

REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

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

Product name: 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

(Composition change)

Sponsor: ADAMA Agan Ltd.

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

Submission date: February 2021

MS Finalisation date: September 2021 (initial Core Assessment)

November 2022, updated December 2022 (final Core Assessment)

Version history

| When | What |
|----------------|---|
| January 2021 | Initial dRR Part B6, version reflecting composition change submitted by applicant |
| September 2021 | Initial zRMS assessment (with regard to the proposed composition change). The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey . Not agreed or not relevant information are struck through and shaded for transparency . |
| November 2022 | Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow . Information no longer relevant is struck through and shaded . At the request of cMS SI the applicant updated the RR to include EFSA model calculations for 2,4-D using updated dermal absorption values. |
| December 2022 | 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 highlighted in green . Information no longer relevant is struck through and shaded . |

DATA PROTECTION CLAIM

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

STATEMENT OF OWNERSHIP

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Reviewer comments:

This part of dossier summarizes data related to the toxicological properties and exposure data for the plant protection product 'Tricera' (ADM.3304.H.1.A.) and has been submitted to support registration according art. art. 33 of 1107/2009 in Poland.

New composition of the preparation for which authorization is requested is given in the Vol 4. The code for the new composition is ADM.3304.H.1.A. New information compared to the previously submitted dRR (AG-CDF1-480 EC) has been added. Toxicological studies were done with the new composition for comparison and classification purposes (study on eye irritation (Verma R., 2019) and dermal absorption (Hassler, S. 2019). For better readability of the document, mentioned above studies have been intentionally **marked in turquoise** by the Reviewer. These studies are presented in dRR Part B, Section 6 and reveal no difference in endpoints compared to the original studies.

Both compositions (previous one and current) are formulated as EC formulations, with 2,4-D EHE, clopyralid and fluroxypyr (meptyl) as active substances. The content of the active substances remains the same and the type of formulation does not change. The possible effect of the composition change on the classification of the new formulation has been discussed in the Vol. 4. Based on available data the composition change does not have any impact on the classification of the product.

Product was not a representative formulation reviewed during the Annex I inclusion/active substances renewal and has not previously been evaluated in any EU countries according to the Uniform Principles.

For the current product registration, APPL provided relevant data on the plant protection product 'Tricera' regarding toxicological assessment of the product, based on *in vivo* tests. ZRMS accepted already existing *in vivo* studies for the purposes of hazard classification and do not request for the new *in vivo* data.

Considering 3R rules and stepwise approach applicant were performed also *in vitro* studies (irritating potential for skin (Gehrke H., 2015) and eye (.....) leading to negative results that were not sufficient for a final assessment, thus *in vivo* studies has been provided which were accepted by the ZRMS. (Note: The numbering of the eye irritating studies reflects the chronology of the submission of the test reports, not the order of stepwise approach)

Reviewer points out that since there are *in vivo* tests already exist the information gained on animal studies are more than just a classification. Existing animal studies allow to identify of effects following a single exposure to the plant protection product can be established. The data is sufficient to indicate the time course and characteristics of the effect with full details of behavioral changes and possible gross pathological findings at post-mortem. These studies are valid for classification and toxicological potential assessment.

The application was for approval of ADM.3304.H.1.A. an emulsifiable concentrate [EC] containing 375 g/L 2,4-D (562.5 g/L as 2,4-D EHE), 30 g/L of Clopyralid, 30 g/L and 75 g/L Fluroxypyr for use as a herbicide to control broadleaved weeds (for details refer dRR part B0).

Change in the penetration of 2,4-D from 3.9% to 6.2% for the diluted product, will not impact on the risk assessment NDE (see ZRMS assessment). All exposure calculations used for estimation of operator, workers and B&R resulting from use of PPP considering all tasks according to the critical use(s), identify safe use of the product 'Tricera' (ADM.3304.H.1.A.).

General remark:

The product AG-CDF1-480-EC and ADM.3304.H.1.A is a herbicide containing the active substance 2,4-D (as the ester variant 2,4-D EHE).

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

6 Mammalian Toxicology (KCP 7)

6.1 Summary

Table 6.1-1: Information on ADM.3304.H.1.A *

| | |
|--|---|
| Product name and code | ADM.3304.H.1.A |
| Formulation type | Emulsifiable Concentrate [Code: EC] |
| Active substance(s) (incl. content) | 2,4-D; 375 g/L (as ester form: 565 g/L 2,4-D EHE) Clopyralid; 30 g/L Fluroxypyr; 75 g/L (108 g/L as meptyl) |
| Function | herbicide |
| Product already evaluated as the ‘representative formulation’ during the approval of the active substance(s) | No |
| Product previously evaluated in another MS according to Uniform Principles | No |

* Information on the detailed composition of ADM.3304.H.1.A can be found in the confidential dRR Part C.

This document reviews the information related to the toxicology and human health properties, the data on application, further information and the classification for the plant protection product AG-CDF1-480 EC, ADM.3304.H.1.A containing the active substances 2,4-D, Clopyralid and Fluroxypyr. As this document is intended to support a change in composition of the product, only new information is given and only new studies are referred to. They are listed in Appendix 1 and are submitted to the zRMS.

The product contains the ester form of the a.i. (2,4-D EHE) but since the reference values are **the same** for the ester and the acid, **the dossier generally refers to 2,4-D**.

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

Clopyralid was included into Annex I of Directive 91/414/EEC according to Commission Regulation (EC) No 451/2000 (renewal of inclusion of the second and third group of active substances in Annex I, see Commission Directive 2006/64/EC of 18 July 2006, Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 that replaced the Directive 2006/64/EC after the application of Regulation 1107/2009, and Commission Implementing Regulation (EU) 2020/421 of 18 March 2020 ~~that fixes the new expiry date of approval to 30/04/2021~~. **Clopyralid was renewed in the meantime, current expiry date is 30.09.2036.**

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

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

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

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

Justified proposals for classification and labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table 6.1-2: Justified proposals for classification and labelling for ADM.3304.H.1.A according to Regulation (EC) No 1272/2008

| | |
|--|--|
| Hazard class(es), categories | Acute Tox. 4, Skin Sens. 1, Skin Irrit 2, Eye dam. 1, STOT SE 3* |
| Hazard pictograms or Code(s) for hazard pictogram(s) | GHS05, GHS07 |
| Signal word | Danger |
| Hazard statement(s) | H302, H315 , H317, H318, H335* |
| Precautionary statement(s) | P102, P270, P261* , P280, P302 + P352, P305 + P351 + P338, P501, P304 + P340 |
| Additional labelling phrases | To avoid risks to man and the environment, comply with the instructions for use. [EUH401] |

***This information has been added to reflect comments from cMS.**

Table 6.1-3: Summary of risk assessment for operators, workers, residents and bystanders for ADM.3304.H.1.A

| | Result | PPE / Risk mitigation measures |
|------------|------------|---|
| Operators | Acceptable | Gloves during mixing/loading Note: regarding hazard assessment (H318) additional personal protection equipment has been proposed during M&L. eye protection/face protection.* |
| Workers | Acceptable | None |
| Residents | Acceptable | None |
| Bystanders | Acceptable | None |

***This information has been added to reflect comments from cMS.**

No unacceptable risk for operators, workers, residents and bystanders was identified when the product is used as intended and provided that the PPE/ risk mitigation measures stated in

| | |
|--|--|
| Hazard class(es), categories | Acute Tox. 4, Skin Sens. 1, Skin Irrit 2, Eye dam. 1, STOT SE 3* |
| Hazard pictograms or Code(s) for hazard pictogram(s) | GHS05, GHS07 |
| Signal word | Danger |
| Hazard statement(s) | H302, H315, H317, H318, H335* |
| Precautionary statement(s) | P102, P270, P261* , P280, P302 + P352, P305 + P351 + P338, P501, P304 + P340 |
| Additional labelling phrases | To avoid risks to man and the environment, comply with the instructions for use. [EUH401] |

*This information has been added to reflect comments from cMS.

Table 6.1-3 are applied. No impact is expected by the formulation change.

A summary of the critical uses and the overall conclusion regarding exposure for operators, workers and residents/bystanders is presented in the following table. There is also no change compared to the current dossier and submission.

Table 6.1-4 Critical uses and overall conclusion of exposure assessment

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
|--------------|--|-------------------------------|---|--|--|-----------------------------|---------|--|--------------------------------------|--------|-----------|-----------|
| Use- No.* | Crops and situation (e.g. growth stage of crop) | F, Fn, Fpn G, Gn, Gpn or I ** | Application | | Application rate | | PHI (d) | Remarks: (e.g. safener/synergist (L/ha)) critical gap for operator, worker, resident or bystander exposure based on [Exposure model] | Acceptability of exposure assessment | | | |
| | | | Method / Kind (incl. application technique *** | Max. number (min. interval between applications) a) per use b) per crop/season | Max. application rate kg as/ha a) 2,4-D b) Clopyralid b) Fluroxypyr | Water L/ha min / max | | | Operator | Worker | Residents | Bystander |
| 1 | Grassland (BBCH 21-39) | F | Spraying, LCTM | 1 ; 1 | a) 0.750 b) 0.060 c) 0.150 | 200 - 400 | n.a. | Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 | R | A | A | A |
| 2, 3 | Cereals (umbrella GAP, BBCH 21-39) | F | Spraying, LCTM | 1 ; 1 | a) 0.750 b) 0.060 c) 0.150 | 200 - 400 | n.a. | Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 | R | A | A | A |

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** 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

*** e.g. LC: low crops, HC: high crop, TM: tractor-mounted, HH: hand-held

Explanation for column 10 "Acceptability of exposure assessment"

| | |
|---|---|
| A | Exposure acceptable without PPE / risk mitigation measures |
| R | Further refinement and/or risk mitigation measures required |
| N | Exposure not acceptable/ Evaluation not possible |

Data gaps

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

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

Member States must pay particular attention to the:

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

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

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

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

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.

6.2 Toxicological Information on Active Substances

~~As the two compositions are regarded to be comparable, no new information compared to the previously submitted dossier is presented.~~

Reviewer comment:

ZRMS decided to summarize information on active substances regarding classification of the active substances. EU endpoints and critical areas of concern identified during the EU review are given in the table below.

Table 6.2-1: Information on active substances

| | 2,4-D | Clopyralid |
|-------------|--------------|-------------------|
| Common Name | 2,4 D | Clopyralid |

| | 2,4-D | Clopyralid |
|--|--|---|
| CAS-No. | 94-75-7 | 1702-17-6 |
| Classification and proposed labelling | | |
| With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended) | Hazard classes, categories: Acute Tox.4, Eye Dam.1 Codes for hazard pictograms: GHS05, GHS07 Signal word: Danger Hazard statements: H302, H318, H317, H335 Precautionary statements: P261, P264, P270, P301+P312, P330, P280, P304 + P340, P305+P351+P338, P310 P501 | Hazard classes, categories: Eye Dam.1 Code for hazard pictogram: GHS05 Signal word: Danger Hazard statement: H318 Precautionary statements: P280, P305+P351+P338, P310 |
| Additional C&L proposal | | Skin Irrit. 2 – H315 ‘Causes skin irritation’ STOT RE 2 – H373 ‘May cause damage to organs through prolonged or repeated exposure’ Repr. 2 – H361d ‘Suspected of damaging the unborn child’ Rewiever comment: ECHA harmonised classification (Annex VI of Reg. 1272/2008 CLP) for clopyralid is available ATP01 (H318; Eye dam 1; Dgr) Additional classification (H361d) has been agreed by the EFSA during experts meeting. (see EFSA Journal 2018;16(8):5389). Mentioned above EFSA proposal has no impact on final hazard classification for the product. |
| Agreed EU endpoints | | |
| AOEL systemic | 0.02 mg/kg bw/d (no correction for oral absorption) | 0.15 mg/kg bw/d (no correction for oral absorption) |
| AAOEL | Not currently set | 0.17 mg/kg bw |
| Reference | EFSA Journal 2014;12(9):3812 | EFSA Scientific Report (2005) 50, 1–65 |
| Conditions to take into account/critical areas of concern with regard to toxicology | | |
| According to Review Report/EFSA Conclusion for active substance | None | None |

| | |
|--|--|
| | Fluroxypyr |
| Common Name | Fluroxypyr |
| CAS-No. | 69377-81-7 |
| Classification and proposed labelling | |
| With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended) | Not classified |
| Additional C&L proposal | None |
| Agreed EU endpoints | |
| AOEL systemic | 0.8 mg/kg bw/d (no correction for oral absorption) |
| AAOEL | Not currently set |
| Reference | EFSA Journal 2011;9(3):2091 |
| Conditions to take into account/critical areas of concern with regard to toxicology | |

| | |
|---|-------------------|
| | Fluroxypyr |
| Review Report/EFSA Conclusion for active substance | None |
| *This information has been adjusted to reflect comments from cMS. | |

6.3 Toxicological Evaluation of Plant Protection Product

A new *in vitro* eye irritation study has been performed with the new composition (Valsad Verma, R., 2019). The new information is added below to the Table of available studies. This study is highlighted in yellow turquoise.

Table 6.3-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for ADM.3304.H.1.A

| Type of test, species, model system (Guideline) | Result | Acceptability | Classification (acc. to the criteria in Reg. 1272/2008) | Reference |
|---|----------------------------|---------------|---|-----------|
| LD50 cut-off oral, rat (OECD 423) | 500 mg/kg bw | Yes | Hazard statement H302 Category 4 | ... |
| LD50 dermal, rat (OECD 402) | > 2000 mg/kg bw | Yes | None | ... |
| LC50 inhalation, rat (OECD 403) | > 5.04 mg/L air | Yes | None | ... |
| Skin irritation, human skin (OECD 439) (<i>in vitro</i> test) | Non-irritant | Yes | None | ... |
| Skin irritation, rabbit (OECD 404) | Non-irritant Irritant * | Yes | None H315 Category 2* | ... |
| Eye irritation, bovine cornea (OECD 437) (<i>in vitro</i> test) | No prediction can be made | Yes | Not applicable | ... |
| Eye irritation, bovine cornea (OECD 437) (<i>in vitro</i> test) | No prediction can be made | Yes | Not applicable | --- |
| Eye irritation, rabbit (OECD 405) | Irritant | Yes | Hazard statement H318 Category 1 | ... |
| Skin sensitization, mouse (OECD 429, LLNA) | Sensitizing | Yes | Hazard statement H317 Category 1B | ... |
| Supplementary studies for combinations of plant protection products | No data – not required | -- | -- | -- |

*for detailed rationale see zRMS assessment dRR point A. 2.5.2

Table 6.3-2: Additional toxicological information relevant for classification/labelling of ADM.3304.H.1.A

| | Substance (concentration in product, % w/w) | Classification of the substance (acc. to the criteria in Reg. 1272/2008) | Reference | Classification of product (acc. to the criteria in Reg. 1272/2008) |
|---|---|---|---|---|
| Toxicological properties of active substance(s) (relevant for classification of product) | Clopyralid (2.9% (w/w)) | Hazard statements (Peer review proposal for harmonised classification): H361d (criteria \geq 3%) H373 (criteria \geq 10%) Hazard classes, categories: Eye Dam.1 Signal word: Danger Hazard statement: H318*** | Reg. 1272/2008 / MSDS*/ EFSA conclusion | Hazard statements: None H361d is not required because the concentration of Clopyralid is below 3% in this mixture.** H373 is not required because the concentration of Clopyralid is below 10% in this mixture.** |
| | 2,4 D (37.5% (w/w))*** | Hazard classes, categories: Acute Tox.4, Eye Dam.1 Codes for hazard pictograms: GHS05, GHS07 Signal word: Danger Hazard statements: H302, H318, H317, H335*** | Reg. 1272/2008 / MSDS | H335 is required because the concentration of 2,4D is above \geq 10 % in this mixture.*** |
| Toxicological properties of non-active substance(s) (relevant for classification of product) | none | none | none | none |
| Further toxicological information | No data – not required | | | |

* Material safety data sheet by the applicant

**** Rewiever comment:**

ECHA harmonised classification (Annex VI of Reg. 1272/2008 CLP) for clopyralid is available ATP01 (H318; Eye dam 1; Dgr). Additional classification (H361d; H373) has been agreed by the EFSA during experts meeting. (see EFSA Journal 2018;16(8):5389).

Mentioned above EFSA proposal has no impact on final hazard classification for the product due to the ECHA decision.

***This information has been added to reflect comments from cMS.

Information on the detailed composition of / ADM.3304.H.1.A can be found in the confidential dossier of this submission (Registration Report - Part C).

