits	10.9 – 16.0	8.68 – 12.6	31.0 – 87.2	12.8 – 27.7	33.8 – 118	10.6 – 24.1
14-day EC <sub>20</sub>	4.95	-	5.15	-	4.26	1.33
95% Conf. Limits	3.85 – 6.11	-	3.36 – 7.43	-	2.62 – 6.43	0.776 – 2.02
14-day EC <sub>10</sub>	-	-	-	-	1.10	-
95% Conf. Limits	-	-	-	-	0.516 – 1.88	-
14-day NOEC	4.39	4.39	4.39	4.39	0.979	0.979
14-day LOEC	16.7	16.7	16.7	16.7	4.39	4.39

(-) Values not reliable, control CV exceeded the effect level.

### C. Validity criteria (from OECD 239)

- The control plants showed uniform growth over the test period and the mean total shoot length increased 3-fold, fresh weight biomass increased more than 4-fold, and mean dry weight biomass more than 4.5-fold.
- The coefficient of variation (C.V.) for control growth based on shoot length, fresh weight and dry weight was 15.1 %, 14.8 % and 9.1 % respectively. The mean C.V. for control yield based on shoot length, fresh weight and for dry weight was 25.5 %, 28.2 % and 17.9 % respectively.

Since shoot biomass and length more than doubled within the test duration, and the C.V. for fresh weight and shoot length yield was below 35 %, the performance of the plants used for this test was considered acceptable.

## III. CONCLUSION

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to 4-Chlorophenol for 14 days, the E<sub>r</sub>C<sub>50</sub> and E<sub>y</sub>C<sub>50</sub> values based on nominal concentrations and total shoot length were 12.9 mg/L and 10.6 mg/L respectively. The NOEC for growth rate and yield based on total shoot length was 4.88 mg/L. Based on geometric mean measured concentrations, the E<sub>r</sub>C<sub>50</sub>, E<sub>y</sub>C<sub>50</sub> and NOEC values were 13.1, 10.4 and 4.39 mg test item/L respectively. The E<sub>r</sub>C<sub>50</sub> and E<sub>y</sub>C<sub>50</sub> values based on nominal concentrations and biomass (fresh weight) were 38.7 mg/L and 17.2 mg/L respectively. The NOEC for growth rate and yield based on biomass (fresh weight) was 4.88 mg/L. Based on geometric mean measured concentrations, the E<sub>r</sub>C<sub>50</sub>, E<sub>y</sub>C<sub>50</sub> and NOEC values were 48.0, 18.2 and 4.39 mg test item/L respectively. The E<sub>r</sub>C<sub>50</sub> and E<sub>y</sub>C<sub>50</sub> values based on nominal concentrations and biomass (dry weight) were 43.8 mg/L and 15.1 mg/L respectively. The NOEC for growth rate and yield based on biomass (dry weight) was 1.53 mg/L. Based on geometric mean measured concentrations, the E<sub>r</sub>C<sub>50</sub>, E<sub>y</sub>C<sub>50</sub> and NOEC values were 56.7, 15.4 and 0.979 mg test item/L respectively.

## **Fluroxypyr acid**

### **Study 1: Toxicity to macrophytes**

Comments of zRMS:	The study below was submitted by the Applicant in support of the evaluation performed for ADM.3304.H.1.A, however it is noted by the zRMS that sufficient EU agreed data are already available and the Applicant for ADM.3304.H.1.A has access to these data via the LoA issued by the authorisation holder.
Reference:	KCP 10.2.1/11
Report	Fluroxypyr acid - Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system. Gonsior, G., (2012a). S11-00188 (report number)
Guideline(s):	OECD Guideline for the Testing of Chemicals, Guideline 221 <i>Lemna</i> sp. Growth Inhibition Test (March 2006) OECD Guidelines for the Testing of Chemicals, Guideline 218 Sediment-Water Chironomid Toxicity Test Using Spiked Sediment (April 2004) AMRAP (Aquatic Macrophyte Risk Assessment for Pesticides) Workgroup (MALTBY et al. 2010)
Deviations:	-
GLP:	Yes
Acceptability:	Not evaluated, sufficient EU agreed data available which the Applicant has access to via the LoA
Duplication (if vertebrate study)	-

## **I. MATERIAL AND METHODS**

### **A. MATERIALS**

- Test Material:** Fluroxypyr acid  
**Description:** Solid  
**Lot/batch, density:** FXPYR(2)-BP8-#2003(V2)  
**Concentration/Purity:** 99.5 ± 0.5 %  
**Stability of test compound:** Expiry date: 28 February 2016
- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART AND BARKO medium; Acetone solvent control
- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*  
**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agroscience Services EcoChem GmbH since November 2010. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.  
  
*M. spicatum* is cultivated under sterile conditions submersed in a modified, aqueous ANDREWS medium containing sucrose.  
  
**Acclimation period:** Seven days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium. Shoot were anchored in an upright position using glass rings and were maintained under controlled environment conditions.

**Culture medium:**

**Test vessel:**

**ANDREWS Medium**

Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with SMART AND BARKO medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.

Sediment used in the test (percentages based on dry weight):

- 4 % sphagnum peat (approximately pH 5.5–6.0; no visible plant remains, finely ground, air dried);
- 20 % kaolin clay (kaolinite content above 30 %);
- 75–76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);
- approximately 0.1 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;
- organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;
- 200 mg ammonium chloride and sodium phosphate per kg sediment (dry weight).

The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.

**SMART AND BARKO medium:**

- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L
- $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L
- $\text{NaHCO}_3$ : 58.4 mg/L
- $\text{KHCO}_3$ : 15.4 mg/L
- pH (air equilibrium) approximately 7.9

**Number of replicates:**

Five replicates per test item concentration and ten replicates for the control and solvent control were used

**Untreated variant:**

Test vessel/medium without test substance

**Reference substance:**

None

**4. Environmental conditions during testing**

**Temperature**

Test solution temperature (range):  $20.3 \pm 0.4$  °C

**pH**

Test solution pH (range):  $8.16 \pm 0.71$

**Oxygen concentration [mg/L]**

The oxygen saturation was determined to be  $117 \pm 20$  %

**Photoperiod**

Photoperiod: 16 h day length

**Light intensity**

Approx. 8000 lux at the water surface

**B. STUDY DESIGN AND METHODS**

**1. In-life dates:**

10.05.2012–02.06.2012

## 2. Experimental design:

Based on the results of preliminary range finding study, test concentrations of Fluroxypyr acid were selected at 19.1, 61.0, 195, 625 and 2000 µg/kg dw. Five replicate vessels per test item concentration and ten for the control and solvent control were used. The test was carried out under static conditions. An appropriate quantity of test item was dissolved in acetone to produce the highest test concentration and a solvent stock series was produced for the lower test concentrations. 2 mL were mixed with 10.43 g of sand per test vessel for each concentration. After the solvent was evaporated, the sand/test item mixture was mixed with the remaining part of dry sediment for each test beaker. The sediment for the solvent control was prepared similar to the test item treated sediment including the solvent, but without test item. The sediment for the control was prepared without solvent and test item. Test vessels were arranged in a randomised design and were re-randomised during the study.

### Test concentrations:

Nominal: 19.1, 61.0, 195, 625 and 2000 µg/kg dw

### Chemical analysis and validation:

HPLC MS/MS

Samples taken: 0 and 14 days

Limit of Detection: 30% of the LOQ = 0.0003 mg/L for test medium and 0.00285 mg/kg dry weight sediment

Limit of Quantitation: 0.00100 mg/L for test medium and 0.00949 mg/kg dry weight sediment

### Test duration:

14 days

## 3. Observations:

Water temperature, pH and dissolved oxygen content were recorded on days 0, 7 and 14. Light intensity at the water surface was measured once during the test with a Luxmeter. The depth of water in each test vessel was measured and topped up with deionised water as necessary, if evaporation had occurred. The main shoot length was measured by use of a ruler on days 0, 7 and 14 during the test. Shoot length, plant fresh weight, plant dry weight and number as well as length of side shoots were assessed on day 14 after harvesting the plants.

## 4. Statistics:

All data were subjected to ANOVA. Before pooling data for the controls and solvent controls, they were statistically tested to see that they are not significantly different, using two-tailed t test. For all endpoints there were no statistically significant difference between the control and solvent control, both control and solvent control were pooled prior to statistical comparisons to the treatments.

A test for normality of the data was carried out by calculating the Shapiro Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's test was performed. A Dunnett's test was performed (SAS® Proprietary Software 9.2), since all data were normally distributed and variance was homogeneous.

## H. RESULTS AND DISCUSSION

### A. Analytical results

The measured concentration of the test item in the sediment at test start ranged between 56 and 76 % of nominal. The mean measured content for all concentrations in the sediment at test start was 66 %. At the end of the test after 14 days 52 % of nominal was measured in the sediment. A geometric mean over the test period of 57 % was calculated.

As the mean content of the test item over the test period was below 80 % of nominal during the test in the sediment, all toxicological endpoints were evaluated using nominal and actual concentrations of the test item.

The analytical data showed that only low amounts of the test item was found in the water.

### B. Biological results

The effect of the test material on the growth of *Myriophyllum spicatum* has been investigated over a 14-day period and gave the results summarized below.

Development of the main shoot length was determined after 7 and 14 days. The plants in the control, solvent control and the treatment groups up to and including 625 µg/kg dw showed a similar uniform growth over the test period of 14 days. The plants at the treatment of 2000 µg/kg dw showed a slightly stronger growth during the first 7 days and a slightly reduced growth between day 7 and day 14. The tables below show the mean values for yield and growth rate based on total shoot length and biomass (fresh and dry weight).

**Table 20: Mean total shoot length including side shoots (cm)**

Nominal concentration [µg/kg dw]	Days after application		Yield [cm]	Reduction in yield [%] <sup>b)</sup>	Growth rate [1/day]	Reduction in growth rate [%] <sup>b)</sup>
	0 <sup>a)</sup>	14				
Control	8.6	24.3	15.7	-	0.0733	-
Solvent control	8.6	24.3	15.7	-	0.0736	-
Pooled control	8.6	24.3	15.7	-	0.0734	-
19.1	8.6	27.2	18.6	-18.5	0.0811	-10.5
61.0	8.6	25.3	16.7	-6.4	0.0761	-3.7
195	8.6	27.1	18.5	-17.8	0.0818	-11.4
625	8.6	22.0	13.4	14.6	0.0663	9.7
2000	8.6	22.0	13.4	14.6	0.0660	10.1

<sup>a)</sup> Based on 10 additional plants, representative of those used in the test.

<sup>b)</sup> Compared to the pooled controls.

**Table 21: Mean total plant fresh weight (g)**

Nominal concentration [µg/kg dw]	Days after application		Yield [g]	Reduction in yield [%] <sup>b)</sup>	Growth rate [1/day]	Reduction in growth rate [%] <sup>b)</sup>
	0 <sup>a)</sup>	14				
Control	0.2425	1.2605	1.0180	-	0.1165	-
Solvent control	0.2425	1.1407	0.8982	-	0.1102	-
Pooled control	0.2425	1.2006	0.9581	-	0.1133	-
19.1	0.2425	1.1773	0.9348	2.4	0.1095	3.4
61.0	0.2425	1.1791	0.9366	2.2	0.1116	1.5
195	0.2425	1.3826	1.1401	-19.0	0.1240	-9.4
625	0.2425	1.0237	0.7812	18.5	0.1026	9.4
2000	0.2425	0.9961	0.7536	21.3	0.0985	13.1

<sup>a)</sup> Based on 10 additional plants, representative of those used in the test.

<sup>b)</sup> Compared to the pooled controls.



**Table 22: Mean total plant dry weight (g)**

Nominal concentration [µg/kg dw]	Days after application		Yield [g]	Reduction in yield [%] <sup>a)</sup>	Growth rate [1/day]	Reduction in growth rate [%] <sup>b)</sup>
	0 <sup>a)</sup>	14				
Control	0.0305	0.1820	0.1515	-	0.1265	-
Solvent control	0.0305	0.1690	0.1385	-	0.1219	-
Pooled control	0.0305	0.1755	0.1450	-	0.1242	-
19.1	0.0305	0.1734	0.1429	1.4	0.1225	1.4
61.0	0.0305	0.1869	0.1564	-7.9	0.1287	-3.6
195	0.0305	0.2075	0.1770	-22.1	0.1363	-9.7
625	0.0305	0.1755	0.1450	0.0	0.1245	-0.2
2000	0.0305	0.1562	0.1257	13.3	0.1143	8.0

<sup>a)</sup> Based on 10 additional plants, representative of those used in the test.

<sup>b)</sup> Compared to the pooled controls.

No statistically significant inhibition was determined up to and including 2000 µg/kg dw after 14 days of exposure, therefore the overall NOEC value after 14 days of exposure was determined to be 2000 µg test item/kg dw with a corresponding LOEC value of > 2000 µg test item/kg dw (nominal). The overall 14-day EC<sub>50</sub> was determined to be > 2000 µg test item/kg dw (nominal). Details are given in the following table.

**Table 23: Summary of biological results based on nominal concentrations (a) total shoot length**

Parameter	Growth rate (total shoot length) [µg/kg dw]	Yield (total shoot length) [µg/kg dw]
14 day EC <sub>50</sub>	> 2000 <sup>a)</sup>	> 2000 <sup>a)</sup>
95 % CI	-	-
14 day NOEC	2000	2000
14 day LOEC	> 2000	> 2000

- = Not applicable.

<sup>a)</sup> No effect > 50% could be observed, therefore the EC<sub>50</sub> was estimated to be > 2000 µg/kg dw.

CI: Confidence interval.

**Table 24: Reduction in growth and yield were seen at 0.0617 ppb so why is 10 and 20 % effect (b) fresh weight**

Parameter	Growth rate (mean total plant fresh weight) [µg/kg dw]	Yield (mean total plant fresh weight) [µg/kg dw]
14 day EC <sub>50</sub>	> 2000 <sup>a)</sup>	> 2000 <sup>a)</sup>
95 % CI	-	-
14 day NOEC	2000	2000
14 day LOEC	> 2000	> 2000

- = Not applicable.

<sup>a)</sup> No effect > 50% could be observed, therefore the EC<sub>50</sub> was estimated to be > 2000 µg/kg dw.

CI: Confidence interval.

**Table 25: Reduction in growth and yield were seen at 0.0617 ppb so why is 10 and 20 % effect (c) dry weight**

Parameter	Growth rate (mean total plant dry weight) [µg/kg dw]	Yield (mean total plant dry weight) [µg/kg dw]
14 day EC <sub>50</sub>	> 2000 <sup>a)</sup>	> 2000 <sup>a)</sup>
95 % CI	-	-
14 day NOEC	2000	2000
14 day LOEC	> 2000	> 2000

- = Not applicable.

<sup>a)</sup> No effect > 50% could be observed, therefore the EC<sub>50</sub> was estimated to be > 2000 µg/kg dw.

CI: Confidence interval.

Qualitative observations on shoot and root development during the test are summarised in the following table. Effects on root and side shoot development could be observed at 625 and 2000 µg test item/kg dw.

**Table 26: Observations on shoot and root development**

Nominal concentration [µg/kg dw]	Shoot development	Root development
control	No effects observed	No effects observed
Solvent control	No effects observed	No effects observed
19.1	No effects observed	No effects observed
61.0	No effects observed	No effects observed
195	No effects observed	No effects observed
625	Reduced number of side shoots	Slightly reduced number of roots; Root development in the upper part of the shoot (above sediment)
2000	Reduced number of side shoots	Reduced number and length of roots; Root development in the upper part of the shoot (above sediment)

### C. Validity criteria (from OECD 239)

The control and solvent control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots.

- The mean total shoot length increase approx. 3 fold mean total shoot fresh biomass increased approx. 5 fold and mean dry weight increased approx. 6 fold.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures was not estimated.

### III. CONCLUSION

The exposure of *Myriophyllum spicatum* to Fluroxypyr acid after 14 days resulted in an  $E_yC_{50}$  and  $E_rC_{50}$  values based on total shoot length of > 2000 µg/kg dw (nominal). The  $E_yC_{50}$  and  $E_rC_{50}$  values based on fresh weight of > 2000 µg/kg dw (nominal). The  $E_yC_{50}$  and  $E_rC_{50}$  values based on dry weight of > 2000 µg/kg dw (nominal). The corresponding  $E_yC_{50}$  and  $E_rC_{50}$  for total shoot length and biomass (fresh and dry weight) based on actual measured concentrations were > 1140 µg/kg dw (actual) and the NOEC was 1140 µg/kg dw (actual).

