REGISTRATION REPORT

Part B
Section 9
Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.06001.H.2.B

Product name(s): EDAPTIS

Chemical active substances:

Mesosulfuron-methyl, 12 g/L

Pinoxaden, 60 g/L

Safener:

Mefenpyr-diethyl, 35 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Sponsor: ADAMA Agan Ltd.

Applicant: Country organisation / representative of ADAMA,

as given in Part A

Submission date: June 2021, updated: September 2022, April 2023

MS Finalisation date: May 2023 (initial Core Assessment) September 2023 (final Core Assessment), updated December 2023

Version history

When	What
June 2021	First version submitted by applicant
September 2022	9.5.2 updated aquatic risk assessment following comments of zRMS regarding PECsw calculations
April 2023	Following comments from Poland the aquatic mixture toxicity assessment for all scenarios was updated. Formulation endpoints for algae and Lemna were recalculated considering mean measured concentration for boh active substances and the assessment was adapted accordingly.
May 2023	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
September 2023	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the Applicant are highlighted in yellow. Information no longer relevant are ~~struck through~~ and shaded for transparency.
December 2023	Final report (Core Assessment updated following the **second** 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 green. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

DATA PROTECTION CLAIM

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

STATEMENT FOR OWNERSHIP

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops) *																				
~~1~~	~~AT, DE, BE, NL, CZ, PL, HU, IE~~	~~Winter wheat, rye, triticale~~	~~F~~	~~ALOMY, APESV, AVESS, BROSS, POAAN, POATR, Broad-leaved weeds~~	~~Foliar, spraying, overall~~	~~BBCH 13-20 (spring)~~	~~a) 1 b) 1~~	~~-~~	~~a) 0.75 L/ha b) 0.75 L/ha~~	~~a) 9 / 45 g/ha b) 9 / 45 g/ha~~	~~80 / 300~~		~~Mefenpyr-diethyl applied as a safener at 26.3 g/ha In PL applied also in tank mix with adjuvat Insert : 0,5-1,0 + 0,2 l/ha (Insert) And with Camaro 306 SE: 0,5 + 0,5 l/ha (Camaro 306 SE)~~							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
2	AT, DE, BE, NL, CZ, PL, HU, IE	Winter wheat, rye, triticale	F	ALOMY, APESV, AVESS, BROSS, POAAN, POATR, Broad-leaved weeds	Foliar, spraying, overall	BBCH 20-39 (spring)	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 12 / 60 g/ha b) 12 / 60 g/ha	80 / 300		Mefenpyr-diethyl applied as a safener at 35.0 g/ha In PL applied also in tank mix with adjuvat Insert : 0,5-1,0 + 0,2 l/ha (Insert) And with Camaro 306 SE: 0,5 + 0,5 l/ha (Camaro 306 SE)	A	A	R	A	A	A	C
3	AT, DE, BE, NL, CZ, PL, HU, IE	Spring wheat	F	ALOMY, APESV, AVESS, BROSS, POAAN, POATR, Broad-leaved weeds	Foliar, spraying, overall	BBCH 13-39 (spring)	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 12 / 60 g/ha b) 12 / 60 g/ha	80 / 300		Mefenpyr-diethyl applied as a safener at 35.0 g/ha In PL applied also in tank mix with adjuvat Insert : 0,5-1,0 + 0,2 l/ha (Insert) And with Camaro 306 SE: 0,5 + 0,5 l/ha (Camaro	A	A	R	A	A	A	C

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													306 SE)							
3*	AT, DE, BE, NL, CZ, PL, HU, IE	Spring wheat	F	ALOMY, APESV, AVESS, BROSS, POAAN, POATR, Broad-leaved weeds	Foliar, spraying, overall	BBCH 13-39 (spring)	a) 1 b) 1	-	a) 0.75 L/ha b) 0.75 L/ha	a) 9 / 45 g/ha b) 9 / 45 g/ha	80 / 300		Mefenpyr-diethyl applied as a safener at 26.3 g/ha	A	A	R	A	A	A	C

* 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

Explanation for column 15 – 21 “Conclusion”

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

***zRMS comments:**

Originally the GAP table presented by the Applicant includes the use no 1. However, the zRMS **in Section 8** asked the Applicant to modified the GAP table, since the use of ADM.06001.H.2.B in winter cereals at BBCH 13-20 is predicted for autumn instead of spring. It must be stressed out that before winter, cereals must develop leaves so it is not possible to apply plant protection products at BBCH 13 during the spring, since this stage will be reached before winter. Consequently, the Applicant was asked to modified the BBCH stages for application to winter cereals for the earliest BBCH stage relevant for spring which is reflected at BBCH 20. Taking this into account the original GAP table has been modified by the zRMS in order the data are consist with information presented in the Core Assessment, Part B, Section 0.

During the commenting period Applicant modified the GAP table for use in spring cereals by considering the additional lower application rate of 0.75 L/ha for the product which thus added in the Table 9-1-1.

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

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

Based on screening step assessments, the acute and chronic risks to small omnivorous birds from exposure to food stuffs contaminated with the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B to winter and spring cereals are acceptable.

The risk to birds from exposure to the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl in drinking water from puddles is acceptable.

No risk for secondary poisoning of earthworm-eating and fish-eating birds is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

Based on screening step assessments, the acute and chronic risks to small herbivorous mammals from exposure to food stuffs contaminated with the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B to winter and spring cereals are acceptable.

The risk to mammals from exposure to the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl in drinking water from puddles is acceptable.

No risk for secondary poisoning of earthworm-eating and fish-eating mammals is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

~~Acceptable risks for aquatic organisms from exposure to ADM.06001.H.2.B following application to winter cereals are indicated with a 10 m no spray buffer with a 10 m vegetative strip or a 5m no spray buffer with 5m VFS mod, except for scenarios D1 and D2. The route of exposure is drainage in these scenarios. Mitigation measures provided at Step 4 will not reduce these PEC_{sw} values.~~
~~Acceptable risks for aquatic organisms from exposure to ADM.06001.H.2.B following application to spring cereals are indicated with a 20 m no spray buffer with a 20 m vegetative strip or a 5m no spray buffer with 5m VFS mod, except for scenarios D1 and D2.~~
~~The scenarios D1 and D2 are not relevant for the central zone.~~

The risk assessment for aquatic organism for pinoxaden and its metabolites as well as for safener mefenpyr diethyl demonstrated an acceptable risk without risk mitigation measures.

Based on the calculations of the risk assessment for aquatic organism for mesosulfuron-methyl the following conclusions has been derived:

1. Winter cereals at BBCH 20-39:

- acceptable risk with no need for risk mitigation measures: D3, D4, D5, R1, R4 scenarios
- scenario R3: risk acceptable with 10 m VFS
- scenarios: D1, D2, D6 an unacceptable risk

2. Winter cereals at BBCH 35-39:

- acceptable risk with no need for risk mitigation measures: D1 (stream), D3, D4, D5, D6, R1 scenarios
- scenario R3: risk acceptable with 10 m VFS
- scenario R4: risk acceptable with 10 m VFS

3. Spring cereals at BBCH 13-39:

- acceptable risk with no need for risk mitigation measures: D3, D4, R4, D5 scenarios
- ~~scenario R4: risk acceptable with 20 m VFS~~
- scenarios D1: risk an unacceptable risk

4. Spring cereals at BBCH 35-39:

- acceptable risk with no need for risk mitigation measures: ~~D1 (ditch)~~, D3, D4, D5 scenarios
- scenario R4: risk acceptable with 20 m VFS
- scenario D1 (stream), D1 (ditch): risk an unacceptable risk

It should be noted that the risk from the mixture toxicity assessment is acceptable for aquatic invertebrates and algae at the screening step.
For aquatic macrophytes the a.s.-mesosulfuron-methyl is driving the toxicity (contributes to more than 90% of the toxicity).

It can be concluded that the mitigations from the active substance mesosulfuron-methyl are relevant and cover mixture toxicity assessment.

Please note that Additional calculations may be required by cMS that do not accept surface water exposure derived using FOCUS models.

The acceptability and applicability of the indicated risk mitigation measures has to be confirmed at the cMS level.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risks to bees from the use of the active substances mesosulfuron-methyl and pinoxaden applied as the formulation ADM.06001.H.2.B to winter and spring cereals is acceptable.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Acceptable in-field risk is indicated based on studies with *Typhlodromus pyri* (standard laboratory test, Tier 1) and *Aphidius rhopalosiphi* (standard and extended laboratory tests, Tier 1 and Tier 2), after application of the formulation ADM.06001.H.2.B. Furthermore, acceptable effects on arthropods are expected in the off-crop area without the consideration of risk mitigation measures, i.e. for the default distance of 1 m.

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

The risks to non-target soil organisms from the use of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B in winter and spring cereals are acceptable.
The risks for soil microorganisms from the use of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B in winter and spring cereals are acceptable.

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

~~The off-field risks to non-target plants from the use of ADM.06001.H.2.B in winter and spring cereals are acceptable without risk mitigation measures.~~

Based on the probabilistic risk assessment the risk for non-target terrestrial plants is considered acceptable with no buffer zone or drift reducing spraying equipment for all proposed uses in cereals.
It is the position of the zRMS-PL that a trigger value of 1 should be used in the probabilistic risk

assessment with a HR5 value; however, it is noted that this is not a Central Zone harmonised position and other member states may consider the use of a different trigger value at National Registration.
Risk mitigation measures based on deterministic assessment of the risk for non-target plants due to the use of ADM.06001. H.2.B at max. application rate of 1 L/ha in cereals are as follows:

- 5 m buffer zone, or alternatively 50% drift reducing spray nozzles and for lower rate 0.75 L/ha the risk mitigation measures are not required.

~~The final decision if risk mitigation measures are left at MS level.~~

The additional calculations of the probabilistic risk for non-target plants performed after commenting period process due to the use of ADM.06001.H.2.B in winter and spring cereals using refined HC_5 endpoint (and lower limit) based on plant dry weight endpoint confidence intervals and including unbound values indicated that the risk mitigation measures are still not required to ensure acceptable risks to non-target plants when trigger value of 1 is applied.

Risk mitigation measures based on probabilistic assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in cereals considering phytotoxicity endpoints are as follows:

- 1 x 0.75 L product/ha using HC5: no mitigations needed
- 1 x 0.75 L product/ha using HC5 lower limit: no mitigations needed
- 1 x 1 L product/ha using HC5: no mitigations needed
- 1 x 1 L product/ha using HC5 lower limit: 5 m buffer zone, or alternatively 50% drift reducing spray nozzles

The conclusion if/which risk mitigations measures are required depends on MS decisions concerning the relevant metric/trigger used for risk assessment.

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

No further data on effects of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl or the formulation ADM.06001.H.2.B on other terrestrial organisms are available.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011). All uses are spring application.

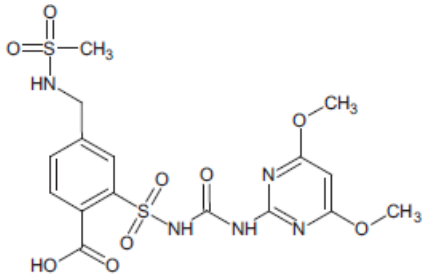
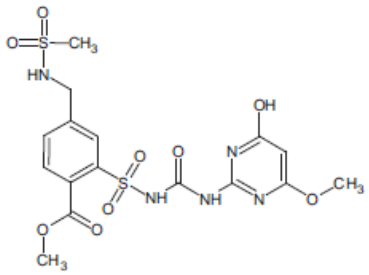
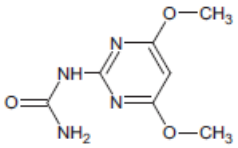
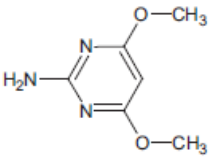
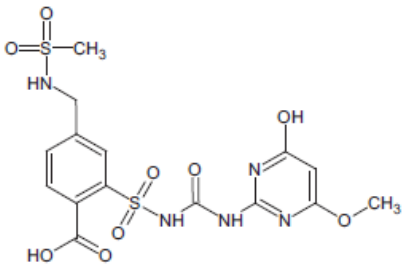
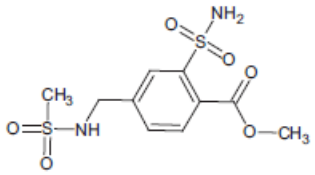
Table 9.1-2: Critical use pattern of ADM.06001.H.2.B

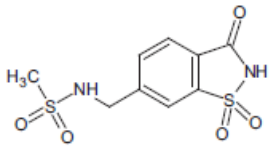
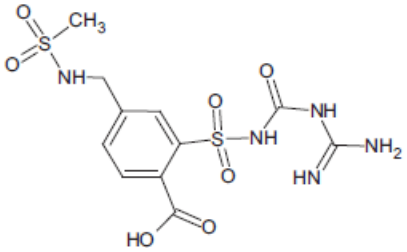
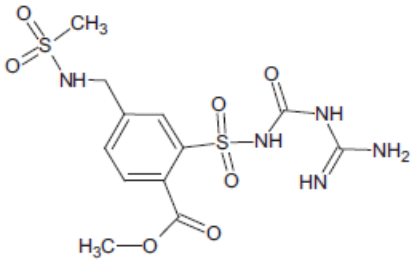
Grouping according to criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Effects on birds and mammals (9.2 and 9.3)			
Cereals	Max. 1 x 1 L product/ha, BBCH 13-39	Crop group according to EFSA/2009/1438	Maximum application rate of 1 L product/ha per season
Effects on aquatic organisms (9.5)			
Winter cereals and spring cereals	Winter cereals: 1 x 1 L product/ha, BBCH 20-39, BBCH 35-39 Spring cereals: 1 x 1 L product/ha, BBCH 13-39, BBCH 35-39	Crop groups according to FOCUS (2001 & 2015): winter cereals and spring cereals	Maximum application rate of 1 L product/ha per season
Effects on bees (9.6)			
Field crops	Max. 1 x 1 L product/ha, BBCH 13-39	Field crops according to ESCORT 2 (2000)	Maximum application rate of 1 L product/ha per season
Effects on non-target arthropods (9.7)			
Field crops	Max. 1 x 1 L product/ha, BBCH 13-39	Field crops according to ESCORT 2 (2000)	Maximum application rate of 1 L product/ha per season
Effects on terrestrial soil meso- and macrofauna (9.8) and (9.9)			
Winter cereals and spring cereals	1 x 1 L product/ha, BBCH 13-39	Crop interception values according EFSA Journal 2014;12(5):3662	BBCH 10-19: 0% crop interception Maximum application rate of 1 L product/ha per season
Effects on terrestrial non-target plants (9.10)			
Field crops	Max. 1 x 1 L product/ha, BBCH 13-39	Field crops according to ESCORT 2 (2000)	Maximum application rate of 1 L product/ha per season

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of ADM.06001.H.2.B is indicated in the table.