Taking into account all submitted data ADM.3304.H.1.A should be labelled with **H302** (Harmful if swallowed), **H315** (Skin Irrit. 2), **H318** (Causes serious eye damage) and **H317** (May cause an allergic skin reaction).

In consequence there is no impact on classification of the product due to the composition change.

6.4 Toxicological Evaluation of Groundwater Metabolites

Reference is made to Part B section 10.

6.5 Dermal Absorption (KCP 7.3)

Dermal absorption of ADM.3304.H.1.A was not evaluated as part of the EU review of 2,4-D, Clopyralid or Fluroxypyr.

For derivation of dermal absorption for the substance 2,4-D EHE a new product specific study has been generated and is submitted with this application (KCP 7.3, Hassler, 2019).

In this study, final dermal absorption values of 0.14% for the concentrate and 6.19% for the spray dilution were derived for 2,4-D risk assessment (mean absorption + ks).

Considering the safety factor in the risk assessment for 2,4-D (exposure between 1 and 39.9% of AOEL), the change in the penetration of 2,4-D EHE from 2.97% to 6.19% for the diluted product will not impact the outcome of the risk assessment performed using the dermal absorption obtained with AG-CDF1-480-EC1.

6.5.1 Justification for proposed values - 2,4-D

For risk assessment a value of 0.4% (concentrate) and 6.19% (dilution) should be taken into account. Considering the safety factor in the risk assessment for 2,4-D (exposure between 1 and 39.9% of AOEL), the change in the penetration of 2,4-D EHE from 3.9% to 6.19% for the diluted product will not impact the outcome of the risk assessment performed using the dermal absorption obtained with AG-CDF1-480-EC1.

Table 6.5.1-1: Summary of the results of submitted dermal absorption studies for 2,4-D

| Test | Concentrate | Spray dilution (dilution factor) | Formulation in study | Acceptability of study | Justification provided on representativity of study formulation for current product | Acceptability of justification | Reference* |
|-------------------------|-------------|----------------------------------|----------------------|------------------------|---|--|------------------|
| <i>In vitro</i> (human) | 0.4% * | 3.9%* (1/188) | AG-CDF1-480 EC1 | Yes | Not required | Endpoint can be used for current product | Hassler S., 2018 |
| <i>In vitro</i> (human) | 0.14% * | 6.19* (1/267) | ADM.3304.H.1.A | Yes | Not required | Endpoint can be used for current product | Hassler S., 2019 |

* values recalculated according to the EFSA Guidance on dermal absorption (2017)

6.5.2 Justification for proposed values - Clopyralid

~~A new exposure assessment for Clopyralid is not required.~~

Reviewer comment:

ZRMS decided to summarize information regarded dermal absorption of Clopyralid in ADM.3304.H.1.A. No data on dermal absorption of Clopyralid is available. Justifications for default values according to Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) are presented in the following table.

Table 6.5.2-1: Default dermal absorption rates for Clopyralid

| | Value | Justification for value | Acceptability of justification |
|-------------|-------|--|--|
| Concentrate | 25% | Default value for organic solvent-based formulations | Rationale for dermal absorption rates is acceptable. |
| Dilution | 70% | Default value for organic solvent-based formulations | Rationale for dermal absorption rates is acceptable. |

6.5.3 Justification for proposed values - Fluroxypyr

~~A new exposure assessment for Fluroxypyr is not required.~~

Reviewer comment:

ZRMS decided to summarize information regarded dermal absorption of Fluroxypyr in ADM.3304.H.1.A. No data on dermal absorption of Fluroxypyr is available. Justifications for default values according to Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) are presented in the following table.

Table 6.5.3-1: Default dermal absorption rates for Fluroxypyr

| | Value | Justification for value | Acceptability of justification |
|-------------|-------|--|--|
| Concentrate | 25% | Default value for organic solvent-based formulations | Rationale for dermal absorption rates is acceptable. |
| Dilution | 70% | Default value for organic solvent-based formulations | Rationale for dermal absorption rates is acceptable. |

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

A risk assessment for the new composition is neither submitted nor required.

Considering the safety factor in the risk assessment for 2,4-D (exposure between 1 and 39.9% of AOEL), the change in the penetration of 2,4-D EHE from 3.9% to 6.2% for the diluted product will not impact the outcome of the risk assessment performed using the dermal absorption obtained with AG-CDF1-480-EC1 (values for the new composition are recalculated according to the EFSA Guidance on dermal absorption (2017) .

6.6.1 Selection of critical use(s) and justification

Please refer to Point 6.6 above

Reviewer comments:

Change in the product composition will not impact on the critical GAP

6.6.2 Operator exposure (KCP 7.2.1)

Please refer to Point 6.6 above

Reviewer comments:

ZRMS additional NDE assessment reflecting new 2,4-D EHE dermal absorption value (**6.2% for the diluted product**) obtained from the new study Hassler, S. 2019.

Table 6.6 2: Exposure models for intended uses

| | |
|-----------------|--|
| Critical use(s) | Cereals (max. 2 L/product/ha) Grassland (max. 2 L/product/ha) |
| Model(s) | EFSA model [Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014; 12 (10):3874] |

Table 6.6 4: Estimated operator exposure (longer term exposure)

| | | 2,4-D | |
|--|--------------------|---------------------------------|--------------------|
| Model data | Level of PPE | Total absorbed dose (mg/kg/day) | % of systemic AOEL |
| Cereals & Grassland Tractor mounted boom spray application outdoors to low crops | | | |
| Application rate | | 0.750 kg a.s./ha | |
| | Potential exposure | 0.0178 | 89% |

| | | | |
|---|---|--------|-----|
| Spray application (AOEM; 75 th percentile) Body weight: 60 kg | Work wear – arms, body and legs covered (no gloves) | 0.0011 | 58% |
| | Additional gloves at mix/loading | 0.0065 | 32% |

Conclusion:
Change in the penetration of 2,4-D EHE from 3.9% to 6.2% for the diluted product, will not impact on the safe use of the ADM.3304.H.1.A.

6.6.2.1 Measurement of operator exposure

Please refer to Point 6.6 above

| |
|--|
| Reviewer comments: Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and consideration of the above mentioned personal protective equipment (PPE), a study to provide measurements of operator exposure was not necessary and was therefore not performed. |
|--|

6.6.3 Worker exposure (KCP 7.2.3)

Please refer to Point 6.6 above

Reviewer comments:
ZRMS additional NDE assessment reflecting new 2,4-D EHE dermal absorption value (6.2% for the diluted product) obtained from the new study Hassler, S. 2019.

Table 6.6.3-1: Exposure models for intended uses

| | |
|-----------------|--|
| Critical use(s) | Cereals (max. 2 L/product/ha) Grassland (max. 2 L/product/ha) |
| Model(s) | EFSA model [Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014; 12 (10):3874] |

Table 6.6.3-2: Estimated worker exposure

| | | | |
|---|---|-------------------------------------|--------------------|
| | | 2,4-D | |
| Model data | Level of PPE | Total absorbed dose (mg/kg/day) | % of systemic AOEL |
| Cereals & Grassland; Inspection, irrigation Outdoor Work rate: 2 hours/day DT50: 30 days; DFR: 3 µg/cm²/kg a.s./ha | | | |
| Number of applications and application rate: | | 0.750 kg a.s./ha | |
| Body weight: 60 kg | Potential TC: 12500 cm²/person/h | 0.058 | 290% |
| | Work wear (arms, body and legs covered) TC: 1400 cm²/person/h | 0.0065 | 32% |
| | Work wear (arms, body and legs covered) and gloves TC: Not available | No TC available for this assessment | -- |

Conclusion:
Change in the penetration of 2,4-D EHE from 3.9% to 6.2% for the diluted product, will not impact on the safe use

of the ADM.3304.H.1.A.

6.6.3.1 Refinement of generic DFR value (KCP 7.2)

Not relevant.

6.6.3.2 Measurement of worker exposure

Please refer to Point 6.6 above

Reviewer comments:

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mention PPE, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

6.6.4 Resident and bystander exposure (KCP 7.2.2)

Please refer to Point 6.6 above

Reviewer comments:

Change in the product will not impact on the critical GAP

6.6.4.1 Estimation of resident exposure

Please refer to Point 6.6 above

Reviewer comments:

ZRMS additional NDE assessment reflecting new 2,4-D EHE dermal absorption value (**6.2% for the diluted product**) obtained from the new study Hassler, S. 2019.

Table 6.6.4.1-1: Exposure models for intended uses

| | |
|-----------------|--|
| Critical use(s) | Cereals (max. 2 L/product/ha) Grassland (max. 2 L/product/ha) |
| Model | EFSA model [Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014; 12 (10):3874] |

Table 6.6.4.1-2: Estimated resident exposure

| | | 2,4-D | |
|--|---|------------------------------------|--------------------|
| Model data | | Total absorbed dose (mg/kg bw/day) | % of systemic AOEL |
| Cereals Tractor mounted boom spray application outdoors to low crops Drift rate: 5.60/4.10 %; Buffer zone: 2-3 (m); Drift reduction technology: no DFR: 3 µg/cm ² /kg a.s./ha | | | |
| Number of applications and application rate | | 1 x 0.750 kg a.s./ha | |
| Resident child Body weight: 10 kg | Drift (75 th perc.) | 0.0063 | 31.58% |
| | Vapour (75 th perc.) | 0.0011 | 5.35% |
| | Surface deposits (75 th perc.) | 0.0013 | 6.43% |

| | | | |
|---|---|----------------------|---------------|
| | Re-entry (75 th perc.) | 0.0078 | 39.23% |
| | All pathways (mean) | 0.0118 | 58.82% |
| Resident adult Body weight: 60 kg | Drift (75 th perc.) | 0.0015 | 7.50% |
| | Vapour (75 th perc.) | 0.0002 | 1.15% |
| | Surface deposits (75 th perc.) | 0.0003 | 1.58% |
| | Re-entry (75 th perc.) | 0.0044 | 21.80% |
| | All pathways (mean) | 0.0047 | 23.26% |
| | Grassland Tractor mounted boom spray application outdoors to low crops Drift rate: 5.60/4.10 %; Buffer zone: 2-3(m); Drift reduction technology: no DFR: 3 µg/cm ² /kg a.s./ha | | |
| Number of applications and application rate | | 1 x 0.750 kg a.s./ha | |
| Resident child Body weight: 10 kg | Drift (75 th perc.) | 0.0063 | 31.58% |
| | Vapour (75 th perc.) | 0.0011 | 5.35% |
| | Surface deposits (75 th perc.) | 0.0013 | 6.43% |
| | Re-entry (75 th perc.) | 0.0062 | 30.76% |
| | All pathways (mean) | 0.0070 | 35.09% |
| Resident adult Body weight: 60 kg | Drift (75 th perc.) | 0.0015 | 7.50% |
| | Vapour (75 th perc.) | 0.0002 | 1.15% |
| | Surface deposits (75 th perc.) | 0.0003 | 1.58% |
| | Re-entry (75 th perc.) | 0.0007 | 3.54% |
| | All pathways (mean) | 0.0019 | 9.42% |

6.6.4.2 Estimation of bystander exposure

Please refer to Point 6.6 above

Reviewer comments:

No acute non-dietary risk assessment is included in this submission for 2,4-D and Fluroxypyr since no AAOEL has currently been set for these active substances. According to EFSA longer term exposure of bystanders is covered by the resident scenario.

6.6.4.3 Measurement of resident and/or bystander exposure

Please refer to Point 6.6 above

Reviewer comments:

Since the resident and bystander exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) for 2,4-D, Clopyralid and Fluroxypyr will not be exceeded under conditions of intended uses and considering above mentioned risk mitigation measures, a study to provide measurements of resident/bystander exposure was not necessary and was therefore not performed.

6.6.5 Combined exposure

Please refer to Point 6.6 above

6.6.5.1 Exposure assessment of 2,4-D, Clopyralid and Fluroxypyr in AG-CDF1-480 EC

Please refer to Point 6.6 above

Reviewer comments:

ZRMS supports following approach. As a first tier, combined exposure is calculated as the sum of the component exposures without regard to the mode of action or mechanism/target of toxicity. Initially, the individual Hazard Quotients (HQ) are calculated for all active substances in the PPP by assessing the exposure according to appropriate models and dividing the individual exposure levels by the respective systemic AOEL. This is equivalent to the predicted exposure as % of systemic AOEL converted to decimal. The Hazard Index (HI) is the sum of the individual HQs.

ZRMS additional HQ assessment reflecting new 2,4-D EHE dermal absorption value (6.2% for the diluted product) obtained from the new study Hassler, S. 2019. Has been highlighted in purple shading.

Table 6.6.5-1: Risk assessment from combined exposure

| Application scenario | Active Ingredient | Estimated exposure / AOEL (HQ) |
|--------------------------------|------------------------------|--------------------------------|
| Operators (work wear + gloves) | 2,4-D | 0.32 |
| | Clopyralid | 0.04 |
| | Fluroxypyr | 0.02 |
| | Combined risk Operators (HI) | 0.43 |
| Workers (work wear) | 2,4-D | 0.32 |
| | Clopyralid | 0.04 |
| | Fluroxypyr | 0.02 |
| | Combined risk Workers (HI) | 0.43 |
| Resident – Child - Cereals | 2,4-D | |
| | Drift | 0.31 |
| | Vapour | 0.05 |
| | Deposits | 0.06 |
| | Re-entry | 0.39 |
| | Sum of all pathways | 0.59 |
| | Clopyralid | |
| | Drift | 0.04 |
| | Vapour | 0.01 |
| | Deposits | 0 |
| | Re-entry | 0.05 |
| | Sum of all pathways | 0.07 |
| | Fluroxypyr | |
| | Drift | 0.02 |
| | Vapour | 0 |
| | Deposits | 0 |
| | Re-entry | 0.02 |
| | Sum of all pathways | 0.03 |
| | Combined risk Resident (HI) | |
| | Drift | 0.37 |

| | | |
|---------------------------------|-----------------------------|-------|
| | Vapour | 0.06 |
| | Deposits | 0.06 |
| | Re-entry | 0.46 |
| | Sum of all pathways | 0.69 |
| Resident – Adult - Cereals | 2,4-D | |
| | Drift | 0.075 |
| | Vapour | 0.01 |
| | Deposits | 0.015 |
| | Re-entry | 0.22 |
| | Sum of all pathways | 0.23 |
| | Clopyralid | |
| | Drift | 0.01 |
| | Vapour | 0 |
| | Deposits | 0 |
| | Re-entry | 0.03 |
| | Sum of all pathways | 0.03 |
| | Fluroxypyr | |
| | Drift | 0.02 |
| | Vapour | 0 |
| | Deposits | 0 |
| | Re-entry | 0.02 |
| | Sum of all pathways | 0.03 |
| | Combined risk Resident (HI) | |
| | Drift | 0.1 |
| | Vapour | 0.01 |
| | Deposits | 0.015 |
| | Re-entry | 0.27 |
| | Sum of all pathways | 0.29 |
| Resident – Child - Grassland | 2,4-D | |
| | Drift | 0.31 |
| | Vapour | 0.05 |
| | Deposits | 0.06 |
| | Re-entry | 0.31 |
| | Sum of all pathways | 0.35 |
| | Clopyralid | |
| | Drift | 0.04 |
| | Vapour | 0.01 |
| | Deposits | 0 |
| | Re-entry | 0.01 |
| | Sum of all pathways | 0.04 |
| | Fluroxypyr | |

| | | |
|--|------------------------------------|-------|
| | Drift | 0.02 |
| | Vapour | 0 |
| | Deposits | 0 |
| | Re-entry | 0 |
| | Sum of all pathways | 0.02 |
| | Combined risk Resident (HI) | |
| | Drift | 0.37 |
| | Vapour | 0.065 |
| | Deposits | 0.065 |
| | Re-entry | 0.32 |
| | Sum of all pathways | 0.41 |
| Resident – Adult - Grassland | 2,4-D | |
| | Drift | 0.075 |
| | Vapour | 0.01 |
| | Deposits | 0.015 |
| | Re-entry | 0.02 |
| | Sum of all pathways | 0.095 |
| | Clopyralid | |
| | Drift | 0.01 |
| | Vapour | 0 |
| | Deposits | 0 |
| | Re-entry | 0 |
| | Sum of all pathways | 0.01 |
| | Fluroxypyr | |
| | Drift | 0 |
| | Vapour | 0 |
| | Deposits | 0 |
| | Re-entry | 0 |
| | Sum of all pathways | 0 |
| | Combined risk Resident (HI) | |
| | Drift | 0.085 |
| | Vapour | 0 |
| | Deposits | 0.015 |
| | Re-entry | 0.02 |
| | Sum of all pathways | 0.1 |