### Methoxypyridine

#### Study 8: Toxicity to macrophytes

Comments of zRMS:	<p>The study was performed in line with OECD 239, with no major deviations. All the validity criteria were met.</p> <p>The replication and the number of plants were modified so there were 10 and 5 replicates per control and test item groups, respectively with one plant each, while the test guideline for water/sediment test indicates that there should be 6 and 4 replicates for control and test item groups, respectively, with 3 shoots per replicate. However, modified replication of the study performed in line with OECD 239 was considered acceptable during the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (see EFSA Supporting publication 2019:EN-1673), so it is not challenged here despite the reduced number of plants per test concentration.</p> <p>Since the test item concentrations were not maintained at 80-120% of nominal, the endpoints from the study were expressed in terms of the actual concentration in sediment, in line with indications of OECD 239.</p> <p>Overall, the study is considered acceptable with following endpoints relevant for the risk assessment (all based on geometric mean measured concentrations):</p> <p><u>Total shoot length, fresh weight, dry weight</u>  <math>E_rC_{50}</math> &gt; 7700 µg pm/kg dw sediment mg pm/L  <math>E_yC_{50}</math> &gt; 7700 µg pm/kg dw sediment mg pm/L</p>
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Reference:	KCP 10.2.1/12
Report	METHOXY: Growth inhibition of <i>Myriophyllum spicatum</i> in a water/sediment system. Gonsior, G., (2012). S12-00026 (report number)
Guideline(s):	AMRAP (Aquatic Macrophyte Risk Assessment for Pesticides) Workgroup (MALTBY et al. 2010), OECD 221 (2006) and OECD 218 (2004)
Deviations:	Minor (see commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIAL AND METHODS

### A. MATERIALS

- Test Material:** Methoxypyridine in the study coded as Methoxy (4-amino-3,5-dichloro-6-fluoro-2methoxypyridine)  
**Description:** Powder/white  
**Lot/batch, density:** FXPYR(3)-BP6-1991(V2)  
**Concentration/Purity:** 98.4 ± 0.5 %  
**Stability of test compound:** Expiry date: 31 December 2015

- Vehicle and/or control:** Untreated sterilised sediment overlaid with SMART AND BARKO medium; Acetone solvent control

- Test animals (Species):** Rooted aquatic macrophyte, *Myriophyllum spicatum*  
**Source:** *Myriophyllum spicatum* plants have been maintained under laboratory conditions at Eurofins Agroscience Services EcoChem GmbH since November 2010. The cultures obtained from Umweltbundesamt Berlin, Germany were based on a culture of the Landesanstalt für Gewässerkunde Koblenz, Germany.

*M. spicatum* is cultivated under sterile conditions submersed in a modified, aqueous ANDREWS medium containing sucrose.

**Acclimation period:** Seven days prior to test initiation, submerged apical shoots of the same size were planted in an aquarium in an artificial sterilised sediment overlaid with SMART AND BARKO medium. Shoot were anchored in an upright position using glass rings and were maintained under controlled environment conditions.

**Culture medium:** ANDREWS Medium

**Test vessel:** Plants were grown in a static water-sediment system using artificial sterilized sediment overlaid with SMART AND BARKO medium under the same conditions as used in the pre-culture. The study was conducted in 2 L glass-beakers measuring approx. 12 cm in diameter and 24 cm height. Only one shoot per test vessel was planted. The volume of added water was recorded, and the level marked on the outside of the test vessels.

Sediment used in the test (percentages based on dry weight):

- 4 % sphagnum peat (approximately pH 5.5 – 6.0; no

- visible plant remains, finely ground, air dried);
- 20 % kaolin clay (kaolinite content above 30 %);
- 75 – 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns);
- approximately 0.1 % calcium carbonate, precipitated extra pure, to adjust the sediment pH to  $7.0 \pm 0.5$  at the start of the test before adding the test item;
- organic carbon content of the final mixture should be 2 % ( $\pm 0.5$  %) and was adjusted by the use of appropriate amounts of peat and sand;
- 200 mg ammonium chloride and sodium phosphate per kg sediment (dry weight).

The dry constituents were blended in the correct proportions and mixed thoroughly in an electric mixer. The dry sediment was sterilised in a heating chamber at 110 °C for at least 2 hours prior to use to minimise algal contamination of the test systems.

SMART AND BARKO medium:

- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ : 91.7 mg/L
- $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ : 69.0 mg/L
- $\text{NaHCO}_3$ : 58.4 mg/L
- $\text{KHCO}_3$ : 15.4 mg/L
- pH (air equilibrium) approximately 7.9

<b>Number of replicates:</b>	Five replicates per test item concentration and ten replicates for the control and solvent control were used
<b>Untreated variant:</b>	Test vessel/medium without test substance
<b>Reference substance:</b>	None

#### 4. Environmental conditions during testing

<b>Temperature</b>	Test solution temperature (range): $20.2 \pm 0.2$ °C
<b>pH</b>	Test solution pH (range): $8.16 \pm 0.71$
<b>Oxygen-concentration [mg/L]</b>	The oxygen saturation was determined to be $117 \pm 20$ %
<b>Photoperiod</b>	Photoperiod: 16 h day length
<b>Light intensity</b>	Approx. 8000 lux at the water surface

## B. STUDY DESIGN AND METHODS

1. **In-life dates:** 10.05.2012 – 02.06.2012

2. **Experimental design:** To quantify the effect of the test item on growth of rooted aquatic macrophyte, a water-sediment study with *Myriophyllum spicatum* was conducted: Five replicates per test item concentration and ten replicates for the control and solvent control were used. The duration of the test was 14 days. The test was performed under static test conditions. A test at nominal concentrations of 10000, 3125, 977, 305 and 95.4 µg/kg dw, control and solvent control was performed. The test item was spiked to the sediment. Test item concentrations in the definitive test were verified at all concentration levels by analysis of the sediment at test start and test end.

**Test concentrations:** Nominal: 10000, 3125, 977, 305 and 95.4 µg test item/kg dw

## Chemical analysis and validation: HPLC-MS/MS

Samples taken: 0 and 14 days

Limit of Detection: 30% of the LOQ (= 0.00120 mg/L for test medium and 0.0141 mg/kg dry weight for sediment).

Limit of Quantitation: 0.00400 mg/L for test medium samples and 0.0469 mg/kg dry weight for sediment samples  
14 days

## Test duration:

## 3. Observations:

On day 14 plants were harvested for assessment of shoot length, plant fresh weight, plant dry weight and number and length of side shoots. Additionally the main shoot length was measured on days 0, 7 and 14 during the test.

## 4. Statistics:

All data were subjected to ANOVA. Before pooling data for the controls and solvent controls, they were statistically tested to see that they are not significantly different, using two-tailed t-test. For all endpoints there were no statistically significant difference between the control and solvent control, both control and solvent control were pooled prior to statistical comparisons to the treatments.

A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. For homogeneity of variances across treatment groups a Bartlett's test was performed. Since all data were normally distributed and variance was homogenous a Dunnett's test was performed (SAS® Proprietary Software 9.2).

# II. RESULTS AND DISCUSSION

## A. Analytical results

The measured concentration of the test item in the sediment at test start ranged between 78 and 86 % of nominal. The mean measured content for all concentrations in the sediment at test start was 83 %. At the end of the test after 14 days a mean content of 73 % of nominal was measured in the sediment. A geometric mean over the test period of 77 % was calculated.

As the mean content of the test item over the test period was below 80 % of nominal in the sediment, all toxicological endpoints were evaluated using nominal and actual concentrations of the test item.

The analytical data showed that only low amounts of Methoxy was found in water.

## B. Biological results

No effects on *Myriophyllum spicatum* could be observed during the test for any parameter, therefore EC<sub>50</sub> values were estimated to be > 10000 µg/kg dw based on nominal concentrations and >7700 µg/kg dw based on actual measured concentrations.

**Table 27: Summary of biological results based on nominal concentrations of the test item and total shoot length**

Parameter	Growth rate (total shoot length in cm) [µg/kg dw mg/L]	Yield (total shoot length in cm) [µg/kg dw mg/L]
<b>Main test</b>		
14-day EC <sub>50</sub>	> 10000*	> 10000*
95 % CI	-	-
14-day NOEC	10000	10000
14-day LOEC	> 10000	> 10000

\* No effect > 50 % could be observed, therefore the EC<sub>50</sub> was estimated to be > 10000 µg/kg dw.

CI: Confidence interval.

**Table 28: Summary of biological results based on nominal concentrations of the test item and fresh weight**

Parameter	Growth rate (fresh weight total shoot length in cm) [µg/kg dw mg/L]	Yield (fresh weight total shoot length in cm) [µg/kg dw mg/L]
<b>Main test</b>		
14-day EC <sub>50</sub>	> 10000*	> 10000*
95 % CI	-	-
14-day NOEC	10000	10000
14-day LOEC	> 10000	> 10000

\* No effect > 50 % could be observed, therefore the EC<sub>50</sub> was estimated to be > 10000 µg/kg dw.

CI: Confidence interval.

**Table 29: Summary of biological results based on nominal concentrations of the test item and dry weight**

Parameter	Growth rate (dry weight total shoot length in cm) [µg/kg dw mg/L]	Yield (dry weight total shoot length in cm) [µg/kg dw mg/L]
<b>Main test</b>		
14-day EC <sub>50</sub>	> 10000*	> 10000*
95 % CI	-	-
14-day NOEC	10000	10000
14-day LOEC	> 10000	> 10000

\* No effect > 50 % could be observed, therefore the EC<sub>50</sub> was estimated to be > 10000 µg/kg dw.

CI: Confidence interval.

### C. Validity criteria (from OECD 239)

The control and solvent control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots.

- The mean total shoot length increase approx. 3-fold, mean total shoot fresh biomass increased approx.5-fold and mean dry weight increased approx. 6-fold.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures was not estimated.

## III. CONCLUSION

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to Methoxy for 14 days, the E<sub>y</sub>C<sub>50</sub> and E<sub>r</sub>C<sub>50</sub> values based on total shoot length were > 10000 µg/kg dw (nominal). The NOEC for yield and growth rate based on total shoot length was 10000 µg/kg dw (nominal).

The E<sub>y</sub>C<sub>50</sub> and E<sub>r</sub>C<sub>50</sub> values based on fresh weight were > 10000 µg/kg dw (nominal). The NOEC for yield and growth rate based on dry weight was 10000 µg/kg dw (nominal).

The E<sub>y</sub>C<sub>50</sub> and E<sub>r</sub>C<sub>50</sub> values based on dry weight was estimated to be > 10000 µg/kg dw (nominal). The NOEC for yield and growth rate based on dry weight was 10000 µg/kg dw (nominal).

The corresponding E<sub>y</sub>C<sub>50</sub> and E<sub>r</sub>C<sub>50</sub> for total shoot length and biomass (fresh and dry weight) based on actual measured concentrations were > 7700 µg/kg dw (actual) and the NOEC was 7700 µg/kg dw (actual).

## Appendix 4 Peer reviewed data for metabolite 4-Chlorophenol – Aquatic organisms

### zRMS comments:

In line with EFSA (2013) it is sufficient to perform the metabolite study with the species that turned out to be most sensitive to the parent. Since available data demonstrate that *Myriophyllum spicatum* is species most sensitive to 2,4-D, the study on toxicity of 4-CP to *M. spicatum* (KCP 10.2.1/10, Gonsior, 2015) is sufficient to resolve the risk and the data below were not evaluated by the zRMS as being not necessary.

A summary of toxicity data obtained from peer reviewed literature data for the metabolite 4-chlorophenol is provided in the following table. The most sensitive endpoint for each of the organism group fish, *Daphnia* and algae is used for the risk assessment as a worst case approach. In addition, for those publications providing the worst case endpoints a short summary and a reliability assessment is presented below.

**Table 1: Ecotoxicological endpoints for non-target organisms exposed to 4-chlorophenol**

Test species	Endpoint	Reference
<i>Oncorhynchus mykiss</i>	96 h LC <sub>50</sub> = 1.9 mg/L	Hodson, P.V., et al. (1984)
<i>Leuciscus idus melanotus</i>	96 h LC <sub>50</sub> = 3.0 mg/L	Dietz, F. & Traud, J. (1978)
<i>Lepomis macrochirus</i>	96 h LC <sub>50</sub> = 3.8 mg/L	Buccafusco, R.J. et al. (1981)
<i>Pimephales promelas</i>	96 h LC <sub>50</sub> = 3.8 mg/L	Mayes, M.A. et al. (1983)
<i>Brahydanio rerio</i>	96 h LC <sub>50</sub> = 5.6 mg/L	Kuiper, J. (1982)
<i>Poecilia reticulata</i>	96 h LC <sub>50</sub> = 8.49 mg/L	Saarikoski J. & Viluksela M. (1982)
<i>Daphnia magna</i>	48 h EC <sub>50</sub> = 2.5 mg/L	Kühn, R. (1989)
<i>Daphnia magna</i>	48 h EC <sub>50</sub> = 4.1 mg/L	LeBlanc, G.A. (1980)
<i>Daphnia magna</i>	48 h LC <sub>50</sub> = 4.82 mg/L	Kopperman, H.L. et al. (1974)
<i>Daphnia magna</i>	48 h LC <sub>50</sub> = 6.8 mg/L	Steinberg, C. et al. (1992)
<i>Daphnia pulex</i>	96 h EC <sub>50</sub> = 3.5 mg/L	Trabalka, J.R. & Burch, M.B. (1978)
<i>Skeletonema costatum</i>	96 h E <sub>h</sub> C <sub>50</sub> = 13.8 mg/L 96 h E <sub>b</sub> C <sub>50</sub> = 11.6 mg/L	Cowgill, U.M. et al. (1989)
<i>Desmodesmus subspicatus</i>	72 h E <sub>h</sub> C <sub>50</sub> = 17 mg/L 72 h E <sub>b</sub> C <sub>50</sub> = 8.3 mg/L	Kühn, R. & Pattard, M. (1990)
<i>Scenedesmus pannonicus</i>	96 h EC <sub>50</sub> = 10 mg/L	Kuiper, J. (1982)
<i>Chlorella vulgaris</i>	96 h EC <sub>50</sub> = 29 mg/L	Shigeoka T. et al. (1988)
<i>Pseudokirchneriella subcapitata</i>	96 h EC <sub>50</sub> = 38 mg/L	Shigeoka T. et al. (1988)

Literature presented in bold provided worst case endpoints that were used in the risk assessment. Summaries and check of reliability of those publications are presented below.

### A 4.1 KCP 10.2 Effects on aquatic organisms

#### A 4.1.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

<b>Report:</b>	KCP 10.2.1/13,
<b>Title:</b>	Measurement of median lethal dose as a rapid indication of contaminant toxicity to fish Hodson P. V., Dixon D.G., Kaiser K.L.E., 1984 Environmental Toxicology and Chemistry, Vol 3: 243–254
<b>Guidelines:</b>	No
<b>GLP</b>	No

### Abstract<sup>16</sup>

A new method was developed to rapidly measure the toxicity of contaminants to fish over 96 h. The method is the measurement of median lethal doses by intraperitoneal injection (IP LD<sub>50</sub>). Contaminants are dissolved in 5% ethanol in saline or in cod liver oil and injected at a rate of 1.0 mL per 100 g of fish.

<sup>16</sup> Quoted from article

The results of parallel bioassays to measure toxicity by oral intubation (OI- $LD_{50}$ ) or aqueous exposure ( $LC_{50}$ ) were closely linked to IP- $LD_{50}$  values.

Data on the non-relevant substances included in the study are not summarized here. Only data of the aqueous exposure test on p-chlorophenol are included below.

## I. MATERIALS AND METHODS

### Test material:

Test item: p-Chlorophenol (= 4-chlorophenol)

Molecular weight 128.6 g/mol

Description: Analytical

Lot/Batch no.: 2450950

Purity: Not stated

Source: BDH Chemicals

Vehicle and/or positive control: None

### Test system:

Organism (Species): Rainbow trout

Age: Not stated

Size: Not stated

Body weight of the animals: 1.2–3.8 g

Source: Goosen's Trout Farm, R.R. #1, Otterville, ON, Canada

Diet/Food: daily with Ewos trout pellets except on weekends

Acclimatisation period: At least 1 week

Medium: Water from Lake Ontario, dechlorinated to less than 10 µg Cl/L with specific composition: acid capacity  $K_{S4,3}$  of 0.8 mmol L<sup>-1</sup>, total hardness of 2,4 mmol/L, a calcium to magnesium ratio of 4:1, a sodium to potassium ratio of 10:1

### Environmental conditions:

Temperature: 14.1–16.5 °C

Light: Not stated

Photoperiod: 16 h light and 8 h dark

pH: 7.6–8.19

Dissolved oxygen: 5.6–9.4

Conductivity: 340 µmhos/cm<sup>2</sup>

Hardness: 86 mg CaCO<sub>3</sub>/L

## STUDY DESIGN

### Experimental treatments

Based on the results of a range finding test at 1.0, 10, 100 and 1,000 mg/L, a definite toxicity test was performed using concentrations of 0 (control), 10, 18, 32, 56 and 100 % of the maximum test concentration. Ten fish were exposed to each concentration and the bioassay was repeated three times. Chemicals were added by a Hamilton syringe pump and dilutions were achieved by a Mount Brungs diluter. Each bioassay tank contains 14 liters of medium and flow per tank varied between tests from 21 to 111 mL/min. Size of test fish was chosen such that the flow rate was always greater than 2 liters per gram of fish per day. During bioassay tanks were not aerated.