Table 9.1-3 Metabolites of mesosulfuron-methyl

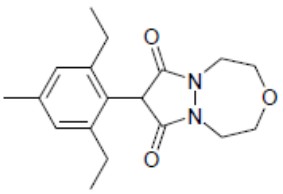
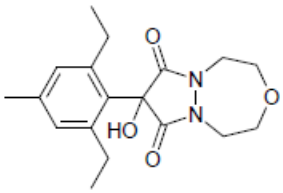
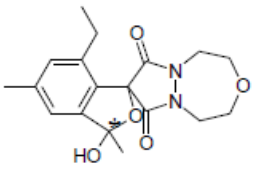
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Mesosulfuron (AE 154851)		489.5	Soil: 16.2% (aerobic soil) Water/Sediment: 4.9% (total system)	Yes, soil and aquatic organisms
AE F160459		489.5	Soil: 8.9% (aerobic, > 5% in > 2 sequential measurements), 25.9% (anaerobic) Water/Sediment: 21.6% (total system)	Yes, soil and aquatic organisms
AE F099095		198.2	Soil: 29.2% (aerobic) Water/Sediment: 0.9% (total system)	Yes, soil and aquatic organisms
AE F092944		155.2	Soil: 10.1% (aerobic) Water/Sediment: 3.2% (total system)	Yes, soil and aquatic organisms
AE F160460		475.5	Soil: 8.6% (aerobic, > 5% in > 2 sequential measurements) Water/Sediment: 8.4% (total system, > 5% in > 2 sequential measurements)	Yes, soil and aquatic organisms
AE F140584		322.4	Soil: 5.1% (aerobic, > 5% in 1 measurement only)	Yes, soil and aquatic organisms

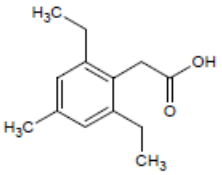
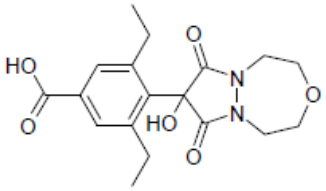
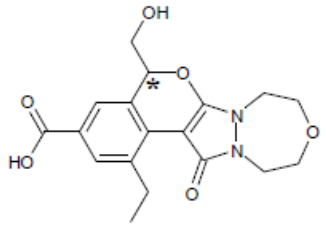
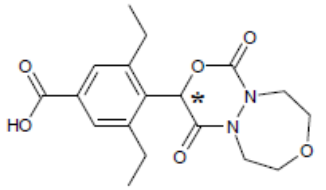
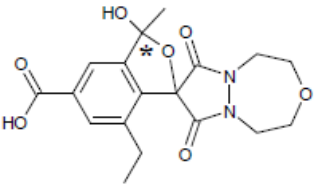
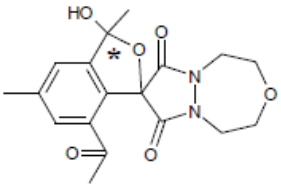
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE F147447		290.3	Soil: 5.8% (aerobic, > 5% in > 2 sequential measurements), 6.5% (anaerobic, maximum of formation not yet reached at the end of the study) Water/Sediment: 10.9% (total system)	Yes, soil and aquatic organisms
BCS-CV14885		393.4	Water/Sediment: 22.0% (total system)	Yes, aquatic organisms
BCS-CO60720		407.4	Water/Sediment: 13.1% (total system)	Yes, aquatic organisms

zRMS comments:

Information regarding metabolites of mesosulfuron-methyl, provided in Table 9.1-3 above is in line with EU agreed endpoints reported in EFSA Journal 2016;14(10):4584.

Table 9.1-4 Metabolites of pinoxaden

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
NOA 407854 (M2)		316.4	Soil: 89.7% (aerobic), 94.4% (anaerobic) Water/Sediment: 98.8% (total system), 86.9% (water), 26.0% (sediment)	Yes, soil and aquatic organisms
NOA 447204 (M3)		332.4	Soil: 30.6% (aerobic) Water/Sediment: 9.7% (total system, > 5% in 2 sequential measurements, < 5% in water or sediment at all sample times) Lysimeter leachate: 0.206 µg/L	Yes, soil and aquatic organisms
SYN 515622			Soil: 20.4% (soil photolysis)	Not relevant according to EFSA Journal 2013;11(8): 3269)

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
NOA 437397			Soil: 6.7% (soil photolysis, maximum of formation not yet reached at the end of the study)	Not relevant according to EFSA Journal 2013;11(8): 3269)
M11		362.4	Lysimeter leachate: 0.06 µg/L	Yes, aquatic organisms
M52		360.3	Lysimeter leachate: 0.130 µg/L	Yes, aquatic organisms
M54		362.4	Lysimeter leachate: 0.150 µg/L	Yes, aquatic organisms
M55		376.4	Lysimeter leachate: 0.134 µg/L	Yes, aquatic organisms
M56		360.4	Lysimeter leachate: 0.266 µg/L	Yes, aquatic organisms

zRMS comments:

Information regarding metabolites of pinoxaden provided in Table 9.1-4 above is in line with EU agreed endpoints reported in EFSA Journal 2013;11(8):3269.

According to the EFSA 2013 conclusion the only ecotoxicologically relevant metabolites are:
Soil: None
Surface water: M2
Groundwater: M2, M3, M11, M52, M54, M55 and M56

The following confirmatory data requirement applies:

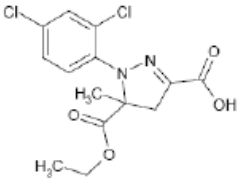
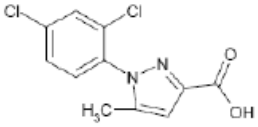
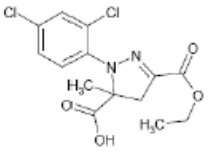
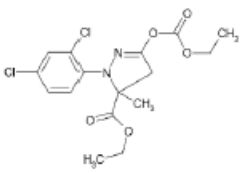
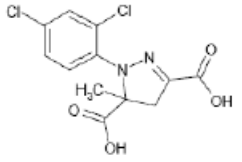
This issue formed part of the confirmatory data requirement set in Commission Implementing Regulation (EU) 2016/370, as follows;

In this overall assessment Member States shall pay particular attention to the protection of groundwater, when the substance is applied in regions with vulnerable soil and/or climatic conditions.
The Member States concerned shall carry out monitoring programmes to verify potential groundwater contamination from the metabolite M2 in vulnerable zones, where appropriate.

The applicant referred to the new studies regarding the metabolites of pinoxaden (earthworms, *Folsomia candida* and *Hypoaspis aculeifer*).

This product assessment should be carried out according to the currently agreed EU endpoints for pinoxaden (EFSA Journal 2013;11(8):3269) and new studies were not taken into account by zRMS in the current Core Dossier.

Table 9.1-5 Metabolites of mefenpyr-diethyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
AE F113225		345.2	Soil: 44.1% (aerobic), 46.7% (anaerobic) Water/Sediment: 74.9% (water), 18.0% (sediment), 82.8% (total system)	Yes, soil and aquatic organisms
AE F094270		271.11	Soil: 72.2% (aerobic), 34.9% (anaerobic) Water/Sediment: 28.5% (water), 33.9% (sediment), 62.4% (total system)	Yes, soil and aquatic organisms
AE F114952		345.18	Soil: 11.5% (aerobic) Water/Sediment: 17.3% (water), 3.8% (sediment), 18.6% (total system)	No, AE F114952 is an isomer of AE F113225, assessment covered by AE F113225
AE F2211046		391.26	Soil: 11% (soil photolysis) Water/Sediment: 40.7% (aqueous photolysis)	Yes, soil and aquatic organisms
AE F109453		317.13	Water/Sediment: 42.0% (water), 5.6% (sediment, > 5% in 2 sequential measurements), 46.5% (total system)	Yes, aquatic organisms

zRMS comments:

Information regarding metabolites of mefenpyr diethyl provided in Table 9.1-5 above is in line with the Monograph (list of endpoints) October 2011.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substances mesosulfuron-methyl and pinoxaden and the pinoxaden metabolite NOA 407854 as well as with the safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU DAR and related documents (Listed in B0).
Effects on birds of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. However, the provision of further data on ADM.06001.H.2.B is not considered essential, because the toxicity of the formulation to birds can be extrapolated from the data on the active substances mesosulfuron-methyl and pinoxaden and the pinoxaden metabolite NOA 407854 as well as from the data on the safener mefenpyr-diethyl.

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

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

Species	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
Colinus virginianus	Mesosulfuron-methyl	Oral Acute	**LD_{50} > 2000 mg a.s./kg bw**	EFSA Conclusion 4584/2016
Anas platyrhynchos	Mesosulfuron-methyl	Oral Acute	**LD_{50} > 2000 mg a.s./kg bw**	EFSA Conclusion 4584/2016
Colinus virginianus	Mesosulfuron-methyl	Dietary Reproductive toxicity	**NOEL = 93 mg a.s./kg bw/d**	EFSA Conclusion 4584/2016
Anas platyrhynchos	Mesosulfuron-methyl	Dietary Reproductive toxicity	NOEL = 126 mg a.s./kg bw/d (maximum test concentration, no effect on growth or reproduction)	EFSA Conclusion 4584/2016
Pinoxaden				
Anas platyrhynchos	Pinoxaden	Oral Acute	**LD_{50} > 2250 mg a.s./kg bw**	EFSA Conclusion 3269/2013
Anas platyrhynchos	NOA 407854	Dietary Reproductive toxicity	NOEL = 150.2 mg/kg bw/d	EFSA Conclusion 3269/2013
Colinus virginianus	NOA 407854	Dietary Reproductive toxicity	**NOEL = 27.8 mg/kg bw/d**	EFSA Conclusion 3269/2013
Mefenpyr-diethyl				
Coturnix coturnix japonica	Mefenpyr-diethyl	Oral Acute	**LD_{50} = 3776 mg/kg bw[a]**	Proposed in Monograph (list of endpoints) Oct 2011[b]
Anas platyrhynchos	Mefenpyr-diethyl	Oral Acute	**LD_{50} = 3776 mg/kg bw[a]**	Proposed in Monograph (list of endpoints) Oct 2011[b]
Coturnix coturnix japonica	Mefenpyr-diethyl	Dietary Reproductive toxicity	**NOEL = 106 mg/kg bw/d**	Proposed in Monograph (list of endpoints) Oct 2011[b]

Endpoints in bold are used in the risk assessment
[a] 10 birds per group; no mortality during study. LD50 value extrapolated with a factor of 1.888 according to EFSA

guidance

[b] Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl.

zRMS comments:

Avian toxicity data are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 for mesosulfuron-methyl and EFSA Journal 2013;11(8):3269 for pinoxaden and its metabolite and

Mixture toxicity

Besides the risk assessment for the active substances mesosulfuron-methyl and pinoxaden as well as the safener mefenpyr-diethyl, risk assessment has been performed for the formulation by calculation of a surrogate LD_{50} for acute effects of the mixture. This surrogate LD_{50} was calculated assuming dose or concentration additivity of toxicity of the active substances and the safener (based on the worst-case assumption that the substances have the same mode of action). Since mefenyr-diethyl is a safener rather than an active substance, a surrogate LD_{50} was additionally calculated for the two active substances mesosulfuron-methyl and pinoxaden alone. For chronic/reproductive toxicity, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment (EFSA/2009/1438). Results of mixture toxicity calculations are presented in the following table.

Table 9.2-2: Mixture toxicity calculation for acute effects (mortality)

Time scale	Substance	Fraction in formulation	LD_{50} (mg/kg bw)	Toxicity per fraction	Contribution to overall toxicity (%)	Surrogate LD_{50} for ADM.06001.H.2.B (mg/kg bw)
acute	Mesosulfuron-methyl	0.112	>2000	17833.3	14.3	2552
	Pinoxaden	0.561	>2250	4012.5	63.6	
	Mefenpyr-diethyl	0.327	3776	11543.8	22.1	
acute	Mesosulfuron-methyl	0.167	>2000	12000	18.4	2204
	Pinoxaden	0.833	>2250	2700	81.6	

zRMS comments:

Acute combined endpoint

The calculations of the acute combined risk assessment have been accepted by zRMS.

Long-term combined endpoint:

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD_{50}, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.
Therefore, the calculated $NOEl_{mix}$ is not required by zRMS in the current risk assessment.
It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to birds. Approach taken with regard to the long-term combined risk assessment represents worst case for mixture of active substances and is in general acceptable.
However, the TER_{mix} approach is also agreed option of combined risk assessment in Central Zone.
For this reason, zRMS used this approach in the combined risk assessment.

9.2.1.1 Justification for new endpoints

No new endpoints were used in the assessment of risk to birds from the use of ADM.06001.H.2.B in accordance with the proposed GAP.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for birds from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

For the formulation ADM.06001.H.2.B, acute risk for birds has been assessed using the surrogate LD_{50} assuming dose or concentration additivity of toxicity (mixture toxicity; please see above) of the active substances and the safener in the virtual compound. The content of the active substances and the safener in the formulation and application rate per hectare have been expressed in terms of this virtual compound, i.e. application rate per hectare is the sum of the application rates of the the active substances and the safener (surrogate application rate: 107 g sum of a.s. + safener/ha). In addition, acute risk for birds has been assessed using the surrogate LD_{50} assuming dose or concentration additivity of toxicity of the active substances only in the virtual compound. In this case, the application rate per hectare is the sum of the application rates of the active substances (surrogate application rate: 72 g sum of a.s./ha).

For pinoxaden, the long-term/reproductive risk for birds has been addressed using the dietary reproductive toxicity endpoint for its metabolite NOA 407854 due to the fast metabolisation of pinoxaden to NOA 407854.

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

The results of the acute and reproductive screening assessments are summarised in the following table.

In accordance with EFSA/2009/1438, the reproductive risk assessments have not been calculated with $LD_{50}/10$ as the NOAEL from the avian reproduction studies are lower than the $LD_{50}/10$ from the avian acute studies.

Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	**Winter and spring cereals, BBCH 13-39**				
Active substance/product	Mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl (safener)/ ADM.06001.H.2.B				
Application rate (g/ha)	12 g a.s./ha (mesosulfuron-methyl), 60 g a.s./ha (pinoxaden), 35 g a.s./ha (mefenpyr-diethyl)/107 g sum of a.s. + safener/ha (surrogate application rate for ADM.06001.H.2.B including safener)/72 g sum of a.s./ha (surrogate application rate for ADM.06001.H.2.B excluding safener)				
Acute toxicity (mg/kg bw)	> 2000 (mesosulfuron-methyl), > 2250 (pinoxaden), 3776 (mefenpyr-diethyl)/ 2552 (surrogate endpoint for ADM.06001.H.2.B including safener)/2204 (surrogate endpoint for ADM.06001.H.2.B excluding safener)				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Mesosulfuron-methyl					
Cereals	Small omnivorous bird	158.8	1	1.906	> 1050

BBCH 13-39	Screening assessment				
Pinoxaden					
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	158.8	1	9.528	> 236
Mefenpyr-diethyl					
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	158.8	1	5.558	679
ADM.06001.H.2.B					
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	158.8	1	16.99	150[a]
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	158.8	1	11.43	193[b]
Reprod. toxicity (mg/kg bw/d)	93 (mesosulfuron-methyl), 27.8 (pinoxaden metabolite NOA 407854), 106 (mefenpyr-diethyl)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Mesosulfuron-methyl					
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	64.8	1 x 0.53	0.412	226
Pinoxaden metabolite NOA 407854					
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	64.8	1 x 0.53	2.061	13.5
Mefenpyr-diethyl					
Cereals BBCH 13-39	Small omnivorous bird Screening assessment	64.8	1 x 0.53	1.202	88.2

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

[a] Calculated with the surrogate endpoint for ADM.06001.H.2.B including safener

[b] Calculated with the surrogate endpoint for ADM.06001.H.2.B excluding safener

Based on screening step assessments, the acute and chronic risks to small omnivorous birds from exposure to food stuffs contaminated with the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B to winter and spring cereals are acceptable.

zRMS comments:

Screening step in the risk assessment

TER_A and TER_{LT} values for the exposure to active substances, pinoxaden metabolite NOA 407854 and safener are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

Acute combined risk assessment:

The calculated the LD_{50}mix values with consideration of relevant toxicity endpoints such as: 2552 mg/kg bw (surrogate endpoint for ADM.06001.H.2.B including safener) and 2204 mg kg/bw (surrogate endpoint for ADM.06001.H.2.B excluding safener, for details, see commenting box in point 9.2.1.1 above) and the acute risk assessment in Table 9.2-3 has been accepted by zRMS.