The Hazard Index is < 1. Thus, combined exposure to all active substances in ADM.3304.H.1.A is not expected to present a risk for operators, workers, residents and bystanders. No further refinement of the assessment is required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

| Data point | Author(s) | Year | Title Company Report No. Source (where different from company) GLP or GEP status Published or not | Vertebrate study Y/N | Owner |
|--------------|-------------|------|---|----------------------|-----------------|
| KCP 7.1.5/01 | Verma, R. | 2019 | <i>In vitro</i> eye irritation test of ADM.3304.H.1.A using bovine corneal opacity and permeability test JRF Department of Toxicology – Valvada – India Sponsor ID: 00103968 Report ID 530-01-23864 GLP Published: no | N | Adama Agan Ltd. |
| KCP 7.3/01 | Hassler, S. | 2019 | 2,4-D EHE – In vitro percutaneous penetration of [¹⁴ C]2,4-D EHE formulated as ADM.3304.H.1.A (Tricera) through Human Skin Membranes Innovative Environmental Services (IES) Ltd, Switzerland Report ID 20190080 Sponsor ID: 000104190 GLP Published: no | N | Adama Agan Ltd. |

Reviewer comments: list of studies summarized below has been considered during dRR evaluation for AG-CDF1-480 EC composition. These studies are relevant to hazard assessment of the new product ADM.3304.H.1.A with new composition. Both compositions (previous one and current) are formulated as EC formulations, with 2,4-D EHE, clopyralid and fluroxypyr (meptyl) as active substances. The content of the active substances remains the same and the type of formulation does not change. The possible effect of the composition change on the classification of the new formulation has been discussed in the Vol. 4. Based on available data the composition change does not have any impact on the hazard assessment of the product. Thus, ZRMS accept these data as relevant for ADM.3304.H.1.A

| 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 7.1.1/01 | ... | 2015 | Acute oral toxicity (Acute Toxic Class Method) in the rat with AG-CDF1-480 EC ... GLP, not published | Y | 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 7.1.2/01 | | 2015 | Acute dermal toxicity (limit test) in the rat with AG-CDF1-480 EC GLP, not published | Y | Adama Agan Ltd. |
| KCP 7.1.3/01 | ... | 2015 | AG-CDF1-480 EC – Acute inhalation toxicity study (nose-only) in the rat GLP, not published | Y | Adama Agan Ltd. |
| KCP 7.1.4/01 | Gehrke, H. | 2015 | <i>In vitro</i> skin irritation: Human skin model test with AG-CDF1-480 EC BSL BIOSERVICE, Scientific Laboratories GmbH, Germany Sponsor ID: R-90017670 Project ID: 144996 GLP, not published | N | Adama Agan Ltd. |
| KCP 7.1.4/02 | ... | 2015 | Acute dermal irritation/corrosion in the rabbit with AG-CDF1-480 EC ... GLP, not published | Y | Adama Agan Ltd. |
| KCP 7.1.5/01 | | 2014 | Screening for the eye irritancy potential using the bovine corneal opacity and Permeability Assay with AG-CDF1-480 EC ... GLP, not published | N | Adama Agan Ltd. |
| KCP 7.1.5/02 | | 2015 | Acute eye irritation / corrosion in the rabbit with AG-CDF1-480 EC ... GLP, not published | Y | Adama Agan Ltd. |
| KCP 7.1.6/01 | ... | 2015 | Test for sensitisation (Local Lymph Node Assay - LLNA) With AG-CDFI -480 EC GLP, not published | Y | Adama Agan Ltd. |
| KCP 7.3/01 | Hassler, S. | 2018 | 2,4-D EHE – In vitro percutaneous penetration of [14C]2,4-D EHE formulated as AG-CDF1-480 EC1 through Human Skin Membranes Innovative Environmental Services (IES) Ltd, Switzerland Sponsor ID: 90021232 Report ID: 20170308 GLP, not published | N | Adama Agan Ltd. |

*The sponsor company ADAMA Agan Ltd. (ADM) is a member of ADAMA Agricultural Solutions.

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

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

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the studies relied upon

A 2.1 Statement on bridging possibilities

| | |
|-------------------|---|
| Comments of zRMS: | Studies summarized below has been considered during dRR evaluation for AG-CDF1-480 EC (previous composition). These studies are relevant to hazard assessment of the new product ADM.3304.H.1.A with new composition. Both compositions (previous one and current) are formulated as EC formulations, with 2,4-D EHE, clopyralid and fluroxypyr (meptyl) as active substances. The content of the active substances remains the same and the type of formulation does not change. The possible effect of the composition change on the classification of the new formulation has been discussed in the Vol. 4. Based on available data the composition change does not have any impact on the hazard assessment of the product. Thus, ZRMS accept these data as relevant for ADM.3304.H.1.A |
|-------------------|---|

A 2.2 Acute oral toxicity (KCP 7.1.1)

| | |
|-------------------|--|
| Comments of zRMS: | Study accepted. See dRR AG-CDF1-480 EC, also refer to our comment to point A2.1. |
|-------------------|--|

~~New studies with the new composition are neither submitted nor required~~

| | |
|--------------------------------------|---|
| Reference | KCP 7.1.1/01 |
| Report | Acute oral toxicity (Acute Toxic Class Method) in the rat with AG-CDF1-480 EC ... |
| Guideline(s) | OECD Guidelines No. 423 (2001) Commission Regulation (EC) No. 440/2008, 8.1 tris EPA OPPTS 870.1000 (2002) EPA OPPTS 870.1100 (2002) |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | No |

Executive summary

The aim of this experiment was to assess the acute oral toxicity of AG-CDF1-480 EC after a single administration to female CRL: (WI) rats. Therefore a group of 3 fasted females was treated by oral gavage administration at a dosage of 2000 mg/kg bw. All three animals treated with the test item at a dose of 2000 mg/kg had to be euthanized for ethical reasons on test day 1.

Two groups, each of three female rats were treated with the test item by oral gavage administration at a dosage of 300 mg/kg body weight. All remaining animals treated with the test item at a dose of 300 mg/kg survived until the end of the study without showing any test item related signs of toxicity.

The median lethal dose of AG-CDF1-480 EC after a single oral administration to female rats, observed over a period of 14 days is: LD₅₀ cut-off (rat): 500 mg/kg bw.

Materials and methods

MATERIALS

| | |
|--|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Yellowish clear liquid |
| Concentration/Purity | 31.47 g/L Clopyralid, 384.52 g/L 2,4 D, 79.31 g/L Fluroxypyr, 579.82 g/L 2,4-D EHE, 114.25 g/L Fluroxypyr-meptyl |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle | Aqua ad injectionem |
| Test animals (Species) | Rat |
| Strain | CRL: (WI) (<i>Rattus norvegicus</i>) |
| Sex | Female |
| Age at test start | 8 - 10 weeks old |
| Weight at test start | Step 1: 165 – 181 g Step 2: 164 – 183 g Step 3: 132 – 140 g |
| Source | Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany |
| Number of animals (per group) | 9 (3 female rats per group) |
| Identification | Individually marked |
| Acclimation period | At least 5 days |
| Diet | <i>Ad libitum</i> : Altromin 1324 diet for rats and mice (lot no.1239) |
| Water | <i>Ad libitum</i> : water |
| Housing and cages | Type III polysulphone cages on Altromin saw fibre bedding, 3 rats/cage |
| Administration | Oral gavage |
| Reference standard | None |
| Environmental conditions during testing | |
| Temperature | 22 ± 3°C |
| Relative humidity | 55 ± 10 % |
| Ventilation | 10 x air exchanges/hour |
| Photoperiod | The rooms were lit and darkened for periods of 12 hours each. |
| STUDY DESIGN AND METHODS | |
| In-life date | 13.11. – 19.12.2014 |
| Animal assignment and treatment | The animals were given the test item, freshly diluted in the vehicle, by oral gavage. |
| Test concentrations | Step 1: 2000 mg/kg bw Step 2 + 3: 300 mg/kg bw |
| Application volume | 10 mL /kg bw |
| Test duration | 14 days |
| Observations: | Clinical observations were performed on the day of treatment at 30 minutes and during 4 hours after application of the test item and once each day for 14 days thereafter. Individual weight of animals was determined prior to the application, on day 8 and 15 after application. |

| | |
|-------------|--|
| | After observation period all the anaesthetized animals were dissected and studied macroscopically. |
| Statistics: | -- |

Results and discussions

Table A 1: Results of acute oral toxicity study in rats of AG-CDF1-480 EC

| Dose (mg/kg bw) | Toxicological results * | Duration of signs | Time of death | LD ₅₀ (mg/kg bw) (14 days) |
|-----------------|-------------------------|-------------------|---------------|---------------------------------------|
| Female rats | | | | |
| 2000 | 3/3/3 | 1 day | Day 1 | < 2000 |
| 300 | 0/0/3 | - | End of study | > 300 |
| 300 | 0/0/3 | - | End of study | > 300 |

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 2: Summary of findings of acute oral toxicity study in rats of AG-CDF1-480 EC

| | |
|--------------------------------|--|
| Mortality | 2000 mg/kg body weight: signs of toxicity and mortality. All three animals treated with the test item at a dose of 2000 mg/kg had to be euthanized for ethical reasons on test day 1. 300 mg/kg body weight: no signs of toxicity or mortality. |
| Clinical signs | 2000 mg/kg body weight: reduced spontaneous activity, piloerection, eyes half closed, prone position, ataxia, wasp waist, kyphosis, lethargy or stupor, bradykinesia and apathy. 300 mg/kg body weight: no specific findings during the whole observation period. |
| Body weight | None of the animals showed weight loss during the observation period. |
| Macroscopic examination | With the exception of acute injection of blood vessels in the abdominal region, which is due to the euthanasia injection, no specific gross pathological changes were recorded for any animal. At necropsy, residues of a milky suspension was found in the stomach in 3 out of 3 animals treated with the test item at a concentration of 2000 mg/kg bw. |

Under the conditions of the present study, a single oral application of the test item AG-CDF1-480 EC to rats at a dose of 2000 mg/kg body weight was associated with signs of toxicity and mortality.

Under the conditions of the present study, a single oral application of the test item AG-CDF1-480 EC to rats at a dose of 300 mg/kg body weight was associated with no signs of toxicity and mortality.

Conclusion

The median lethal dose of AG-CDF1-480 EC after a single oral administration to female rats, observed over a period of 14 days is: LD₅₀ cut-off (rat): 500 mg/kg bw.

Therefore AG-CDF1-480 EC should be classified Acute Toxicity Category 4 H302, in accordance with Regulation (EC) 1272/2008.

A 2.3 Acute percutaneous (dermal) toxicity (KCP 7.1.2)

| | |
|-------------------|--|
| Comments of zRMS: | Study accepted. See dRR AG-CDF1-480 EC, also refer to our comment to point A2.1. |
|-------------------|--|

New studies with the new composition are neither submitted nor required

| | |
|--------------|---|
| Reference | KCP 7.1.2/01 |
| Report | Acute dermal toxicity (limit test) in the rat with AG-CDF1-480 EC ... |
| Guideline(s) | OECD Guidelines 402 (1987) Commission Regulation (EC) No. 440/2008, L142 |

| | |
|--------------------------------------|---------------------------|
| | EPA OPPTS 870.1200 (1998) |
| | EPA OPPTS 870.1000 (2002) |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | No |

Executive Summary

The aim of this experiment was to assess the acute dermal toxicity of AG-CDF1-480 EC after a single administration to 5 female and 5 male rats. Therefore the undiluted test substance was applied to 10 rats on a shaved dorsal area in a dose of 2000 mg per kilogram of body weight for 24 h. After substance application, no clinical signs or local dermal signs were observed in animals during the 14 days observation period. No mortality occurred through the observation period. The body weight and body weight gain of AG-CDF1-480 EC treated animals was within the expected range.

After 14-day observation period anaesthetized animals were dissected and studied macroscopically. There was no evidence of the test item-related observations at a dose level of 2000 mg/kg bw at necropsy. With the exception of acute injection of blood vessels in the abdominal region, which is due to the euthanasia injection, no specific gross pathological changes were recorded for any animal.

The acute dermal lethal dose, LD₅₀ of AG-CDF1-480 EC was determined to be LD₅₀ > 2000 mg/kg body weight.

Materials and methods

| MATERIALS | |
|---------------------------------|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC - Lot/Batch No.: Clopyralid: 1702-17-6 2,4-D-2EHE: 1928-43-4 Fluroxypyr-meptyl: 81406-37-3 |
| Description | Yellowish clear liquid |
| Concentration/Purity | 31.47 g/L Clopyralid, 384.52 g/L 2,4-D, 79.31 g/L Fluroxypyr, 579.82 g/L 2,4-D-EHE, 114.25 g/L Fluroxypyr-meptyl |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle and/or positive control | -- |
| Test animals (Species) | Rat |
| Strain | CRL: (WI) Wistar rats (<i>Rattus norvegicus</i>) |
| Sex | Male and female |
| Age at test start | Males: 09-11 weeks Females: 12-13 weeks |
| Weight at test start (mean) | Males: 263g – 273 g Females: 167g – 182 g |
| Source | Charles River (Europe) Laboratories Inc. |
| Number of animals (per group) | Total 10 (5 rats per sex) |
| Acclimation period | 5 days |

| | |
|--|---|
| Diet | <i>Ad libitum</i> : Altromin 1324 diet for rats and mice (lot no.1239) |
| Fasting | The animals were not fasted before the experiment |
| Water | <i>Ad libitum</i> : water |
| Housing and cages | Type III H polysulphone cages on Altromin saw fibre bedding (lot no. 02102140831), 1 rat / cage |
| Dose | 2000 mg/kg |
| Administration | The back of each animal was shaved (approx.10 % area of the total body surface) approximately 24 hours prior to treatment. The test item was applied as a single dose, uniformly over an area which was approximately 10% of the total body surface. The test item was held in contact with the skin by a dressing throughout a 24-hour period. The dressing consisted of a gauze-dressing and non-irritating tape and was fixed with an additional dressing in a suitable manner. At the end of the exposure period the residual test item was removed using aqua ad injectionem. All animals were observed for 14 days after dosing. |
| Reference standard | None |
| Environmental conditions during testing | |
| Temperature | 22 ± 3°C |
| Relative humidity | 55± 10 % |
| Ventilation | 10 x air exchange/hour |
| Photoperiod | The rooms were lit and darkened for periods of 12 hours each. |
| STUDY DESIGN AND METHODS | |
| In-life dates | 11.12.2014 – 21.01.2015 |
| Animal assignment and treatment | The animals were individually marked. Undiluted substance was applied over dorsal area in a dose of 2000 mg/kg bw remained in contact with the skin for the 24- hour exposure period. Test group consisted of 5 males and 5 females. The animals were observed during 14 days. |
| Test concentrations | 2000 mg/kg bw |
| Test duration | 14 days |
| Observations | Clinical observations were performed on the day of treatment at 30 minutes and during 4 hours after application of the test item and once each day for 14 days thereafter. Individual weight of animals was determined prior to the application, on day 8 and 15 after application. After observation period all the anaesthetized animals were dissected and studied macroscopically. |
| Statistics | -- |

Results and discussions

Table A 3: Results of acute dermal toxicity study in rats of AG-CDF1-480 EC

| Dose (mg/kg bw) | Toxicological results * | Duration of signs | Time of death | LD ₅₀ (mg/kg bw) (14 days) |
|-----------------|-------------------------|--|---------------|---------------------------------------|
| Male rats | | | | |
| 2000 | 0/0/5 | - | End of study | > 2000 |
| Female rats | | | | |
| 2000 | 0/3/5 | Reversible within the observation period | End of study | > 2000 |

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 4: Summary of findings of acute dermal toxicity study in rats of AG-CDF1-480 EC

| | |
|--------------------------------|--|
| Mortality | No mortality occurred. |
| Clinical signs | After application of AG-CDF1-480 EC at a dose level of 2000 mg/kg bw, the following observations were done: In female animals erythema grade 1 was observed in 1 of 5 animals, crust in 3 of 5 animals and scratches in 2 of 5 animals. All signs of irritation were reversible within the observation period. |
| Body weight | Body weight gain was considered to be normal. |
| Macroscopic examination | With the exception of acute injection of blood vessels in the abdominal region, which is due to the euthanasia injection, no specific gross pathological changes were recorded for any animal. |

Conclusion

Under the conditions of the present study, single dermal application of the test item AG-CDF1-480 EC to rats at a dose of 2000 mg/kg body weight was associated with neither mortality nor signs of toxicity but very slight signs of irritation. The dermal LD₅₀ was determined to be > 2000 mg AG-CDF1-480 EC/ kg body weight.