### Observations

After 96 h the number of animals in the control and test solutions was assessed for mortality.

### Statistical calculations

$LC_{50}$  values were calculated from records of percent mortality by computerized Probit analysis. When the number of partial mortalities was too low for probit analysis, a graphical method was chosen.

$LC_{50}$  values are based on mean measured concentration.



## II. RESULTS AND DISCUSSION

### Findings

The determined LC values for rainbow trout are given in the table below.

**Table 2: Determined LC<sub>50</sub> values for p-chlorophenol on rainbow trout**

Test period in h	Test period in h	LC <sub>50</sub>
p-chlorophenol	96	14.8 $\mu\text{mol/L}$ corresponding to 1.9 mg/L

## III. CONCLUSIONS

Data generated for the toxicity of p-chlorophenol to rainbow trout resulted in a 96 h LC<sub>50</sub> of 1.9 mg/L.

Reliability Assessment of Ecotoxicity Studies Based on ToxRTool (Schneider et al., 2009)			
Criteria		Score	Evaluator's comments on criteria (optional)
No.	Cut-off criteria		
1	Was the test substance identified?	+	-
2	Is the species given?	+	-
3	Is the administration route given?	+	-
4	Are doses administered or concentrations in application media given?	+	stated that preliminary tests at 1.0, 10, 100 and 1000 mg/L gave conc. around LC <sub>50</sub> and bioassay was conducted at 0 (control), 10,18, 32, 56 and 100% of maximum test concentration
5	Are frequency and duration of exposure as well as time points of observations explained?	+	-
6	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required)?	+	not required (fish acute test)
7	Is the number of replicates and/or organisms per group given?	+	-
8	Is the study design chosen appropriate for obtaining the substance specific data aimed at?	+	-
-	Subtotal Test Substance	8	If not 8, study is not reliable.
-	Criteria Group I: Test substance identification		
9	Is the purity of the substance given?	0	stated chemical was purchased at the highest degree of purity
10	Is information on the source/origin of the substance given?	+	-
11	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data?	+	-
-	Subtotal Test Substance	2	-
-	Criteria Group II: Test organism characterisation		
12	Is the sex or the sex ratio of the test organisms given?	0	not required (fish acute test)
13	Is information given on the source or strain of test organisms plus, if considered necessary to judge the study, other specifications?	+	-
14	Is age, life stage, growth stage, body weight of the test organisms at the start of the study given?	+	fish weighed between 1 and 4 g each
15	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	+	no repeated dose study
-	Subtotal Test Organisms	3	-
-	Criteria Group III: Study design description		
16	Is the test media clearly described (water, soil, plant)?	+	-
17	Are sufficient details of the administration scheme given to judge the study?	+	-
18	For repeated dose toxicity studies only (give point for other study types): Were exposure concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	+	no repeated dose study
-	Subtotal Study design / Test method	3	-
-	Criteria Group IV: Study results documentation		

19	Are the study endpoint(s) and their method(s) of determination clearly described?	1	-
20	Is the description of the study results for all endpoints investigated transparent and complete?	0	control mortality for the chemical compound is not stated
21	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable)?	1	-
-	<b>Subtotal Study Result Documentation</b>	<b>2</b>	-
-	<b>Criteria Group V: Plausibility of study design and results</b>		
22	Are the quantitative study results reliable?	1	-
-	<b>Subtotal Plausibility</b>	<b>1</b>	-
-	<b>Total Score</b>	<b>19</b>	-
-	<b>A Numerical result leads to initial Category:</b>	<b>1</b>	-
-	<b>B Cut-off criteria restricts Category:</b>	<b>No</b>	Minimal requirements given
-	<b>C Evaluator's proposed Category:</b>	<b>2</b>	-
-	<b>D Justification in case evaluator deviates from B:</b>	information on used concentrations and percentage of mortality are scarce	
Date/period of evaluation:		16.11.2015	

Qualitative assessment		
Study assessment	Score	Rationale
<b>Reliability/Repeatability</b>	Klimisch 2	Reliable with restrictions. <ul style="list-style-type: none"> <li>19 out of 22 reliability criteria are met according to ToxRTool (Schneider et al. 2009)</li> <li>Well documented study with a few exceptions</li> </ul>
<b>Limitations</b>		Information on the concentrations used in the definitive test are scarce and mortality for each concentration used is not reported
<b>GLP</b>		No
<b>Relevance</b>	Data for risk assessment of fish	Data will be used in risk assessment.

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<b>Report:</b>	KCP 10.2.1/14;
<b>Title:</b>	Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to <i>Daphnia magna</i> Kühn R., Pattard M., Pernak K. D., Winter A., 1989 Water Research, Vol 23 (4): 495–499
<b>Guidelines:</b>	DIN 38412, part II
<b>GLP</b>	No

### Abstract<sup>17</sup>

Using the acute *Daphnia* test (according to DIN 38412, Part II), the EC<sub>50</sub>, EC<sub>0</sub> and EC<sub>100</sub> after 24 and 48 h test time were calculated or determined for 70 substances—phenols, anilines and aliphatic compounds. The results of the tests are given in three tables according to substance group. Evaluation showed that the toxicity of the substances (particularly in the case of anilines) may be higher or even substantially greater when the test period is extended from 24 to 48 h.

Data on the non relevant substances included in this study are not summarized here. Only data on p-chlorophenol are included below.

## I. MATERIALS AND METHODS

### Test material:

Test item:	p-Chlorophenol (= 4-chlorophenol)
Description:	Analytical
Lot/Batch no.:	Not stated
Purity:	Not stated
Source:	Not stated

<sup>17</sup> Quoted from article

<b>Vehicle and/or positive control:</b>	None
<b>Test system:</b>	
<b>Organism (Species):</b>	<i>Daphnia magna</i>
<b>Source:</b>	Own culture
<b>Medium:</b>	Water with specific composition: acid capacity $K_{s4,3}$ of 0.8 mmol L <sup>-1</sup> , total hardness of 2,4 mmol L <sup>-1</sup> , a calcium to magnesium ratio of 4 : 1, a sodium to potassium ratio of 10 : 1
<b>Environmental conditions:</b>	
<b>Temperature:</b>	20°C in an incubator
<b>Light:</b>	Not stated
<b>Photoperiod:</b>	Not stated
<b>pH:</b>	Start of the test: 8.0 ± 0.2 End of the test: > 7.0
<b>Dissolved oxygen:</b>	End of the test: > 4.0 mg O <sub>2</sub> /L
<b>Conductivity:</b>	Not stated

## STUDY DESIGN

### Experimental treatments

The effects of p-chlorophenol on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (2 replicates of ten 6–24 h-old animals per test beaker) were exposed per concentration step and control. Concentration steps were selected so that 3–4 EC values were in the range between EC<sub>0</sub> and EC<sub>100</sub>, with at least one value below and one above EC<sub>50</sub>. The ratio between concentrations was 1:1.4.

### Observations

After 24 h and 48 h, the number of animals in the control and test solutions that could still swim were counted.

### Statistical calculations

Not stated how data were statistically evaluated.

## II. RESULTS AND DISCUSSION

### Findings

The determined EC values for *Daphnia magna* are given in the table below.

**Table 3: Determined EC values for p-chlorophenol on *Daphnia magna***

Test period in h	EC <sub>50</sub> in mg/L	CI 95% in mg/L	EC <sub>0</sub> in mg/L	EC <sub>100</sub> in mg/L	EC <sub>50</sub> 48 h: 24 h
24	3.4	2.8–4.0	1.5	11	1 : 1.4
48	2.5	2.3–2.7	1.5	4	

### Observations

The ratio between 24 h EC<sub>50</sub> and 48 h EC<sub>50</sub> of *Daphnia magna* for p-chlorophenol was calculated. Results show that toxicity to *Daphnia magna* increased by a factor of 1.4 if test period is prolonged from 24 h to 48 hours.

## III. CONCLUSIONS

Data generated for the toxicity of p-chlorophenol to *Daphnia magna* resulted in a 48 h EC<sub>50</sub> = 2.5 mg/L.

Reliability Assessment of Ecotoxicity Studies Based on ToxRTool (Schneider et al., 2009)				
Criteria		Score	Evaluator's comments on criteria (optional)	
No.	Cut-off criteria			
1	Was the test substance identified?	+	-	
2	Is the species given?	+	-	
3	Is the administration route given?	+	-	

4	Are doses administered or concentrations in application media given?	+	stated that concentration steps were selected so that 3-4 EC values were in the range between EC 0 and EC 100, of which at least one value was below and one above EC 50
5	Are frequency and duration of exposure as well as time points of observations explained?	+	-
6	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required)?	+	not required
7	Is the number of replicates and/or organisms per group given?	+	-
8	Is the study design chosen appropriate for obtaining the substance specific data aimed at?	+	-
-	<b>Subtotal Test Substance</b>	<b>8</b>	<b>If not 8, study is not reliable.</b>
-	<b>Criteria Group I: Test substance identification</b>		
9	Is the purity of the substance given?	0	not stated
10	Is information on the source/origin of the substance given?	0	not stated
11	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data?	0	no information on the physico-chemical properties are given (solubility)
-	<b>Subtotal Test Substance</b>	<b>0</b>	-
-	<b>Criteria Group II: Test organism characterisation</b>		
12	Is the sex or the sex ratio of the test organisms given?	+	not necessary for daphnia
13	Is information given on the source or strain of test organisms plus, if considered necessary to judge the study, other specifications?	0	not stated
14	Is age, life stage, growth stage, body weight of the test organisms at the start of the study given?	+	-
15	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	+	no repeated dose study
-	<b>Subtotal Test Organisms</b>	<b>4</b>	-
-	<b>Criteria Group III: Study design description</b>		
16	Is the test media clearly described (water, soil, plant)?	+	-
17	Are sufficient details of the administration scheme given to judge the study?	+	-
18	For repeated dose toxicity studies only (give point for other study types): Were exposure concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	+	no repeated dose study
-	<b>Subtotal Study design / Test method</b>	<b>3</b>	-
-	<b>Criteria Group IV: Study results documentation</b>		
19	Are the study endpoint(s) and their method(s) of determination clearly described?	0	information about effected daphnids per concentration step is missing
20	Is the description of the study results for all endpoints investigated transparent and complete?	+	-
21	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable)?	0	no information
-	<b>Subtotal Study Result Documentation</b>	<b>1</b>	-
-	<b>Criteria Group V: Plausibility of study design and results</b>		
22	Are the quantitative study results reliable?	+	-
-	<b>Subtotal Plausibility</b>	<b>1</b>	-
-	<b>Total Score</b>	<b>17</b>	-
-	<b>A Numerical result leads to initial Category:</b>	<b>2</b>	-
-	<b>B Cut-off criteria restricts Category:</b>	<b>No</b>	Minimal requirements given
-	<b>C Evaluator's proposed Category:</b>	-	-
-	<b>D Justification in case evaluator deviates from B:</b>	-	-
Date/period of evaluation:		10.11.2015	

Qualitative assessment		
Study assessment	Score	Rationale
Reliability/Repeatability	Klimisch 2	Reliable with restrictions: • 17 out of 22 reliability criteria are met according to ToxRTool (Schneider et al. 2009) • Well documented study with a few exceptions

Qualitative assessment		
Study assessment	Score	Rationale
Limitations		Information on the chemical property of the substance are scarce as well as information on the concentrations used in the definitive test
GLP		No
Relevance	Data for risk assessment of aquatic invertebrates	Data will be used in risk assessment

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Report:	KCP 10.2.1/15;
Title:	Toxicity of nine benchmark chemicals to <i>Skeletonema costatum</i> a marine diatom Cowgill, U., 1989 Environmental Toxicology and Chemistry, Vol 8: 451–455
Guidelines:	No standard guideline used.
GLP	No

### Abstract<sup>18</sup>

The purpose of this study was to determine the sensitivity of a marine diatom to eight common chemicals and one herbicide. The 50% reduction in the number of cells per milliliter and that of total cell volume  $\times 10^4 \mu\text{m}^3/\text{ml}$  was estimated in relation to each of the nine chemicals. Nominal concentrations of triclopyr triethylamine salt (Garlon 3A),  $\text{K}_2\text{Cr}_2\text{O}_7$ , 4-chlorophenol and phenol were slightly toxic ( $>10 \text{ mg/L}$ ) according to the U.S. Environmental Protection Agency classificatory scheme, while diethanolamine, chlorobenzene, chloroform, acetone and ethanol were classified as practically nontoxic ( $>100 \text{ mg/L}$ ). No observed effect levels were found for each of the two cell measurements in relation to each of the chemicals tested for the 5-d period of the test. The range was found to be from 1 to 6,000 mg/L for total cell count and from 0.65 to 6,000 mg/L for total cell volume. Data on the sensitivities of other organisms to the group of common chemicals are also included. The marine diatom proved to be less sensitive to  $\text{K}_2\text{Cr}_2\text{O}_7$  and diethanolamine than the green alga *Selenastrum capricornutum*.

Data on the non relevant substances included in this study are not summarized here. Only data on 4-Chlorophenol are included below.

## I. MATERIALS AND METHODS

### Test material:

Test item: 4-chlorophenol  
Description: Analytical  
Lot: AOD  
Purity: Not stated  
Source: Eastman Kodak, CAS No. 106-48-9  
Vehicle and/or positive control: None

### Test system:

Organism (Species): Marine diatom (*Skeletonema costatum*)  
Source: Bigelow Laboratory for Ocean Sciences at West Boothbay Harbor, Maine  
Medium: Provasoli, revised ASP-12 medium. Revision consists of addition of SE ( $\text{Na}_2\text{SeO}_4$ , 0.00479 g/L) and Cu ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.06707 g/L) and doubling of the amount of cyanocobalamine (0.00040 g/L)

### Environmental conditions:

Temperature: 19.5–20.6 °C  
Light: 4296–4318 lux  
Photoperiod: 14h light/ 10 h dark

<sup>18</sup> Quoted from article

## STUDY DESIGN

### Experimental treatments

At the beginning of the experiment a range finding test was conducted by exposing the diatom to three concentrations of 4-chlorophenol and a control with two replicates per concentration. Concentrations were set an order of magnitude apart, 0.1, 1, 10, 100, 1,000 mg/L and so on. Cell density was 100,000 cells/mL at the beginning of the range finding test and in the definitive test. The definitive test consisted of 5 or more concentrations and a control replicated three times. For each concentration a counting blank was included. Total cell count and cell volume were measured by the use of a cell counter. Additionally, initial and final pHs of control, low, middle and high test item rates were measured. Each test lasted 5 days.

### Observations

In all tests temperature and light intensity was assessed daily until day 5.

### Statistical calculations

Not stated how data were statistical evaluated.

## II. RESULTS AND DISCUSSION

### Findings

The determined EC<sub>50</sub> and NOEL values for *Skeletonema costatum* are given in the table below.

**Table 4: Determined EC<sub>50</sub> and NOEL values for 4-chlorophenol**

4-chlorophenol	Total cell count	Total cell volume
120 EC <sub>50</sub> (95 % CI)	13.8 mg/L ( 16.0, 43.5)	11.6 mg/L ( 18.9, 42.2)
120 NOEL	1.08 mg/L	0.39 mg/L

### Observations

According to the EPA criteria 4-chlorophenol is considered to be slightly toxic. Furthermore, *Skeletonema costatum* is more sensitive to phenol than to chlorophenol.

## III. CONCLUSIONS

Data generated for the toxicity of 4-chlorophenol to *Skeletonema costatum* resulted in an EC<sub>50</sub> = 13.8 mg/L and a NOEL = 1.08 mg/L for total cell count and an EC<sub>50</sub> = 11.6 mg/L and NOEL = 0.39 mg/L.