Combined risk assessment:

TER_{mix} approach was taken by zRMS for combined risk assessment with regard the active substance mesosulfuron-methyl, pinoxaden metabolite NOA 407854 and safener.

The relevant calculations are provided below:

TERmix assessment of long-term/reproductive risk for birds due to the use of ADM.06001.H.2.B in winter and spring cereals.

						Σ1/TER	Σ1/TER^{-}	Trigger
Mesosulfuron-methyl		**Pinoxaden metabolite NOA 407854**		**Mefenpyr-diethyl**				
226 [1)]	0.0044	13.5 [1)]	0.0740	88.2[1)]	0.011	0.0894	11.18 [1)]	5

[1)] TER_{LT} values calculated at screening step.

Overall, based on performed calculations of refined TER_{mix} value acceptable combined long-term risk may be concluded for birds.

9.2.2.2 Higher-tier risk assessment

Higher tier risk assessments for the uses of ADM.06001.H.2.B in winter and spring cereals are not required as the screening step risk assessments indicate acceptable risks for both acute and chronic exposure to birds.

9.2.2.3 Drinking water exposure

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

Leaf scenario
Since ADM.06001.H.2.B is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

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

With a K(f)oc of 64 mL/g (geometric mean) for mesosulfuron-methyl, 323 mL/g (median) for pinoxaden and 619 mL/g (median) for mefenpyr-diethyl, mesosulfuron-methyl and pinoxaden belong to the group of less sorptive substances and mefenpyr-diethyl belongs to the group of more sorptive substances. The pinoxaden metabolite NOA 407854 has a K(f)oc of 6.0 mL/g (median) and, therefore, also belong to the group of less sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for birds from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Mesosulfuron-methyl				
Effective application rate (g/ha)	=	12		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.006
Reprod. toxicity (mg/kg bw/d)	=	93	quotient =	0.129
Pinoxaden				
Effective application rate (g/ha)	=	60 (pinoxaden)		
Acute toxicity (mg/kg bw)	=	2250 (pinoxaden)	quotient =	0.027
Reprod. toxicity (mg/kg bw/d)	=	27.8 (NOA 407854)	quotient =	2.158
Mefenpyr-diethyl				
Effective application rate (g/ha)	=	35		
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.009
Reprod. toxicity (mg/kg bw/d)	=	106	quotient =	0.330

No specific calculations of exposure and TER are necessary as the ratios of effective application rate to acute and reprotoxic endpoints for birds are less than 50 in the case of mesosulfuron-methyl and pinoxaden (and its metabolite NOA 407854) and less than 3000 in the case of mefenpyr-diethyl.

zRMS comments:

The leaf scenario does not have to be considered taking into account the proposed uses (cereals).
The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is < 50 in case of mesosulfuron-methyl and pinoxaden (and its metabolite) and <3000 in the case of mefenpyr-diethyl.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of mesosulfuron-methyl ranges between 1.90 at pH 4 and -2.10 at pH 10 (25°C; EFSA Journal 2016;14(10):4584) and thus does not exceed the trigger value of 3. For pinoxaden, the log Pow is 3.2 (25°C, pH effect not determined as no dissociation observed; EFSA Journal 2013;11(8):3269) and is therefore slightly above the trigger value of 3. The log P_{ow} of mefenpyr-dietyl amounts to 3.83 (21°C, pH 6.3; proposed in Monograph (list of endpoints) Oct 2011 - voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl) and thus exceeds the trigger value of 3. Therefore, a risk assessment for effects due to secondary poisoning is formally required for pinoxaden and mefenpyr-diethyl.

In the case of pinoxaden, assessment of the risk for earthworm- and fish-eating birds via secondary poisoning is however not considered required due to the following reasons. Pinoxaden degrades rapidly in soil (DT_{50}: 1.01 days, EFSA Conclusion 3269/2013) and water (DT_{50}: 0.28 days, EFSA Conclusion 3269/2013). Therefore, neither long-term exposure of earthworms and fish nor bioaccumulation in these species are expected for pinoxaden. For this reason, the dietary reproductive toxicity study was performed with the pinoxaden metabolite NOA 407854. Exposure of birds to the pinoxaden metabolite NOA 407854 via consumption of earthworms and fish is however expected to be low as this metabolite is not considered to bioaccumulate in fat tissue due to its log P_{ow} of -1.1 (EFSA Conclusion 3269/2013). Consequently, no bioconcentration factor in fish is available for the pinoxaden metabolite NOA 407854, which is a prerequisite for calculation of risk for fish-eating birds via secondary poisoning. For completeness purposes and since all input data have been available, the risk for earthworm-eating birds via secondary poisoning has been calculated for the pinoxaden metabolite NOA 407854.

Risk assessment for earthworm-eating birds via secondary poisoning
According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil based on experimental data (*cf.* Section 8, Chapter 8.7.2).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for

the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for birds from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

~~**Table 9.2-4: Assessment of the risk for earthworm-eating birds due to exposure to the pinoxaden metabolite NOA 407854 via bioaccumulation in earthworms (secondary poisoning) for the intended use of ADM.06001.H.2.B in winter and spring cereals**~~

~~Parameter~~	~~Pinoxaden metabolite NOA 407854~~	~~Comments~~
~~PEC_{soil} (twa = 21 d) (mg/kg soil)~~	~~0.056~~	
~~log P_{ow} / P_{ow}~~	~~-1.1 / 0.0794~~	
~~Koc~~	~~6.0~~	~~Median (n = 12)~~
~~foc~~	~~0.02~~	~~Default~~
~~BCF_{worm}~~	~~7.01~~	~~$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$~~
~~PEC_{worm}~~	~~0.39~~	~~$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$~~
~~Daily dietary dose (mg/kg bw/d)~~	~~0.41~~	~~$DDD = PEC_{worm} \times 1.05$~~
~~NOEL (mg/kg bw/d)~~	~~27.8~~	
~~TER_{lt}~~	~~67.6~~	~~TER criterion: 5~~

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

Table 9.2-5: Assessment of the risk for earthworm-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms (secondary poisoning) for the intended use of ADM.06001.H.2.B in winter and spring cereals

Parameter	Mefenpyr-diethyl	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.014	
log P_{ow} / P_{ow}	3.83 / 6760.8	
Koc	619	Median (n = 6)
foc	0.02	Default
BCF_{worm}	6.62	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.09	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.10	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	106	
TER_{lt}	1089	TER criterion: 5

TER values shown in bold fall below the relevant trigger.

Based on TER_{lt} values above the trigger of 5 for both pinoxaden and mefenpyr-diethyl, no risk for secondary poisoning of earthworm-eating birds is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).
Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.
No unacceptable effects to earthworm-eating birds are expected following application of ADM.06001.H.2.B according to the proposed use pattern.
Overall, acceptable risk of secondary exposure from all relevant compounds could be concluded for birds.

Risk assessment for fish-eating birds via secondary poisoning

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for birds from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Table 9.2-6: Assessment of the risk for fish-eating birds due to exposure to mefenpyr-diethyl via bioaccumulation in fish (secondary poisoning) for the intended use of ADM.06001.H.2.B in winter and spring cereals

Parameter	Mefenpyr-diethyl	Comments
PEC_{sw}[a] (mg/L)	0.000391	
BCF_{fish}	362	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.142	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0225	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	106	
TER_{lt}	4710	

TER values shown in bold fall below the relevant trigger.

[a] Maximum PECsw of Step 3 (worst case: R4 scenario for application to spring cereals) worst case also for updated modelling

Based on a TER_{lt} value above the trigger of 5 for mefenpyr-diethyl, no risk for secondary poisoning of fish-eating birds is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

zRMS comments:

zRMS agrees with the above calculations and risk assessment approach with some remarks:

- Koc of pinoxaden metabolite NOA 407854 and mefenpyr-diethyl reported represents the median value, instead of the geomean value. Usually, it is preferable to use the geomean value (NOA 407854 Koc = 10.6 and mefenpyr-diethyl = 610); however, since the median value is worst case the risk assessment in acceptable and it is deemed correct.
- In risk assessment via secondary poisoning for fish-eating birds, the Maximum PECsw of Step 3 (worst case: R4 scenario for application to spring cereals) is used for mefenpyr diethyl; calculations should have been carried out using the Step1 21days TWA.
  However, the zRMS has verified that even with the global max PEC STEP 1 (6.756 µg/L), TER is 273.

No unacceptable effects to fish-eating birds are expected following application of ADM.06001.H.2.B according to the proposed use pattern.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

Based on screening step assessments, the acute and chronic risks to small omnivorous birds from exposure to food stuffs contaminated with the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B to winter and spring cereals are acceptable. The risk to birds from exposure to the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl in drinking water from puddles is acceptable.
Acceptable risk for secondary poisoning of earthworm-eating and fish-eating birds is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with the active substances mesosulfuron-methyl and pinoxaden as well as with the safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. However, the provision of further data on ADM.06001.H.2.B is not considered essential, because the toxicity of the formulation to mammals can be extrapolated from the data on the active substances mesosulfuron-methyl and pinoxaden as well as from the data on the safener mefenpyr-diethyl.

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

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

Species	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
Rat	Mesosulfuron-methyl	Oral Acute	**LD_{50} > 5000 mg a.s./kg bw**	EFSA Conclusion 4584/2016
Rat	Mesosulfuron-methyl	Dietary Reproductive toxicity Two-generation study	**NOEL = 840 mg a.s./kg bw/d** (maximum test concentration, no effect on reproduction and on parental or neonatal parameters)	EFSA Conclusion 4584/2016
Pinoxaden				
Rat	Pinoxaden	Oral Acute	**LD_{50} > 5000 mg a.s./kg bw**	EFSA Conclusion 3269/2013
Rat	Pinoxaden	Multi-generation, gavage	NOAEL Offspring = 250 mg a.s./kg bw/d Parental = 50 mg a.s./kg bw Repro = 500 mg a.s./kg bw	EFSA Conclusion 3269/2013
Rabbit	Pinoxaden	Developmental	**NOAEL (pups) = 30 mg a.s./kg bw/d** (based on early resorptions)	EFSA Conclusion 3269/2013
Mefenpyr-diethyl				
Rat	Mefenpyr-diethyl	Oral Acute	**LD_{50} > 5000 mg/kg bw**	Proposed in Monograph (list of endpoints) Oct 2011[a]
Rat	Mefenpyr-diethyl	Dietary Reproductive toxicity Two-generation study	**NOAEL = 88.8 mg/kg bw/d**	Proposed in Monograph (list of endpoints) Oct 2011[a]

Endpoints in bold are used in the risk assessment

[a] Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl.

zRMS comments:

Mammalian toxicity data are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 for mesosulfuron-methyl and EFSA Journal 2013;11(8):3269 for pinoxaden and

Mixture toxicity

Besides the risk assessment for the active substances mesosulfuron-methyl and pinoxaden as well as the safener mefenpyr-diethyl, risk assessment has been performed for the formulation by calculation of a surrogate LD_{50} for acute effects of the mixture. This surrogate LD_{50} is calculated assuming dose or concentration additivity of toxicity of the active substances including and excluding the safener (based on the worst-case assumption that the substances have the same mode of action). In the present case, it is not necessary to calculate the surrogate LD_{50} because LD_{50} values for acute effects (mortality) are the same for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl, i.e. >5000 mg/kg bw.
Consequently, the surrogate LD_{50} for acute effects of the formulation ADM.06001.H.2.B (including and excluding the safener) is also >5000 mg/kg bw.

For chronic/reproductive toxicity, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment (EFSA/2009/1438).

zRMS comments:

Acute combined endpoint:

zRMS agrees with the LD_{50} mix approach.

Long term combined endpoint:

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD50, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values. Therefore, the calculated $NOEL_{mix}$ is not considered by zRMS in the current risk assessment.
It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to mammals.
TER_{mix} approach is agreed option of combined risk assessment. For this reason, zRMS used this approach in the combined risk assessment.

9.3.1.1 Justification for new endpoints

No new endpoints were used in the assessment of risk to mammals from the use of ADM.06001.H.2.B in accordance with the proposed GAP.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for mammals from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

For the formulation ADM.06001.H.2.B, acute risk for mammals has been assessed using the surrogate LD_{50} assuming dose or concentration additivity of toxicity (mixture toxicity; please see above) of the active substances including and excluding the safener in the virtual compound. The content of the active substances and the safener in the formulation and application rate per hectare have been expressed in terms of this virtual compound, i.e. application rate per hectare is the sum of the application rates of the the active substances including and excluding the safener (surrogate application rate: 107 g sum of a.s. + safener/ha and 72 g sum of a.s./ha).

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

The results of the acute and reproductive screening assessments are summarised in the following table.

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	Winter and spring cereals, BBCH 13-39				
Active substance/product	Mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl (safener)/ ADM.06001.H.2.B				
Application rate (g/ha)	12 g a.s./ha (mesosulfuron-methyl), 60 g a.s./ha (pinoxaden), 35 g a.s./ha (mefenpyr-diethyl)/107 g sum of a.s. + safener/ha (surrogate application rate for ADM.06001.H.2.B including safener)/72 g sum of a.s./ha (surrogate application rate for ADM.06001.H.2.B excluding safener)				
Acute toxicity (mg/kg bw)	> 5000 (mesosulfuron-methyl), > 5000 (pinoxaden), > 5000 (mefenpyr-diethyl)/ > 5000 (surrogate endpoint for ADM.06001.H.2.B including and excluding safener)				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Mesosulfuron-methyl					
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	118.4	1	1.421	> 3519
Pinoxaden					
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	118.4	1	7.104	> 703.8
Mefenpyr-diethyl					
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	118.4	1	4.144	> 1207
ADM.06001.H.2.B					
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	118.4	1	12.67	> 394.7[a]
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	118.4	1	8.525	> 586.5[b]
Reprod. toxicity (mg/kg bw/d)	840 (mesosulfuron-methyl), 30 (pinoxaden), 88.8 (mefenpyr-diethyl)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Mesosulfuron-methyl					
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	48.3	1 x 0.53	0.307	2734
Pinoxaden					
Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	48.3	1 x 0.53	1.536	19.5
Mefenpyr-diethyl					

Cereals BBCH 13-39	Small herbivorous mammal Screening assessment	48.3	1 x 0.53	0.896	99.1

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

[a] Calculated with the surrogate endpoint for ADM.06001.H.2.B including safener

[b] Calculated with the surrogate endpoint for ADM.06001.H.2.B excluding safener

Based on screening step assessments, the acute and chronic risks to small herbivorous mammals from exposure to food stuffs contaminated with the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B to winter and spring cereals are acceptable.

zRMS comments:

Screening step in the risk assessment:

TER_A and TER_{LT} values for the exposure to active substances, pinoxaden metabolite NOA 407854 and safener are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for mammals.

Acute combined risk assessment:

The LD_{50}mix values >5000 mg /kg bw with consideration of relevant toxicity endpoints such as: 5000 mg/kg bw (surrogate endpoint for ADM.06001.H.2.B including safener) and >5000 mg kg/bw (surrogate endpoint for ADM.06001.H.2.B excluding safener) and the acute risk assessment in Table 9.3-2 have been accepted by zRMS.

Combined long-term risk assessment:

TER_{mix} approach was taken by zRMS to combined risk assessment with regard the active substance mesosulfuron-methyl, pinoxaden metabolite and safener.

The relevant calculations are provided below:

TER_{mix} assessment of long-term/reproductive risk for mammals due to the use of ADM.06001.H.2.B in winter and spring cereals

Mesosulfuron-methyl		**Pinoxaden metabolite NOA 407854**		**Mefenpyr-diethyl**		**Σ1/TER**	**Σ1/TER$^-$**	**Trigger**
2734[1)]	0.000365	19.5 [1)]	0.051	99.1 [1)]	0.010	0.061	16.4 [1)]	5

[1)] TER_{LT} values calculated at screening step.