According to Regulation (EC) 1272/2008, the formulation AG-CDF1-480 EC does not warrant classification and labelling regarding acute dermal toxicity.

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

| | |
|-------------------|--|
| Comments of zRMS: | Study accepted. See dRR AG-CDF1-480 EC, also refer to our comment to point A2.1. |
|-------------------|--|

~~New studies with the new composition are neither submitted nor required.~~

| | |
|--------------------------------------|---|
| Reference | KCP 7.1.3/01 |
| Report | AG-CDF1-480 EC – Acute inhalation toxicity study (nose-only) in the rat ... |
| Guideline(s) | OECD guideline 403 (2009) EPA OPPTS 870.1300 (1998) EC 440/2008, Annex Part B, B.2 (2008) |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | No |

Executive Summary

The objective of this study was to assess the acute inhalation toxicity of AG-CDF1-480 EC when administered to rats by a single four-hour nose-only inhalation exposure. The results of the study serve as a basis for hazard assessment and classification and labelling of the product.

Under the experimental conditions of this study, no mortality occurred when exposed to a test atmosphere concentration of 5.04 mg/L for 4 hours. The acute inhalation median lethal concentration (LC₅₀) of AG-CDF1-480 EC in Wistar Crl:WI rats was therefore considered to be above 5.04 mg/L.

Materials and methods

MATERIALS

| | |
|--|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Yellowish liquid |
| Concentration/Purity | Clopyralid: 2.90 % (w/w); 31.40 g/L 2,4 D: 35.44 % (w/w); 384.50 g/L (as 2,4-D Ethylhexyl ester: 53.44 % (w/w)) Fluroxypyr: 7.31 % (w/w); 79.30 g/L (as Fluroxypyr meptyl: 10.53 % (w/w)) |
| Stability of test compound | Expiry date 30.06.2016 |
| Vehicle and/or positive control | -- |
| Test animals (Species) | Rat |
| Strain | CRL: (WI) Wistar rats (<i>Rattus norvegicus</i>) |
| Sex | Male and female |
| Age at test start | Approx. 10 weeks |
| Weight at test start (mean) | Males: 387 - 425 g Females: 211 - 232 g |
| Source | Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany |
| Number of animals (per group) | 6 animals/sex |
| Acclimation period | Animals were acclimated to laboratory conditions for 14 days (sighting study) or 20 days (main study) prior to involvement in the study. Animals were also acclimatized to the test apparatus (restrain procedures) for a short period (60 min) prior to testing in order to lessen the stress during exposure. |
| Diet | <i>Ad libitum</i> : ssniff SM R/M “Autoclavable Complete Feed for Rats and Mice – Breeding and Maintenance” (ssniff Spezialdiäten GmbH, Germany ⁹) |
| Fasting | The animals were not fasted before the experiment |
| Water | <i>Ad libitum</i> : tap water |
| Housing and cages | Group caging (5 animals, by sex, per cage), during the sighting study individual caging. Polycarbonate solid floor cages (type II or III) with stainless steel mesh lids. |
| Reference standard | None |
| Environmental conditions during testing | |
| Temperature | 19.4 – 25.0°C |
| Relative humidity | 35 - 68 % |
| Ventilation | At least 15 air exchanges/hour |
| Photoperiod | 12 hours of continuous artificial light in each twenty-four hour period (from 6.00 a.m. to 6.00 p.m.) |
| STUDY DESIGN AND METHODS | |
| In-life dates | 01.10. – 29.10.2014 (experimental phase) |
| Animal assignment and treatment | The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of two concentric anodised aluminum chambers and a computer control system incorporating pressure detectors and mass flow controllers. |
| Test concentrations | Target concentration 5 mg/L |

| | |
|--|--|
| Test duration | Duration of exposure: 4 hours/animal Observation: 14 days after application |
| Generation of the test atmosphere | The test item was aerosolized using a stainless steel concentric jet nebulizer (TSE Systems GmbH, Bad Homburg, Germany) located at the top of the exposure chamber. The rate of test item usage was controlled by a syringe pump. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the nebulizer. |
| Observations | <p><u>Mortality:</u> Animals were checked hourly during exposure, 1 hour after exposure and twice daily during the 14-day observation period for morbidity and/or mortality.</p> <p><u>Clinical signs:</u> All animals were observed for clinical signs at hourly intervals during exposure. Following exposure, clinical observations were performed twice on the day of exposure and subsequently once daily for 14 days.</p> <p><u>Bodyweight:</u> Individual bodyweights were recorded once during acclimation period (day -1), prior to treatment on the day of exposure (day 0) and on days 1, 3, 7 and 14.</p> <p><u>Necropsy:</u> At the end of the 14-day observation period, anaesthetized animals were dissected and studied macroscopically. Macroscopic study of internal organs was performed.</p> |
| Statistics | -- |

Results and discussions

Table A 5: Concentrations and exposure conditions

| Group | Target conc. (mg/L air) | Nominal conc. (mg/L air) | Actual conc. (mg/L air) | MMAD * (µm) | GSD ** (µm) |
|---|----------------------------|-----------------------------|----------------------------|----------------|----------------|
| Sighting exposure: Group 0.1 | 5 | 17.17 | 5.08 | 2.00 | 1.90 |
| Main study: Group 1 | 5 | 16.08 | 5.04 | 2.01 | 1.90 |

* MMAD = Mass Median Aerodynamic Diameter

** GSD = Geometric Standard Deviation

The mean achieved atmosphere concentrations were 5.08 and 5.04 in Group 0.1 and Group 1, respectively. The mass median aerodynamic diameter (MMAD) was 2.00 µm and 2.01 µm with geometric standard deviation (GSD) 1.90 and 1.90 in the sighting group and the main group, respectively.

Table A 6: Results of acute inhalation toxicity study in rats of AG-CDF1-480 EC

| Concentration (mg/L air) | Toxicological results * | Duration of signs | Time of death | LC ₅₀ (mg/L air) (14 days) |
|-----------------------------|-------------------------|--|------------------|--|
| Male rats | | | | |
| 5.04 | 0/5/5 | Reversible from Day 7 at the latest | End of the study | > 5.04 |
| Female rats | | | | |
| 5.04 | 0/5/5 | Reversible from Day 7 at the latest | End of the study | > 5.04 |

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 7: Summary of findings of acute inhalation toxicity study in rats of AG-CDF1-480 EC

| | |
|-----------------------|--|
| Mortality | No mortality was noted in the animals following exposure to the test atmosphere at a mean aerosol concentration of 5.04 mg/L. |
| Clinical signs | <p><u>Group 0.1:</u> laboured respiration (slight) and increased respiratory rate (slight) were recorded in both animals as well as noisy respiration (slight) in the male animal was noted on Day 0-1. Red-brown staining and/or ruffled fur were also seen in both animals on Day 0-1, but these observations were considered to be related to the restraint and exposure procedures and not to be toxicologically significant. Both rats were symptoms-free from Day 2.</p> |

| | |
|--------------------------------|--|
| | <p>Group 1: laboured respiration (slight) was recorded in all rats from Day 0 up to Day 2. Noisy respiration (slight to moderate) was observed in all males and in one female during the period of Day 0-2 and increased respiratory rate (slight) was also noted in three females on the day of the exposure. Slight sneezing began in all animals on Day 1 or Day 2 and persisted up to Day 6. Additionally, decreased activity (slight) was shown by a single male on Day 0 and Day 1.</p> <p>Red-brown staining in 4 males and 1 female and ruffled fur in 1 female were observed on Day 0-1 which were considered to be related to the restraint and exposure procedures and not to be toxicologically significant.</p> <p>All animals were symptom-free from Day 7 at the latest.</p> |
| Body weight | <p>Slight body weight loss (2.3-5.4%) was recorded in both animals on Day 1 in Group 0.1. The animals gained back their initial body weight values by Day 7 at the latest.</p> <p>In Group 1, slight to moderate body weight loss (0.5-10.9%) or low body weight gain was noted in all animals on Day 1-3. With exception of 2 males, all rats of this group returned to their initial body weight values by up to Day 7. The two males gained back their starting body weights between Day 7 and Day 14.</p> |
| Macroscopic examination | No external or internal findings were noted at necropsy in all animals. |

Conclusion

Under the experimental conditions of this study, no mortality occurred when exposed to a test atmosphere concentration of 5.04 mg/L for 4 hours. The acute inhalation median lethal concentration (LC₅₀) of AG-CDF1-480 EC in Wistar CrI:WI rats was therefore considered to be above 5.04 mg/L.

According to Regulation (EC) 1272/2008, the formulation AG-CDF1-480 EC does not warrant classification and labelling regarding acute inhalation toxicity.

A 2.5 Skin irritation (KCP 7.1.4)

| | |
|-------------------|--|
| Comments of zRMS: | Study accepted. See dRR AG-CDF1-480 EC, also refer to our comment to point A2.1. |
|-------------------|--|

~~New studies with the new composition are neither submitted nor required.~~

Study 1

| | |
|--------------------------------------|---|
| Reference | KCP 7.1.4/01 |
| Report | <i>In vitro</i> skin irritation: Human skin model test with AG-CDF1-480 EC Gehrke H., 2015 Project ID 144996, Sponsor ID R-90017670 |
| Guideline(s) | OECD guideline 439 |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | n.a. |

Executive Summary

In the present study the skin irritant potential of AG-CDF1-480 EC was analysed. The EPISKIN- Standard Model™ (EPISKIN-SMTM), a reconstituted three-dimensional human epidermis model, was used as a replacement for the Draize Skin Irritation Test (OECD TG 404) to distinguish between UN GHS “Category

2” skin irritating test substances and not categorized test substances (“No Category”) which may be considered as non-irritant. Hereby, the test item was applied topically. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT after a 15 min. exposure and 42 h post incubation period and compared to those of the concurrent negative controls. In this study under the given conditions the test item showed no irritant effects. The relative mean tissue viability after 15 min. of exposure and 42 h post incubation was > 50%. The test item is therefore classified as “non-irritant” in accordance with UN GHS “No Category”.

Materials and methods

| MATERIALS | |
|---------------------------------|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Yellowish clear liquid |
| Concentration/Purity | Clopyralid 31 g/L, 2,4-D-EHE 383 g/L, Fluroxypyr-meptyl 79 g/L |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle and/or positive control | Negative control: Phosphate Buffered Saline (PBS; Gibco, Cat. No.: 14040-091, Lot No.: 1581502). Positive control: 5% sodium dodecyl sulfate (SDS; CAS No.: 151-21-3, AppliChem, Art.-No.: A1112,0500, Lot No.: 1V010396) in aqua dest. |
| Test organism | Adult human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis. |
| Source | The EPISKIN-SM TM tissues were provided as kits (SkinEthic) |
| STUDY DESIGN AND METHODS | |
| In-life dates | 18.11. – 15.12.2014 |
| Experimental procedure | <p>Upon receipt of the EPISKIN-SMTM, the tissues were transferred into 12-well plates containing 2 mL prewarmed maintenance medium per well. The 12-well plates were incubated in a humidified incubator at 37 ± 1 °C, 5.0% CO₂ for at least 24 h.</p> <p>After this pre-incubation the tissues were treated with each dose group in triplicate, starting with the negative control. Then the tissues were incubated at room temperature for 15 ± 0.5 min. Afterwards, the tissues were washed with PBS to remove any residual test item. Excess PBS was removed by blotting bottom with blotting paper. The inserts were placed in a prepared 12-well plate containing 2 mL prewarmed fresh maintenance medium and post-incubated at 37 ± 1 °C, 5.0% CO₂ for 42 ± 1 h.</p> <p>After this incubation period the plates were placed for 15 ± 2 min. on a plate shaker. Then the inserts were transferred in a prepared 12-well plate containing 2 mL prewarmed MTT medium and further incubated for 3 h ± 5 min. at 37 ± 1°C, 5.0% CO₂.</p> <p>After the 3 h MTT incubation period the tissues were placed on blotting paper to dry the tissues. Afterwards a total biopsy of the epidermis by using the special biopsy punch was performed and the epidermis was separated from the collagen matrix with the aid of forceps. Both parts (epidermis and collagen matrix) were transferred into suitable tubes and 500 µL of acidic isopropanol were added. Extraction was carried out protected from light over the weekend at 2 - 8°C.</p> <p>At the end of the formazan extraction period the tubes were mixed by vortexing until solution colour became homogeneous</p> <p>If any visible cell/tissue fragments were in suspension, the tubes were centrifuged at 500 rpm to eliminate the fragments and avoid further possible interference with the absorbance readings.</p> <p>Per each tissue 2 x 200 µL aliquots of the extract were transferred into a 96-well plate and OD was measured at 550 nm without reference wavelength in a plate spectrophotometer</p> |

Results and discussions

Pre-experiments

The mixture of 10 µL test item per 2 mL MTT medium showed no reduction of MTT compared to the solvent. The mixture did not turn blue/purple.

The mixture of 10 µL of the test item per 90 µL aqua dest. showed no blue/purple colouring detectable by unaided eye-assessment.

Experiment

Table A 8 Result of the Test Item AG-CDF1-480 EC

| Name | Negative Control | | | Positive Control | | | Test Item | | |
|--|------------------|----------------|----------------|------------------|----------------|----------------|----------------|----------------|----------------|
| Tissue | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| absolute OD550 | 0.945 0.905 | 0.990 1.000 | 0.906 0.897 | 0.241 0.236 | 0.125 0.127 | 0.188 0.193 | 0.717 0.712 | 0.835 0.793 | 0.831 0.787 |
| OD550 (blank- corrected) | 0.901 0.861 | 0.946 0.956 | 0.862 0.853 | 0.197 0.192 | 0.081 0.083 | 0.144 0.149 | 0.673 0.668 | 0.791 0.749 | 0.787 0.743 |
| mean OD550 of the duplicates (blank-corrected) | 0.881 | 0.951 | 0.858 | 0.195 | 0.082 | 0.147 | 0.670 | 0.770 | 0.765 |
| total mean OD550 of 3 replicate tissues (blank-corrected) | 0.897* | | | 0.141 | | | 0.735 | | |
| SD OD550 | 0.049 | | | 0.057 | | | 0.056 | | |
| relative tissue viabilities [%] | 98.2 | 106.1 | 95.7 | 21.7 | 9.1 | 16.4 | 74.8 | 85.9 | 85.3 |
| mean relative tissue viability [%] | 100.0 | | | 15.7** | | | 82.0 | | |
| SD tissue viability [%]** | 5.4 | | | 6.3 | | | 6.3 | | |
| CV [% viability] | 5.4 | | | 40.1 | | | 7.6 | | |

* Corrected mean OD550 of the negative control corresponds to 100% absolute tissue viability.

** Mean relative tissue viability of the three positive control tissues is ≤ 40%.

*** The standard deviation (SD) obtained from the three concurrently tested tissues is < 18%.

Quality criteria

Table 6.6 9 Quality criteria

| | Value | Cut off | pass/fail |
|---------------------------------------|---------|-------------------------------|-----------|
| Mean OD550 nm Blank | 0.044 | < 0.1 | pass |
| Mean Absolute OD550 nm NC | 0.941 | $0.6 \leq \text{NC} \leq 1.5$ | pass |
| Mean Relative Viability [%] PC | 15.7 | ≤ 40% | pass |
| SD of Viability [%] | 5.4-6.3 | < 18% | pass |

Historical Data

Table A 10 **Historical data**

| | OD550 blank | Absolute OD550 NC | Relative Viability [%] PC | SD of Viability [%] |
|-------------|-------------|-------------------|---------------------------|---------------------|
| Mean | 0.043 | 0.871 | 11.3 | 7.0 |
| SD | 0.002 | 0.131 | 7.319 | 7.603 |
| n | 35 | 35 | 35 | 141 |

Discussion

The potential of the test item to induce skin irritation was analysed by using the three-dimensional human skin model EPISKIN-SM™ (SkinEthic) comprising a reconstructed epidermis with a functional *stratum corneum*.

In the present study AG-CDF1-480 EC was applied topically to the EPISKIN-SM™ tissue for 15 min. followed by a 42 h post incubation period and immediate determination of cytotoxic effects via MTT reduction assay.