Reliability Assessment of Ecotoxicity Studies Based on ToxRTool (Schneider et al., 2009)			
Criteria		Score	Evaluator's comments on criteria (optional)
No.	Cut-off criteria		
1	Was the test substance identified?	+	-
2	Is the species given?	+	-
3	Is the administration route given?	+	-
4	Are doses administered or concentrations in application media given?	+	concentrations of a range finding test are given and definitive test consisted of five or more concentrations minimum of 5
5	Are frequency and duration of exposure as well as time points of observations explained?	+	-
6	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required)?	+	-
7	Is the number of replicates and/or organisms per group given?	+	-
8	Is the study design chosen appropriate for obtaining the substance specific data aimed at?	+	-
-	Subtotal Test Substance	8	If not 8, study is not reliable.
-	Criteria Group I: Test substance identification		
9	Is the purity of the substance given?	0	stated that purity is similar to those used in previous studies
10	Is information on the source/origin of the substance given?	+	-

11	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data?	0	no information about solubility are given
-	<b>Subtotal Test Substance</b>	1	-
-	<b>Criteria Group II: Test organism characterisation</b>		
12	Is the sex or the sex ratio of the test organisms given?	1	not required
13	Is information given on the source or strain of test organisms plus, if considered necessary to judge the study, other specifications?	1	-
14	Is age, life stage, growth stage, body weight of the test organisms at the start of the study given?	1	initial cell density = 100,000cells/mL
15	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1	no repeated dose study
-	<b>Subtotal Test Organisms</b>	4	-
-	<b>Criteria Group III: Study design description</b>		
16	Is the test media clearly described (water, soil, plant)?	1	-
17	Are sufficient details of the administration scheme given to judge the study?	1	-
18	For repeated dose toxicity studies only (give point for other study types): Were exposure concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	1	no repeated dose study
-	<b>Subtotal Study design / Test method</b>	3	-
-	<b>Criteria Group IV: Study results documentation</b>		
19	Are the study endpoint(s) and their method(s) of determination clearly described?	1	-
20	Is the description of the study results for all endpoints investigated transparent and complete?	1	-
21	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable)?	0	not described how EC <sub>50</sub> and NOEL were obtained
-	<b>Subtotal Study Result Documentation</b>	2	-
-	<b>Criteria Group V: Plausibility of study design and results</b>		
22	Are the quantitative study results reliable?	1	-
-	<b>Subtotal Plausibility</b>	1	-
-	<b>Total Score</b>	19	-
-	<b>A-Numerical result leads to initial Category:</b>	1	-
-	<b>B-Cut-off criteria restricts Category:</b>	No	Minimal requirements given
-	<b>C-Evaluator's proposed Category:</b>	2	-
-	<b>D-Justification in case evaluator deviates from B:</b>	Information on the concentrations in the definitive test are scarce	
Date/period of evaluation:		09.11.2015	

Qualitative assessment		
Study assessment	Score	Rationale
Reliability/Repeatability	Klimisch 2	Reliable with restrictions. <ul style="list-style-type: none"> <li>19 out of 22 reliability criteria are met according to ToxRTool (Schneider et al. 2009)</li> <li>Well documented study with a few exceptions</li> </ul>
Limitations		Information on the chemical property of the substance are scarce as well as information on the concentrations used in the definitive test
GLP		No
Relevance	Data for risk assessment of algae	Data will be used in risk assessment.

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Report:	KCP 10.2.1/16;
Title:	Results of the harmful effects of water pollutants to green algae ( <i>Scenedesmus subspicatus</i> ) in the cell multiplication inhibition test. Kühn, R., Pattard, M., 1990 Water Research, Vol 24 (1): 31–38
Guidelines:	DIN 38 412

GLP	No
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### Abstract<sup>19</sup>

In the *Scenedesmus* cell multiplication inhibition test, 68 potentially hazardous substances were examined to determine the effect concentrations (EC). The tests were conducted in accordance with the test procedure DIN 38 412, Part 9 (draft standard). The green alga *Scenedesmus subspicatus* CHODAT was cultivated as the test organism. Twenty five substances were examined according to the standardized test procedure. The procedure was modified somewhat for the examination of volatile and/or strongly smelling substances. For 21 of the tested substances the concentrations for the 72 h and/or 48 h  $E_{B/C_{10}}$  values were in the concentration range  $0.0001 - 2 \text{ mg l}^{-1}$ . Thus, they prove very/highly toxic to the cell multiplication of *Scenedesmus subspicatus*. When compared with the results of the 21d *Daphnia* reproduction test, monobromoacetic, monochloroacetic acid, chloroacetaldehyde and chloramine T proved very harmful to green algae.

Data on the non-relevant substances included in this study are not summarized here. Only data on 4-chlorophenol are included below.

### I. MATERIALS AND METHODS

Test material:

Test item: 4-chlorophenol

Description: Analytical

Lot: Not stated

Purity: Not stated

Source: Not stated

Vehicle and/or positive control: None

Test system:

Organism (Species): Green algae (*Scenedesmus subspicatus*)

Source: Own culture

Medium: Nutrient solution

Dissolve in double distilled water:

496 mg sodium nitrate,  $\text{NaNO}_3$ , AR;

39 mg dipotassium hydrogen phosphate,  $\text{K}_2\text{HPO}_4$ -anhydrous, high purity;

75 mg magnesium sulphate,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , AR;

36 mg calcium chloride,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , AR;

40 mg sodium metasilicate,  $\text{NaSiO}_3$ ;

58 mg sodium carbonate,  $\text{Na}_2\text{CO}_3$ , anhydrous, AR;

3 mg citric acid,  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ , AR;

3 mg iron (III)-citrate,  $\text{C}_6\text{H}_5\text{FeO}_7 \cdot 5\text{H}_2\text{O}$ ;

10 mg disodium salt of ethylene diamine tetracetic acid,  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$

Add 10 mL of the trace elements operating solution, complete to 1 L with double distilled water

Stock solution (trace elements)

Dissolve in double distilled water:

2.86 g boric acid  $\text{H}_3\text{BO}_3$ , AR;

220 mg zinc sulphate,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , AR; 80 mg copper (II) sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , AR;

80 mg copper (II) sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , AR;

24 mg sodium molybdate  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , AR

40 mg cobalt (II) chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , AR

Complete solution with double distilled water to 1 litre in a

<sup>19</sup> Quoted from article



volumetric flask  
*Operating solution of trace elements*  
Complete 4 ml of stock solution with double distilled water to 100 ml in a volumetric flask

Environmental conditions:

Temperature:

19.5—20.6 °C

Light:

Osram L 25/40 fluorescent tubes with irradiance of 17.0 W/m<sup>2</sup>, each

Photoperiod:

Constant

pH:

Not stated

## STUDY DESIGN

### Experimental treatments

The toxicity of 4-chlorophenol to the green alga *Scenedesmus subspicatus* was determined in a 96-hour, static test. The test incorporated nominal concentrations of 4-chlorophenol between 0.16 and 20 mg/L with a dilution factor of 2 and a control. The test was performed in 300 mL Erlenmeyer flasks or in the case of a volatile or strongly smelling substance in 250 mL wide-necked bottles. Control preparations were prepared from dilution water, nutrient solution and algal suspension whereas test flasks or bottles were prepared with stock solution, nutrient solution and algal suspension. Cell density was 100,000 cells/mL at the beginning of the test.

Biomass was measured 24, 48, 72 and 96 h when using Erlenmeyer flasks or 24 and 48 hours when using bottles with an Eppendorf digital photometer.

### Observations

Data were evaluated for growth rate  $E_rC$  and inhibition of cell multiplication  $E_bC$ .

### Statistical calculations

No statistical calculations.

## II. RESULTS AND DISCUSSION

### Findings

The determined  $EC_{50}$  and NOEL values for *Scenedesmus subspicatus* are given in the table below.

**Table 4:** Determined  $EC_{50}$  values for 4-chlorophenol

Test period in hours	Inhibition of cell multiplication in mg/L		Growth rate in mg/L	
	$E_bC_{10}$	$E_bC_{50}$	$E_rC_{10}$	$E_rC_{50}$
0-48	2.2	11	5.5	19
0-72	1.9	8.3	5.8	17
0-96	3.0	8.0	-	-

## III. CONCLUSIONS

Data generated for the toxicity of 4-chlorophenol to *Scenedesmus subspicatus* resulted in a 72-hour  $E_rC_{50}$  for growth rate of 17 mg/L and in a 96-h  $E_bC_{50}$  for inhibition of cell multiplication of 8 mg/L.

Reliability Assessment of Ecotoxicity Studies Based on ToxRTool (Schneider et al., 2009)			
Criteria		Score	Evaluator's comments on criteria (optional)
No.	Cut-off criteria		
1	Was the test substance identified?	+	-
2	Is the species given?	+	-
3	Is the administration route given?	+	-
4	Are doses administered or concentrations in application media given?	+	tested concentration range between 0.16 and 20 mg/L, with a dilution factor of 2

5	Are frequency and duration of exposure as well as time-points of observations explained?	+	-
6	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required)?	+	not required
7	Is the number of replicates and/or organisms per group given?	+	-
8	Is the study design chosen appropriate for obtaining the substance specific data aimed at?	+	-
-	<b>Subtotal Test Substance</b>	<b>8</b>	<b>If not 8, study is not reliable.</b>
-	<b>Criteria Group I: Test substance identification</b>		
9	Is the purity of the substance given?	0	not stated
10	Is information on the source/origin of the substance given?	0	not stated
11	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data?	0	water solubility not given
-	<b>Subtotal Test Substance</b>	<b>0</b>	-
-	<b>Criteria Group II: Test organism characterisation</b>		
12	Is the sex or the sex ratio of the test organisms given?	+	not required
13	Is information given on the source or strain of test organisms plus, if considered necessary to judge the study, other specifications?	+	-
14	Is age, life stage, growth stage, body weight of the test organisms at the start of the study given?	+	-
15	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	+	no repeated dose study
-	<b>Subtotal Test Organisms</b>	<b>4</b>	-
-	<b>Criteria Group III: Study design description</b>		
16	Is the test media clearly described (water, soil, plant)?	+	-
17	Are sufficient details of the administration scheme given to judge the study?	0	it is not clearly stated how test with 4-chlorophenol was conducted
18	For repeated dose toxicity studies only (give point for other study types): Were exposure concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	+	no repeated dose study
-	<b>Subtotal Study design / Test method</b>	<b>2</b>	-
-	<b>Criteria Group IV: Study results documentation</b>		
19	Are the study endpoint(s) and their method(s) of determination clearly described?	+	-
20	Is the description of the study results for all endpoints investigated transparent and complete?	0	unclear
21	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable)?	+	-
-	<b>Subtotal Study Result Documentation</b>	<b>2</b>	-
-	<b>Criteria Group V: Plausibility of study design and results</b>		
22	Are the quantitative study results reliable?	+	-
-	<b>Subtotal Plausibility</b>	<b>1</b>	-
-	<b>Total Score</b>	<b>17</b>	-
-	<b>A Numerical result leads to initial Category:</b>	<b>2</b>	-
-	<b>B Cut-off criteria restricts Category:</b>	<b>No</b>	Minimal requirements given
-	<b>C Evaluator's proposed Category:</b>	<b>2</b>	-
-	<b>D Justification in case evaluator deviates from B:</b>	-	-
-	Date/period of evaluation:	16.11.2015	

Qualitative assessment		
Study assessment	Score	Rationale
Reliability/Repeatability	Klimisch 2	Reliable with restrictions: <ul style="list-style-type: none"> <li>17 out of 22 reliability criteria are met according to ToxRTool (Schneider et al. 2009)</li> <li>Well documented study with a few exceptions</li> </ul>
Limitations		Information on the chemical property of the substance are scarce as well as information on the concentrations used in the definitive test. In addition, administration scheme is not well reported.

Qualitative assessment		
Study assessment	Score	Rationale
GLP		No
Relevance	Supplemental data for risk assessment of algae	Data might be used to give supplemental information

## Appendix 5 Studies performed on active substances/metabolites in support of the evaluation – Terrestrial organisms

### 2,4-D METABOLITES

#### 4-Chlorophenol

#### A 5.1 KCP 10.4 Effects on no target soil meso-and macrofauna

#### A 5.1.1 KCP 10.4.1 Earthworms

#### Study 1: Sub-lethal toxicity to Earthworms

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>It was noted that the maximum temperature during the study (23.9°C) was higher than the maximum recommended by the guideline (20°C). However, this deviation was short-term (20 minutes only) and is considered to have no impact on the test results since all validity criteria were met.</p> <p>The test design was not relevant to derive both NOEC and EC<sub>x</sub> values as there should have been 8 concentrations tested with 4 replicates per treatment, 8 replicates for control, and the spacing factor should have not exceeded 1.8. In the study there were 5 concentrations tested with 4 replicates per treatment, 8 replicates for control, and the spacing factor was 2. Therefore, the test design was relevant to derive only the NOEC value.</p> <p>From the second lowest test concentration the promotion of the juveniles production was observed. However, no clear dose-response relationship could be observed and for this reason this effects is considered to be not relevant for derivation of an endpoint.</p> <p>As all the validity criteria were met, overall the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>56 d NOEC (reproduction, mortality, biomass) ≥ 10 mg pm/kg dw soil</p> <p>As no effects &gt;10% were observed, the EC<sub>x</sub> value could not be determined/</p>
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Reference:	KCP 10.4.1/02
Report	4-chlorophenol: Sublethal Toxicity to the Earthworm, <i>Eisenia fetida</i> (Annelida, Lumbricidae) in Artificial Soil with 10 % Peat. Wagenhoff, E. (2015). S15-00154 (report number)
Guideline(s):	OECD 222
Deviations:	-
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test Material:	4-chlorophenol
Description:	Not specified
Lot/batch:	TSN304318/MKBJ7452V
Concentration/Purity:	100 %

<b>Stability of test compound:</b>	Re-certification date: 26 January 2017
<b>2. Vehicle and/or control:</b>	Vehicle control: deionized water Positive control: Twist WP 60 % w/w (active ingredient: carbendazim)
<b>3. Test animals (Species):</b>	<i>Eisenia fetida</i>
<b>Age:</b>	They were at least two months old, but not older than one year with a clitellum (age of the worms did not differ by more than four weeks)
<b>Mean weight:</b>	Body weight at test start: 366 to 600 mg
<b>Source:</b>	Bred under standardised conditions at the test facility
<b>Feeding:</b>	Feeding: weekly up to the 28-day assessment with dried and finely ground cow manure
<b>Acclimation period:</b>	One day before exposure, the adult earthworms were selected and transferred from the rearing medium into moist, untreated artificial soil for acclimatisation.
<b>Animals per test concentration:</b>	See below
<b>Number of replicates:</b>	Four replicates/test substance treatment and eight replicates/control. 10 earthworms per replicate
<b>Artificial soil components:</b>	Artificial soil, percentage distribution on dry weight basis): <ul style="list-style-type: none"><li>• Sphagnum peat: 10%</li><li>• Kaolin clay (kaolinite content &gt; 30%): 20%</li><li>• Fine quartz sand (&gt; 50% particles of 50 – 200 µm): 69.5%</li><li>• &lt; 1% calcium carbonate – precipitated extra pure (the soil pH is adjusted to <math>6.0 \pm 0.5</math> at the start of the test before the addition of the test item)</li></ul> <p>The dry components were blended and mixed thoroughly in an electric mixer. After mixing the mean maximum water holding capacity (<math>WHC_{max.}</math>) was determined to be 48.92 % and the pH value was 5.9.</p>
<b>Test unit:</b>	Immediately after mixing, the test substrate of each treatment group was split and 634.6 g (corresponding to 500 g dry substrate) were placed into the test units (BELAPLAST, 17 cm × 12.5 cm × 6 cm; 1000 cm <sup>3</sup> , filling height approximately 5 cm). <p>The individual weights of the earthworms were recorded after washing them shortly before use. Ten randomly selected earthworms were placed onto the soil surface of each test container in a manner which ensures that they are homogenously distributed throughout the treatment groups with regard to the mean body weight per replicate. The test containers were closed with a perforated lid to allow gaseous exchange between the medium and the atmosphere.</p>
<b>Untreated variant:</b>	The control substrate was left untreated
<b>Reference standard:</b>	Twist WP 60 % w/w (active ingredient: carbendazim)

#### 4. Environmental conditions

<b>Temperature:</b>	20.0 – 23.9 °C (the temperature was outside the required range of $20 \pm 2$ °C for approx. 20 minutes only; this should have had no impact on the outcome and integrity of the study)
<b>pH:</b>	pH at initiation: 5.8 to 5.9 pH at termination: 6.0
<b>Humidity (Moisture content of the soil):</b>	Water content at initiation: 28.3 – 28.9 % Water content at termination: 28.9 – 30.6 %
<b>Photoperiod:</b>	16 hour light to 8 hour dark photoperiod
<b>Light intensity:</b>	600 – 700 lux

### B. STUDY DESIGN AND METHODS

- In-life dates:** 10.03.2015 – 07.05.2015
- Experimental design:**

Adult earthworms were exposed to artificial soil mixed with the test substance at five concentrations or remaining untreated (control) for a period of four weeks. After this period, the adults were removed from the test vessels and their survival, behavioural effects and growth (body weight change) were determined. The cocoons and juvenile earthworms remained in the test vessels for additional four weeks. The reproduction rate was determined by counting the number of offspring hatched from the cocoons after this additional test period of four weeks.