Overall, based on performed calculations TER_{mix} value acceptable combined long-term risk may be concluded for mammals.

9.3.2.2 Higher-tier risk assessment

Higher tier risk assessments for the uses of ADM.06001.H.2.B in winter and spring cereals are not required as the screening step risk assessments indicate acceptable risks for both acute and chronic exposure to mammals.

9.3.2.3 Drinking water exposure

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

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

With a K(f)oc of 64 mL/g (geometric mean) for mesosulfuron-methyl, 323 mL/g (median) for pinoxaden and 619 mL/g (median) for mefenpyr-diethyl, mesosulfuron-methyl and pinoxaden belong to the group of less sorptive substances and mefenpyr-diethyl belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for mammals from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Mesosulfuron-methyl				
Effective application rate (g/ha)	=	12		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.002
Reprod. toxicity (mg/kg bw/d)	=	840	quotient =	0.014
Pinoxaden				
Effective application rate (g/ha)	=	60		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.012
Reprod. toxicity (mg/kg bw/d)	=	30	quotient =	2.0
Mefenpyr-diethyl				
Effective application rate (g/ha)	=	35		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.007
Reprod. toxicity (mg/kg bw/d)	=	88.8	quotient =	0.394

No specific calculations of exposure and TER are necessary as the ratios of effective application rate to acute and reprotoxic endpoints for mammals are less than 50 in the case of mesosulfuron-methyl and pinoxaden and less than 3000 in the case of mefenpyr-diethyl.

zRMS comments:

The leaf scenario does not have to be considered taking into account the proposed uses (cereals).

The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not re-quired since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is < 50 in case of mesosulfuron-methyl and pinoxaden (and its metabolite) and <3000 in the case of mefenpyr-diethyl.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of mesosulfuron-methyl ranges between 1.90 at pH 4 and -2.10 at pH 10 (25°C; EFSA Journal 2016;14(10):4584) and thus does not exceed the trigger value of 3. For pinoxaden, the log Pow is 3.2 (25°C, pH effect not determined as no dissociation observed; EFSA Journal 2013;11(8):3269) and is therefore slightly above the trigger value of 3. The log P_{ow} of mefenpyr-dietyl amounts to 3.83 (21°C, pH 6.3; proposed in Monograph (list of endpoints) Oct 2011 - voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl) and thus exceeds the trigger value of 3. Therefore, a risk assessment for effects due to secondary poisoning is formally required for pinoxaden and mefenpyr-diethyl.

In the case of pinoxaden, assessment of the risk for earthworm- and fish-eating mammals via secondary poisoning is however not considered required due to the following reasons. Pinoxaden degrades rapidly in soil (DT_{50}: 1.01 days, EFSA Conclusion 3269/2013) and water (DT_{50}: 0.28 days, EFSA Conclusion 3269/2013). Therefore, neither long-term exposure of earthworms and fish nor bioaccumulation in these

species are expected for pinoxaden. Consequently, no bioconcentration factor in fish is available for pinoxaden, which is a prerequisite for calculation of risk for fish-eating mammals via secondary poisoning. For completeness purposes and since all input data have been available, the risk for earthworm-eating mammals via secondary poisoning has been calculated for pinoxaden. As a worst-case assumption, input parameters for the pinoxaden metabolite NOA 407854 have been used together with the NOEL for chronic toxicity of pinoxaden in mammals (no NOEL for chronic toxicity of NOA 407854 in mammals is available).

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil based on experimental data (*cf.* Section 8, Chapter 8.7.2).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for mammals from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

~~Table 9.3-3: Assessment of the risk for earthworm-eating mammals due to exposure to pinoxaden / NOA 407854 via bioaccumulation in earthworms (secondary poisoning) for the intended use of ADM.06001.H.2.B in winter and spring cereals~~

~~Parameter~~	~~Pinoxaden / pinoxaden metabolite NOA 407854~~	~~Comments~~
~~PEC_{soil} (twa = 21 d) (mg/kg soil)~~	~~0.056 (NOA 407854)~~	
~~log P_{ow} / P_{ow}~~	~~-1.1 / 0.0794 (NOA 407854)~~	
~~Koc~~	~~6.0 (NOA 407854)~~	~~Median (n = 9)~~
~~foc~~	~~0.02~~	~~Default~~
~~BCF_{worm}~~	~~7.01 (NOA 407854)~~	~~$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$~~
~~PEC_{worm}~~	~~0.3924 (NOA 407854)~~	~~$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$~~
~~Daily dietary dose (mg/kg bw/d)~~	~~0.5023 (NOA 407854)~~	~~$DDD = PEC_{worm} \times 1.28$~~
~~NOEL (mg/kg bw/d)~~	~~30 (pinoxaden)~~	
~~TER_{lt}~~	~~59.7~~	~~TER criterion: 5~~

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

Table 9.3-4: Assessment of the risk for earthworm-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in earthworms (secondary poisoning) for the intended use of ADM.06001.H.2.B in winter and spring cereals

Parameter	Mefenpyr-diethyl	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.014	
log P_{ow} / P_{ow}	3.83 / 6760.8	
Koc	619	Median (n = 6)
foc	0.02	Default
BCF_{worm}	6.62	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.09	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.12	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	88.8	
TER_{lt}	748	TER criterion: 5

TER values shown in bold fall below the relevant trigger.

Based on TER_{lt} values above the trigger of 5 for both pinoxaden / NOA 407854 and mefenpyr-diethyl, acceptable risk for secondary poisoning of earthworm-eating mammals is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS.

Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.

No unacceptable effects to earthworm-eating mammals are expected following application of ADM.06001.H.2.B according to the proposed use pattern.

Overall, acceptable risk of secondary exposure from all relevant compounds could be concluded for mammals.

Risk assessment for fish-eating mammals via secondary poisoning

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for mammals from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Table 9.3-5: Assessment of the risk for fish-eating mammals due to exposure to mefenpyr-diethyl via bioaccumulation in fish (secondary poisoning) for the intended use of ADM.06001.H.2.B in winter and spring cereals

Parameter	Mefenpyr-diethyl	Comments
PEC_{sw}[a] (mg/L)	0.000391	
BCF_{fish}	362	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.142	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0201	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	88.8	
TER_{lt}	4418	TER criterion: 5

TER values shown in bold fall below the relevant trigger.

[a] Maximum PECsw of Step 3 (worst case: R4 scenario for application to spring cereals) , worst case also for updated modelling

Based on a TER_{lt} value above the trigger of 5 for mefenpyr-diethyl, acceptable risk for secondary poisoning of fish-eating mammals is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

zRMS comments:

zRMS agrees with the above calculations and risk assessment approach with some remarks:

- Koc of pinoxaden metabolite NOA 407854 and mefenpyr-diethyl reported represents the median value, instead of the geomean value. Usually, it is preferable to use the geomean value (NOA 407854 Koc = 10.6 and mefenpyr-diethyl = 610); however, since the median value is worst case the risk assessment in acceptable and it is deemed correct.
- In risk assessment via secondary poisoning for fish-eating mammals, the Maximum PECsw of Step 3 (worst case: R4 scenario for application to spring cereals) is used for mefenpyr diethyl; calculations should have been carried out using the Step1 21days TWA.
  However, the zRMS has verified that even with the global max PEC STEP 1 (6.756 µg/L), TER is 255.

Overall, no unacceptable effects to fish-eating birds are expected following application of ADM.06001.H.2.B according to the proposed use pattern.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

Based on screening step assessments, the acute and chronic risks to small herbivorous mammals from exposure to food stuffs contaminated with the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B to winter and spring cereals are acceptable.

The risk to mammals from exposure to the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl in drinking water from puddles is acceptable.

Acceptable risk for secondary poisoning of earthworm-eating and fish-eating mammals is indicated for the use of the formulation ADM.06001.H.2.B in winter and spring cereals.

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

No additional data was submitted as part of the active substance renewals of mesosulfuron-methyl and pinoxaden and as part of the Monograph (list of endpoints) Oct 2011 of mefenpyr-diethyl (voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing the safener mefenpyr-diethyl). No further data is presented in this product submission.

zRMS comments:

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

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with the active substances mesosulfuron-methyl and pinoxaden and/or their relevant metabolites as well as with the safener mefenpyr-diethyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mesosulfuron-methyl and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Mesosulfuron-methyl	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Lepomis macrochirus	Mesosulfuron-methyl	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Cyprinodon variegates	Mesosulfuron-methyl	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Oncorhynchus mykiss	AE F092944	96 h, s	**LC_{50} > 97 mg/L $_{mm}$[a]**	EFSA Conclusion 4584/2016
Oncorhynchus mykiss	AE F099095	96 h, s	**LC_{50} = 70.7 mg/L $_{nom}$[b]**	EFSA Conclusion 4584/2016
Oncorhynchus mykiss	Mesosulfuron-methyl	28 d, ss	**NOEC = 32 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Pimephales promelas	Mesosulfuron-methyl	32 d (ELS), f	NOEC = 95 mg a.s./L $_{nom}$	EFSA Conclusion 4584/2016
Daphnia magna	Mesosulfuron-methyl	48 h, s	**EC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Mysidopsis bahia	Mesosulfuron-methyl	48 h, s	**EC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Crassostrea virginica	Mesosulfuron-methyl	96 h, f	**EC_{50} > 100 mg a.s./L $_{nom}$** (mortality/shell deposition)	EFSA Conclusion 4584/2016
Daphnia magna	AE F092944	48 h, s	EC_{50} = 223 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Daphnia magna	AE F092944	48 h, s	**EC_{50} > 100 mg/L $_{mm}$[a]**	EFSA Conclusion 4584/2016
Daphnia magna	AE F099095	48 h, s	**EC_{50} > 100 mg/L $_{nom}$[b]**	EFSA Conclusion 4584/2016
Daphnia magna	Mesosulfuron-methyl	21 d, ss	**NOEC = 1.8 mg a.s./L $_{nom}$**	EFSA Conclusion 4584/2016
Daphnia magna	AE F092944	21 d, ss	**NOEC = 24.9 mg/L $_{mm}$[a]**	EFSA Conclusion 4584/2016

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	Mesosulfuron-methyl	72 h, s	**E_rC_{50} > 0.29 mg a.s./L $_{mm}$** E_bC_{50} = 0.18 mg a.s./L $_{mm}$ NOE_rC = 0.018 mg a.s./L $_{mm}$	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	Mesosulfuron-methyl	72 h, s	E_rC_{50} = 3.99 mg a.s./L $_{nom}$ NOE_rC = 0.143 mg a.s./L $_{nom}$	EFSA Conclusion 4584/2016
Navicula pelliculosa	Mesosulfuron-methyl	72 h, s	E_rC_{50} > 74.9 mg a.s./L $_{mm}$ E_bC_{50} > 74.9 mg a.s./L $_{mm}$ NOE_rC = 74.9 mg a.s./L $_{mm}$	EFSA Conclusion 4584/2016
Anabaena flos-aquae	Mesosulfuron-methyl	96 h, s	E_rC_{50} = 4.1 mg a.s./L $_{nom}$ E_bC_{50} = 2.4 mg a.s./L $_{nom}$ NOE_rC = 1 mg a.s./L $_{nom}$	EFSA Conclusion 4584/2016
Skeletonema costatum	Mesosulfuron-methyl	72 h, s	E_rC_{50} > 100 mg a.s./L $_{nom}$ E_bC_{50} = 82 mg a.s./L $_{nom}$ NOE_rC = 60 mg a.s./L $_{nom}$	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	Mesosulfuron	72 h, s	**E_rC_{50} = 38 mg/L $_{mm}$**	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	AE F160459	72 h, s	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} = 92 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	AE F099095	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	AE F099095	72 h, s	**E_rC_{50} = 99.1 mg/L[c]** E_bC_{50} = 41.1 mg/L[c]	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	AE F092944	72 h, s	E_rC_{50} > 120 mg/L $_{nom}$[a] E_bC_{50} > 120 mg/L $_{nom}$[a] NOE_rC = 7.5 mg/L $_{nom}$[a]	EFSA Conclusion 4584/2016
Desmodesmus subspicatus	AE F092944	72 h, s	**E_rC_{50} > 100 mg/L[d]** E_bC_{50} > 100 mg/L[d] NOEC = 100 mg/L $_{nom}$[d]	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	AE F147447	72 h, s	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	BCS-CO60720	72 h, s	**E_rC_{50} > 10 mg/L $_{nom}$**	EFSA Conclusion 4584/2016
Pseudokirchneriella subcapitata	BCS-CO60721	72 h, s	E_rC_{50} > 10 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Lemna gibba	Mesosulfuron-methyl	7 d, ss	E_rC_{50} = 0.001717	EFSA Conclusion

Species	Substance	Exposure System	Results	Reference
			mg a.s./L $_{twa}$ E_bC_{50} = 0.001863 mg a.s./L $_{twa}$ NOEC < 0.00077 mg a.s./L $_{twa}$	4584/2016
Lemna gibba	Mesosulfuron	7 d, s	**E_rC_{50} = 0.11 mg/L $_{nom}$**	EFSA Conclusion 4584/2016
Lemna gibba	AE F160459	7 d, s	**E_rC_{50} = 2.6 mg/L $_{nom}$** E_bC_{50} = 1.7 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Lemna gibba	AE F099095	7 d, s	**E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Conclusion 4584/2016
Lemna gibba	AE F092944	7 d, ss	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Lemna gibba	AE F160460	7 d, ss	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Lemna gibba	AE F140584	7 d, ss	**E_rC_{50} > 10 mg/L $_{nom}$**	EFSA Conclusion 4584/2016
Lemna gibba	AE F147447	7 d, ss	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Lemna gibba	BCS-CO60720	7 d, s	**E_rC_{50} > 11.8 mg/L $_{nom}$**	EFSA Conclusion 4584/2016
Lemna gibba	BCS-CO60721	7 d, s	E_rC_{50} > 10 mg/L $_{nom}$	EFSA Conclusion 4584/2016
Higher-tier studies (micro- or mesocosm studies)				
Aquatic macrophytes (9 species) *Elodea canadensis* *Potamogeton pectinatus* *Pontederia cordata* *Nymphaea odorata* *Cabomba caroliniana* *Cerat. demersum* *Glyceria maxima* *Mentha aquatica* *Myriophyll.heterophyllum*	Mesosulfuron-methyl	outdoor growth inhibition, static 8 weeks	Lowest NOAEC = 0.00057 mg a.s./L $_{mm}$ (shoot length/dry weight)	EFSA Conclusion 4584/2016
Lemna gibba	Mesosulfuron-methyl	growth inhibition, mimicking exposure of outdoor study 8 weeks	7 d E_rC_{50} = 0.00161 mg a.s./L $_{nom}$ (frond number) **7 d E_rC_{50} = 0.00129 mg a.s./L $_{nom}$** (frond area) 7 d NOE_rC = 0.00039 mg a.s./L $_{nom}$ 8 wk NOE_rC = 0.000388 mg a.s./L $_{nom}$ / 0.00026 mg a.s./L $_{mm}$ (frond number) 8 wk NOE_rC = 0.000388 mg a.s./L $_{nom}$ / 0.00026 mg a.s./L $_{mm}$ (frond area)	EFSA Conclusion 4584/2016

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

twa: time-weighted average
Endpoints in bold are used in the risk assessment
[a] Refer to the EFSA conclusion on the peer review of the active substance flupyrsulfuron-methyl, EFSA (2014a)
[b] Refer to the EFSA conclusion on the peer review of the active substance orthosulfamuron, EFSA (2014b)
[c] Refer to the EFSA conclusion on the peer review of the active substance bensulfuron, EFSA (2009)
[d] Refer to the EFSA conclusion on the peer review of the active substance flazasulfuron, EFSA (2016c)

zRMS comments:

Endpoints presented in Table 9.5-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584.