Irritant potential of the test item was predicted from the relative mean tissue viabilities obtained compared to the corresponding negative control tissues concurrently treated with PBS.

The test item showed no irritant effects. The mean relative tissue viability (% negative control) was > 50% (82.0%) after 15 min. treatment and 42 h post incubation.

The controls confirmed the validity of the study. The mean OD₅₅₀ of the six blank values was < 0.1. The mean absolute OD₅₅₀ of the three negative control tissues was ≥ 0.6 and ≤ 1.5. The mean relative tissue viability (% negative control) of the positive control was ≤ 40% (15.7%). The maximum standard deviation of viability of replicate tissues of all dose groups was < 18% (5.4% - 6.3%).

Conclusion

In this study under the given conditions the test item showed no irritant effects. The relative mean tissue viability after 15 min. of exposure and 42 h post incubation was > 50%. The test item is therefore classified as “non-irritant” in accordance with UN GHS “No Category”.

Negative results of *in-vitro* studies are not sufficient for a final assessment of PPP's. Therefore, an *in vivo* study was performed.

Study 2

Reviewer comment:

Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol, the OECD 404 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted. Considering following result:

(..)The subsequent clinical observations showed irritant but no corrosive effects on the intact skin of three female rabbits (strain NZW) after a contact time of 4 hours, which were completely reversible within 8 days in animal no. 1, 72 hours in animal no. 2 and 9 days in animal no. 3 (refer study report KCP 7.1.4-01)

Discussing evaluation of dermal irritation - prolonged observation period in the rabbit 1 and 2, we can notice well defined erythema in the animal 1 (day 4 to 5) and slight erythema (day 6 to 8). In animal 2 slight erythema was observed (day4 to 8), when inflammation persists to the end of the observation period in 2 or more test animals, material shall be considered to be an irritant (Reg. 1272/2008 EC) Thus in the reviewer opinion product should be classified with hazard category Skin Irrit. 2 H315, also refer to our comment to point A2.1.

| | |
|--------------------------------------|--|
| Reference | KCP 7.1.4/02 |
| Report | Acute dermal irritation/corrosion in the rabbit with AG-CDF1-480 EC ... |
| Guideline(s) | OECD Guideline, No. 404 Commission Regulation (EC) No 440/2008, Method 8.4 EPA Health Effects Test Guidelines, OPPTS 870.2500 and 870.1000 |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | No |

Executive Summary

An *in vitro* skin irritation (Human Skin Model Test, OECD guideline number 439) was performed with AG-CDF1-480 EC (BSL Project No. 144996). Based on the results of this test further *in vivo* testing for assessing acute dermal irritation/corrosion was necessary.

Under the conditions of the present study (following OECD guideline number 404), the single dermal application of the test item AG-CDF1-480 EC to three rabbits at a dose of 0.5 mL showed irritant but no corrosive effects in animal no. 1 directly after patch removal which were fully reversible within 8 days. The other animals also showed irritant but no corrosive effects which were completely reversible within 72 hours in animal no. 2 and within 9 days in animal no. 3. Neither mortalities nor significant clinical signs of toxicity were observed.

Materials and methods

| MATERIALS | |
|---------------------------------|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Yellowish clear liquid |
| Concentration/Purity | Clopyralid 31.47 g/L, 2,4-D-EHE 384.52 g/L, Fluroxypyr-meptyl 114.25 g/L |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle and/or positive control | None |
| Test organism | New Zealand White Rabbits CrI: KBL (NZW) |
| Source | Charles River Deutschland, 97633 Sulzfeld, Germany |
| Number of animals | 3 |
| STUDY DESIGN AND METHODS | |
| In-life dates | 25.01. – 12.02.2015 (experimental phase) |
| Experimental procedure | The test item was applied at a single dose to a small area (approximately 6 cm ²) of skin on one side of the dorsal area and covered with a gauze patch, which was held in place with a non-irritating tape. The untreated other side served as control. The test item was applied to the patch first and then applied to the skin. The patch was fixed with a semi-occlusive dressing. The limits of the application site were marked with an ink marker. |
| Dose level | A dose of 0.5 mL of the test item was applied to each test site. |

| | |
|-----------------------------|--|
| Exposure period | The test item was held in contact with the skin throughout a 4-hour period. At the end of the exposure period, the residual test item was removed with tap water. |
| Observation period | Due to the persistent lesions 72 hours after the patch removal, the observation period was extended in order to evaluate the reversibility or irreversibility of the lesions. Therefore, animal no. 1 was observed for 8 days and animal no. 3 was observed for 9 days |
| Clinical observation | The animals were examined for signs of erythema and oedema 1 hour after the patch removal. For the determination of classification-relevant values, the animals were examined for signs of erythema and oedema 24, 48 and 72 hours after the patch removal. Dermal irritation was scored and recorded. Any other signs such as hyperplasia, scaling, discolouration, fissures and scabs or any systemic effects were also recorded. For the initial test in one animal, the test site was also examined immediately after the patch had been removed. |

Results and discussions

Evaluation of dermal irritation

Initial test in animal no. 1: The examination of the test site immediately after the patch removal revealed erythema grade 1.

The subsequent clinical observations showed irritant but no corrosive effects on the intact skin of three female rabbits (strain NZW) after a contact time of 4 hours, which were completely reversible within 8 days in animal no. 1, 72 hours in animal no. 2 and 9 days in animal no. 3.

Table A 11: Skin irritation of AG-CDF1-480 EC

| Animal No. | | Scores after treatment * | | | | Mean scores (24-72 h) | Reversible (day) |
|------------|----------|--------------------------|------|------|------|-----------------------|------------------|
| | | 1 h | 24 h | 48 h | 72 h | | |
| 1 | Erythema | 1 | 1 | 2 | 2 | 1.67 | 8 |
| | Oedema | 1 | 1 | 0 | 0 | 0.33 | |
| 2 | Erythema | 1 | 1 | 1 | 0 | 0.67 | 3 |
| | Oedema | 0 | 1 | 0 | 0 | 0.33 | |
| 3 | Erythema | 1 | 2 | 2 | 1 | 1.67 | 9 |
| | Oedema | 1 | 1 | 0 | 0 | 0.33 | |

* scores in the range of 0 to 4

Table A 12 Individual data – Evaluation of dermal irritation – Prolonged observation period

| Animal No. | Timepoint | Irritation | | | |
|------------|-----------|------------|---------|-----------|---------|
| | | Oedema | | Erythema | |
| | | Test item | Control | Test item | Control |
| 1 | Day 4 | 0 | 0 | 2 | 0 |
| | Day 5 | 0 | 0 | 2 | 0 |
| | Day 6 | 0 | 0 | 1 | 0 |
| | Day 7 | 0 | 0 | 1 | 0 |
| | Day 8 | 0 | 0 | 0 | 0 |
| 3 | Day 4 | 0 | 0 | 1 | 0 |
| | Day 5 | 0 | 0 | 1 | 0 |
| | Day 6 | 0 | 0 | 1 | 0 |
| | Day 7 | 0 | 0 | 1 | 0 |
| | Day 8 | 0 | 0 | 1 | 0 |
| | Day 9 | 0 | 0 | 0 | 0 |

0-4 grade

Conclusion

Under the conditions of the present study, the single dermal application of the test item AG-CDF1-480 EC to 3 rabbits at a dose of 0.5 mL showed irritant but no corrosive effects in animal no. 1 directly after patch

removal which were fully reversible within 8 days. The other animals also showed irritant but no corrosive effects which were completely reversible within 72 hours in animal no. 2 and within 9 days in animal no. 3.

Neither mortalities nor significant clinical signs of toxicity were observed.

According to Regulation (EC) 1272/2008, the formulation AG-CDF1-480 EC does not warrant classification and labelling regarding skin irritation.

A 2.6 Eye irritation (KCP 7.1.5)

| | |
|-------------------|--|
| Comments of zRMS: | Studies 1 and 2 are accepted. See dRR AG-CDF1-480 EC, also refer to our comment to point A2.1. |
|-------------------|--|

A 2.6.1 Study 1

| | |
|--------------------------------------|---|
| Reference | KCP 7.1.5/01 |
| Report | Screening for the eye irritancy potential using the bovine corneal opacity and Permeability Assay with AG-CDF1-480 EC ... |
| Guideline(s) | Yes, OECD 437 ICCVAM Test Method Evaluation Report: Current Validation Status of In Vitro Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products Appendix 81, NIH Publication No. 10-7553, 2010 |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | n.a. |

Executive Summary

The eye irritancy potential of AG-CDF1-480 EC was investigated in the bovine corneal opacity and permeability assay.

The *in vitro* irritation score obtained with the positive control (i.e. 51.83) fell within the two standard deviations of the current historical mean and therefore this assay is considered to be valid.

No prediction can be made regarding the classification of the test substance AG-CDF1-480 EC according to the evaluation criteria. Further testing in another suitable method is required.

Materials and methods

| MATERIALS | |
|---------------------------------|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Yellowish clear liquid |
| Concentration/Purity | Clopyralid 31 g/L, 2,4-D-EHE 383 g/L, Fluroxypyr-meptyl 79 g/L |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle and/or positive control | <u>Negative control</u> : physiological saline 0.9% NaCl <u>Positive control</u> : ethanol 100% |

| | |
|---------------------------------|---|
| Test organism | The assay uses isolated corneas obtained as a by-product from animals freshly slaughtered at the abattoir A. Moksel AG, Buchloe, Germany. |
| STUDY DESIGN AND METHODS | |
| In-life dates | 19.11.2014 |

Results and discussions

The eye irritancy potential of AG-CDF1-480 EC was investigated in the bovine corneal opacity and permeability assay.

Preparation of the test item: tested as provided by the sponsor

The following mean *in vitro* irritation score (IVIS) was calculated: 51.83

Classification:

- ☐ UN GHS No Category
- ☒ No prediction can be made
- ☐ UN GHS Category 1

Table 6.6 13 *In vitro* irritation score

| Cornea No. | Test Item | Corrected Opacity | Corrected OD490 Value | IVIS |
|------------|------------------|-------------------|-----------------------|-------|
| 1 | Negative Control | 1.00 | 0.001 | 0.48 |
| 2 | | 1.00 | 0.005 | |
| 3 | | -1.00 | 0.023 | |
| MV | | 0.33 | 0.010 | |
| 4 | Positive Control | 49.67 | 1.770 | 80.21 |
| 5 | | 46.67 | 2.028 | |
| 6 | | 56.67 | 2.042 | |
| MV | | 51.00 | 1.947 | |
| 7 | Test Item | 53.67 | 0.035 | 51.83 |
| 8 | | 46.67 | 0.074 | |
| 9 | | 51.67 | 0.122 | |
| MV | | 50.67 | 0.077 | |

MV = mean value

No prediction can be made regarding the classification of the test substance AG-CDF1-480 EC according to the evaluation criteria. Further testing in another suitable method is required.

The *in vitro* irritation score obtained with the positive control fell within the two standard deviations of the current historical mean and therefore this assay is considered to be valid.

Conclusion

No prediction can be made regarding the classification of the test substance AG-CDF1-480 EC according to the evaluation criteria. Further testing in another suitable method is required. Study 3

A 2.6.2 Study 2

| | |
|-----------|--|
| Reference | KCP 7.1.5/02 |
| Report | Acute eye irritation / corrosion in the rabbit with AG-CDF1-480 EC |

| | |
|--------------------------------------|--|
| | ... |
| | ... |
| Guideline(s) | OECD Guideline No 405 Commission Regulation (EC) No 440/2008, Method B.5 EPA OPPTS 870.2400 and 870.1000 |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | No |

Executive Summary

A Bovine Corneal Opacity and Permeability Assay (OECD Guideline number 437) with AG-CDF1-480 EC was performed (BSP Project No. 144997). Based on the results of this test further *in vivo* testing for assessing acute eye irritation/corrosion was necessary.

Under the conditions of the present study, a single ocular application of the test item AG-CDF1-480 EC to rabbits at a dose of 0.1 mL produced irritant effects, which were fully reversible within 8 days (animal no. 1) and 4 days (animal no. 2) but were not reversible within 21 days in animal no. 3. Neither mortalities nor significant clinical signs of toxicity were observed.

Materials and methods

| MATERIALS | |
|---|--|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Yellowish clear |
| Concentration/Purity | Clopyralid 31 g/L, 2,4-D-EHE 383 g/L, Fluroxypyr-meptyl 79 g/L |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle and/or positive control | Untreated eye |
| Test animals | |
| Species | New Zealand White Rabbits, CrI: KBL (NZW) |
| Age at test start | Animal no. 1: 27 weeks old; Animals no. 2 and 3: 28 weeks old |
| Body weight at start of treatment | 3.5 – 4.6 kg |
| Total number of animals | 3 females (nulliparous and non pregnant) |
| Source | Charles River Deutschland, 97633 Sulzfeld, Germany |
| Acclimation period | At least 5 days |
| Diet | Free access to irradiated hay briquettes and to Altromin 2123 maintenance diet for rabbits (lot no. 0923), rich in crude fibre |
| Water | Free access to tap water (drinking water, municipal residue control, microbiological controls at regular intervals) |
| Housing | Semi barrier in an air-conditioned room. |
| Environmental conditions during testing | |

| | |
|--|---|
| Temperature (mean ± S.D.) | 18 C ± 3°C |
| Relative humidity | 55 % ± 10 % |
| Air changes | At least 10 times per hour |
| Photoperiod | Alternating 12-hour light and 12 hours dark per day (about 350 lux). |
| STUDY DESIGN AND METHODS | |
| In-life dates | 16.02. – 17.03.2015 (experimental phase) |
| Animal assignment and treatment | The test item was applied at a single dose in the conjunctival sac of one eye of each test animal after pulling the lower lid away from the eyeball. The lids were then gently held together for about 1 second in order to prevent loss of the material. The untreated contralateral eye served as control. The eyes were not rinsed to remove residues of the test item, foreign bodies or incrustation 24 hours after application. |
| Test doses | 0.1 mL test item |
| Test duration | The animals were observed for 72 hours after dosing. To determine the reversibility of the observed effects, the observation period was extended up to 8 days for animal no. 1, 4 days for animal no. 2 and 21 days for animal no. 3 after dosing. |
| Observations | The eyes were examined for signs of irritation throughout the observation period. The eye irritation was scored and recorded. Individual reactions for each animal were recorded according to the scoring system at each time of observation. For the calculation only the 24, 48 and 72-hour readings were used. Nature, severity and duration of clinical observations were described. The body weight changes were summarized in a tabular form. |

Results and discussions

After the application into the eyes of three female rabbits the test item produced irritant but not corrosive effects in all animals, which were fully reversible within 8 days (animal no. 1) and 4 days (animal no. 2) but were not reversible within 21 days in animal no. 3. Neither mortalities nor significant clinical signs of toxicity were observed.

Upon fluorescein examinations at all time points starting with and after 24 h corneal lesions (cornea opacification) were found in all animals.

Table A 14: Eye irritation of AG-CDF1-480 EC

| Animal No. | | Scores after treatment * | | | | Mean scores (24-72 h) | Reversible (day) |
|------------|-----------------------|--------------------------|------|------|------|--------------------------|---------------------|
| | | 1 h | 24 h | 48 h | 72 h | | |
| 1 | Corneal opacity | 0 | 1 | 1 | 0 | 0.67 | 3 |
| | Iritis | 0 | 0 | 0 | 0 | 0.00 | - |
| | Redness conjunctivae | 1 | 2 | 2 | 1 | 1.67 | 8 |
| | Chemosis conjunctivae | 1 | 2 | 2 | 1 | 1.67 | 6 |
| 2 | Corneal opacity | 0 | 1 | 1 | 1 | 1.00 | 4 |
| | Iritis | 0 | 0 | 0 | 0 | 0.00 | - |
| | Redness conjunctivae | 1 | 3 | 2 | 1 | 2.00 | 4 |
| | Chemosis conjunctivae | 1 | 2 | 1 | 0 | 1.00 | 3 |
| 3 | Corneal opacity | 0 | 1 | 1 | 1 | 1.00 | > 21 |
| | Iritis | 0 | 0 | 0 | 0 | 0.00 | - |
| | Redness conjunctivae | 1 | 2 | 2 | 1 | 1.67 | > 21 |
| | Chemosis conjunctivae | 1 | 2 | 1 | 0 | 1.00 | > 21 |

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis

Table 6.6 15: Findings at Fluorescein examination

| Time Post-Application | Animal No.1 | Animal No.2 | Animal No.3 |
|-----------------------|---|--|---|
| 24 hours | cornea opacification (approx. ø 0.5 cm) | cornea opacification (approx. ø 0.5 cm) | cornea opacification (approx. ø 0.5 cm) |
| 48 h | cornea opacification (narrow band approx. 1 cm) | cornea opacification (narrow band approx. 1 cm) | cornea opacification (spot approx. ø 0.3 mm) |
| 72 h | nsf | cornea opacification (small spot approx. ø 1 mm) | cornea opacification (spot approx. ø 2 mm) |
| 4 d | nsf | nsf | cornea opacification (spot approx. ø 2 mm) |
| 5 d | nsf | - | cornea opacification (spot approx. ø 2 mm) |
| 6 - 8d | nsf | - | cornea opacification (narrow band approx. 0.5 cm) |
| 9 – 12 d | - | - | cornea opacification (narrow band approx. 0.5 cm) |
| 13 d | - | - | cornea opacification (small spot approx. ø 2 mm) |
| 14– 15 d | - | - | cornea opacification (small spot ø 2 mm) |
| 16 d | - | - | cornea opacification (small spot ø 4 mm) |
| 17– 21 d | - | - | cornea opacification (small spot ø 1 mm) |

d= day; nsf = no specific findings

Conclusion

Under the conditions of the present study, a single ocular application of the test item AG-CDF1-480 EC to rabbits at a dose of 0.1 mL produced irritant effects, which were fully reversible within 8 days (animal no. 1) and 4 days (animal no. 2) but were not reversible within 21 days in animal no. 3. Neither mortalities nor significant clinical signs of toxicity were observed.