The test substance was applied to the artificial soil via deionized water.

**Test concentrations:** Control (deionised water), 0.625, 1.25, 2.50, 5.00, 10.0 mg test item/kg soil dry weight.

**Test duration:** 8 weeks (4 weeks adult mortality; 4 weeks juvenile development)
- Observations:**

Parameters reported are mortality, body weight change and reproduction. All of them are given as NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration). Reproduction is also given as EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub>, if possible. Additionally, changes in behaviour (e.g. food consumption) and morphology (e.g. pathological symptoms) of the adult earthworms were recorded if observed. Mortality is given in percent per treatment group after four weeks. Earthworm body weight was recorded individually at test initiation and per replicate after four weeks of exposure. Body weight change is reported as absolute weight change [in mg] and in percent per treatment group compared to the initial weight. Reproduction was evaluated as the number of juveniles per replicate and the mean number per treatment group after eight weeks.

The pH and water of the soil was measured for all treatment groups and the control at the start and at the end of the test.
- Statistics:** Calculation of treatment means and standard deviations. Level of significance  $\alpha = 0.05$  for each of the tests.

Analysis of mortality data using multiple Fisher's Exact Test with Bonferroni-Holm Adjustment. Analysis of reproduction and body weight change data for normality (Shapiro-Wilk's Test) and homoscedasticity (Levene's Test), followed by Dunnett's t-Test (one-sided smaller for reproduction, two-sided for bodyweight change). The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> could not be calculated.

## II. RESULTS AND DISCUSSION

### A. Biological results

There were no pathological symptoms of the adult earthworms observed during the first four weeks of exposure to the test item. Food consumption of the adult earthworms was estimated to be similar in all treatment groups compared to the control group during the first four weeks of the study.

Exposure of *E. fetida* to 4-chlorophenol had no effect on mortality, body weight or reproduction. Therefore, the LOEC for all measurement endpoints was greater than 10 mg/kg soil dry weight, and the NOEC was 10 mg/kg soil dry weight, the highest concentration tested. Since there was no dose-response relationship, and as the effect on reproduction was below 10 % for each of the concentrations tested, the EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> for reproduction were assumed to be greater than 10 mg/kg soil dry weight, the highest concentration tested in this study.

**Table 1: Effects of 4-chlorophenol on earthworm survival and biomass and reproduction**

Test concentrations (mg/kg soil dry weight)	% Mortality after 28 days	% Bodyweight change after 28 days	Mean no. of juveniles at day 56	% Reduction in number of juveniles compared to control <sup>1)</sup>
Control	2.5	-8.6	312.9	--
0.625	2.5	-11.3	304.8	2.6
1.25	0.0	-6.9	367.8	-17.5
2.50	0.0	-8.1	357.5	-14.3
5.00	5.0	-7.4	342.8	-9.6
10.0	0.0	-9.6	340.3	-8.8

\* Statistically different from the control.

<sup>1)</sup> Negative values indicate higher reproduction compared to the control.

### B. Validity criteria

All validity criteria for the control were met:

- Adult mortality required  $\leq$  10%. An adult mortality of 2.5 % was observed in the test.
- Mean number of juveniles per vessel required  $\geq$  30. A mean of 312.9 juveniles was obtained in the test.
- Coefficient of variation (CV) of reproduction required  $\leq$  30 %. The CV in the test was 20.9 %.

## III. CONCLUSION

No effects of 4-chlorophenol could be demonstrated on *E. fetida* for any of the investigated endpoints and test item concentrations including the highest tested concentration of 10 mg/kg soil dry weight in this study. Accordingly, the NOECs for mortality, body weight change and reproduction were all considered to be 10 mg/kg soil dry weight, the highest concentration tested. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> for reproduction were assumed to be greater than 10 mg/kg soil dry weight.

## **FLUROXYPYR METABOLITES**

### **Methoxyypyridine**

#### **Study 1: Sub-lethal toxicity to Earthworms**

Comments of zRMS:	The Applicant for ADM.3304.H.1.A has access to fluroxypyr EU agreed data via the LoA and study on effects of methoxyypyridine on earthworm reproduction was not required to finalise the soil risk assessment since sufficient EU agreed data are available. Furthermore, the study summarised below have not produced adverse data. Taking this into account, the study was not validated by the zRMS and its summary below is struck through.
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Reference:	KCP 10.4.1/03
Report	Effects of Methoxy (Fluroxypyr.) on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil. Witte, B., (2014). 92411022 (report number)
Guideline(s):	OECD 222 (2004), ISO 11268-2 (1998)
Deviations:	-
GLP:	Yes
Acceptability:	Not evaluated, sufficient EU agreed toxicity data available to which the Applicant has LoA
Duplication (if vertebrate study)	-

## **I. MATERIALS AND METHODS**

### **A. MATERIALS**

1. **Test Material:** Methoxyypyridine  
**Description:** Not specified  
**Lot/batch:** FXPYR(3) BP6 1991(V2a)  
**Concentration/Purity:** 98.4 ± 0.5 %  
**Stability of test compound:** Stable under storage conditions (room temperature).  
Expiry date: 22.12.2015
2. **Vehicle and/or control:** Vehicle control: Untreated quartz sand –  
Positive control: The reference substance Luxan Carbendazim 500 FC is tested in a dose response study at least once a year. The most recent reference substance study performed from August to October 2013 resulted in an EC<sub>50</sub> for reproduction of 1.32 mg carbendazim/kg dry soil. This value is in the range of the five most recent studies, in which EC<sub>50</sub> values between 1.11 and 1.59 mg carbendazim/kg soil were determined.
3. **Test animals (Species):** Earthworm (*Eisenia fetida* (Savigny 1826))  
**Age:** Approximately eight months old adults with well-developed clitellum (age of the worms did not differ by more than four weeks) –  
**Mean weight:** Body weight at test start: 300 to 516 mg  
**Source:** Bred under standardised conditions at the test facility  
**Feeding:** Finely ground cattle manure was used as food source during the study. The horse manure was fed one day after application and once a week during the exposure phase of the adults when the food of the previous week had almost been consumed. Per test unit, 5 g were scattered on the soil surface and moistened with deionised water. If the food



	<p>was not quite fully consumed, the added amount of food was adjusted to replace the visually estimated consumption. Four weeks after application, the food was mixed into the substrate following removal of the adult worms</p> <p>For one day prior to test start, the test organisms were acclimatised to the artificial soil and test conditions</p> <p>See below</p> <p>Four replicates/test substance treatment and eight replicates/control. 10 earthworms per replicate</p> <p>Artificial soil was prepared according to OECD Guideline 222 (percentage distribution on dry weight basis):</p> <ul style="list-style-type: none"> <li>▪ Sphagnum peat: 10 %</li> <li>▪ Kaolin clay (kaolinite content &gt; 30 %): 20 %</li> <li>▪ Fine quartz sand (&gt; 50 % particles of 50–200 µm): 69.5 %</li> <li>▪ Calcium carbonate (CaCO<sub>3</sub>): 0.5 % (for adjustment to pH 6.0 ± 0.5)</li> </ul> <p>The artificial soil was moistened to approximately half of the final water content one day before application. The additional water required to achieve the final water content was added when applying the test substance</p>
<b>Acclimation period:</b>	
<b>Animals per test concentration:</b>	
<b>Number of replicates:</b>	
<b>Artificial soil components:</b>	
<b>Test unit:</b>	<p>Plastic boxes (18.3 cm × 13.6 cm × 6 cm, soil surface 189.75 cm<sup>2</sup>) were used as test vessels. The test vessels were covered by transparent lids to prevent worms from escaping and to minimise evaporation from the artificial soil. The lids were perforated to allow air exchange. The layer depth of the artificial soil in the test vessels was approximately 4–5 cm. Each container was filled with 652.8 g of artificial soil (500 g dry weight plus 152.8 g deionised water)</p>
<b>Untreated variant:</b>	The control substrate was left untreated
<b>Reference standard:</b>	Luxan Carbendazim 500 FC
<b>4. Environmental conditions</b>	
<b>Temperature:</b>	20 ± 2 °C
<b>pH:</b>	The pH value in the test substance treatments and control was between 5.7 and 5.8 at the start of the test and between 5.9 and 6.0 at the end of the test
<b>Humidity (Moisture content of the soil):</b>	After application, the soil moisture content in each test vessel was adjusted to 31.8–32.7 % (corresponding to 53.9–55.4 % of the maximum water holding capacity, WHC <sub>max</sub> ) by addition of deionised water. The soil moisture content at study end was 32.7–35.7 % (55.4–60.6 % of WHC <sub>max</sub> )
<b>Photoperiod:</b>	16 hour light to 8 hour dark photoperiod
<b>Light intensity:</b>	400–800 lux

## B. STUDY DESIGN AND METHODS

- In-life dates:** 26.06.2014 to 22.08.2014
- Experimental design:** Adult earthworms were exposed to artificial soil treated with the test substance at five concentrations or remaining

untreated (control) for a period of four weeks. After this period, the adults were removed from the test vessels and their survival, behavioural effects and growth (body weight change) were determined. The cocoons and juvenile earthworms remained in the test vessels for additional four weeks. The reproduction rate was determined by counting the number of offspring hatched from the cocoons after this additional test period of four weeks.

The test substance was applied to the artificial soil via treated quartz sand. The application solution for the highest test concentration was prepared by dissolving 164.0 mg of methoxypyridine in 20 mL acetone. This application solution was serially diluted to obtain the application solutions of the lower test concentrations. From each application solution, 9 mL were added to 20 g fine quartz sand. The treated sand was left for at least one hour in a fume hood until the solvent had evaporated and was mixed. Then, each treated sand sample was added to a soil aliquot equivalent to 2030 g dry weight. The control was treated in the same way but using quartz sand to which untreated acetone was added. While mixing the artificial soil in a laboratory mixer, the soil of each treatment group was moistened with deionised water. Each group was treated in one batch (two in the control) which was then split into the replicates.

After soil treatments, batches of ten weighed test organisms were placed on the soil surface of each test vessel (test start). The different batches were sorted into four classes on the basis of the total weight and one batch of each weight class was assigned to each treatment group (two batches for the control) to ensure that weights were homogeneous.

**Test concentrations:**

Methoxypyridine was tested at 2.25, 4.5, 9.0, 18 and 36 mg/kg dry soil. A control (receiving untreated quartz sand) was tested in parallel

**Test duration:**

8 weeks (4 weeks adult mortality; 4 weeks juvenile development)

**3. Observations:**

For each test vessel, adult mortality and the difference in mean body wet weight of the surviving test organisms between start and end of the 4 week exposure period were calculated. Mean mortality and growth (body weight change) was calculated for each treatment group and the control.

The feeding activity during the test period was recorded as the cumulative amount of food added to each test container.

At test termination after eight weeks, the reproduction rate was calculated as the mean number of juvenile worms per test vessel of each treatment group and the control. Furthermore, the reproduction rate in each test substance

treatment was given as percentage of the reproduction rate in the control.

At the start of the test, soil moisture was measured for all treatment groups and the control. Once per week, the test vessels were weighed and water evaporation losses were compensated for by adding deionised water, if necessary, in order to ensure that the difference in water content between study start and end was < 10 %. At the end of the test, the soil moisture was determined again for all treatment groups and the control.

The pH of the soil was measured for all treatment groups and the control at the start and at the end of the test.

#### 4. Statistics:

The body weight change and reproduction rate were tested for normal distribution and homogeneity of variance ( $\alpha = 0.05$ ) using the Shapiro Wilk's test and the Levene's test, respectively. As the data for body weight change and reproduction rate were normally distributed and homogeneous in both cases, the Williams t test was used to compare treatment and control values (multiple comparison, two-sided for body weight change and one-sided smaller for reproduction rate,  $\alpha = 0.05$ ). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH.

## H. RESULTS AND DISCUSSION

### A. Biological results

Mean mortality of adult test organisms in the control and in the five test substance treatments was 1.3% and 0 % after four weeks, respectively. Thus, the survival rate of the earthworms after four weeks of exposure to methoxy pyridine was not affected up to and including the highest test concentration of 36 mg/kg dry soil. Moreover, no behavioural abnormalities were observed in adult test organisms at any test concentration.

The mean body weight of adult worms in the control had increased during the 4 week exposure period on average by 54.8 % of the mean initial weight. At all test concentrations of methoxy pyridine, the mean increase in body weight ranged from 50.7 % to 59.3 % and was not statistically significantly different from the control. Furthermore, food consumption was 25.0 g in all test groups and was therefore not affected by the test substance.

In the control, a mean reproduction rate of 290 juveniles per replicate was found. In the test substance treatments, the mean reproduction rate ranged between 261 and 295 juveniles per replicate which corresponds with 89.8 – 101.6 % of the control value. The mean reproduction rate at all test concentrations and was not statistically significantly different from the control. Thus, the reproduction rate of the earthworms after eight weeks of exposure to methoxy pyridine was not affected up to and including the highest test concentration of 36 mg/kg dry soil.

Based on these results, the NOEC and LOEC of methoxy pyridine for growth and reproduction of *Eisenia fetida andrei* were determined to be  $\geq 36$  mg/kg dry soil and  $> 36$  mg/kg dry soil, respectively. The  $EC_{50}$  for growth and reproduction could not be determined but was clearly  $> 2.25$  mg/kg dry soil.

**Table 2: Effects of methoxy pyridine on earthworm survival, growth and reproduction**

Treatment [mg/kg dry soil]	Mortality after 4 weeks of exposure [%]	Change in body fresh weight after 4 weeks of exposure (mean $\pm$ SD) [%]	Reproduction rate after 8 weeks (mean $\pm$ SD)	
			[juveniles/test vessel]	[% of control]
Control	1.3	54.8 $\pm$ 9.6	290 $\pm$ 45	-
2.25	0.0	52.8 $\pm$ 4.1	288 $\pm$ 17	99.3
4.5	0.0	55.0 $\pm$ 12.4	295 $\pm$ 32	101.6
9.0	0.0	59.3 $\pm$ 12.0	261 $\pm$ 21	89.8
18	0.0	56.3 $\pm$ 7.3	263 $\pm$ 34	90.5
36	0.0	50.7 $\pm$ 9.7	277 $\pm$ 41	95.4
<b>Endpoints [mg/kg dry soil]</b>				
EC <sub>50</sub> (growth and reproduction)		$\geq 36$		
NOEC (growth and reproduction)		$\geq 36$		
LOEC (growth and reproduction)		$\geq 36$		

Note: Test substance treatments were not statistically significantly different from the control (results of a Williams t test, multiple comparison, two-sided for body weight change and one-sided smaller for reproduction rate,  $\alpha = 0.05$ ).

## B. Validity criteria

The validity of the test was fulfilled since each control replicate produced  $\geq 220$  juveniles (required  $\geq 30$  juveniles) and the coefficient of variance of the reproduction rate per test vessel in the control was 15.5 % (required  $\leq 30$  %). Furthermore, mortality of the adults in the control was 1.3 % (required  $\leq 10$  %).

## III. CONCLUSION

The overall NOEC of methoxy pyridine for *Eisenia fetida* was determined to be 36 mg/kg dry soil based on survival, growth and reproduction of exposed adults. The EC<sub>50</sub> for growth and reproduction could not be determined but was clearly  $> 36$  mg/kg dry soil.