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Pinoxaden	96 h, f	**LC_{50} = 10.3 mg a.s./L $_{mm}$**	EFSA Conclusion 3269/2013
Cyprinodon variegates	Pinoxaden	96 h, f	LC_{50} = 16 mg a.s./L $_{mm}$	EFSA Conclusion 3269/2013
Pimephales promelas	Pinoxaden	96 h, f	LC_{50} = 20 mg a.s./L $_{mm}$	EFSA Conclusion 3269/2013
Oncorhynchus mykiss	NOA 407854	96 h, s	**LC_{50} > 100 mg/L $_{nom}$**	EFSA Conclusion 3269/2013
Oncorhynchus mykiss	NOA 447204	96 h, s	**LC_{50} > 120 mg/L $_{nom}$**	EFSA Conclusion 3269/2013
Pimephales promelas	NOA 407854	32 d (ELS), f	**NOEC = 1.0 mg/L $_{nom}$**	EFSA Conclusion 3269/2013
Daphnia magna	Pinoxaden	48 h, f	EC_{50} = 52 mg a.s./L $_{mm}$	EFSA Conclusion 3269/2013
Americamysis bahia	Pinoxaden	48 h, f	LC_{50} = 8.3 mg a.s./L $_{mm}$	EFSA Conclusion 3269/2013
Crassostrea virginica	Pinoxaden	48 h, f	**EC_{50} > 0.88 mg a.s./L $_{mm}$ (shell deposition)**	EFSA Conclusion 3269/2013
Daphnia magna	NOA 407854	48 h, s	**EC_{50} > 100 mg/L $_{nom}$**	EFSA Conclusion 3269/2013
Daphnia magna	NOA 447204	48 h, s	**EC_{50} > 120 mg/L $_{nom}$[a]**	EFSA Conclusion 3269/2013
Daphnia magna	NOA 407854	21 d, ss	**NOEC = 6.25 mg/L $_{nom}$**	EFSA Conclusion 3269/2013
Navicula pelliculosa	Pinoxaden	96 h, s	72 h E_rC_{50} = 14 mg a.s./L $_{nom}$ 96 h E_bC_{50} = 10.2 mg a.s./L $_{mm}$	EFSA Conclusion 3269/2013
Anabaena flos-aquae	Pinoxaden	96 h, s	E_rC_{50} = 16.4 mg a.s./L $_{nom}$ E_bC_{50} = 5.0 mg a.s./L $_{nom}$	EFSA Conclusion 3269/2013
Skeletonema costatum	Pinoxaden	96 h, s	**E_rC_{50} = 1.3244 mg a.s./L $_{im}$** E_bC_{50} = 0.9086 mg a.s./L $_{im}$	EFSA Conclusion 3269/2013
Pseudokirchneriella subcapitata	Pinoxaden	96 h, s	E_rC_{50} = 41 mg a.s./L $_{nom}$	EFSA Conclusion 3269/2013

Species	Substance	Exposure System	Results	Reference
			E_bC_{50} = 16 mg a.s./L $_{nom}$	
Pseudokirchneriella subcapitata	NOA 407854	72 h, s	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} = 100 mg/L $_{nom}$	EFSA Conclusion 3269/2013
Pseudokirchneriella subcapitata	NOA 447204	96 h, s	**E_rC_{50} > 120 mg/L $_{nom}$** E_bC_{50} = 89.9 mg/L $_{nom}$	EFSA Conclusion 3269/2013
Lemna gibba	Pinoxaden	7 d, s	E_rC_{50} = 9.73 mg a.s./L $_{im}$[a] E_bC_{50} = 3.5 mg a.s./L $_{im}$	EFSA Conclusion 3269/2013
Phragmytes australis	Pinoxaden	20 d, s	**E_rC_{50} = 8.5 mg a.s./L $_{nom}$**	EFSA Conclusion 3269/2013
Lemna gibba	NOA 407854	7 d, s	**E_rC_{50} = 14.6 mg/L $_{nom}$** E_bC_{50} = 10.6 mg/L $_{nom}$	EFSA Conclusion 3269/2013
Lemna gibba	NOA 447204	7 d, s	**E_rC_{50} > 100 mg/L $_{nom}$** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Conclusion 3269/2013
Higher-tier studies (micro- or mesocosm studies)				
Not available				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations
Endpoints in bold are used in the risk assessment

[a] Calculated on the basis of nominal E_rC_{50} of 13.9 mg/L; lowest initial measured concentration was 70% of nominal (DAR, 2005)

zRMS comments:

Endpoints presented in Table 9.5-2 are in line with the EU agreed endpoints reported in EFSA Journal 2013;11(8):3269.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mefenpyr-diethyl and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Cyprinus carpio	Mefenpyr-diethyl	96 h, s	**LC_{50} = 2.4 mg/L $_{nom}$[c]**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	Mefenpyr-diethyl	96 h, s	LC_{50} = 4.2 mg/L $_{nom}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Lepomis macrochirus	AE F113225[a]	96 h, s	**LC_{50} > 100 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	AE F109453	96 h, s	**LC_{50} > 100 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	AE F094270	96 h, s	LC_{50} > 100 mg/L $_{nom}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Brachydanio rerio	AE F094270	96 h, f	**LC_{50} > 72 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]

Species	Substance	Exposure System	Results	Reference
Cyprinus carpio	AE F2211046[b]	96 h, s	**LC_{50} = 0.24 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	AE F2211046[b]	96 h, s	LC_{50} = 0.42 mg/L $_{nom}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	Mefenpyr-diethyl	28 d, f	**NOEC = 0.1 mg/L $_{nom}$[c]**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	AE F113225[a]	28 d, f	**NOEC = 32 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Brachydanio rerio	AE F094270	8 d, f	NOEC = 10 mg/L $_{nom}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Brachydanio rerio	AE F094270	206 d, f	**NOEC = 3.2 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Oncorhynchus mykiss	AE F2211046[b]	28 d, f	**NOEC = 0.01 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	Mefenpyr-diethyl	48 h, s	**EC_{50} = 5.5 mg/L $_{mm}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F113225[a]	48 h, s	**EC_{50} > 100 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F109453	48 h, s	**EC_{50} > 100 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F094270	48 h, s	**EC_{50} > 60.3 mg/L $_{mm}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F2211046[b]	48 h, s	**EC_{50} = 0.55 mg/L $_{mm}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	Mefenpyr-diethyl	21 d, ss	**NOEC = 0.32 mg/L $_{nom}$[c]**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F113225[a]	21 d, ss	**NOEC = 3.2 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F094270	21 d, ss	**NOEC = 32 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Daphnia magna	AE F2211046[b]	21 d, ss	**NOEC = 0.032 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Chironomus riparius	AE F094270	28 d, s (spiked water)	**NOEC = 50 mg/L $_{nom}$**	Proposed in Monograph (list of endpoints) Oct 2011[d]
Pseudokirchneriella subcapitata	Mefenpyr-diethyl	96 h, s	E_rC_{50} = 10.71 mg/L $_{mm}$	Proposed in Monograph (list of

Species	Substance	Exposure System	Results	Reference
			E_bC_{50} = 4.86 mg/L $_{mm}$	endpoints) Oct 2011[d]
Navicula pelliculosa	Mefenpyr-diethyl	72 h, s	**E_rC_{50} = 3.12 mg/L $_{mm}$** E_bC_{50} = 1.39 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Pseudokirchneriella subcapitata	AE F113225[a]	72 h, s	**E_rC_{50} > 100 mg/L $_{mm}$** E_bC_{50} > 100 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Pseudokirchneriella subcapitata	AE F109453	96 h, s (in neutralised medium)	**E_rC_{50} > 100 mg/L $_{mm}$** E_bC_{50} > 100 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Pseudokirchneriella subcapitata	AE F094270	96 h, s	**E_rC_{50} = 40.2 mg/L $_{nom}$** E_bC_{50} = 30.8 mg/L $_{nom}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Pseudokirchneriella subcapitata	AE F2211046[b]	96 h, s	E_rC_{50} = 1.071 mg/L $_{mm}$ E_bC_{50} = 0.486 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Navicula pelliculosa	AE F2211046[b]	72 h, s	**E_rC_{50} = 0.312 mg/L $_{mm}$** E_bC_{50} = 0.139 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Lemna gibba	Mefenpyr-diethyl	7 d, ss	**E_rC_{50} > 7.6 mg/L $_{mm}$** E_bC_{50} > 7.6 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Lemna gibba	AE F2211046[b]	7 d, ss	**E_rC_{50} > 0.76 mg/L $_{mm}$** E_bC_{50} > 0.76 mg/L $_{mm}$	Proposed in Monograph (list of endpoints) Oct 2011[d]
Higher-tier studies (micro- or mesocosm studies)				
Not available				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
Endpoints in bold are used in the risk assessment

[a] Also endpoint value of the isomeric metabolite AE F114952

[b] Metabolite considered 10 times more toxic than mefenpyr-diethyl as there are no data available

[c] Endpoints expressed as the sum of mefenpyr-diethyl and its 2 metabolites AE F113225 and AE F109453

[d] Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl.

zRMS comments:

Endpoints presented in Table 9.5-3 are agreed in the document: Monograph (list of endpoints) Oct 2011d.
Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorization of plant protection products containing safener mefenpyr-diethyl. Therefore, zRMS agrees with the endpoint selected for the safener.
In case of absence of toxicity endpoint for the metabolite AE F2211046, 10 times higher toxicity than the parent is assumed.
In addition, in case of mesosulfuron metabolite BCS-CV14885 the toxicity of this metabolite was considered to be equal to the a.s. thus the risk is covered by the parent a.s.
Moreover, it was agreed during the EU review that "BCS-CV14885 has lost the toxophore and that BCS-CV14885 should be less toxic than the active substance.

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – ADM.06001.H.2.B

Species	Substance	Exposure System	Results	Reference
Daphnia magna	ADM.06001.H.2.B	48 h, ss	EC_{50} = 79.5 mg product/L $_{nom}$	Seidel U. and Mollandin G., 2021a, 140711220 (000105363)
Raphidocelis subcapitata	ADM.06001.H.2.B	72 h, s	E_rC_{50} = 54.8 mg product/L $_{mm}$ E_yC_{50} = 27.8 mg product/L $_{mm}$	Seidel U. and Mollandin G., 2021b, 140711210 (000105364)
Lemna gibba	ADM.06001.H.2.B	7 d, ss	E_rC_{50} = 0.074 mg product/L $_{twa}$ E_yC_{50} = 0.035 mg product/L $_{twa}$	Seidel U. and Mollandin G., 2021c, 140711240 (000105365)
Higher-tier studies (micro- or mesocosm studies)				
Not available				

s: static; ss: semi-static; nom: based on nominal concentrations; mm: based on mean measured concentrations; twa: time-weighted average

No acute fish study with the formulation has been conducted due to animal welfare. Pinoxaden and mesosulfuron-methyl are herbicides and therefore primary producers are considered to be most at risk.
For mesosulfuron-methyl *Lemna* is the most sensitive species with an E_rC_{50} of 1.29 µg a.s./L. In the mixture with pinoxaden and mefenpyr-diethyl the active substance mesosulfuron-methyl is clearly driving the risk assessment. The fish endpoint with an LD_{50} of 100'000 µg a.s./L is by a magnitude of 10'000 less toxic than *Lemna*.
For pinoxaden the eastern oyster *Crassostrea virginica* is the most sensitive species with an EC_{50} of >880 µg a.s./L. The fish endpoint with an LD_{50} of 10300 µg a.s./L is therefore by a magnitude of 10 less toxic than the eastern oyster. However, the eastern oyster endpoint is a 'greater than' endpoint and the definite toxicity endpoint might be even higher as no mortalities have been observed up to and including 0.88 µg a.s./L, the maximum rate tested. The most sensitive endpoints for algae (E_rC_{50} of 1324.4 µg a.s./L for *Skeletonema costatum*) and aquatic plants (E_rC_{50} of 8500 µg a.s./L for *Phragmytes australis*) are also ~~less~~ higher toxic than fish, by a factor of 7.8 and 1.2, respectively.
In Table 9.5.2-34 below, the predicted mixture toxicity was calculated based on the active substances including the safener mefenpyr-diethyl and using the same species for each trophic level. The calculated $ECx_{mix\text{-}CA}$ was 7494 µg/L for fish, 14010 µg/L for aquatic invertebrates, 2321 µg/L for algae and 11.49 µg/L for aquatic plants. The calculated mixture toxicity for *Lemna* is therefore ~650 times more toxic than the calculated mixture toxicity for fish. As *Lemna* was expected to be clearly the most sensitive species for the product ADM.06001.H.2.B, an acute formulation study with fish was not conducted due to animal welfare. According to information provided in the DAR, Section B9, page 34, a long-term risk assessment with pinoxaden was not deemed appropriate due to the rapid degradation of pinoxaden ((DT50 < 1 day) resulting in limited potential for exposure of aquatic organisms.
In case of a long - term risk assessment performed for major metabolite M02 for fish and Daphnia magna species is considered acceptable and sufficient to protective aquatic organism.
Formulation studies have been conducted with *Daphnia*, algae and *Lemna* (see Table 9.5-4). The results of these studies confirmed that *Lemna* is clearly the most sensitive species (by a magnitude of 1000 more toxic than *Daphnia* and algae).

zRMS comments:

Studies on toxicity of ADM.06001.H.2.B to aquatic organisms were evaluated by the zRMS and are considered acceptable. For details of evaluation please refer to Appendix 2.

9.5.1.1 Justification for new endpoints

New studies and endpoints are provided on acute toxicity in *Daphnia magna* and on effects on algae and aquatic plant growth of ADM.06001.H.2.B to address current data requirements.

9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for aquatic organisms from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).
In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.
Additional aquatic risk assessments have been provided using the updated PECsw calculations - evaluated and in Section 8.
For more information on PECs please refer to this dRR Part B8, point 8.8.