In conformity with the EC criteria for classification and labelling requirements for dangerous substances and preparations according to Annex VI of Commission Directive 2001/59/EC, the test item AG-CDF1-480 EC has obligatory labelling requirement for eye irritation with R41.

According to Annex I of Regulation (EC) 1272/2008, the formulation AG-CDF1-480 EC has obligatory labelling requirement for eye irritation and has to be classified into Category 1, H318.

A 2.6.3 Study 3

| | |
|-------------------|--|
| Comments of zRMS: | New study submitted by the applicant reflecting composition change. Study has been reviewed for compliance with the current requirements. There is no deviation from studies protocol. The <i>in vitro</i> OECD 437 procedure fully implements the 3R rules (replacement) thus study is in line with the suggestions of point 5 of Regulation 284/2013. Study accepted. No prediction can be made regarding the classification of the test substance AG-CDF1-480 EC according to the evaluation criteria. Therefore, an <i>in vivo</i> study was performed (Study 2; ...). |
|-------------------|--|

Reference

KCP 7.1.5/03

Report

In vitro eye irritation test of ADM.3304.H.1.A using bovine corneal opacity and permeability test

| | |
|--------------------------------------|---|
| | ... |
| | ... |
| Guideline(s) | OECD 437 (2017) EU Method B47 (2007) |
| Deviations | No |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | n.a. |

Executive Summary

This study was conducted to evaluate the ocular irritancy of ADM.3304.H.1.A, as measured by the potential to induce opacity and increase permeability in isolated bovine cornea. The BCOP test can identify test items classified as UN GHS Category 1 (severe eye irritants) or UN GHS no category. Isolated bovine cornea (three corneas per set) were treated with normal saline (Set 1 - control), undiluted dimethylformamide (Set 2 - positive control) and ADM.3304.H.1.A (Set 3 – test group). After a 10 minute application, corneas were washed and incubated for approximately 2 h at 32 ± 1 °C. At the end of incubation, opacity readings were recorded, and the corneas were applied with 1 mL of fluorescein sodium solution (4 mg/mL) on to the anterior surface of the cornea followed by incubation for approximately 90 min at 32 °C. At the end of incubation, the Optical Density (OD) was measured at 490 nm from fluid collected from the posterior chamber. The negative and positive controls met the acceptance criteria, as described in the study plan, and confirmed the reliability of the test procedure. No prediction for eye irritancy of ADM.3304.H.1.A can be made, based on the mean in vitro irritancy score of 13.54, determined under the specified experimental conditions of this BCOP assay

Materials and methods

| MATERIALS | |
|-------------------------------|---|
| Test material (Lot/Batch No.) | ADM.3304.H.1.A (Lot/Batch No. N6812-A) |
| Description | Clear orange liquid |
| Concentration/Purity | Clopyralid 29.5 g/L, 2,4-D 370 g/L as acid equiv., Fluroxypyr-meptyl 75 g/L |
| Stability of test compound | Expiry date: June 2021 |
| Test system | Isolated cornea from the eyes of freshly slaughtered cattle obtained as a by-product from animals freshly slaughtered at the abattoir Deonar Abattoir slaughterhouse, Mumbai, Maharashtra |
| Positive control | N,N-dimethylformamide (99.96%) |
| Negative control | Sodium Chloride (0.9%) |
| Vehicle/Dilution | None |
| STUDY DESIGN AND METHODS | |
| Number samples | 3 per group |
| Application of the test item | 750 µL of the test item (or of control) were applied to the cornea for 10 minutes \pm 30 seconds at 32 ± 1 °C. |
| Additional rinsing | At the end of exposure period, the test item, positive control and negative control were removed from their respective anterior chamber and the corneal epithelium washed until no visual evidence of test item, positive control or negative control was observed using EMEM |

| | |
|----------------------------|--|
| Post- application exposure | After rinsing, the corneas were incubated for an additional period of approximately 2 hours \pm 10 minutes at $32 \pm 1^\circ\text{C}$. |
|----------------------------|--|

Results and discussions

The eye irritancy potential of ADM.3304.H.1.A was investigated in the bovine corneal opacity and permeability assay. The following mean *in vitro* irritation score (IVIS) was calculated:13.54 This value falls between the cut-off values for predicting serious eye damage or no classification. according to the UN GHS; therefore, no prediction can be made.

Table 6.6 16 *In vitro* irritation score

| Cornea No. | Test Item | Final Opacity value | Final Corrected OD ₄₉₀ Value | IVIS |
|------------|------------------|---------------------|---|--------------|
| 1 | Negative Control | -0.09 | 0.004 | -0.03 |
| 2 | | 0.68 | 0.059 | 1.57 |
| 3 | | 0.30 | 0.06 | 1.20 |
| MV/SD | | 0.30/0.39 | 0.041/ 0.032 | 0.91/ 0.84 |
| 8 | Positive Control | 79.99 | 1.567 | 103.50 |
| 12 | | 75.76 | 1.703 | 101.31 |
| 13 | | 72.60 | 1.548 | 95.82 |
| MV / SD | | 76.12/ 3.71 | 1.606 / 0.085 | 100.21/ 3.96 |
| 4 | Test Item | 15.05 | 0.046 | 15.74 |
| 5 | | 11.41 | 0.053 | 12.21 |
| 6 | | 8.94 | 0.249 | 12.68 |
| MV/ SD | | 11.80/ 3.07 | 0.116 / 0.115 | 13.54 / 1.92 |

MV = mean value
SD= Standard Deviation

The *in vitro* irritation score obtained with the positive control fell within the two standard deviations of the current historical mean and therefore, this assay is considered to be valid.

Conclusion

No prediction can be made regarding the classification of the test substance ADM.3304.H.1.A according to the evaluation criteria. Further testing in another suitable method is required.

A 2.7 Skin sensitisation (KCP 7.1.6)

| | |
|-------------------|--|
| Comments of zRMS: | Study accepted. See dRR AG-CDF1-480 EC, also refer to our comment to point A2.1. |
|-------------------|--|

~~New studies with the new composition are neither submitted nor required.~~

| | |
|---------------|--|
| Reference | KCP 7.1.6/01 |
| Report | Test for sensitisation (Local Lymph Node Assay - LLNA) With AG-CDFI - 480 EC |
| Guideline(s) | OECD Guideline No. 429 |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication | No |

(if vertebrate study)

Executive Summary

Based on the results of the prescreen test the test item was assessed for sensitising properties at concentrations of 50%, 25% and 12.5% (v/v), each diluted with AOO.

At the daily clinical observation the animals did not show any visible clinical symptoms and no case of mortality was observed.

One of the three tested concentrations of the test item reached the stimulation index of 3. The EC3 value (derived by linear interpolation) was calculated to be at a test item concentration of 39.29.

Consequently, according to OECD 429 solutions or preparations containing more than 39.29% AG-CDF1-480 EC are expected to have a stimulation index of > 3 and are therefore considered to be dermal sensitisers.

Materials and methods

| MATERIALS | |
|---|---|
| Test material (Lot/Batch No.) | AG-CDF1-480 EC (Lot/Batch No. D-N6406) |
| Description | Liquid |
| Concentration/Purity | 31.4 g/L Clopyralid, 384.52 g/L 2,4-D, 79.31 g/L Fluroxypyr, 579.82 g/L 2,4-2EHE, 114.25 g/L Fluroxypyr-meptyl |
| Stability of test compound | Expiry date: June 2016 |
| Vehicle | AOO (4:1 (v/v) acetone / olive oil) |
| Test animals (Species) | healthy mice (Mus musculus) |
| Strain | CBA/CaOlaHsd |
| Sex | Females (nulliparous and non-pregnant) |
| Age at test start | 8 - 9 weeks |
| Weight at test start | 18 - 21 g |
| Source | Harlan Laboratories GmbH, 5800 AN Venray, The Netherlands |
| Number of animals (per group) | 5 mice / group 10 mice / prescreen test |
| Acclimation period | Not stated |
| Diet | <i>Ad libitum</i> : Altromin 1324 |
| Fasting | -- |
| Water | <i>Ad libitum</i> : tap water |
| Housing and cages | The animals were kept in groups of 5 animals in IVC cages, type II L, poly-sulphone cages on Altromin saw fibre bedding (prescreen test and main study: lot no. 0210214083) |
| Reference standard | -- |
| Environmental conditions during testing | |
| Temperature | 22 ± 3°C |
| Relative humidity | 55 ± 10 % |
| Ventilation | Air change at least 10 x / hour |

| | |
|---------------------------------|--|
| Photoperiod | Artificial light, sequence being 12 hours light, 12 hours dark |
| STUDY DESIGN AND METHODS | |
| In-life dates | 03.12.2014 – 26.01.2015 (experimental phase) |
| Test duration | 6 days |
| Observations | <p>Clinical Observation Prior to the application and once a day thereafter all animals were observed in order to detect signs of toxicity, including dermal irritation at site of application.</p> <p>Weight Assessment The animals were weighed prior to the application and at the end of the test period (prior to the treatment with 3HTdR).</p> |
| Statistics | -- |

Results and discussions

One of the three tested concentrations of the test item reached the stimulation index of 3.

The stimulation index at a concentration of 12.5 % was **1.2**

The stimulation index at a concentration of 25 % was **2.6**

The stimulation index at a concentration of 50 % was **3.3**

All animals survived throughout the test period without showing any clinical signs.

Table A 16: Results of skin sensitisation study of AG-CDF1-480 EC

| | No. of animals | Concentration (%) | DPM / group | Stimulation index (SI) |
|----------------------------|----------------|-------------------|-------------|------------------------|
| AG-CDF1-480 EC | 5 | 12.5 | 1944.6 | 1.2 |
| | 5 | 25 | 4157.0 | 2.6 |
| | 5 | 50 | 5197.0 | 3.3 |
| Test vehicle control group | 5 | 100 | 1598.0 | 1.0 |
| Positive control | - | 1 | n.a. | > 5.2 |

Table A 17: Results of the Last 10 Positive-Control Experiments with 1% Phenylenediamine in DMSO

| Project No. | Date | Stimulation Index |
|-------------|----------------|-------------------|
| 144653 D | December 2014 | 6.4 |
| 144653 C | November 2014 | 5.4 |
| 144653 B | October 2014 | 8.5 |
| 144653 A | September 2014 | 11.9 |
| 142473 J | August 2014 | 8.0 |
| 142473 F | July 2014 | 5.2 |
| 142473 B | June 2014 | 8.8 |
| 142473 A | May 2014 | 10.0 |
| 134702 X | April 2014 | 10.3 |
| 134702 U | March 2014 | 8.4 |

Body Weight Development

All animals showed the expected weight development, which includes a weight loss of up to 2 g throughout the study.

Measurement of Ear Thickness

Directly prior to the first application, approximately 48 hours after the first application and shortly before excising the lymph nodes the thickness of both ears from all animals was measured. The means of the ear thickness per group showed no relevant difference compared to the negative control.

| The mean ear thickness on | day 1 | day 3 | day 6 |
|------------------------------------|---------|---------|---------|
| for the 12.5% test group was | 0.18 mm | 0.18 mm | 0.18 mm |
| for the 25% test group was | 0.18 mm | 0.19 mm | 0.18 mm |
| for the 50% test group was | 0.18 mm | 0.18 mm | 0.18 mm |
| for the negative control group was | 0.18 mm | 0.18 mm | 0.18 mm |

Conclusion

The EC3 value (derived by linear interpolation) was calculated to be at a test item concentration of 39.29. Consequently, according to OECD 429 solutions or preparations containing more than 39.29 % AG-CDF1-480 EC are expected to have a stimulation index of > 3 and are therefore considered to be dermal sensitizers.

According to Regulation (EC) 1272/2008 the formulation AG-CDF1-480 EC has obligatory labelling requirement for skin sensitisation and is classified into Category 1B H317.

According to the EPA Health Effects Guidelines (US-EPA, 2002) the test item AG-CDF1-480 EC at the tested concentration is positive for sensitization

A 2.8 Supplementary studies for combinations of plant protection products (KCP 7.1.7)

Not applicable since AG-CDF1-480 EC is not intended to use in combination with other products.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.9.1 Material safety data sheet for each co-formulant

Information regarding material safety data sheets of the co-formulants can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.9.2 Available toxicological data for each co-formulant

Available toxicological data for each co-formulant can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.10 Studies on dermal absorption (KCP 7.3)

A 2.10.1 2,4-D in AG-CDF1-480 EC

The following dermal absorption *in vitro* study performed with 2,4-D EHE applied as ADM.3304.H.1.A to human skin is provided in support of the assessment.

| | |
|-------------------|---|
| Comments of zRMS: | New study submitted by the applicant reflecting composition change. Study were conducted according to OECD Guideline 428 and in compliance with GLP. All the recoveries where between the recovery boundaries mentioned in the dermal absorption guidance (EFSA Journal 2012;10(4):2665). Studies are considered to be acceptable and the dermal absorption for [14C]2,4-D EHE are covered by this study. |
|-------------------|---|

| | |
|--------------------------------------|---|
| Report | 2,4-D EHE – <i>In vitro</i> percutaneous penetration of [¹⁴ C]2,4-D EHE formulated as ADM.3304.H.1.A through Human Skin Membranes Hassler S., 2019 Project ID 20190008, Sponsor ID 00104190 |
| Guideline(s) | OECD Guideline No 428 (2004) Commission Regulation (EC) No 440/2008, Method B.45 (2008) |
| Deviations | n.a. |
| GLP | Yes |
| Acceptability | Yes |
| Duplication (if vertebrate study) | n.a. |

Executive summary

The percutaneous penetration of 2,4-D EHE formulated as ADM.3304.H.1.A, was investigated *in vitro* using split-thickness human skin membranes. The skin membranes were set up in flow-through diffusion cells and formulated [¹⁴C]2,4-D EHE was applied onto the skin membranes at two dose levels. The high dose level reflects the commercial formulation concentrate 560 g a.i./L. The low dose reflects the lowest in use field concentration i.e. 1.5 L product/ha in 400 L water corresponding 2 g a.i./L. The exposure of the test item was performed under non-occluded conditions over an exposure time of 8 hours. Thereafter, the remaining test item was removed by washing the skin membranes with a mild soap solution. Further penetration of the remaining test item after removal was measured for additional 16 hours. At end of experiment, i.e. 24 hours after application, an additional skin membrane wash was performed. Test item remaining in/on the application site was removed by tape stripping of the application site until the epidermis was removed. During the experimental period the receptor fluid (phosphate buffered physiological saline with the addition of 5 % (w/v) bovine serum albumin (BSA)) was collected in hourly intervals between 0-8 hours and thereafter in 2 hours intervals until the end of the experiments.