## A 5.1.2 KCP 10.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

### Study 1: Sub-lethal toxicity to Collembolan

Comments of zRMS:	The Applicant for ADM.3304.H.1.A has access to fluroxypyr EU agreed data via the LoA and study on effects of methoxy pyridine on <i>Folsomia candida</i> reproduction was not required to finalise the soil risk assessment since sufficient EU agreed data are available. Furthermore, the study summarised below have not produced adverse data. Taking this into account, the study was not validated by the zRMS and its summary below is struck through.
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Reference:	KCP 10.4.2/04
Report	Methoxy pyridine: Effects on the reproductive output of the springtail <i>Folsomia candida</i> Willem (Collembola, Isotomidae) using an artificial soil test with 5 % peat content. Höhn, P. (2012). S12-00021 (report number)
Guideline(s):	OECD 232 (2009)
Deviations:	-
GLP:	Yes
Acceptability:	Not evaluated, sufficient EU agreed toxicity data available to which the Applicant has LoA
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

1—Test Material:	Methoxypyridine
—Active ingredients / purity:	98.4 ± 0.5 %
—Description:	Not stated
—Lot/Batch no.:	FXPYR(3) BP6-1991(V2)
2—Reference toxicant:	
—Purity / Active ingredient	The reference item boric acid (99.5–100.5 % analysed)
3—Test Animals:	
—Species:	Collembolan <i>Folsomia candida</i> (Willem)
—Age/growth stage:	9–12 days (juvenile collembolans)
—Source:	Laboratory rearing stock at the testing facility
—Acclimation:	Not stated
—Food:	Granulated dry yeast
Number of animals / test vessel	10
Number of collembolans	80 (control group), 40 (treated group)
4—Environmental Conditions	
—Temperature	20.1–23.9 °C
—Photoperiod	Light : dark = 16 h : 8 h
—Light intensity	400–450 lux
Composition of artificial soil	<ul style="list-style-type: none"><li>• 5 % peat, air dried, finely ground</li><li>• 20% kaolin clay (kaolinite content above 30 %)</li><li>• 75 % air dried industrial sand, predominantly fine sand with more than</li><li>• 50% of the particles between 0.05 mm and 0.2 mm.</li></ul> The dry components were blended and mixed thoroughly. After mixing, the water holding capacity of the substrate was determined to be 32.89 % and the pH value was 5.87. No addition of CaCO <sub>2</sub> was necessary

### B. STUDY DESIGN AND METHODS

1. In-life dates:	18.08.2015–15.09.2015
2. Experimental design:	On the day of the test start, the test item was introduced in the test substrate: Due to poor solubility of the test item in water but solubility in organic solvent, the test item was dissolved in acetone as a vehicle. An acetone solution was prepared for each test item and an aliquot of 1 mL solution was mixed with 8 g of fine quartz sand per test item treatment. The highest application rate was applied directly to the sand and 1 mL acetone was added. An acetone-treated control was also included in the test design. The acetone was eliminated by evaporation under a fume hood. This mixture of quartz sand and test item was added to the pre-moistened soil and thoroughly mixed by adding the necessary amount of deionised water to obtain a water content of 60 % of the total water holding capacity. The final mixture was given into the test vessels immediately after mixing.

	<del>The test was started with transferring ten juvenile springtails, <i>Folsomia candida</i>, to the exposure units. 28 days after introducing the juvenile springtails on the test and control substrates, effects on mortality and reproductive output were evaluated.</del>
<b>Test concentrations:</b>	<del>23.9, 31.1, 40.4, 52.5, 68.3, 88.8, 115.4, 150 mg test item/kg soil d.w.</del>
<b>Test duration:</b>	<del>28 days</del>
<b>3. Observations:</b>	<p><del>The pH and water content of the test substrate were determined at the start and at the end of the test.</del></p> <p><del>Collembolans in the test and control vessels were counted: The test substrate of each replicate was poured into an individual 700 mL container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. The numbers of parental and juvenile collembolans floating on the surface were determined. To facilitate counting, photos of the water surface were taken with a digital camera and counting was done by means of a pen tablet and a mousotron software program (mousotron 5.0) was used to count the springtails. Juvenile springtails were counted twice by different persons and a mean value was calculated.</del></p>
<b>4. Statistics:</b>	<p><del>Statistical analysis on mortality was conducted using Fisher's Exact Test (Bonferroni Holms corrected, one-sided, <math>p \sim 0.05</math>). Concerning reproductive output, the test item treatment groups were compared to the acetone control using Jonckheere Terpstra Test (one-sided, <math>p \sim 0.05</math>). Data were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). The <math>LC_{50}</math> and <math>EC_{50}</math> were calculated using Probit analysis (Logistic and Gompertz procedure). Statistical analyses were conducted using the program SAS INSTITUTE INC. 2002 2008. SAS® Proprietary Software 9.2.</del></p>

## II. RESULTS AND DISCUSSION

### A. Biological results

Mortality rates of 2.5 to 95 % were recorded in the test item treatment groups. Mortality in the combined controls was 6.9 %. Statistically significant effects (Fisher's Exact Test, Bonferroni Holms corrected, one-sided,  $p \leq 0.05$ ) on parental mortality were recorded at 200 to 800 mg test item/kg soil d.w. The 28-day NOEC in terms of mortality was determined to be 100 mg test item/kg soil dry weight. The  $LC_{50}$  was determined to be 159 mg test item/kg soil dry weight. Individuals from the test item treatment groups with 200, 400 and 800 mg Methoxyypyridine/kg soil dry weight were undersized compared to the combined controls.

After 28 days of exposure the mean number of juveniles in the acetone control was 942.8 per replicate. The mean numbers of juvenile springtails in the test item treatment groups at 50, 100, 200, 400 and 800 mg Methoxyypyrieline/kg soil dry weight were 802.8, 620.4, 0.0, 0.0 and 0.0, resulting in a reduction in reproductive output of 14.9, 34.2, 100.0, 100.0 and 100.0 %, respectively compared to the acetone control. Differences for each test item treatment group were statistically significantly different compared to the acetone control group. Therefore, the NOEC for reproductive output could not be determined. The

LOEC for reproductive output was determined to be 50 mg Methoxy pyridine/kg soil dry weight. The 28-day EC<sub>50</sub> of the test item was calculated as 106 mg Methoxy pyridine/kg soil dry weight (95 % confidence limits: 97 to 115 mg Methoxy pyridine/kg soil dry weight, Gompertz Model). No physiological symptoms or abnormal behaviour of the juvenile springtails was observed.

**Table 3:** ~~Effect of Methoxy pyridine on *Folsomia candida* on mortality and reproductive output~~

Treatment	Rate [mg/kgsdw]	Mean mortality [%]	Corrected mortality [%] <sup>1)2)</sup>	Mean no. of juvenile springtails	Reduction in reproductive output [%] <sup>3)</sup>
Acetone control	0	7.5	-	942.8	-
Water control	0	6.3	-	n.a.	n.a.
Combined controls	0	6.9	-	n.a.	n.a.
Methoxy pyridine	50	2.5	-4.7	802.8**	14.9
	100	17.5	11.4	620.4**	34.2
	200	77.5*	75.8	0.0**	100
	400	90.0*	89.3	0.0**	100
	800	95.0*	94.6	0.0**	100
LC <sub>50</sub>	159 mg Methoxy pyridine/kg sdw (upper 95 % confidence limit: 134 mg Methoxy pyridine/kg sdw lower 95 % confidence limit: 187 mg Methoxy pyridine/kg sdw)				
EC <sub>50</sub>	106 mg Methoxy pyridine/kg sdw (upper 95 % confidence limit: 97 mg Methoxy pyridine/kg sdw lower 95 % confidence limit: 115 mg Methoxy pyridine/kg sdw)				

\* Statistically significantly increased compared to the combined controls (Fisher's Exact Test, Bonferroni-Holms corrected, one-sided,  $p \leq 0.05$ ).

\*\* Statistically significantly reduced compared to the acetone control (Jonckheere-Terpstra, one-sided,  $p \leq 0.05$ ).

1) Corrected mortality according to SC-NEIDER-ORELLI (1947).

2) A negative value indicates lower mortality in the test item treatment group compared to the combined controls.

3) Reduction in reproductive output according to ABBOTT (1925) compared to the acetone control.

sdw: Soil dry weight.

n: Number of replicates per treatment group.

n.a.: Not applicable.

## A. Validity criteria

The validity criteria for the control group were accomplished:

- Mean adult mortality:  $\leq 20$  % (observed: 6.9 %).
- Mean number of juveniles per test vessel:  $\leq 100$  (observed: average of 942.8/vessel).
- Coefficient of variation for the mean number of juveniles:  $< 30$  % (observed: 7.4 %).

## III. CONCLUSION

The NOEC for mortality of the parental collembolans and for reproduction was determined to be 88.8 mg test item/kg soil dry weight. The LC<sub>50</sub> value for mortality was calculated to be 139.9 mg test item/kg soil dry weight. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (based on reproduction) were calculated to be 103.0, 115.8 and 144.8 mg test item/kg soil dry weight, respectively.

Methoxy pyridine caused statistically significant effects on mortality of *Folsomia candida* from 200 to 800 mg Methoxy pyridine/kg soil dry weight. Therefore, the 28-day NOEC was determined to be 100 mg Methoxy pyridine/kg soil dry weight and the 28-day LOEC for mortality was 200 mg Methoxy pyridine/kg soil dry weight.

The 28-day LC<sub>50</sub> of the test item was calculated as 159 mg Methoxy pyridine/kg soil dry weight (95 % confidence limit: 134 mg to 187 mg Methoxy pyridine/kg sdw).

Statistically significant differences in reproductive output between the acetone control and the test item groups were observed for each rate. Therefore, the NOEC for reproductive output could not be determined. The LOEC was determined to be 50 mg Methoxy pyridine/kg soil dry weight.

The 28-day EC<sub>50</sub> of the test item was calculated as 106 mg Methoxyypyridine/kg soil dry weight (95 %-confidence limit: 97 mg to 115 mg Methoxyypyridine/kg sdw).

Two amendments of the study Höhn (2012) are provided in support of the assessment.

Reference:	Amendment to KCP 10.4.2/04
Report	Methoxyypyridine: Effects on the reproductive output of the springtail <i>Folsomia candida</i> Willem (Collembola, Isotomidae) using an artificial soil test with 5% peat content. — Report Amendment No. 1 to Study S12-00021 (from Höhn, 2012). Wagenhoff, E. (2015). S12-00021 (report number)
Guideline(s):	OECD 232 (2009)
Deviations:	-
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Based on the available results, the EC<sub>10</sub> and EC<sub>20</sub> values were calculated via Probit analysis (Gompertz Model). The EC<sub>10</sub> was determined by extrapolation to a value of 54 mg Methoxyypyridine/kg dry weight soil (95% confidence limits: 44 – 62 mg Methoxyypyridine/kg d.w. soil).

Reference:	Amendment 2 to KCP 10.4.2/04
Report:	Methoxyypyridine: Effects on the reproductive output of the springtail <i>Folsomia candida</i> Willem (Collembola, Isotomidae) using an artificial soil test with 5% peat content. — Report Amendment No. 2 to Study S12-00021 (from Höhn, 2012). Wagenhoff, E. (2017)
Guideline(s):	OECD 232 (2009)
Deviations:	-
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Based on the available results, the EC<sub>10</sub> and EC<sub>20</sub> values were recalculated using ToxRat 3.2.1.

The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> for reproduction were calculated using Weibull analysis (linear maximum likelihood regression) based on single replicate values. The EC<sub>10</sub> was calculated by extrapolation. Statistical analyses were conducted using the program SAS INSTITUTE INC. 2002-2008. SAS® Proprietary Software 9.2 (hypothesis testing and LC50 calculation) and ToxRat Professional 3.2.1 (EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> calculation).

The EC<sub>10</sub>, calculated by extrapolation, was determined to be 57.9 mg Methoxyypyridine/kg soil (upper 95 %-confidence limit: 47.4 mg Methoxyypyridine/kg soil, lower 95 %-confidence limit: 66.1 mg Methoxyypyridine/kg soil).

#### Results of the effects of Methoxyypyridine on reproduction of springtails after 28 days

Treatment	Rate [mg/kg sdw]	Mean no. of juvenile springtails	Mean mortality (%)	Corrected mortality (%) 1,2	Reduction in reproductive output (%) 3
Acetone control	0	942.8	7.5	-	-
Water control	0	n.a.	6.3	-	n.a.
Combined controls	0	n.a.	6.9	-	n.a.
Methoxyypyridine	50	802.8**	2.5	4.7	14.9
	100	620.4**	17.5	11.4	34.2
	200	0.0**	77.5*	75.8	100.0
	400	0.0**	90.0*	89.3	100.0



	800	0.0**	95.0*	94.6	100.0
EC <sub>10</sub> <sup>4</sup>	57.9 mg Methoxyypyridine/kg sdw (upper 95% confidence limit: 47.4 mg Methoxyypyridine/kg sdw lower 95% confidence limit: 66.1 mg Methoxyypyridine/kg sdw)				
EC <sub>20</sub>	76.1 mg Methoxyypyridine/kg sdw (upper 95% confidence limit: 66.8 mg Methoxyypyridine/kg sdw lower 95% confidence limit: 83.3 mg Methoxyypyridine/kg sdw)				
EC <sub>50</sub>	115 mg Methoxyypyridine/kg sdw (upper 95% confidence limit: 107 mg Methoxyypyridine/kg sdw lower 95% confidence limit: 124 mg Methoxyypyridine/kg sdw)				

\* Statistically significantly increased compared to the combined controls (Fisher's Exact Test, Bonferroni-Holms corrected, one-sided,  $p \leq 0.05$ )

\*\* Statistically significantly reduced compared to the acetone control (Jonckheere-Terpstra, one-sided,  $p \leq 0.05$ )

<sup>1</sup>) Corrected mortality according to SCHNEIDER-ORELLI (1947)

<sup>2</sup>) A negative value indicates lower mortality in the test item treatment group compared to the combined controls

<sup>3</sup>) Reduction in reproductive output according to ABBOTT (1925) compared to the acetone control

<sup>4</sup>) The EC<sub>10</sub> was calculated by extrapolation

n.a.: Not applicable

## Study 2: Sub-lethal toxicity to Collembolan

Comments of zRMS:	The Applicant for ADM.3304.H.1.A has access to fluroxypyr EU agreed data via the LoA and study on effects of methoxyypyridine on <i>Folsomia candida</i> reproduction was not required to finalise the soil risk assessment since sufficient EU agreed data are available. Furthermore, the study summarised below have not produced adverse data. Taking this into account, the study was not validated by the zRMS and its summary below is struck through.
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Reference:	KCP 10.4.2/06
Report	FXP-2FXP-211-6MeO – A laboratory test to determine the effects of fresh residues on the springtail <i>Folsomia candida</i> (Collembola, Isotomidae). Geary, N. (2016). 90019202 (report number)
Guideline(s):	OECD 232 (2016); ISO 11267
Deviations:	-
GLP:	Yes
Acceptability:	Not evaluated, sufficient EU agreed toxicity data available to which the Applicant has LoA
Duplication (if vertebrate study)	-

## I. MATERIALS AND METHODS

### A. MATERIALS

#### 1. Test material

Designation	FXP-211-6MeO (Methoxyypyridine)
Characteristics	Not specified
Lot no.	171-2674
Active ingredient(s)/Content	3,5-dichloro-2-fluoro-6-methoxy-pyridin-4-amine (99.3 %)
Storage conditions	21.1 – 27.4°C
Stability (expiry date)	31 December 2017

2. Vehicle control:	Control: acetone - and purified water-treated control Reference item: Betosip 114 (114 g/L phenmedipham)
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#### 3. Test System

Species	<i>Folsomia candida</i> (Willem 1902)
Age	11 days old
Source	In-house culture
Test units	Glass vessels of approximately 125 mL volume (diameter: 4.5 cm)
Diet	Fed with granulated dry yeast

Soil	LUFA 2.2. $1.61 \pm 0.15$ % organic matter content. Water content 50% of the total water-holding capacity, pH 5.59 – 6.14
<b>4. Test Conditions</b>	
Housing	Eggs were transferred 12 days before the test started from the breeding containers to freshly prepared breeding substrate. After 48 hours, the egg clusters were removed and the instars which hatched were used for the test.
Temperature	19.1 – 20.5 °C
Photoperiod	16 ours, 590 – 740 lux

## B. STUDY DESIGN AND METHODS

- 1. In-life dates** 21 September to 21 October 2016
- 2. Treatment**

The toxicity of Methoxyypyridine was tested at concentrations of 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 mg/kg d.w. soil. Four replicates for the test item treatment and eight replicates for the water and solvent controls treatments were used. The test item was dissolved in acetone as a vehicle, due to poor solubility of the test item in water but solubility in organic solvent.

To make up each rate of treated soil 2.0 mL volume of the appropriate acetone solution of the test item was mixed with an aliquant of sand (16.00 g) in a glass vessel and the acetone was allowed to evaporate away for 60 - 65 minutes. Once the treated sand was dry, it was thoroughly mixed with 200 g dry weight of natural soil (LUFA 2.2) equivalent to an actual weight of 219.3 g. Purified water was mixed with this treated natural soil to achieve a final soil moisture content of 50% of the soil's maximum water-holding capacity (WHC). No direct measurement was made of test item homogeneity in the soil, but care was taken to mix both the test item dilution and the treated soil thoroughly.
- 2. Observations**

At the start and at the end of the test, soil pH-value of the test medium was determined in additional containers for each treatment group.

After 28 days, mortality of adults and number of juveniles were determined. The effect of the test item was assessed by comparing mortality data with the combined results of both control groups and the number of juveniles in the test item treatment groups with the acetone control.