Mesosulfuron-methyl

Table 9.5.2-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. Subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} 100000	NOEC 32000	EC_{50} 100000	NOEC 1800	E_rC_{50} 290	E_rC_{50} 1.29
AF		100	10	100	10	10	10
RAC (µg/L)		1000	3200	1000	180	29	0.129
FOCUS Scenario	PEC $_{gl\ max}$ (µg/L)						
Step 1							
-	3.796	0.0038	0.0012	0.0038	0.0211	0.1309	**29.4264**
Step 2							
N-Europe	0.795	-	-	-	-	-	**6.1628**
S-Europe	1.492	-	-	-	-	-	**11.5659**
Step 3							
D1/ditch	0.176	-	-	-	-	-	**1.3643**
D1/stream	0.114	-	-	-	-	-	0.8837
D2/ditch	1.413	-	-	-	-	-	**10.9535**
D2/stream	0.902	-	-	-	-	-	**6.9922**
D3/ditch	0.078	-	-	-	-	-	0.6047
D4/pond	0.032	-	-	-	-	-	0.2481
D4/stream	0.061	-	-	-	-	-	0.4729
D5/pond	0.014	-	-	-	-	-	0.1085
D5/stream	0.054	-	-	-	-	-	0.4186
D6/ditch	0.085	-	-	-	-	-	0.6589
R1/pond	0.008	-	-	-	-	-	0.0620
R1/stream	0.276	-	-	-	-	-	**2.1395**
R3/stream	0.248	-	-	-	-	-	**1.9225**
R4/stream	0.213	-	-	-	-	-	**1.6512**

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

Table 9.5.2-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on updated FOCUS Steps 3 calculations for the use of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha, BBCH 20-39)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. Subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >100000	NOEC 32000	EC_{50} >100000	NOEC 1800	E_rC_{50} 290	E_rC_{50} 1.29
AF		100	10	100	10	10	10
RAC (µg/L)		>1000	3200	>1000	180	29	0.129
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	3.796	0.0038	0.0012	0.0038	0.0211	0.1309	**29.4264**
Step 2							
N-Europe	0.795	-	-	-	-	-	**6.1628**
S-Europe	1.492	-	-	-	-	-	**11.5659**
Step 3							
D1/ditch	0,508	-	-	-	-	-	**3.9380**
D1/stream	0,318	-	-	-	-	-	**2.4651**
D2/ditch	1,432	-	-	-	-	-	**11.1008**
D2/stream	0,900	-	-	-	-	-	**6.9767**
D3/ditch	0,078	-	-	-	-	-	0.6047
D4/pond	0,032	-	-	-	-	-	0.2481
D4/stream	0,059	-	-	-	-	-	0.4574
D5/pond	0,016	-	-	-	-	-	0.1240
D5/stream	0,066	-	-	-	-	-	0.5116
D6/ditch	0,462	-	-	-	-	-	**3.5814**
R1/pond	0,005	-	-	-	-	-	0.0388
R1/stream	0,080	-	-	-	-	-	0.6202
R3/stream	0,209	-	-	-	-	-	**1.6202**
R4/stream	0,050	-	-	-	-	-	0.3876

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

Table 9.5.2-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on updated FOCUS Steps 3 calculations for the use of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha, BBCH 35-39)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. Subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >100000	NOEC 32000	EC_{50} >100000	NOEC 1800	E_rC_{50} 290	E_rC_{50} 1.29
AF		100	10	100	10	10	10
RAC (µg/L)		>1000	3200	>1000	180	29	0.129
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	3.796	0.0038	0.0012	0.0038	0.0211	0.1309	**29.4264**
Step 2							
N-Europe	0.795	-	-	-	-	-	**6.1628**
S-Europe	1.492	-	-	-	-	-	**11.5659**
Step 3							
D1/ditch	0.176	-	-	-	-	-	**1.3643**
D1/stream	0.114	-	-	-	-	-	0.8837
D2/ditch	2.099	-	-	-	-	-	**16.2713**
D2/stream	1.345	-	-	-	-	-	**10.4264**
D3/ditch	0.078	-	-	-	-	-	0.6047
D4/pond	0.032	-	-	-	-	-	0.2481
D4/stream	0.062	-	-	-	-	-	0.4806
D5/pond	0.014	-	-	-	-	-	0.1085
D5/stream	0.066	-	-	-	-	-	0.5116
D6/ditch	0.086	-	-	-	-	-	0.6667
R1/pond	0.006	-	-	-	-	-	0.0465
R1/stream	0.100	-	-	-	-	-	0.7752
R3/stream	0.137	-	-	-	-	-	**1.0620**
R4/stream	0.213	-	-	-	-	-	**1.6512**

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

Table 9.5.2-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. Subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} 100000	NOEC 32000	EC_{50} 100000	NOEC 1800	E_rC_{50} 290	E_rC_{50} 1.29
AF		100	10	100	10	10	10
RAC (µg/L)		1000	3200	1000	180	29	0.129
FOCUS Scenario	PEC gl max (µg/L)						
Step 1							
-	3.796	0.0038	0.0012	0.0038	0.0211	0.1309	**29.4264**
Step 2							
N-Europe	0.795	-	-	-	-	-	**6.1628**
S-Europe	1.492	-	-	-	-	-	**11.5659**
Step 3							
D1/ditch	0.231	-	-	-	-	-	**1.7907**
D1/stream	0.199	-	-	-	-	-	**1.5426**
D3/ditch	0.078	-	-	-	-	-	0.6047
D4/pond	0.028	-	-	-	-	-	0.2171
D4/stream	0.066	-	-	-	-	-	0.5116
D5/pond	0.014	-	-	-	-	-	0.1085
D5/stream	0.070	-	-	-	-	-	0.5426
R4/stream	0.321	-	-	-	-	-	**2.4884**

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

Table 9.5.2-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on updated FOCUS Steps 3 calculations for the use of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha, BBCH 13-39)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. Subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} >100000	NOEC 32000	EC_{50} >100000	NOEC 1800	E_rC_{50} 290	E_rC_{50} 1.29
AF		100	10	100	10	10	10
RAC (µg/L)		>1000	3200	>1000	180	29	0.129
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	3.796	0.0038	0.0012	0.0038	0.0211	0.1309	**29.4264**
Step 2							
N-Europe	0.795	-	-	-	-	-	**6.1628**
S-Europe	1.492	-	-	-	-	-	**11.5659**
Step 3							
D1/ditch	0.249	-	-	-	-	-	**1.9302**
D1/stream	0.207	-	-	-	-	-	**1.6047**
D3/ditch	0.079	-	-	-	-	-	0.6124
D4/pond	0.036	-	-	-	-	-	0.2791
D4/stream	0.065	-	-	-	-	-	0.5039
D5/pond	0.014	-	-	-	-	-	0.1085
D5/stream	0.065	-	-	-	-	-	0.5039
R4/stream	0.050	-	-	-	-	-	0.3876

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

Table 9.5.2-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl for each organism group based on updated FOCUS Steps 3 calculations for the use of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha, BBCH 35-39)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. Subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} ≥100000	NOEC 32000	EC_{50} ≥100000	NOEC 1800	E_rC_{50} 290	E_rC_{50} 1.29
AF		100	10	100	10	10	10
RAC (µg/L)		≥1000	3200	≥1000	180	29	0.129
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	3.796	0.0038	0.0012	0.0038	0.0211	0.1309	**29.4264**
Step 2							
N-Europe	0.795	-	-	-	-	-	**6.1628**
S-Europe	1.492	-	-	-	-	-	**11.5659**
Step 3							
D1/ditch	0.242	-	-	-	-	-	**1.8760**
D1/stream	0.207	-	-	-	-	-	**1.6047**
D3/ditch	0.078	-	-	-	-	-	0.6047
D4/pond	0.033	-	-	-	-	-	0.2558
D4/stream	0.065	-	-	-	-	-	0.5039
D5/pond	0.014	-	-	-	-	-	0.1085
D5/stream	0.070	-	-	-	-	-	0.5426
R4/stream	0.321	-	-	-	-	-	**2.4884**

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

For the intended uses on cereals, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by an E_rC_{50} for *Lemna gibba* of 1.29 µg a.s./L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

~~**Table 9.5.2-7: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha)**~~

~~**Intended use**~~ ~~**Active substance**~~ ~~**Application rate (g/ha)**~~		~~Winter cereals~~ ~~Mesosulfuron-methyl~~ ~~1 x 12 g a.s./ha~~		
~~**Nozzle reduction**~~	~~**No-spray buffer (m)**~~	~~5~~	~~10~~	~~20~~
	~~**Vegetated filter strip (m)**~~	~~5 (includes 5m VFS-mod)~~	~~10~~	~~20~~
~~None~~	~~D1 ditch~~	~~0.176~~	~~0.176~~	~~0.176~~
~~None~~	~~D2 ditch~~	~~1.413~~	~~1.413~~	~~1.413~~
~~None~~	~~D2 stream~~	~~0.902~~	~~0.902~~	~~0.902~~
~~None~~	~~R1 stream~~	~~0.018~~	~~0.125~~	~~0.065~~
~~None~~	~~R3 stream~~	~~0.026~~	~~0.110~~	~~0.057~~
~~None~~	~~R4 stream~~	~~0.018~~	~~0.097~~	~~0.051~~
~~**RAC (µg/L)**~~ ~~0.129~~		~~**PEC/RAC ratio**~~		
~~None~~	~~D1 ditch~~	~~**1.3643**~~	~~**1.3643**~~	~~**1.3643**~~
~~None~~	~~D2 ditch~~	~~**10.9535**~~	~~**10.9535**~~	~~**10.9535**~~
~~None~~	~~D2 stream~~	~~**6.9922**~~	~~**6.9922**~~	~~**6.9922**~~
~~None~~	~~R1 stream~~	~~0.1395~~	~~0.9690~~	~~0.5039~~
~~None~~	~~R3 stream~~	~~0.2016~~	~~0.8527~~	~~0.4419~~
~~None~~	~~R4 stream~~	~~0.1395~~	~~0.7519~~	~~0.3953~~

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

Table 9.5.2-8: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl based on updated FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha, BBCH 20-39)

Intended use **Active substance** **Application rate (g/ha)**		Winter cereals Mesosulfuron-methyl 1 x 12 g a.s./ha
Nozzle reduction	**No-spray buffer (m)**	**10**
	Vegetated filter strip (m)	**10**
None	D1 ditch	0.508
None	D1 stream	0.318
None	D2 ditch	1.432
None	D2 stream	0.900
None	D6 ditch	0.467
None	R3 stream	0.094
RAC (µg/L) 0.129		**PEC/RAC ratio**
None	D1 ditch	**3.9380**
None	D1 stream	**2.4651**
None	D2 ditch	**11.1008**
None	D2 stream	**6.9767**
None	D6 ditch	3.6814
None	R3 stream	0.7287

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

An acceptable risk for R3 is achieved with a buffer zone (VFS) of 10 meters for use in winter cereals at BBCH 20-39. The drainage scenarios D1, D2 and D6 are not relevant to the central zone.

An acceptable risk for R3 is achieved with a buffer zone (VFS) of 10 meters for use in winter cereals at BBCH 20-39. The drainage scenarios D1, D2 and D6 are not relevant to the central zone.

Table 9.5.2-7-1: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl based on updated FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha, BBCH 35-39)

Intended use **Active substance** **Application rate (g/ha)**		Winter cereals Mesosulfuron-methyl 1 x 12 g a.s./ha
Nozzle reduction	**No-spray buffer (m)**	**10**
	Vegetated filter strip (m)	**10**
None	D1 ditch	0.160
None	D1 stream	0.102
None	D2 ditch	2.074
None	D2 stream	1.329
None	R3 stream	0.066
None	R4 stream	0.099
RAC (µg/L) 0.129		**PEC/RAC ratio**
None	D1 ditch	**1.25**
None	D1 stream	0.80
None	D2 ditch	**16.077**
None	D2 stream	**10.30**
None	R3 stream	0.511
None	R4 stream	0.77

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

An acceptable risk for R3 and R4 is achieved with a buffer zone (VFS) of 10 meters. The drainage scenarios D1 (ditch), D2 are not relevant to the central zone.

Table 9.5.2-8: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha at BBCH 13-39)

Intended use **Active substance** **Application rate (g/ha)**		Spring cereals Mesosulfuron-methyl 1 x 12 g a.s./ha		
Nozzle reduction	**No-spray buffer (m)**	5	10	20
	Vegetated filter strip (m)	5 (includes 5m VFS-mod)	10	20
None	D1 ditch	0.231 0.249	0.231 0.249	0.231 0.249
None	D1 stream	0.190 0.207	0.190 0.207	0.190 0.207
None	R4 stream	0.010	0.145	0.076
RAC (µg/L) 0.129		**PEC/RAC ratio**		
None	D1 ditch	1.7907 **1.9302**	1.7907 **1.9302**	1.7907 **1.9302**
None	D1 stream	1.5426 **1.6047**	1.5426 **1.6047**	1.5426 **1.6047**
None	R4 stream	0.1395	1.1240	0.5891

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

Acceptable risks for aquatic organisms from exposure to mesosulfuron-methyl following application to winter cereals are indicated with a 10-m no-spray buffer with a 10-m vegetative strip or a 5m no-spray buffer with 5m VFS-mod, except for scenarios D1 and D2. The route of exposure is drainage in these scenarios. Mitigation measures provided at Step 4 will not reduce these PEC_{SW} values.

Acceptable risks for aquatic organisms from exposure to mesosulfuron-methyl following application to spring cereals at 13-39 BBCH are indicated at Step 3 without mitigations, except for scenarios D1 and D2. The route of exposure is drainage in these scenarios. Mitigation measures provided at Step 4 will not reduce these PEC_{SW} values.

~~Acceptable risks for aquatic organisms from exposure to mesosulfuron-methyl following application to spring cereals are indicated with a 20-m no-spray buffer with a 20-m vegetative strip or a 5m no-spray buffer with 5m VFS-mod, except for scenarios D1 and D2.~~

~~Risk assessments for aquatic organisms following application to winter and spring cereals using updated PECsw values indicate a safe use for all scenarios with 10m VFS.~~

Table 9.5.2-9-1: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron-methyl based on FOCUS Step 4 calculations and toxicity data for aquatic plants with mitigation of spray drift and run-off for the use of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha) at BBCH 35-39.

Intended use **Active substance** **Application rate (g/ha)**		Winter cereals Mesosulfuron-methyl 1 x 12 g a.s./ha
Nozzle reduction	**No-spray buffer (m)**	**10**
	Vegetated filter strip (m)	**10**
None	D1 ditch	0.231
None	D1 stream	0.199
None	R4 stream	0.147
RAC (µg/L) 0.129		**PEC/RAC ratio**
None	D1 ditch	**1.79**
None	D1 stream	**1.54**
None	R4 stream	**1.14**
Nozzle reduction	**No-spray buffer (m)**	20
	Vegetated filter strip (m)	20
None	D1 ditch	0.231
None	D1 stream	0.199
None	R4 stream	0.077
RAC (µg/L) 0.129		**PEC/RAC ratio**
None	D1 ditch	**1.79**
None	D1 stream	**1.54**
None	R4 stream	0.60

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

Acceptable risks for aquatic organisms from exposure to mesosulfuron-methyl following application to ~~winter~~ spring- cereals are indicated with a 20-m no-spray buffer with a 20-m vegetative strip except for scenarios D1.

zRMS comments:

Based on the calculations of the risk assessment for aquatic organism for mesosulfuron-methyl the following conclusions has been derived:

1. **Winter cereals at BBCH 20-39:**
   - acceptable risk with no need for risk mitigation measures: D3, D4, D5, R1, R4 scenarios
   - scenario R3: risk acceptable with 10 m VFS
   - scenarios: D1, D2, D6 an unacceptable risk
2. **Winter cereals at BBCH 35-39:**
   - acceptable risk with no need for risk mitigation measures: D1 (stream), D3, D4, D5, D6, R1 scenarios
   - scenario R3: risk acceptable with 10 m VFS
   - scenario R4: risk acceptable with 10 m VFS
3. **Spring cereals at BBCH 13-39:**
   - acceptable risk with no need for risk mitigation measures: D3, D4, D5, R4 scenarios
   - ~~scenario R4: risk acceptable with 20 m VFS~~
   - scenarios D1: risk an unacceptable risk
4. **Spring cereals at BBCH 35-39:**
   - acceptable risk with no need for risk mitigation measures: ~~D1 (ditch),~~ D3, D4, D5 scenarios
   - scenario R4: risk acceptable with 20 m VFS
   - scenario D1 (stream), D1 (ditch): risk an unacceptable risk

Metabolites of mesosulfuron-methyl

Table 9.5.2-10: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for mesosulfuron (AE F154851) following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish/ Invertebrates	Algae	Aquatic plants
Test species		***O.mykiss/D.daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >10 0000	ErC_{50} 38000	ErC_{50} 110
AF		100	10	10
RAC (µg/L)		>100	3800	11
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.76	0.0076	0.0002	0.0691

*In the absence of a toxicity endpoint for the metabolite 10 times higher toxicity than the parent is assumed, in line with the EFSA Conclusion (2016)

Table 9.5.2-11: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for AE F160459 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish/ Invertebrates	Algae	Aquatic plants
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >10 0000	ErC_{50} 100000	ErC_{50} 2600
AF		100	10	10
RAC (µg/L)		100	10000	260
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	1.18	0.0118	0.0001	0.0045

*In the absence of a toxicity endpoint for the metabolite 10 times higher toxicity than the parent is assumed, in line with the EFSA Conclusion (2016)

Table 9.5.2-12: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for AE F099095 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 70700	EC_{50} 100000	ErC_{50} 99100	ErC_{50} 100000
AF		100	100	10	10
RAC (µg/L)		707	1000	9910	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
	0.247	0.0003	0.0002	<0.0001	<0.0001

Table 9.5.2-13: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for AE F092944 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish acute	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 97000	EC_{50} 100000	NOEC 24900	ErC_{50} 100000	ErC_{50} 100000
AF		100	100	10	10	10
RAC (µg/L)		970	1000	2490	10000	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	0.073	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 9.5.2-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for AE F160460 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish/ Invertebrates	Algae	Aquatic plants
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >10 0000*	ErC_{50} 29*	ErC_{50} 100000
AF		100	10	10
RAC (µg/L)		100	2.9	10000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
	0.641	0.0064	0.2210	<0.0001

* Since no toxicity endpoint for the metabolite is available, the toxicity endpoint for the parent divided by 10 was used.