At the end of the exposure period the bulk of radioactivity could be removed from the application site by skin membrane wash. After skin wash at 24 hours a small part of applied test item was remaining on the skin membrane. The majority could be removed from the surface of the skin membranes by applying the first two tape strips. After tape stripping very low amounts remained in the skin membrane. The average of total recovery for the performed experiments was found at 97.87% and 98.69% for the low and high dose level, respectively. The totally absorbed test item accounted for 1.50% for the low dose (spray dilution) and 0.02% for the high dose (concentrate). Due to the fact that the Absorption t0.5 was found at 44% and 36% the potential absorption (incl. the tape strip 3-10) was calculated to be 3.94 % for the low dose level and 0.09 % for the high dose level.

Material and Methods

The dermal absorption of 2,4-D EHE, contained in the product ADM.3304.H.1.A, was evaluated on human skin membranes (split thickness 400 µm), using [Ring-¹⁴C-(U)]2,4-D EHE, in a flow-through diffusion cell system.

The integrity of each skin disc was checked by determination of the permeation of tritiated water and was within the acceptability criteria ($K_p \leq 3 \cdot 10^{-3}$ cm/h).

One group of 10 human skin discs (5 different donors) was exposed to the undiluted formulation of ADM.3304.H.1.A containing 560 g/L 2,4-D EHE and one groups of 9 human skin discs (6 different donors) were exposed to the aqueous dilution of ADM.3304.H.1.A containing 2 g/L 2,4-D EHE (equivalent to a field dilution rate of 1.5L product in 400 L water) for 8 hours under non-occlusion conditions. Thereafter, the remaining test item was removed by washing the skin membranes with a mild soap solution. Further

penetration of the remaining test item after removal was measured for additional 16 hours. At end of experiment, i.e. 24 hours after application, an additional skin membrane wash was performed. Test item remaining in/on the application site was removed by tape stripping of the application site until the epidermis was removed. During the experimental period the receptor fluid (phosphate buffered physiological saline with the addition of 5 % (w/v) bovine serum albumin (BSA)) was collected in hourly intervals between 0-8 hours and thereafter in 2 hours intervals until the end of the experiments.

| Dose Level | Species | Conc. [mg/cm ³] | Appl. Dose [µg/cm ²] | Number of Replicates | Number of Donors | Collection period [h] |
|----------------|---------|--------------------------------|-------------------------------------|-------------------------|---------------------|--------------------------|
| Low Dose (A1) | Human | 2.066 | 20.7 | 9 | 5 | 0-24 h |
| High Dose (A2) | | 557 | 5571 | 10 | 6 | |

The amount of radioactivity was determined by liquid scintillation counting at the donor site in the skin (tape stripping and total skin) and in the receptor fluid samples as well as donor and receptor cells.

Results and discussions

The amounts of 2,4-D EHE recovered at each dose in the receptor fluids, skin membrane wash, tape strips, skin membranes, and donor cell washes are summarized in Table below.

| Dose group | High dose | | Low dose | |
|--|---------------------------------|-------|--------------------------------|-------|
| | (Formulation concentrate) n=9 * | | (Spray dilution 1:267) n=10 | |
| Applied dose [µg/cm²] | 5571 | | 20.7 | |
| | Recovery [%] | | Recovery [%] | |
| | Mean | S.D. | Mean | S.D. |
| Dislodgeable dose | | | | |
| Skin washing after 8 h | 97.65 | 1.78 | 66.41 | 12.61 |
| Skin washing after 24 h | 0.42 | 0.27 | 6.62 | 2.92 |
| Donor chamber wash | 0.08 | 0.04 | 2.73 | 2.35 |
| Dose associated to skin | | | | |
| Tape strips: 1 st sample, strips 1 + 2 | 0.45 | 0.37 | 16.18 | 10.01 |
| Tape strips: 2 nd sample; strip 3 | 0.06 | 0.06 | 2.43 | 3.08 |
| Skin preparation | <0.01 | <0.01 | 0.75 | 0.61 |
| Absorbed dose | | | | |
| Receptor fluid 0-8 h | <0.01 | <0.01 | 0.12 | 0.05 |
| Receptor fluid 8-12 h | <0.01 | <0.01 | 0.19 | 0.25 |
| Receptor fluid 0-12 h | <0.01 | <0.01 | 0.31 | 0.26 |
| Receptor fluid 12-24 h | <0.01 | <0.01 | 0.40 | 0.38 |
| Receptor chamber wash | <0.01 | <0.01 | 0.05 | 0.06 |
| Total recovery ¹ | 98.69 | 1.99 | 97.87 | 2.21 |
| Absorption essentially complete at end of study (>75% absorption within half the study duration) [% Absorption at t _{0.5}] | No [36%] | | No [44%] | |
| If no: Absorption estimates = absorbed dose + skin preparation + tape strips sample 2) ² | 0.09 | 0.06 | 3.94 | 3.12 |
| If yes: Absorption estimates = absorbed dose + skin preparation | 0.02 | 0.02 | 1.50 | 0.90 |
| Absorption estimates used for risk assessment ³ | 0.14 | | 6.19 | |

* One cell (Cell 3) was excluded from the calculation

¹ Values may not calculate exactly due to rounding of figures

² In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873), the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study.

³ In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873), total absorption = mean + k*SD, where k= 0.77 based on the number of replicates employed (n=9), or k= 0.72 based on the number of replicates employed (n=10).

SD: standard deviation

The average of total recovery for the performed experiments was found at 97.87% for the low dose spray dilution and 98.69% for the high dose concentrate.

The totally absorbed test item accounted for 1.50% for the low dose and 0.02% for the high dose. Due to the fact that the Absorption t_{0.5} was found at 44% and 36% the potential absorption (incl. the tape strip 3-10) was calculated to be 3.94 % for the low dose level and 0.09 % for the high dose level.

Conclusion/endpoint:

In summary, 2,4-D EHE, applied as ADM.3304.H.1.A formulation to human skin membranes, penetrated at a very low extent for both concentrations tested.

To address variability between replicates, dermal absorption should be calculated as follows: Absorption (mean value) + ks, where s is the sample standard deviation and k the multiplication factor correlated to the

number of replicates. There are 9 replicates for the concentrate and 10 replicates for the in-use dilution leading to multiplication factors of 0.77 and 0.72, respectively.

Thus, the relevant absorption estimates in human skin are set at 0.14% for the concentrate and 6.19% for the field dilution after rounding.

A 2.11 Other/Special Studies

Not available.

Appendix 3 Exposure calculations

~~No exposure calculations are submitted since the dermal absorption study comparable absorption values.~~

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for 2,4-D

Table A 18: Operator exposure, 2,4-D, without RPE/PPE (potential) / with PPE – Cereals and grass-land

| | | | | | |
|--|---|--|--|--------------------------------|---|
| Substance | 2,4-D | Formulation = Soluble concentrates, emulsifiable concentrate, etc. | Application rate-0.75 kg a.s. /ha | Spray dilution = 3.75 g a.s./l | Vapour pressure = low volatile substances having a vapour pressure of |
| Scenario | Cereals / Outdoor / Downward spraying / Vehicle-mounted | | | Buffer = 2-3 | Number applications = 1, Application interval = 365 days |
| Percentage Absorption | Dermal for product = 0.4 | Dermal for in use dilution = 6.2 | Oral = 100 | Inhalation = 100 | |
| RVNAS | 0.02 mg/kg bw/day | | RVAAS | mg/kg bw/day | |
| DFR | 3 µg a.s./cm2 per kg a.s./ha | | DT50 | 30 days | |
| Operator Model Mixing, loading and application AOEM | | | | | |
| Potential exposure | Longer term systemic exposure mg/kg bw/day | | 0.0178 | % of RVNAS | 89.21% |
| | Acute systemic exposure mg/kg bw/day | | 0.0860 | % of RVAAS | |
| Mixing and Loading | Gloves = Yes | | Clothing = Work wear - arms, body and legs covered | RPE = None | Soluble bags = No |
| Application | Gloves = No | | Clothing = Work wear - arms, body and legs covered | RPE = None | Closed cabin = No |
| Exposure (including PPE options above) | Longer term systemic exposure mg/kg bw/day | | 0.0065 | % of RVNAS | 32.34% |
| | Acute systemic exposure mg/kg bw/day | | 0.0368 | % of RVAAS | |

Operator exposure for TRICERA outdoor spray applications

| | | |
|--------------------------------------|--|------------------------|
| Application rate of active substance | 0.75 kg a.s./ha | <i>i_AppRate</i> |
| Assumed area treated | 50 ha/day | <i>d_AreaTreated</i> |
| Amount of active substance applied | 37.5 kg a.s./day | <i>i_AmountAS</i> |
| Dermal absorption of the product | 0.40% | <i>i_AbsorpProduct</i> |
| Dermal absorption of in-use dilution | 6.20% | <i>i_AbsorInuse</i> |
| Formulation type | Soluble concentrates, emulsifiable concentrate, etc. | |
| Indoor or Outdoor application | Outdoor | |
| Application method | Downward spraying | |
| Application equipment | Vehicle-mounted | |
| Season | not relevant | |

| Mixing and loading | Exposure values | µg exposure/day mixed and loaded | | Reference | Comment |
|--------------------|---|---|--------------------------|---------------------|------------------------------|
| | | 75 th centile | 95 th centile | | |
| | Hands | 79085 | 299200 | AOEM | |
| | Body | 45579 | 206430 | AOEM | |
| | Head | 1946 | 10671 | AOEM | |
| | Protected hands (gloves) | 364 | 7428 | AOEM | |
| | Protected body (workwear or protective garment and sturdy footwear) | 590 | 5484 | AOEM | |
| | Protected head (hood and face shield) | 31 | 604 | AOEM | |
| | Inhalation | 11 | 31 | AOEM | |
| | Protective Equipment | Select for inclusion | | Penetration factor | Inhalation Protection factor |
| | Gloves | Yes | | Incl. in AOEM model | |
| | Clothing | Work wear - arms, body and legs covered | | Incl. in AOEM model | |
| | Head and respiratory PPE | None | | 1 | 1 |
| | Water soluble bag | No | | 1 | |

| Application | Exposure values | µg exposure/day applied | | Reference | Comment |
|-------------|---|---|--------------------------|--------------------------------------|------------------------------|
| | | 75 th centile | 95 th centile | | |
| | Hands | 5562 | 32584 | AOEM | |
| | Body | 3110 | 16032 | AOEM | |
| | Head | 147 | 443 | AOEM | |
| | Protected hands (gloves) | 303 | 5086 | AOEM | |
| | Protected body (workwear or protective garment and sturdy footwear) | 85 | 209 | AOEM | |
| | Inhalation | 6 | 23 | AOEM | |
| | Protective Equipment | Select for inclusion | | Penetration factor | Inhalation Protection factor |
| | Gloves | No | | | |
| | Clothing | Work wear - arms, body and legs covered | | Incl. in AOEM model | |
| | Head and respiratory PPE | None | | 1 | 1 |
| | Closed cab | No | | vehicle mounted upward spraying only | |

1. Total

| | Without RPE/PPE | With RPE/PPE | |
|--|-----------------|--------------|--|
| Longer term | | | |
| Total systemic exposure from mixing, loading and application (mg a.s./day) | 1.0704733 | 0.3881057 | |
| Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day) | 0.0178412 | 0.0064684 | |
| % of RVNAS | 89.21% | 32.34% | |
| Acute | | | |
| Total systemic exposure from mixing, loading and application (mg a.s./day) | 5.1615450 | 2.2096740 | |
| Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day) | 0.0860258 | 0.0368279 | |
| % of RVAAS | #DIV/0! | #DIV/0! | |

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for 2,4-D

Table A 21: Worker exposure, 2,4-D, Cereals & Grassland, without / with PPE

| | | | | | |
|-----------------------|---|--|-----------------------------------|--------------------------------|---|
| Substance | 2,4-D | Formulation = Soluble concentrates, emulsifiable concentrate, etc. | Application rate-0.75 kg a.s. /ha | Spray dilution = 3.75 g a.s./l | Vapour pressure = low volatile substances having a vapour pressure of |
| Scenario | Cereals / Outdoor / Downward spraying / Vehicle-mounted | | | Buffer = 2-3 | Number applications = 1, Application interval = 365 days |
| Percentage Absorption | Dermal for product = 0.4 | Dermal for in use dilution = 6.2 | Oral = 100 | Inhalation = 100 | |
| RVNAS | 0.02 mg/kg bw/day | | RVAAS | mg/kg bw/day | |
| DFR | 3 µg a.s./cm ² per kg a.s./ha | | DT50 | 30 days | |

| | | | | |
|--|--|--------|------------|---------|
| Worker - Inspection, irrigation | Potential exposure mg/kg bw/day | 0.0581 | % of RVNAS | 290.63% |
| | Working clothing mg/kg bw/day | 0.0065 | % of RVNAS | 32.55% |
| | Working clothing and gloves mg/kg bw/day | | % of RVNAS | |

Worker exposure from residues on foliage for TRICERA

| | | |
|--|-------------------------------------|-----------------|
| Crop type | Cereals | |
| Indoor or outdoor | Outdoor | |
| Application method | Downward spraying | |
| Application equipment | Vehicle-mounted | |
| Worker's task | Inspection, irrigation | |
| Main body parts in contact with foliage | Hand and body | |
| Application rate of active substance | 0.75 kg a.s./ha | i_AppRate |
| Number of applications | 1 | i_AppNo |
| Interval between multiple applications | 365 days | i_AppInt |
| Half-life of active substance | 30 days | d_HalfLifeAS |
| Multiple application factor | 1.0 | d_MAF |
| Dermal absorption of the product | 0.40% | i_AbsorpProduct |
| Dermal absorption of the in-use dilution | 6.20% | i_AbsorpInuse |
| Dislodgeable foliar residue (i_AppRate*i_DFR) | 2.25 µg a.s./cm ² | d_DFR |
| Working hours | 2 hr | d_WorkHr |
| Dermal transfer coefficient - Total potential exposure | 12500 cm ² /hr | d_DermTcUCV |
| Dermal transfer coefficient - arms, body and legs covered | 1400 cm ² /hr | d_DermTcCV1 |
| Dermal transfer coefficient - hands, arms, body and legs covered | no TC available for this assessment | d_DermTcCV2 |
| Inhalation transfer coefficient for automated applications | NA ha/hr*10 ⁻³ | d_InhalTcAut |
| Inhalation transfer coefficient for cutting ornamentals | NA ha/hr*10 ⁻³ | d_InhalTcCut |
| Inhalation transfer coefficient for sorting / bundling ornamentals | NA ha/hr*10 ⁻³ | d_InhalTcSort |

| | | | | |
|---|--------------------|---|-------------------------------------|----------|
| 1. Total | | | | |
| | Potential exposure | Work wear - arms, body and legs covered | Working wear and gloves | Comments |
| Total systemic exposure (mg a.s./day) | 3.4875000 | 0.3906000 | no TC available for this assessment | |
| Total systemic exposure per kg body weight (mg/kg bw/day) | 0.0581250 | 0.0065100 | | |
| % of RVNAS | 290.63% | 32.55% | | |

A 3.3 Resident and bystander exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for 2,4-D