## Statistics

To estimate the *median lethal concentration* ( $LC_{50}$ ), Probit regression analysis (Finney, 1952) was performed on the numbers of surviving adult springtails in the individual treatments at 28 days. For the analysis, the test-item concentrations were log<sub>10</sub>-transformed and the data for the individual replicates were entered separately. The level of background mortality (the natural response rate estimate) was estimated by including combined control data (log<sub>10</sub> concentration value entered manually as 0). The 95% confidence intervals for the  $LC_{50}$  value were also calculated and a Chi-square test for goodness of fit ( $\alpha = 0.05$ ) was performed on the Probit line. Only data from what was considered to be the active part of the response-curve were included in the analysis.

*Derivation of the NOEC and LOEC for the number of juveniles.* Following a check for normal distribution of the data (Shapiro-Wilk test,  $\alpha = 0.05$ ), the numbers of juveniles in the solvent control were compared to those in the water control using an independent samples t-test ( $\alpha = 0.05$ ), which included Levene's test for equality of variances ( $\alpha = 0.05$ ) (Fowler & Cohen, 1990). Since there was no significant difference between the two controls, the data were pooled for comparison with the test-item treatments. Following a check for normal distribution of the data (Shapiro-Wilk test,  $\alpha = 0.05$ ) and for equality of variances (Levene's test,  $\alpha = 0.05$ ), the test-item treatments and toxic reference was compared to the combined control treatment results by t-test for independent samples ( $\alpha = 0.05$ ) (Fowler & Cohen, 1990). It was intended that the results of these analyses would be used to determine the LOEC and NOEC with respect to effects on reproduction.

*Derivation of the  $EC_x$  for the F1 generation.* Probit regression analysis was performed on the data for the numbers of progeny in the test item, in order to derive the *median effect concentration* ( $EC_{50}$ ) and also values for the  $EC_{20}$  and  $EC_{10}$ . For the Probit analysis, data for the individual test-item treatment replicates were entered separately, having been first converted to values for the percentage change in reproductive success, relative to the mean combined control value (any negative values were substituted with a zero). The test-item concentrations were log<sub>10</sub>-transformed prior to analysis of the data. The 95% confidence intervals for the  $EC_x$  value were also calculated and a Chi-square test for goodness of fit ( $\alpha = 0.05$ ) was performed on the Probit line. Only data from what was considered to be the active part of the response-curve were included in the analysis. Statistical analyses were performed using validated computer software (SPSS, 2013).

## II. RESULTS AND DISCUSSION

### B. Mortality

Details and corrected mortality are shown in the following table.

**Table 4: Results of the effects of Methoxy pyridine on mortality of springtails after 28 days**

Treatment	Concentration [mg/kg sdw]	Mean number of surviving adults	±SD	Mean mortality [%] <sup>a</sup>	Corrected mortality [%] <sup>b</sup>
Acetone control	-	9.4	1.1	6	-
Water control	-	9.5	0.8	5	-
Combined controls	-	9.4	0.9	6	-
FXP-211-6MeO (Methoxy pyridine)	3.9	8.8	0.5	13	7
	7.8	8.5	1.0	15	10
	15.6	8.5	0.6	15	10
	31.3	7.8	1.0	23 *	18
	62.5	0.5	1.0	95 *	95
	125	0.0	0.0	100 *	100
	250	0.3	0.5	98 *	97
	500	0.0	0.0	100 *	100
LC <sub>50</sub> <sup>c</sup>	33.3 mg Methoxy pyridine/kg sdw (upper 95%-confidence limit: 44.4 mg Methoxy pyridine/kg sdw lower 95% confidence limit: 23.5 mg Methoxy pyridine/kg sdw)				

<sup>a</sup> Mortality of springtails originally introduced, recorded at 28 days. Treatments were compared using Fisher's Exact Test ( $\alpha = 0.05$ ). An asterisk (\*) indicates a result that differed significantly from the combined control.

<sup>b</sup> Corrected mortality according to Abbott

<sup>c</sup> Lethal effect concentration (LC<sub>50</sub>) and 95% confidence limits were derived by Probit regression analysis. Only data from what was considered to be the active part of the response curve (shaded values) were included in this analysis.

sdw = Soil dry weight

SD = Standard Deviation

## C. Reproduction

The summary data are shown in the following table.

**Table 5: Results of the effects of Methoxy pyridine on reproduction of springtails after 28 days**

Treatment	Concentration [mg/kg sdw]	Mean no. of juvenile springtails (controls: n=8, test item: n=4) <sup>a</sup>	±SD	Coefficient of variation [%]	Reduction in reproductive output [%]
Acetone control	-	303.1	47.1	15.6	-
Water control	-	322.5	64.0	19.9	-
Combined controls	-	312.8	55.2	17.7	-
FXP-211-6MeO (Methoxy pyridine)	3.9	303.5	33.0	-	3
	7.8	289.3	32.8	-	8
	15.6	276.8	27.4	-	12
	31.3	284.5	40.6	-	9
	62.5	3.3 *	3.0	-	99
	125	0.0 *	0.0	-	100
	250	0.0 *	0.0	-	100
	500	0.0 *	0.0	-	100
NOEC	31.3 mg Methoxy pyridine/kg sdw				
EC <sub>50</sub> <sup>b</sup>	32.2 mg Methoxy pyridine/kg sdw (upper 95%-confidence limit: 50.2 mg Methoxy pyridine/kg sdw lower 95% confidence limit: 22.1 mg Methoxy pyridine/kg sdw)				
EC <sub>20</sub> <sup>b</sup>	16.1 mg Methoxy pyridine/kg sdw (upper 95%-confidence limit: 23.3 mg Methoxy pyridine/kg sdw lower 95% confidence limit: 8.9 mg Methoxy pyridine/kg sdw)				
EC <sub>10</sub> <sup>b</sup>	11.2 mg Methoxy pyridine/kg sdw (upper 95%-confidence limit: 17.0 mg Methoxy pyridine/kg sdw lower 95% confidence limit: 5.1 mg Methoxy pyridine/kg sdw)				

<sup>a</sup> The mean number of juveniles produced per replicate. The results for reproduction in the individual treatments were compared by t-test for independent samples ( $\alpha = 0.05$ ). Values marked with an asterisk (\*) differed significantly from the combined control.

<sup>b</sup> Key effect concentrations for number of progeny (EC<sub>50</sub>, EC<sub>20</sub> and EC<sub>10</sub>) and their 95% confidence limits were derived by Probit regression analysis. Only data from what was considered to be the active part of the response curve (shaded values) were included in this analysis.

sdw Soil dry weight

SD Standard Deviation

n number of replicates

### III. CONCLUSION

The effects of FXP-211-6MeO on the springtail *Folsomia candida* were assessed following application to a natural sandy loam soil (LUFA 2.2.). In terms of effects on springtail survival, the 28-day LC<sub>50</sub> was 33 mg test item/kg soil dry weight, the LOEC was 31.3 mg test item/kg soil dry weight and the NOEC was 15.6 mg test item/kg soil dry weight. In terms of effects on reproduction, the 28-day EC<sub>50</sub>, EC<sub>20</sub> and EC<sub>10</sub> values were 32, 16 and 11 mg test item/kg soil dry weight, respectively. The LOEC for reproduction was 62.5 mg test item/kg soil and the NOEC 31.3 mg test item/kg soil dry weight.

#### A 5.2 KCP 10.5 Effects on soil nitrogen transformation

##### Pyridinol

##### Study 1: Toxicity to the soil microflora

Comments of zRMS:	The Applicant for ADM.3304.H.1.A has access to fluroxypyr EU agreed data via the LoA and study on effects of pyridinol on soil microbial activity was not required to finalise the soil risk assessment since sufficient EU agreed data are available. Furthermore, the study summarised below have not produced adverse data, since the lower endpoints are results of the selection of the test concentrations and not more severe effects observed comparing to the EU agreed study evaluated as Confirmatory Data in 2014. Taking this into account, the study was not validated by the zRMS and its summary below is struck through.
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Reference:	KCP 10.5/02
Report	Effects of Pyridinol on the activity of the soil microflora - nitrogen transformation test. Schöbinger, U., (2012). S12-00189 (report number)
Guideline(s):	OECD 216 (2000)
Deviations:	-
GLP:	Yes
Acceptability:	Not evaluated, sufficient EU agreed toxicity data available to which the Applicant has LoA
Duplication (if vertebrate study)	-

### ~~I. MATERIALS AND METHODS~~

#### ~~A. MATERIALS~~

1. ~~Test Material:~~ Pyridinol  
~~Description:~~ -  
~~Lot/batch:~~ FXPYR(2)-BP9-1992  
~~Concentration/Purity:~~ 99.2 ± 0.5 %  
~~Stability of test compound:~~ Expiry date: 31 December 2015
  2. ~~Vehicle and/or control:~~ Untreated soil
  3. ~~Test animals (Species):~~ Soil microorganisms  
~~Replicates:~~ 3 per control (water control, solvent control) and test item  
~~Test vessel:~~ 4 portions of soil of weight 3300 g; one water control group, one solvent control group and 2 groups containing test material. All portions were divided into 3 replicates of weight 1100 g.
- ~~In order to avoid drying out of the soil, the glass bottles in which soil was kept were closed loosely with screw caps.~~
- ~~Test soil:~~ A common agricultural soil type was used for the study; soil parameters were in accordance with the recommendations of OECD guideline 216. Soil moisture content: 40 % WHC<sub>max</sub>.

**Soil parameter:**

Approx. 1 100 g soil for each study group  
Soil batch: F2.3 5011 (medium loamy sand):

Particle size distribution [%] according to DIN 4220	
– Sand	54.4
– Silt	36.6
– Clay	9.0
Soil dry weight [%]	87.5
pH (CaCl <sub>2</sub> )	7.25*
Density [g/L]	1297
Total Organic Carbon (TOC; %) based on soil dry weight	0.96
CEC [meq/100 g]	11.2
WHC <sub>m</sub> [%]	42.48
Nitrogen content [% d.w.]	0.097
Nitrate concentration [mg/kg sdw]	11.0**

\* pH value of solvent control from nitrogen turnover sample at t = 0 days

\*\* Mean NO<sub>3</sub>-N value from nitrogen turnover solvent control sample at t = 0 days

**Untreated variant:**  
**Reference standard:**

Untreated soil with lucerne meal  
Sodium-Chloride (tested in a separate study)

**4. Environmental conditions**

**Temperature:** 19.2 – 22 °C  
**Photoperiod:** Incubation in the dark  
**pH** 7.25  
**Soil moisture:** 40 % (adjusted once a week by re-weighing)

**B. STUDY DESIGN AND METHODS**

**1. In-life dates:** 20.03.2012 – 02.05.2012

**2. Experimental design:** The test soil was sieved to particle size of 2 mm. The soil was amended with lucernemeal (sieve size < 1 mm, C/N ratio 15:1) and then thoroughly mixed (concentration in soil 0.5 %).

The test item was dissolved in acetone, for application, the test solution was added on 28.9 g quartz sand. After evaporation of the solvent the quartz sand was mixed with the appropriate amount of pre moistened soil to get the assigned nominal test concentration. Accordingly, solvent treated quartz sand and untreated quartz sand were mixed with the appropriate amount of soil for the control treatments. Soil moisture content was adjusted to 40 % WHC<sub>max</sub> (maximum water holding capacity).

**Test concentrations:** Untreated and solvent controls, 0.018 and 0.09 mg/kg soil dry weight

**Test duration:** 42 days

**3. Observations:** During the nitrogen transformation test samples of soil (from each replicate of treatments and control) were collected after 0, 7, 14, 28 and 42 days of incubation. pH value and water content were analysed, soil nitrification was determined. The procedures for the determination of the water content and the pH are described in DIN ISO 11465 and DIN ISO 10390, respectively. Soil nitrification was determined by measuring the NO<sub>3</sub> contents of aqueous soil extracts by means of calibrated ion sensitive electrode and the WTW inoLab pH/ION 735.

#### 4. Statistics:

Calculation of treatment means and deviation from solvent control in %. Test on normality and homogeneity of data using Shapiro-Wilk's and Levene's test followed by Dunnett's t Test (based on mg nitrate N/kg soil dry weight/day and per sampling interval)

## II. RESULTS AND DISCUSSION

### A. Nitrogen Transformation

The effects of Pyridinol on nitrate N content and rate of nitrate N formation in the course of the study of toxicity evaluation of Pyridinol on nitrogen transformation process in soil and the percent deviation to the control is shown in **Table A2.5.1-1**.

Because the variations between treatments and control are  $\leq 25\%$ , the obtained results in nitrogen transformation test can be evaluated as valid. During nitrogen transformation study no significant differences in nitrogen turnover were observed at both test item concentrations, 42 days after test initiation. Deviations at test end were 5.74 % and 9.80 % from the solvent control, respectively.

No statistically significantly differences from the solvent control were observed for the nitrate formation rates for the whole study period (day 0 to 42) and the last sampling interval (day 28 to 42), respectively.

The toxic reference item (Sodium Chloride), tested in a separate study (Study code: S11-03710) had significant effects on the soil nitrogen turnover (decrease of the nitrogen level of 65.9 %, decrease of the nitrate formation rate for the interval of 0 to 28 days of 125 %, decrease of the nitrate formation rate for the interval of 14 to 28 days of 106 %) in a field soil tested at a concentration of 20.0 g a.s./kg soil dry weight.

**Table 4:** Summary of the effects of Pyridinol on nitrate-N content and rate of nitrate-N formation in soil

Test item [mg/kg-sdw]	NO <sub>3</sub> -N levels (day 42)		NO <sub>3</sub> -N formation rate (day 0 to 42)		NO <sub>3</sub> -N formation rate (day 28 to 42)	
	[mg/kg-sdw]	% Deviation from solvent control <sup>a</sup>	[mg/kg-sdw]	% Deviation from solvent control <sup>a</sup>	[mg/kg-sdw]	% Deviation from solvent control <sup>a</sup>
Solvent Control	29.6	—	0.436	—	1.19	—
Water Control	29.0	-2.03	0.429	-1.61	1.14	-4.20
0.018	27.9	-5.74	0.398	-8.72	1.09	-8.40
0.09	26.7	-9.80	0.369	-15.4	1.04	-12.6

<sup>a</sup> + = stimulation; — = inhibition.

— = no value calculable.

### B. Validity criteria

The results of the study can be regarded to be valid, since the variation between replicate control samples was less than 15 %.

## III. CONCLUSION

Taking into account the obtained results it was assessed that Pyridinol in concentrations of 0.018 and 0.09 mg/kg of soil can be evaluated as having no long term influence on nitrogen transformation in soil.

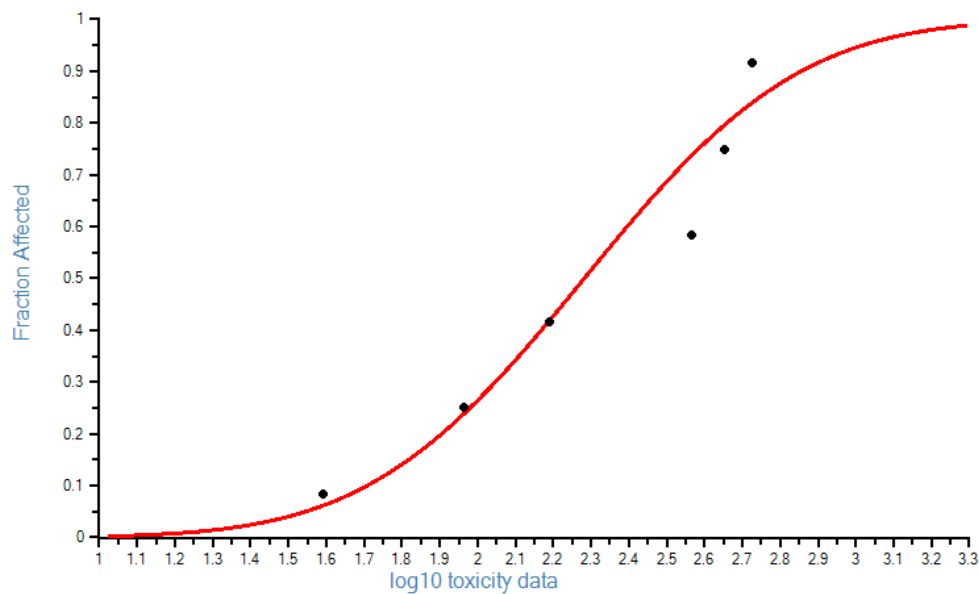
## Appendix 6 Non-target plants - SSD Graphs and goodness of fit toxicity data

### zRMS comments:

Applicants' calculation of HC<sub>5</sub> value was not agreed by the zRMS since it was based on endpoints derived from studies performed with the old variant of the formulation, while results for the new variant of the formulation, which will be placed on the market, are available and in case of the critical parameter (vegetative vigour) they are lower comparing to the old variant. For discussion regarding this issue, please refer to point 9.10 of this document.