Table 9.5.2-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for AE F140584 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish/ Invertebrates	Algae	Aquatic plants
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >10 0000*	ErC_{50} 29*	ErC_{50} 10000
AF		100	10	10

Group		Fish/ Invertebrates	Algae	Aquatic plants
RAC (µg/L)		100	2.9	1000
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	0.232	0.00232	0.0800	0.0002

* Since no toxicity endpoint for the metabolite is available, the toxicity endpoint for the parent divided by 10 was used.

Table 9.5.2-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for AE F147447 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		**Fish/ Invertebrates**	**Algae**	**Aquatic plants**
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >10 0000*	ErC_{50} 100000	ErC_{50} 100000
AF		100	10	10
RAC (µg/L)		100	10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.390	0.00390	<0.0001	<0.0001

***** Since no toxicity endpoint for the metabolite is available, the toxicity endpoint for the parent divided by 10 was used.

~~**Table 9.5.2-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CV14885 following application of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha)**~~

~~**Group**~~		~~**Algae**~~	~~**Aquatic plants**~~
~~**Test species**~~		~~***Pseudokirchn. subcapitata***~~	~~***Lemna gibba***~~
~~**Endpoint (µg/L)**~~		~~E_rC_{50}~~ ~~290*~~	~~E_rC_{50}~~ ~~1.29*~~
~~**AF**~~		~~10~~	~~10~~
~~**RAC (µg/L)**~~		~~29~~	~~0.129~~
~~**FOCUS Scenario**~~	~~**PEC $_{gl\text{-}max}$ (µg/L)**~~		
~~**Step 1**~~			
~~-~~	~~0.843~~	~~0.0291~~	~~**6.5349**~~
~~**Step 2**~~			
~~N-Europe~~	~~0.176~~	~~-~~	~~**1.3643**~~
~~S-Europe~~	~~0.333~~	~~-~~	~~**2.5814**~~
~~**Step 3**~~			
~~D1/ditch~~	~~0.056~~	~~-~~	~~0.4341~~
~~D1/stream~~	~~0.039~~	~~-~~	~~0.3023~~
~~D2/ditch~~	~~0.061~~	~~-~~	~~0.4729~~
~~D2/stream~~	~~0.280~~	~~-~~	~~**2.1705**~~
~~D3/ditch~~	~~0.060~~	~~-~~	~~0.4651~~
~~D4/pond~~	~~0.012~~	~~-~~	~~0.0930~~
~~D4/stream~~	~~0.051~~	~~-~~	~~0.3953~~
~~D5/pond~~	~~0.098~~	~~-~~	~~0.7597~~
~~D5/stream~~	~~0.038~~	~~-~~	~~0.2946~~
~~D6/ditch~~	~~0.027~~	~~-~~	~~0.2093~~
~~R1/pond~~	~~0.002~~	~~-~~	~~0.0155~~
~~R1/stream~~	~~0.007~~	~~-~~	~~0.0543~~
~~R3/stream~~	~~0.016~~	~~-~~	~~0.1240~~
~~R4/stream~~	~~0.007~~	~~-~~	~~0.0543~~

~~* Mesosulfuron-methyl endpoints used as information indicates that the toxophore is lost, please refer to EFSA Conclusion 4584/2016~~

Table 9.5.2-18: Aquatic organisms: Updated PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CV14885 following application of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha, BBCH 20-39)

Group		**Fish/ Invertebrates**	**Algae**	**Aquatic plants**
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >100 0000*	E_rC_{50} 290*	E_rC_{50} 1.29*
AF		100	10	10
RAC (µg/L)		1000	29	0.129
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.843	0.000843	0.0291	**6.5349**
Step 2				
N-Europe	0.176		-	**1.3643**
S-Europe	0.333		-	**2.5814**
Step 3				
D1/ditch	0.057		-	0.4419
D1/stream	0.051		-	0.3953
D2/ditch	0.077		-	0.5969
D2/stream	0.279		-	**2.1628**
D3/ditch	0.060		-	0.4651
D4/pond	0.123		-	0.9535
D4/stream	0.054		-	0.4186
D5/pond	0.099		-	0.7674
D5/stream	0.038		-	0.2946
D6/ditch	0.034		-	0.2636
R1/pond	0.002		-	0.0155
R1/stream	0.002		-	0.0155
R3/stream	0.014		-	0.1085
R4/stream	<0.001		-	<0.0078

* Mesosulfuron-methyl endpoints used as information indicates that the toxophore is lost, please refer to EFSA Conclusion 4584/2016.

In the absence of a toxicity endpoint for the metabolite the available toxicity endpoint of the parent compound was used since from the available information the toxophore appear to be lost.

Table 9.5.2-19: Aquatic organisms: Updated PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CV14885 following application of ADM.06001.H.2.B in winter cereals (1 x 12 g a.s./ha, BBCH 35-39)

Group		**Fish/ Invertebrates**	**Algae**	**Aquatic plants**
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >100 0000*	E_rC_{50} 290*	E_rC_{50} 1.29*
AF		100	10	10
RAC (µg/L)		1000	29	0.129
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
	0.843	0.000843	0.0291	**6.5349**
Step 2				
N-Europe	0.176		-	**1.3643**
S-Europe	0.333		-	**2.5814**
Step 3				
D1/ditch	0.056		-	0.4341
D1/stream	0.039		-	0.3023
D2/ditch	0.151		-	**1.1705**
D2/stream	0.414		-	**3.2093**
D3/ditch	0.060		-	0.4651
D4/pond	0.119		-	0.9225
D4/stream	0.052		-	0.4031
D5/pond	0.094		-	0.7287
D5/stream	0.037		-	0.2868
D6/ditch	0.025		-	0.1938
R1/pond	0.002		-	0.0155
R1/stream	0.003		-	0.0233
R3/stream	0.010		-	0.0775
R4/stream	0.007		-	0.0543

* Mesosulfuron-methyl endpoints used as information indicates that the toxophore is lost, please refer to EFSA Conclusion 4584/2016

In the absence of a toxicity endpoint for the metabolite the available toxicity endpoint of the parent compound was used since from the available information the toxophore appear to be lost.

Step 4 calculations with mitigation are not provided for metabolite BCS CV14885 because the only scenario that fails the risk assessment is D2 Stream. The exposure route is drainage, so mitigation measures provided at Step 4 will not reduce the PEC_{SW} value. D2 is not relevant to the central zone.

~~**Table 9.5.2-20: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CV14885 following application of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha)**~~

~~**Group**~~		~~**Algae**~~	~~**Aquatic plants**~~
~~**Test species**~~		~~***Pseudokirchn. subcapitata***~~	~~***Lemna gibba***~~
~~**Endpoint (µg/L)**~~		~~E_rC_{50} 290*~~	~~E_rC_{50} 1.29*~~
~~**AF**~~		~~10~~	~~10~~
~~**RAC (µg/L)**~~		~~29~~	~~0.129~~
~~**FOCUS Scenario**~~	~~**PEC $_{gl-max}$ (µg/L)**~~		
~~**Step 1**~~			
~~-~~	~~0.843~~	~~0.0291~~	~~**6.5349**~~
~~**Step 2**~~			
~~N-Europe~~	~~0.176~~	~~-~~	~~**1.3643**~~
~~S-Europe~~	~~0.333~~	~~-~~	~~**2.5814**~~
~~**Step 3**~~			
~~D1/ditch~~	~~0.025~~	~~-~~	~~0.1938~~

Group		Algae	Aquatic plants
D1/stream	0.030	-	0.2326
D3/ditch	0.050	-	0.3876
D4/pond	0.061	-	0.4729
D4/stream	0.025	-	0.1938
D5/pond	0.084	-	0.6512
D5/stream	0.032	-	0.2481
R4/stream	0.011	-	0.0853

* Mesosulfuron-methyl endpoints used as information indicates that the toxophore is lost, please refer to EFSA Conclusion 4584/2016

Table 9.5.2-21: Aquatic organisms: Updated PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CV14885 following application of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha, BBCH 13-39)

Group		**Fish/ Invertebrates**	**Algae**	**Aquatic plants**
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >100 0000*	E_rC_{50} 290*	E_rC_{50} 1.29*
AF		100	10	10
RAC (µg/L)		1000	29	0.129
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	0.843	0.000843	0.0291	**6.5349**
Step 2				
N-Europe	0.176		-	**1.3643**
S-Europe	0.333		-	**2.5814**
Step 3				
D1/ditch	0.006		-	0.0465
D1/stream	0.017		-	0.1318
D3/ditch	<0.001		-	<0.0078
D4/pond	0.007		-	0.0543
D4/stream	0.001		-	0.0078
D5/pond	0.007		-	0.0543
D5/stream	0.001		-	0.0078
R4/stream	0.001		-	0.0078

* Mesosulfuron-methyl endpoints used as information indicates that the toxophore is lost, please refer to EFSA Conclusion 4584/2016

In the absence of a toxicity endpoint for the metabolite the available toxicity endpoint of the parent compound was used since from the available information the toxophore appear to be lost.

Table 9.5.2-22: Aquatic organisms: Updated PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CV14885 following application of ADM.06001.H.2.B in spring cereals (1 x 12 g a.s./ha, BBCH 35-39)

Group		**Fish/ Invertebrates**	**Algae**	**Aquatic plants**
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >100 0000*	E_rC_{50} 290*	E_rC_{50} 1.29*
AF		100	10	10
RAC (µg/L)		>1000	29	0.129
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	0.843	0.000843	0.0291	**6.5349**
Step 2				
N-Europe	0.176	0.000176	-	**1.3643**
S-Europe	0.333	0.000333	-	**2.5814**

Group		Fish/ Invertebrates	Algae	Aquatic plants
Step 3				
D1/ditch	0.036	-	-	0.2791
D1/stream	0.033	-	-	0.2558
D3/ditch	0.055	-	-	0.4264
D4/pond	0.097	-	-	0.7519
D4/stream	0.038	-	-	0.2946
D5/pond	0.084	-	-	0.6512
D5/stream	0.032	-	-	0.2481
R4/stream	0.011	-	-	0.0853

*Mesosulfuron-methyl endpoints used as information indicates that the toxophore is lost, please refer to EFSA Conclusion 4584/2016.

In the absence of a toxicity endpoint for the metabolite the available toxicity endpoint of the parent compound was used since from the available information the toxophore appear to be lost.

Table 9.5.2-103: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for BCS CO60720 following application of ADM.06001.H.2.B in winter and spring cereals (1 x 12 g a.s./ha)

Group		Fish/ Invertebrates	Algae	Aquatic plants
Test species		***O.mykiss/Daphnia****	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint (µg/L)		LC_{50}/EC_{50} >10 0000*	ErC_{50} 10000	ErC_{50} 11800
AF		100	10	10
RAC (µg/L)		100	1000	1180
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**			
Step 1				
	0.436	0.00436	0.0004	0.0004

zRMS comments:

The risk assessment for mesosulfuron-methyl metabolites is considered acceptable.

Pinoxaden

The risk assessment relies on step 1 and 2 modelling, therefore updated modelling not considered further.

Table 9.5.2-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pinoxaden for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 60 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species		***Oncorhynchus mykiss***	***Crassostrea virginica***	***Skeletonema costatum***	***Phragmytes australis***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		10300	880	1324.4	8500
AF		100	100	10	10
RAC (µg/L)		103	8.8	132.44	850
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	14.851	0.1442	**1.6876**	0.1121	0.0175
Step 2					
N-Europe	0,552	-	0.0627	-	-
S-Europe	0,552	-	0.0627	-	-

The risks to aquatic organisms from exposure to pinoxaden are acceptable without mitigation.

Metabolites of pinoxaden

Table 9.5.2-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite NOA 407854 (M2) for each organism group based on FOCUS Step 1 calculations for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 60 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Invertb. prolonged	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		100000	1000	100000	6250	100000	14600
AF		100	10	100	10	10	10
RAC (µg/L)		1000	100	1000	625	10000	1460
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	31.596	0.0316	0.3160	0.0316	0.0506	0.0032	0.0216

Table 9.5.2-26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite NOA 447204 (M3) for each organism group based on FOCUS Step 1 calculations for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 60 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae	Aquatic plants
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		120000	120000	120000	100000
AF		100	100	10	10
RAC (µg/L)		1200	1200	12000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	32.339	0.0269	0.0269	0.0027	0.0032

The risks to aquatic organisms from exposure to pinoxaden metabolites are acceptable without mitigation.

zRMS comments:

The calculations provided for pinoxaden and two potentially relevant surface water metabolites of pinoxaden such as: M2 (NOA407854) and M3 (NOA447204) presented in the Tables 9.5.2 24 and 9.5.2-25 are validated by zRMS.
Based on FOCUS Step 1 or 2, all PEC/RAC ratios for pinoxaden and pinoxaden metabolites are less than 1, indicating an acceptable risk for aquatic organism.

Mefenpyr-diethyl

The risk assessment relies on step 1 and 2 modelling, therefore updated modelling not considered further

Table 9.5.2-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefenpyr-diethyl for each organism group based on FOCUS Step 1 calculations for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 35 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		***Cyprinus carpio***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 2400	NOEC 100	EC_{50} 5500	NOEC 320	E_rC_{50} 3120	E_rC_{50} 7600
AF		100	10	100	10	10	10
RAC (µg/L)		24	10	55	32	312	760
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	6.756	0.2815	0.6756	0.1228	0.2111	0.0217	0.0089

The risks to aquatic organisms from exposure to mefenpyr-diethyl are acceptable without mitigation.

Metabolites of mefenpyr-diethyl

Table 9.5.2-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite AE F113225 for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 35 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
Test species		***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint (µg/L)		LC_{50} 100000	NOEC 32000	EC_{50} 100000	NOEC 3200	E_rC_{50} 100000
AF		100	10	100	10	10
RAC (µg/L)		1000	3200	1000	320	10000
FOCUS Scenario	**PEC gl-max (µg/L)**					
Step 1						
	12.187	0.0122	0.0038	0.0122	0.0381	0.0012

Table 9.5.2-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite AE F109453 for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 35 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint (µg/L)		LC_{50} 100000	EC_{50} 100000	E_rC_{50} 100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	4.676	0.0047	0.0047	0.0005

Table 9.5.2-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite AE F094270 for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 35 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sediment
Test species		***Brachydanio rerio***	***Brachydanio rerio***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 72000	NOEC 3200	EC_{50} 60300	NOEC 32000	E_rC_{50} 40200	NOEC 50000
AF		100	10	100	10	10	10
RAC (µg/L)		720	320	603	3200	4020	5000
FOCUS Scenario	**PEC gl-max (µg/L)**						
Step 1							
	9.374	0.0130	0.0293	0.0155	0.0029	0.0023	0.0019

Table 9.5.2-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite AE F2211046 for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 35 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plants**
Test species		***Cyprinus carpio***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 240*	NOEC 10*	EC_{50} 550*	NOEC 32*	E_rC_{50} 312*	E_rC_{50} 760*
AF		100	10	100	10	10	10
RAC (µg/L)		2.4	1	5.5	3.2	31.2	76
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	0.487	0.2029	0.4870	0.0885	0.1522	0.0156	0.0064

* Since no toxicity endpoint for the metabolite is available, the toxicity endpoint for the parent divided by 10 was used.