Table A 24: Resident exposure, 2,4-D, Cereals

| | | | | | |
|-----------------------|---|--|-----------------------------------|--------------------------------|---|
| Substance | 2,4-D | Formulation = Soluble concentrates, emulsifiable concentrate, etc. | Application rate-0.75 kg a.s. /ha | Spray dilution = 3.75 g a.s./l | Vapour pressure = low volatile substances having a vapour pressure of |
| Scenario | Cereals / Outdoor / Downward spraying / Vehicle-mounted | | | Buffer = 2-3 | Number applications = 1, Application interval = 365 days |
| Percentage Absorption | Dermal for product = 0.4 | Dermal for in use dilution = 6.2 | Oral = 100 | Inhalation = 100 | |
| RVNAS | 0.02 mg/kg bw/day | | RVAAS | mg/kg bw/day | |
| DFR | 3 µg a.s./cm ² per kg a.s./ha | | DT50 | 30 days | |

| | | | | |
|-------------------------|---|--------|------------|--------|
| Resident - child | Spray drift (75th percentile) mg/kg bw/day | 0.0063 | % of RVNAS | 31.58% |
| | Vapour (75th percentile) mg/kg bw/day | 0.0011 | % of RVNAS | 5.35% |
| | Surface deposits (75th percentile) mg/kg bw/day | 0.0013 | % of RVNAS | 6.43% |
| | Entry into treated crops (75th percentile) mg/kg bw/day | 0.0078 | % of RVNAS | 39.23% |
| | All pathways (mean) mg/kg bw/day | 0.0118 | % of RVNAS | 58.82% |
| Resident - adult | Spray drift (75th percentile) mg/kg bw/day | 0.0015 | % of RVNAS | 7.50% |
| | Vapour (75th percentile) mg/kg bw/day | 0.0002 | % of RVNAS | 1.15% |
| | Surface deposits (75th percentile) mg/kg bw/day | 0.0003 | % of RVNAS | 1.58% |
| | Entry into treated crops (75th percentile) mg/kg bw/day | 0.0044 | % of RVNAS | 21.80% |
| | All pathways (mean) mg/kg bw/day | 0.0047 | % of RVNAS | 23.26% |

| Resident exposure for TRICERA | | | | | |
|---|--|------------------------------------|--|---------------------|--------------------------|
| Croptype | Cereals | | | | |
| Application method | Downward spraying | | | | |
| Application equipment | Vehicle-mounted | | | | <i>i_AppEquip</i> |
| Formulation type | Soluble concentrates, emulsifiable concentrate, etc. | | | | <i>i_FormVal</i> |
| Buffer strip | 2-3 m | | | | <i>i_Buffer</i> |
| Application rate of the product | 0.75 kg a.s./ha | | | | <i>i_AppRate</i> |
| Concentration of active substance (in-use dilution for liquid applications) | 3.75 g a.s./l | | | | <i>d_ConcAS</i> |
| Dermal absorption of product | 0.40% | | | | <i>i_AbsorpProduct</i> |
| Dermal absorption of in-use dilution | 6.20% | | | | <i>i_Absorplnuse</i> |
| Oral absorption | 100.00% | | | | <i>i_AbsorpOrallnuse</i> |
| Dislodgeable foliar residue (<i>i_AppRate</i> * <i>i_DFR</i>) | 2.25 µg a.s./cm ² | | | | <i>d_DFR</i> |
| Vapour pressure of in-use dilution | low volatile substances having a vapour pressure of <5*10 ⁻³ Pa | | | | <i>i_Volat</i> |
| Concentration in air | 0.001 mg/m ³ | | | | <i>d_AirCon</i> |
| Resident dermal spray drift exposure 75th percentile - adult | 0.47 ml spray dilution/person | | | | |
| Resident dermal spray drift exposure 75th percentile - child | 0.327 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure 75th percentile - adult | 0.00010 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure 75th percentile - child | 0.00022 ml spray dilution/person | | | | |
| Resident dermal spray drift exposure mean - adult | 0.22318 ml spray dilution/person | | | | |
| Resident dermal spray drift exposure mean - child | 0.18 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure mean - adult | 0.00009 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure mean - child | 0.00017 ml spray dilution/person | | | | |
| Exposure duration dermal | 2 hours | | | | <i>d_ReExpDur</i> |
| Exposure duration inhalation | 24 hours | | | | <i>d_ReExpDurInhal</i> |
| Exposure duration entry into treated crops | 0.25 hours | | | | <i>d_ExpDurTreatCrop</i> |
| Light clothing adjustment factor | 18.0% | | | | <i>d_ClothAF</i> |
| Breathing rate adult | 0.23 m ³ /day/kg | | | | <i>d_BreathRAd</i> |
| Breathing rate child (1-3 year old) | 1.07 m ³ /day/kg | | | | <i>d_BreathRCh</i> |
| Drift percentage on surface (75th percentile) | 5.60% | | | | |
| Drift percentage on surface (mean) | 4.10% | | | | |
| Turf transferable residues percentage | 5.00% | | | | <i>d_Turf</i> |
| Transfer coeff. of surface deposits-adult | 7300 cm ² /hour | | | | <i>d_ReTCAd</i> |
| Transfer coeff. of surface deposits-child (1-3 year old) | 2600 cm ² /hour | | | | <i>d_ReTCCh</i> |
| Saliva extraction percentage | 50.00% | | | | <i>d_SalExt</i> |
| Surface area of hands mouthed | 20 cm ² | | | | <i>d_AreaHM</i> |
| Frequency of hand to mouth activity | 9.5 events/hour | | | | <i>d_ReFreqHM</i> |
| Ingestion rate for mouthing of grass per day | 25 cm ² | | | | <i>d_MouthGrass</i> |
| Dislodgeable residues percentage transferability for object to mouth | 20.00% | | | | <i>d_DRP</i> |
| Transfer coefficient for entry into treated crops (75th percentile) - ad | 7500 cm ² /h | | | | <i>d_TcEntryAd</i> |
| Transfer coefficient for entry into treated crops (75th percentile) - chi | 2250 cm ² /h | | | | <i>d_TcEntryCh</i> |
| Transfer coefficient for entry into treated crops (mean) - adult | 5980 cm ² /h | | | | <i>d_TcEntryAd</i> |
| Transfer coefficient for entry into treated crops (mean) - child | 1794 cm ² /h | | | | <i>d_TcEntryCh</i> |
| 1. Total | | | | | |
| 1.1 1-3 year old child | | | | | |
| Spray drift (75th percentile) | Vapour (75th percentile) | Surface deposits (75th percentile) | Entry into treated crops (75th percentile) | All pathways (mean) | |
| Total systemic exposure (mg a.s./day) | 0.0631676 | 0.0107000 | 0.0128604 | 0.0784688 | 0.1176359 |
| Total systemic exposure per kg body weight (mg a.s./day/kg) | 0.0063168 | 0.0010700 | 0.0012860 | 0.0078469 | 0.0117636 |
| % of RVNAS | 31.58% | 5.35% | 6.43% | 39.23% | 58.82% |
| 1.2 Adult | | | | | |
| Spray drift | Vapour | Surface deposits | Entry into treated crops | All pathways (mean) | |
| Total systemic exposure (mg a.s./day) | 0.0899805 | 0.0138000 | 0.0190092 | 0.2615625 | 0.2791567 |
| Total systemic exposure per kg body weight (mg a.s./day/kg) | 0.0014997 | 0.0002300 | 0.0003168 | 0.0043594 | 0.0046526 |
| % of RVNAS | 7.50% | 1.15% | 1.58% | 21.80% | 23.26% |

Table A 25: Resident exposure, 2,4-D, Grassland

| | | | | | |
|-----------------------|---|--|-----------------------------------|--------------------------------|---|
| Substance | 2,4-D | Formulation = Soluble concentrates, emulsifiable concentrate, etc. | Application rate-0.75 kg a.s. /ha | Spray dilution = 3.75 g a.s./l | Vapour pressure = low volatile substances having a vapour pressure of |
| Scenario | Grassland and lawns / Outdoor / Downward spraying / Vehicle-mounted | | | Buffer = 2-3 | Number applications = 1, Application interval = 365 days |
| Percentage Absorption | Dermal for product = 0.4 | Dermal for in use dilution = 6.2 | Oral = 100 | Inhalation = 100 | |
| RVNAS | 0.02 mg/kg bw/day | | RVAAS | mg/kg bw/day | |
| DFR | 3 µg a.s./cm ² per kg a.s./ha | | DT50 | 30 days | |

| | | | | |
|-------------------------|---|--------|------------|--------|
| Resident - child | Spray drift (75th percentile) mg/kg bw/day | 0.0063 | % of RVNAS | 31.58% |
| | Vapour (75th percentile) mg/kg bw/day | 0.0011 | % of RVNAS | 5.35% |
| | Surface deposits (75th percentile) mg/kg bw/day | 0.0013 | % of RVNAS | 6.43% |
| | Entry into treated crops (75th percentile) mg/kg bw/day | 0.0062 | % of RVNAS | 30.76% |
| | All pathways (mean) mg/kg bw/day | 0.0070 | % of RVNAS | 35.09% |
| Resident - adult | Spray drift (75th percentile) mg/kg bw/day | 0.0015 | % of RVNAS | 7.50% |
| | Vapour (75th percentile) mg/kg bw/day | 0.0002 | % of RVNAS | 1.15% |
| | Surface deposits (75th percentile) mg/kg bw/day | 0.0003 | % of RVNAS | 1.58% |
| | Entry into treated crops (75th percentile) mg/kg bw/day | 0.0007 | % of RVNAS | 3.54% |
| | All pathways (mean) mg/kg bw/day | 0.0019 | % of RVNAS | 9.42% |

| Resident exposure for TRICERA | | | | | |
|---|--|--------------------------|------------------------------------|--|---------------------|
| Croptype | Grassland and lawns | | | | |
| Application method | Downward spraying | | | | |
| Application equipment | Vehicle-mounted | | | | i_AppEquip |
| Formulation type | Soluble concentrates, emulsifiable concentrate, etc. | | | | i_FormVal |
| Buffer strip | 2-3 m | | | | i_Buffer |
| Application rate of the product | 0.75 kg a.s./ha | | | | i_AppRate |
| Concentration of active substance (in-use dilution for liquid applications) | 3.75 g a.s./l | | | | d_ConcAS |
| Dermal absorption of product | 0.40% | | | | i_AbsorpProduct |
| Dermal absorption of in-use dilution | 6.20% | | | | i_Absorplnuse |
| Oral absorption | 100.00% | | | | i_AbsorpOrallnuse |
| Dislodgeable foliar residue (i_AppRate*i_DFR) | 2.25 µg a.s./cm² | | | | d_DFR |
| Vapour pressure of in-use dilution | low volatile substances having a vapour pressure of <5*10-3Pa Pa | | | | i_Volat |
| Concentration in air | 0.001 mg/m³ | | | | d_AirCon |
| Resident dermal spray drift exposure 75th percentile - adult | 0.47 ml spray dilution/person | | | | |
| Resident dermal spray drift exposure 75th percentile - child | 0.327 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure 75th percentile - adult | 0.00010 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure 75th percentile - child | 0.00022 ml spray dilution/person | | | | |
| Resident dermal spray drift exposure mean - adult | 0.22318 ml spray dilution/person | | | | |
| Resident dermal spray drift exposure mean - child | 0.18 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure mean - adult | 0.00009 ml spray dilution/person | | | | |
| Resident inhal. spray drift exposure mean - child | 0.00017 ml spray dilution/person | | | | |
| Exposure duration dermal | 2 hours | | | | d_ReExpDur |
| Exposure duration inhalation | 24 hours | | | | d_ReExpDurInhal |
| Exposure duration entry into treated crops | 0.25 hours | | | | d_ExpDurTreatCrop |
| Light clothing adjustment factor | 18.0% | | | | d_ClothAF |
| Breathing rate adult | 0.23 m³/day/kg | | | | d_BreathRAD |
| Breathing rate child (1-3 year old) | 1.07 m³/day/kg | | | | d_BreathRCh |
| Drift percentage on surface (75th percentile) | 5.60% | | | | |
| Drift percentage on surface (mean) | 4.10% | | | | |
| Turf transferable residues percentage | 5.00% | | | | d_Turf |
| Transfer coeff. of surface deposits-adult | 7300 cm²/hour | | | | d_ReTCAd |
| Transfer coeff. of surface deposits-child (1-3 year old) | 2600 cm²/hour | | | | d_ReTCCh |
| Saliva extraction percentage | 50.00% | | | | d_SalExt |
| Surface area of hands mouthed | 20 cm² | | | | d_AreaoHM |
| Frequency of hand to mouth activity | 9.5 events/hour | | | | d_ReFreqHM |
| Ingestion rate for mouthing of grass per day | 25 cm² | | | | d_MouthGrass |
| Dislodgeable residues percentage transferability for object to mouth | 20.00% | | | | d_DRP |
| Transfer coefficient for entry into treated crops (75th percentile) - ad | 7500 cm²/h | | | | d_TcEntryAd |
| Transfer coefficient for entry into treated crops (75th percentile) - chi | 2250 cm²/h | | | | d_TcEntryCh |
| Transfer coefficient for entry into treated crops (mean) - adult | 5980 cm²/h | | | | d_TcEntryAd |
| Transfer coefficient for entry into treated crops (mean) - child | 1794 cm²/h | | | | d_TcEntryCh |
| | | | | | |
| 1. Total | | | | | |
| 1.1 1-3 year old child | | | | | |
| Spray drift (75th percentile) | | Vapour (75th percentile) | Surface deposits (75th percentile) | Entry into treated crops (75th percentile) | All pathways (mean) |
| Total systemic exposure (mg a.s./day) | 0.0631676 | 0.0107000 | 0.0128604 | 0.0615188 | 0.0701827 |
| Total systemic exposure per kg body weight (mg a.s./day/kg) | 0.0063168 | 0.0010700 | 0.0012860 | 0.0061519 | 0.0070183 |
| % of RVNAS | 31.58% | 5.35% | 6.43% | 30.76% | 35.09% |
| | | | | | |
| 1.2 Adult | | | | | |
| Spray drift | | Vapour | Surface deposits | Entry into treated crops | All pathways (mean) |
| Total systemic exposure (mg a.s./day) | 0.0899805 | 0.0138000 | 0.0190092 | 0.0424313 | 0.1130355 |
| Total systemic exposure per kg body weight (mg a.s./day/kg) | 0.0014997 | 0.0002300 | 0.0003168 | 0.0007072 | 0.0018839 |
| % of RVNAS | 7.50% | 1.15% | 1.58% | 3.54% | 9.42% |
| | | | | | |

Appendix 4 Detailed evaluation of exposure and/or DFR studies relied upon (KCP 7.2, KCP 7.2.1.1, KCP 7.2.2.1, KCP 7.2.3.1)

Not relevant:

Reviewer comment: This additional NDE zRMS assessment consider new dermal absorption value calculated for 2,4D to reflect composition change:

| | | | | | |
|--|---|--|--|--------------------------------|--|
| Substance | 2,4D | Formulation = Soluble concentrates, emulsifiable concentrate, etc. | Application rate- 0,75 kg a.s. /ha | Spray dilution = 3,75 g a.s./l | Vapour pressure = low volatile substances having a vapour pressure of <5*10 ⁻³ Pa |
| Scenario | Cereals / Outdoor / Downward spraying / Vehicle-mounted | | | Buffer = 2-3 | Number applications = 1, Application interval = 365 days |
| Percentage Absorption | Dermal for product = 0,4 | Dermal for in use dilution = 6,2 | Oral = 100 | Inhalation = 100 | |
| RVNAS | 0,02 mg/kg bw/day | | RVAAS | mg/kg bw/day | |
| DFR | 3 µg a.s./cm ² per kg a.s./ha | | DT50 | 30 days | |
| | | | | | |
| Operator Model | | Mixing, loading and application AOEM | | | |
| Potential exposure | Longer term systemic exposure mg/kg bw/day | | 0,0178 | % of RVNAS | 89,21% |
| | Acute systemic exposure mg/kg bw/day | | 0,0860 | % of RVAAS | |
| Mixing and Loading | | Gloves = Yes | Clothing = Work wear - arms, body and legs covered | RPE = None | Soluble bags = No |
| Application | | Gloves = No | Clothing = Work wear - arms, body and legs covered | RPE = None | Closed cabin = No |
| Exposure (including PPE options above) | Longer term systemic exposure mg/kg bw/day | | 0,0065 | % of RVNAS | 32,34% |
| | Acute systemic exposure mg/kg bw/day | | 0,0368 | % of RVAAS | |
| | | | | | |
| Worker - Inspection, irrigation | Potential exposure mg/kg bw/day | | 0,0581 | % of RVNAS | 290,63% |
| | Working clothing mg/kg bw/day | | 0,0065 | % of RVNAS | 32,55% |
| | Working clothing and gloves mg/kg bw/day | | | % of RVNAS | |
| | | | | | |
| Resident - child | Spray drift (75th percentile) mg/kg bw/day | | 0,0063 | % of RVNAS | 31,58% |
| | Vapour (75th percentile) mg/kg bw/day | | 0,0011 | % of RVNAS | 5,35% |
| | Surface deposits (75th percentile) mg/kg bw/day | | 0,0013 | % of RVNAS | 6,43% |
| | Entry into treated crops (75th percentile) mg/kg bw/day | | 0,0078 | % of RVNAS | 39,23% |
| | All pathways (mean) mg/kg bw/day | | 0,0118 | % of RVNAS | 58,82% |
| Resident - | Spray drift (75th percentile) mg/kg bw/day | | 0,0015 | % of RVNAS | 7,50% |

| | | | | |
|--------------|---|--------|------------|--------|
| adult | Vapour (75th percentile) mg/kg bw/day | 0,0002 | % of RVNAS | 1,15% |
| | Surface deposits (75th percentile) mg/kg bw/day | 0,0003 | % of RVNAS | 1,58% |
| | Entry into treated crops (75th percentile) mg/kg bw/day | 0,0044 | % of RVNAS | 21,80% |
| | All pathways (mean) mg/kg bw/day | 0,0047 | % of RVNAS | 23,26% |