Information below is struck through as not agreed.

SSD Graph





## Vegetative vigour — plants height

### Input data

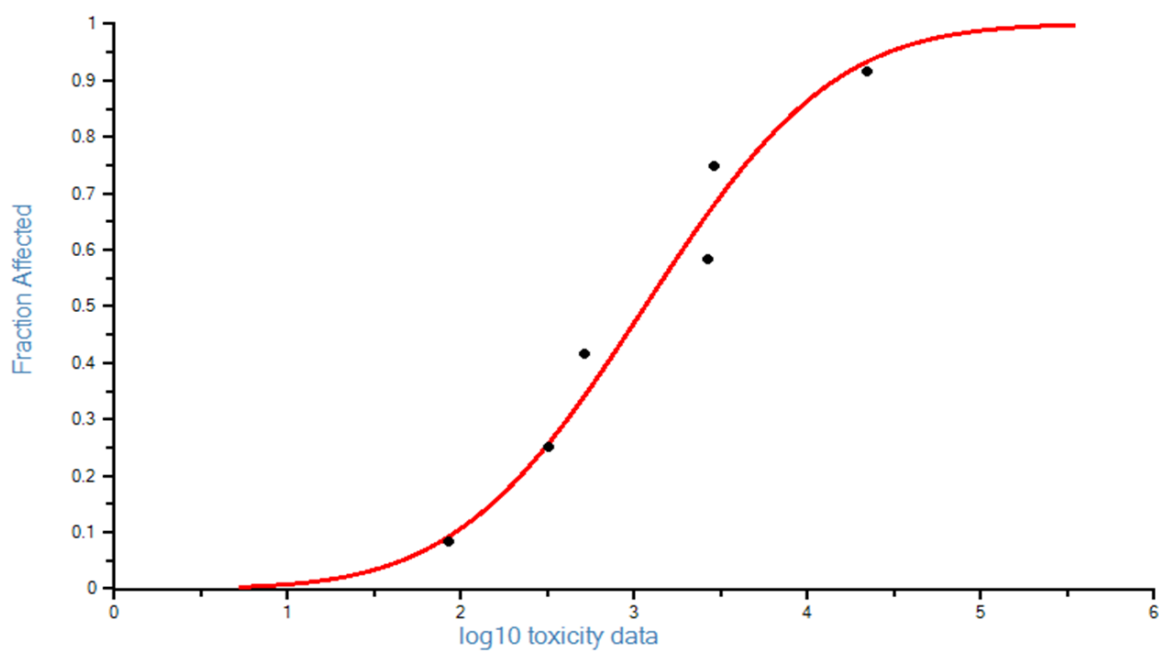
Input toxicity data		
Data no	Toxicity data	Label
1	2877	field bean
2	517	OSR
3	2639	Carrot
4	84	Soya bean
5	320	Lettuce
6	21880	Turnip

Data no	Toxicity data	Label
1	2877	field bean
2	517	OSR
3	2639	Carrot
4	84	Soya bean
5	320	Lettuce
6	21880	Turnip
7		
8		
9		
10		

## Goodness of fit data

Anderson-Darling test for normality					
Sign. level	Critical	Normal?			
0.1	0.631	Accepted			
0.05	0.752	Accepted		AD Statistic:	0.23073106
0.025	0.873	Accepted		n:	6
0.01	1.035	Accepted			
Kolmogorov-Smirnov test for normality					
Sign. level	Critical	Normal?			
0.1	0.819	Accepted			
0.05	0.895	Accepted		KS Statistic:	0.4561739
0.025	0.995	Accepted		n:	6
0.01	1.035	Accepted			
Cramer von Mises test for normality					
Sign. level	Critical	Normal?			
0.1	0.104	Accepted			
0.05	0.126	Accepted		CM Statistic:	0.01880384
0.025	0.148	Accepted		n:	6
0.01	0.179	Accepted			

## SSD Graph



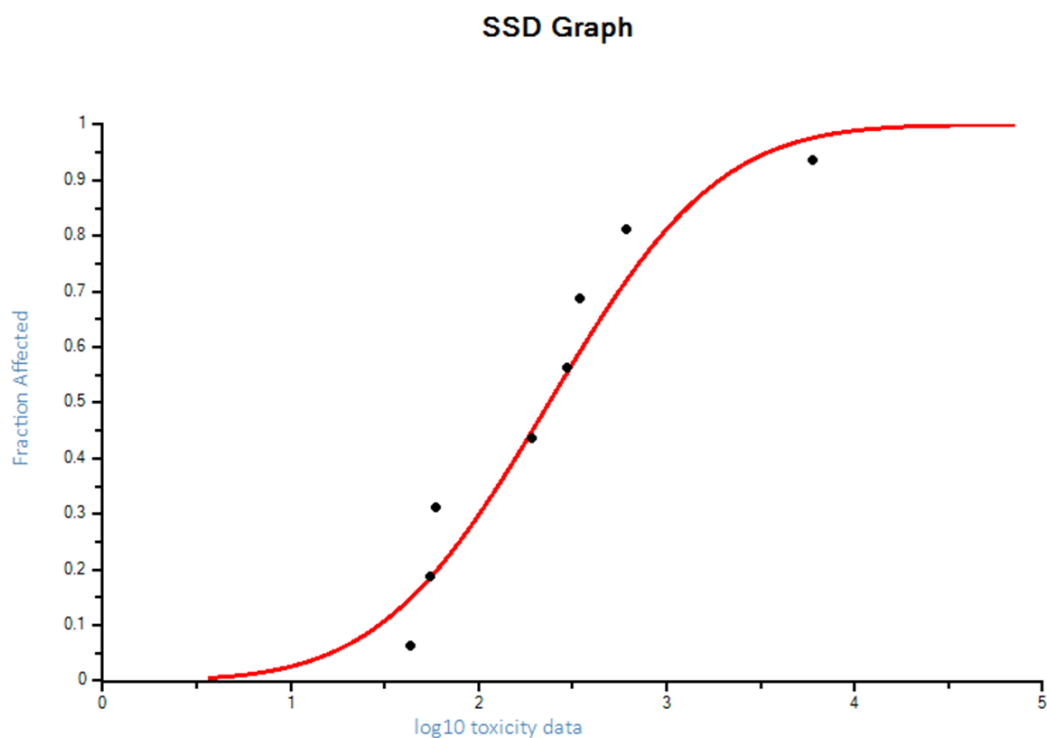
## Vegetative vigour — plants weight

### Input data

Input toxicity data		
Data no	Toxicity data	Label
1	297	field bean
2	55	OSR
3	342	Carrot
4	190	Soya bean
5	43	Lettuce
6	59	Turnip
7	605	onion
8	6000	oats

### Goodness of fit data

Anderson-Darling test for normality					
Sign. level	Critical	Normal?			
0.1	0.631	Accepted			
0.05	0.752	Accepted		AD Statistic:	0.42012533
0.025	0.873	Accepted		n:	8
0.01	1.035	Accepted			
Kolmogorov-Smirnov test for normality					
Sign. level	Critical	Normal?			
0.1	0.819	Accepted			
0.05	0.895	Accepted		KS Statistic:	0.55582111
0.025	0.995	Accepted		n:	8
0.01	1.035	Accepted			
Cramer von Mises test for normality					
Sign. level	Critical	Normal?			
0.1	0.104	Accepted			
0.05	0.126	Accepted		CM Statistic:	0.04327461
0.025	0.148	Accepted		n:	8
0.01	0.179	Accepted			



### Seedling emergence—seedling emergence

#### Input data

Input toxicity data		
Data no	Toxicity data	Label
1	5337	field bean
2	1064	OSR
3	6653	Carrot
4	4885	Soya bean
5	254	Lettuce
6	2449	Turnip
7	1367	onion

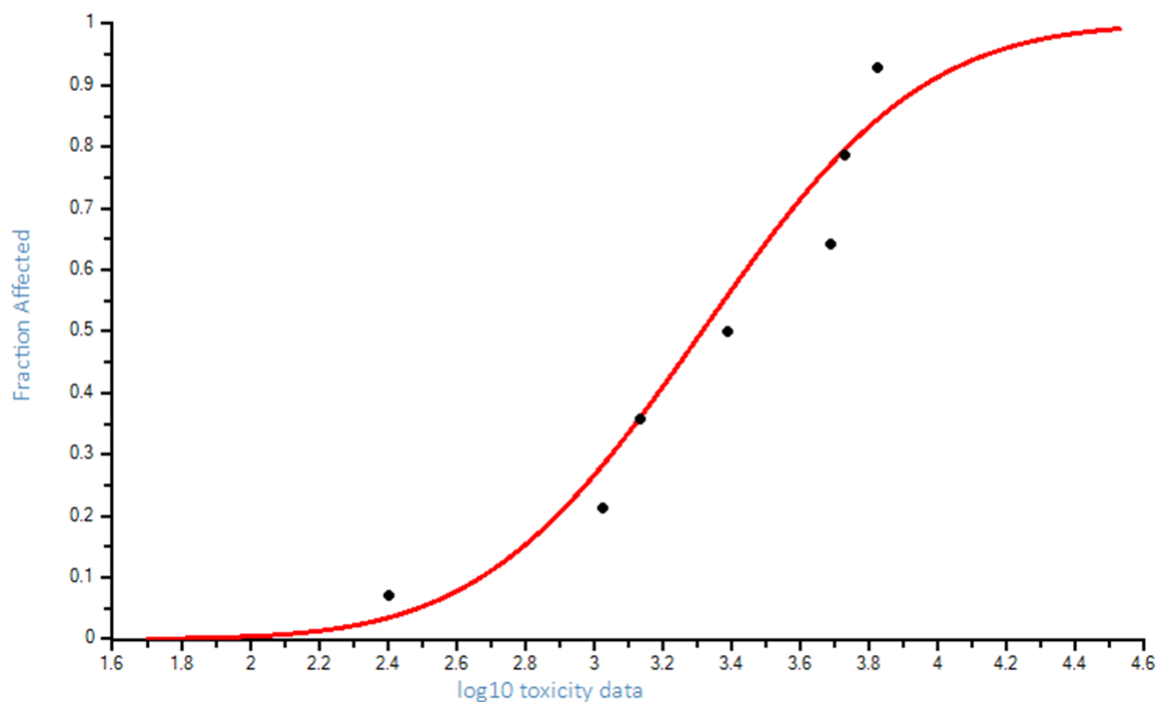
Input toxicity data		
Data no	Toxicity data	Label
1	5337	field bean
2	1064	OSR
3	6653	Carrot
4	4885	Soya bean
5	254	Lettuce
6	2449	Turnip
7	1367	onion

Input toxicity data		
Data no	Toxicity data	Label
1	5337	field bean
2	4064	OSR
3	6653	Carrot
4	4885	Soya bean
5	254	Lettuce
6	2449	Turnip
7	4367	onion
8		
9		
10		

### Goodness of fit data

Anderson-Darling test for normality				
Sign. level	Critical	Normal?		
0.1	0.631	Accepted		
0.05	0.752	Accepted	AD Statistic:	0.37086562
0.025	0.873	Accepted	n:	7
0.01	1.035	Accepted		
Kolmogorov-Smirnov test for normality				
Sign. level	Critical	Normal?		
0.1	0.819	Accepted		
0.05	0.895	Accepted	KS Statistic:	0.59377586
0.025	0.995	Accepted	n:	7
0.01	1.035	Accepted		
Cramer von Mises test for normality				
Sign. level	Critical	Normal?		
0.1	0.104	Accepted		
0.05	0.126	Accepted	CM Statistic:	0.0360556
0.025	0.148	Accepted	n:	7
0.01	0.179	Accepted		

## SSD Graph



## Seedling emergence—plants height

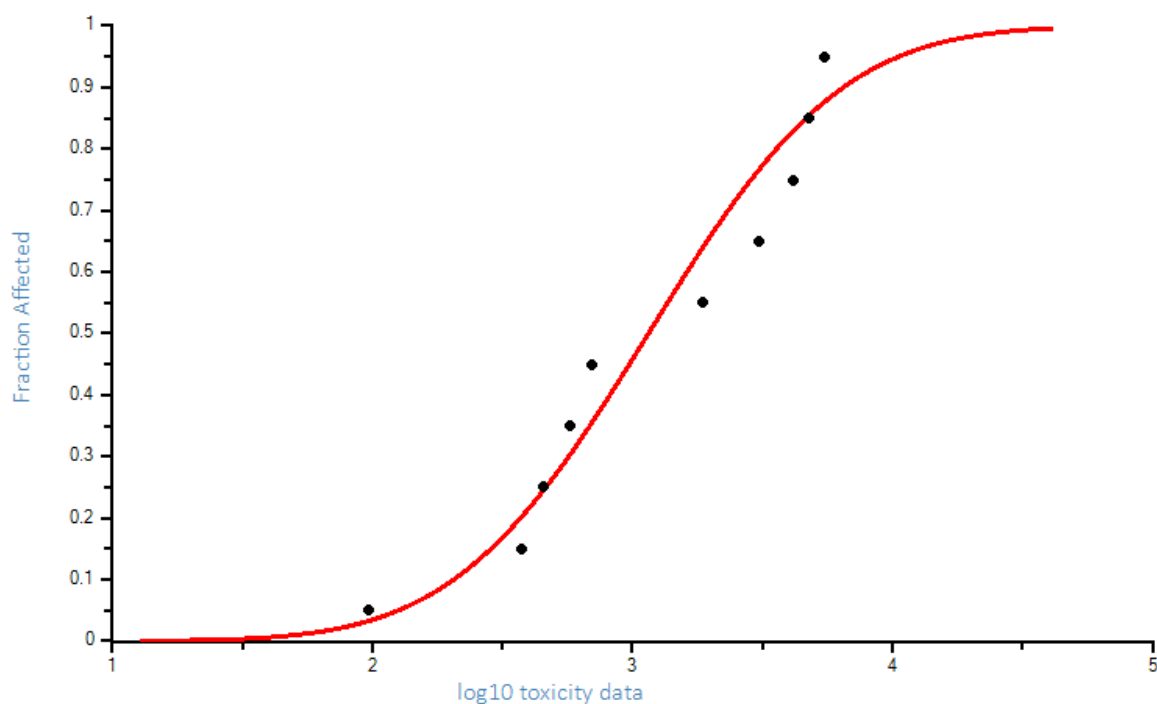
### Input data

Input toxicity data		
Data no	Toxicity data	Label
1	456	field bean
2	698	OSR
3	375	Carrot
4	575	Soya bean
5	96	Lettuce
6	3059	Turnip
7	4785	oats
8	1867	onion
9	4150	rye grass
10	5503	corn

## Goodness of fit data

Anderson-Darling test for normality					
Sign. level	Critical	Normal?			
0.1	0.631	Accepted			
0.05	0.752	Accepted		AD Statistic:	0.39046634
0.025	0.873	Accepted		n:	10
0.01	1.035	Accepted			
Kolmogorov-Smirnov test for normality					
Sign. level	Critical	Normal?			
0.1	0.819	Accepted			
0.05	0.895	Accepted		KS Statistic:	0.56722027
0.025	0.995	Accepted		n:	10
0.01	1.035	Accepted			
Cramer von Mises test for normality					
Sign. level	Critical	Normal?			
0.1	0.104	Accepted			
0.05	0.126	Accepted		CM Statistic:	0.04980902
0.025	0.148	Accepted		n:	10
0.01	0.179	Accepted			

## SSD Graph



## Seedling emergence — plants weight

### Input data

Input toxicity data		
Data no	Toxicity data	Label
1	368	field bean
2	450	OSR
3	92	Carrot
4	533	Soya bean
5	39	Lettuce
6	155	Turnip
7	2109	oats
8	605	onion
9	1625	rye grass
10	4352	corn

### Goodness of fit data

Anderson-Darling test for normality				
Sign. level	Critical	Normal?		
0.1	0.631	Accepted		
0.05	0.752	Accepted	AD Statistic:	0.18512841
0.025	0.873	Accepted	n:	10
0.01	1.035	Accepted		
Kolmogorov-Smirnov test for normality				
Sign. level	Critical	Normal?		
0.1	0.819	Accepted		
0.05	0.895	Accepted	KS Statistic:	0.47125916
0.025	0.995	Accepted	n:	10
0.01	1.035	Accepted		
Cramer von Mises test for normality				
Sign. level	Critical	Normal?		
0.1	0.104	Accepted		
0.05	0.126	Accepted	CM Statistic:	0.02071059
0.025	0.148	Accepted	n:	10
0.01	0.179	Accepted		



SSD Graph