Table 9.5.2-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite AE F114952 for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 35 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**
Test species		***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint (µg/L)		LC_{50} 100000*	NOEC 32000*	EC_{50} 100000*	NOEC 3200*	E_rC_{50} 100000*
AF		100	10	100	10	10
RAC (µg/L)		1000	3200	1000	320	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**					
Step 1						
	3.260	0.0033	0.0010	0.0033	0.0102	0.0003

* Since no toxicity endpoint for the metabolite is available, the toxicity endpoints for AE F113225 were used since AE F114952 is an isomer of AE F113225.

The risks to aquatic organisms from exposure to mefenpyr-diethyl metabolites are acceptable without mitigation.

zRMS comments:

For mefenpyr-diethyl, the risk assessment is based on FOCUS Step 1 and 2 PEC_{SW} values.
Four potentially relevant surface water metabolites of mefenpyr-diethyl such as AE F113225, AE F109453, AE F094270, AE F114952 have been identified which need to be considered in the aquatic risk assessment.
Based on FOCUS Step 1, all PEC/RAC ratios for the potentially relevant surface water metabolites of mefenpyr-diethyl are less than 1 indicating an acceptable risk.

Formulated product ADM.06001.H.2.B

PEC_{SW} values were calculated for formulation ADM.06001.H.2.B following application to cereals, based on a standard FOCUS ditch scenario and Ganzelmeier drift values, please refer to this dRR document Part B8, point 8.9.2. Endpoints for toxicity studies on aquatic organisms using formulation ADM.06001.H.2.B are provided in this document, Table 9.5-4.

Table 9.5.2-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for formulation ADM.06001.H.2.B for the use of ADM.06001.H.2.B in winter and spring cereals (1 x 1 L product/ha)

Group		Inverteb. acute	Algae	Aquatic plants
Test species		***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***
Endpoint (µg/L)		EC_{50} 79500	E_rC_{50} 54800	E_rC_{50} 74
AF		100	10	10
RAC (µg/L)		7950	5480	7.4
Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
No buffer	7.7899	0.00098	0.0014	**1.0527**
5m buffer	2.1115	-	-	0.2853

Mixture toxicity assessment

In accordance with "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", the measured mixture toxicity should be compared to the calculated toxicity to determine if the combined toxicity of the components is additive, synergistic or antagonistic. The mixture toxicity for each aquatic trophic level was calculated as follows:

$$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

Model deviation ratios were calculated using the following equation:

$$MDR = \frac{ECx_{mix-CA}\ (\text{calculated mixture toxicity})}{ECx_{PPP}\ (\text{measured mixture toxicity})}$$

Results are presented in the table below.

For the mixture assessment the same species were used for each trophic level (Oncorhynchus mykiss for fish, Daphnia magna for aquatic invertebrates, Pseudokirchneriella subcapitata for algae and Lemna gibba for aquatic plants).

Table 9.5.2-34: Calculation of Model Deviation Ratio (with safener)

Substance	Concentration in formulation (g/L)	Relative fraction of mixture	Aquatic endpoint			
			Fish acute LD_{50} (µg/L) (*O. mykiss*)	**Inverteb. acute EC_{50} (µg/L) (*Daphnia magna*)**	**Algae E_rC_{50} (µg/L) (*P. subcapitata*)**	**Aq. Plants E_rC_{50} (µg/L) (*Lemna gibba*)**
Mesosulfuron-methyl	12	0.11	100000	100000	290	1.29
Pinoxaden	60	0.56	10300	52000	~~1324.4~~ 41000	9730
Mefenpyr-diethyl	35	0.33	4200	5500	~~5120~~ 10710	7600
ECx_{mix-CA}			7494	14010	~~1093~~ 2321	11.49
ECx_{PPP}			na	79500	54800	74.00
$ECx_{PPPcorr}$[e*]			na	8770	6045	8.163

Substance	Concentration in formulation (g/L)	Relative fraction of mixture	Aquatic endpoint			
			Fish acute LD_{50} (µg/L) (*O. mykiss*)	Inverteb. acute EC_{50} (µg/L) (*Daphnia magna*)	Algae E_rC_{50} (µg/L) (*P. subcapitata*)	Aq. Plants E_rC_{50} (µg/L) (*Lemna gibba*)
MDR			na	1.598	~~0.181~~ 0.384	1.407

~~[b] *Daphnia magna* endpoint used instead of *Crassostrea virginica* endpoint to allow comparison of toxicity to the same species for each substance~~

~~[c] *Lemna gibba* endpoint used instead of *Phragmytes australis* endpoint to allow comparison of toxicity to the same species for each substance~~

[e*] Corrected for density of 0.97 g/L and sum of active substances of 107 g/L

The MDR values for aquatic invertebrates, algae and aquatic plants are between 0.2 and 5, indicating that observed and calculated mixture toxicities are in agreement.

~~The MDR value for algae is slightly below 0.2, indicating that the formulated product with safener is slightly less toxic than predicted.~~

The MDR calculation is presented again below, without mefenpyr-diethyl as this is a safener rather than an active substance.

Table 9.5.2-35: Calculation of Model Deviation Ratio (without safener)

Substance	Concentration in formulation (g/L)	Relative fraction of mixture	Aquatic endpoint			
			Fish acute LD_{50} (µg/L) (*O. mykiss*)	Inverteb. acute EC_{50} (µg/L) (*Daphnia magna*)	Algae E_rC_{50} (µg/L) (*P. subcapitata*)	Aq. Plants E_rC_{50} (µg/L) (*Lemna gibba*)
Mesosulfuron-methyl	12	0.17	100000	100000	290	1.29
Pinoxaden	60	0.83	10300	52000	~~1224.4~~ 41000	9730
$ECx_{mix\text{-}CA}$			12111	56522	830.6	7.735
ECX_{PPP}			na	79500	54800	74.00
$ECx_{PPPcorr}$[c]			na	5901	4068	5.493
MDR			na	9.578	~~0.204~~ 0.413	1.408

~~[b] *Daphnia magna* endpoints used instead of *Crassostrea virginica* endpoint to allow comparison of toxicity to the same species for each substance~~

~~[c] *Lemna gibba* endpoint used instead of *Phragmytes australis* endpoint to allow comparison of toxicity to the same species for each substance~~

[e*] Corrected for density of 0.97 g/L and sum of active substances of 107 g/L

The MDR values for algae and aquatic plants are between 0.2 and 5, indicating that observed and calculated mixture toxicities are in agreement. The MDR value for aquatic invertebrates is above 5, indicating that the formulated product with safener is slightly more toxic than predicted.

The full calculations including all exposure scenarios and GAP scenarios have been performed with the excel based Aquatic Mixtox calculation tool recommended by the Central/Northern Zones (v.1.22).
Calculations have been made with and without considering the safener. Without considering the safener the MDR was above 5 indicating synergistic effects for aquatic invertebrates.
However, these effects can be explained by the safener mefenpyr-diethyl. Therefore, the mixture calculations including the safener are relevant. For transparency reasons the calculations without safener are provided as well.

Winter cereals including safener BBCH 20-39

KCP102~1.XLS

Conclusion:

Steps	Conclusion on the Steps			
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)				Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?				
Step 2: apparent synergism or antagonism?				
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment				

Winter cereals including safener BBCH 35-39

KCP102~2.XLS

Conclusion:

Steps	Conclusion on the Steps			
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)				Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?				
Step 2: apparent synergism or antagonism?				
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment				

Spring cereals including safener BBCH 13-39

KCP102~3.XLS

Conclusion:

Steps		Conclusion on the Steps		
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)				Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?				
Step 2: apparent synergism or antagonism?				
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios in FOCUS step 1-3.
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment				

Spring cereals including safener BBCH 35-39

KCP102~4.XLS

Conclusion:

Steps		Conclusion on the Steps		
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)				Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?				
Step 2: apparent synergism or antagonism?				
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment				

Winter cereals without safener BBCH 20-39

KCCC50~1.XLS

Conclusion:

Steps	Conclusion on the Steps			
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)		No driver detected, go to Step 1.		Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?		Endpoints available for a.s. and the ppp, go to Step 2.		
Step 2: apparent synergism or antagonism?		The MDR is >5. Thus, synergism is indicated, go to Step 10.		
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment		Measured mixture toxicity plausible: go to Step 3 *		

* the potential synergism can be explained by the safener mefenpyr-diethyl. When including the safener in the mixture toxicity assessment the synergism disappears. For the calculations including the safener mefenpyr-diethyl please refer to the assessment "Winter cereals including safener BBCH 20-39" above

Winter cereals without safener BBCH 35-39

Conclusion:

Steps	Conclusion on the Steps			
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)		No driver detected, go to Step 1.		Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?		Endpoints available for a.s. and the ppp, go to Step 2.		
Step 2: apparent synergism or antagonism?		The MDR is >5. Thus, synergism is indicated, go to Step 10.		
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment		Measured mixture toxicity plausible: go to Step 3 *		

* the potential synergism can be explained by the safener mefenpyr-diethyl. When including the safener in the mixture toxicity assessment the synergism disappears. For the calculations including the safener mefenpyr-diethyl please refer to the assessment "Winter cereals including safener BBCH 35-39" above

Spring cereals without safener BBCH 13-39

Conclusion:

Steps		Conclusion on the Steps		
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)		No driver detected, go to Step 1.		Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?		Endpoints available for a.s. and the ppp, go to Step 2.		
Step 2: apparent synergism or antagonism?		The MDR is >5. Thus, synergism is indicated, go to Step 10.		
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios in FOCUS step 1-3.
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment		Measured mixture toxicity plausible: go to Step 3 *		

* the potential synergism can be explained by the safener mefenpyr-diethyl. When including the safener in the mixture toxicity assessment the synergism disappears. For the calculations including the safener mefenpyr-diethyl please refer to the assessment "Spring cereals including safener BBCH 13-39" above

Spring cereals without safener BBCH 35-39


KCA925~1.XLS

Conclusion:

Steps		Conclusion on the Steps		
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)		No driver detected, go to Step 1.		Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?		Endpoints available for a.s. and the ppp, go to Step 2.		
Step 2: apparent synergism or antagonism?		The MDR is >5. Thus, synergism is indicated, go to Step 10.		
Step 3: mixture similar or not?				
Step 4: ETRmix assessment (ECxPPP)				
Step 5: driver available?				There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment				Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment				
Step 8b: RQmix assessment				
Step 9: antagonism assessment				
Step 10: synergism assessment		Measured mixture toxicity plausible: go to Step 3 *		

* the potential synergism can be explained by the safener mefenpyr-diethyl. When including the safener in the mixture toxicity assessment the synergism disappears. For the calculations including the safener mefenpyr-diethyl please refer to the assessment "Spring cereals including safener BBCH 35-39" above

Mixture toxicity conclusion:

The risk from the mixture toxicity assessment is acceptable for fish, aquatic invertebrates and algae at the screening step. For aquatic macrophytes it could be demonstrated that mesosulfuron-methyl is clearly driving the toxicity (contributes to more than 90% of the toxicity), therefore the respective mitigations from the active substance mesosulfuron-methyl are relevant.

zRMS comments:

The mixture calculations including the safener and without safener have been validated by zRMS.
The risk from the mixture toxicity assessment is acceptable for fish, aquatic invertebrates and algae at the screening step. For aquatic macrophytes the a.s.- mesosulfuron-methyl is driving the toxicity (contributes to more than 90% of the toxicity).

It can be concluded that the mitigations from the active substance mesosulfuron-methyl are relevant and cover mixture toxicity assessment.

The next step is to check whether the mixture composition in the ECx_{ppp} is similar to the mixture composition of at the PEC_{mix}. The PEC_{mix} is calculated using the formula below:

$$PEC_{mix} = \sum_{i=1}^{n} PEC_i$$

The PEC_{sw} values for scenario D2 ditch were used as a worst case, giving a PEC_{mix} of 2.023 µg/L.
The PEC_{mix} values without safener, using values from scenario D2 ditch, is 1.798 µg/L.
The PEC_{sw} values for BBCH 20-39 scenario D2 ditch were used as a worst case, giving a PEC_{mix} of 1.952 µg/L.
The PEC_{mix} values for BBCH 20-39 without safener, using values from scenario D2 ditch, is 1.729 µg/L.
The PEC_{sw} values for BBCH 35-39 scenario D2 ditch were used as a worst case, giving a PEC_{mix} of 2.728 µg/L.
The PEC_{mix} values for BBCH 35-39 without safener, using values from scenario D2 ditch, is 2.484 µg/L.
The relative proportions of individual components in the PEC_{mix} are shown in the table below and used to calculate $ECx_{mix\text{-}CA}$ using the formulation as described above. These $ECx_{mix\text{-}CA}$ values are compared to the previously $ECx_{mix\text{-}CA}$, which were calculated using the proportions of individual components in the formulation, using the following equation:

$ECx_{mix\text{-}CA}$ (a.s. in PPP)/$ECx_{mix\text{-}CA}$ (a.s. in PEC_{mix})

Table 9.5.2-36: Calculation of $ECx_{mix\text{-}CA}$ using proportions of the PEC_{mix} (with safener)

Substance	Relative fraction of PECmix	Aquatic endpoint		
		Inverteb. acute EC_{50} (µg/L)	Algae E_rC_{50} (µg/L)	Aq. Plants E_rC_{50} (µg/L)
Mesosulfuron-methyl	0.698	100000	290	1.29
Pinoxaden	0.190	52000[a]	1324.4	9730[b]
Mefenpyr-diethyl	0.111	5500	3120	7600
$ECx_{mix\text{-}CA}$		32398	386.4	1.847

[a] *Daphnia magna* endpoints used instead of *Crassostrea virginica* endpoint to allow comparison of toxicity to the same species for each substance
[b] *Lemna gibba* endpoint used instead of *Phragmytes australis* endpoint to allow comparison of toxicity to the same species for each substance

Table 9.5.2-37: Calculation of $ECx_{mix\text{-}CA}$ using proportions of the PEC_{mix} (with safener)

Substance		Relative fraction of PECmix	Aquatic endpoint		
			Inverteb. acute EC_{50} (µg/L)	Algae E_rC_{50} (µg/L)	Aq. Plants E_rC_{50} (µg/L)
Mesosulfuron-methyl		0.786	100000	290	1.29
Pinoxaden		0.214	52000[a]	1324.4	9730[b]
	$ECx_{mix\text{-}CA}$		83496	348.2	1.641

[a] *Daphnia magna* endpoints used instead of *Crassostrea virginica* endpoint to allow comparison of toxicity to the same species for each substance

[b] *Lemna gibba* endpoint used instead of *Phragmytes australis* endpoint to allow comparison of toxicity to the same species for each substance

Table 9.5.2-38: Comparison of values calculated using proportions of the formulation and proportions of the PEC_{mix} (with safener)

Substance	$ECx_{mix\text{-}CA}$ calculated with Rel. proportions in PPP	$ECx_{mix\text{-}CA}$ calculated with Rel. proportions in PEC_{mix}	Comparison
Aquatic invertebrates	14010	32398	0.432
Algae	1093	386.4	2.828
Aquatic plants	11.49	1.847	6.221

All comparison values are greater than 1.2 or less than 0.8, therefore the mixtures are not considered to be similar.

The comparison of relative proportions is presented again without consideration of mefenpyr-diethyl as this is a safener rather than an active substance.

Table 9.5.2-39: Comparison of values calculated using proportions of the formulation and proportions of the PEC_{mix} (without safener)

Substance	$ECx_{mix\text{-}CA}$ calculated with Rel. proportions in PPP	$ECx_{mix\text{-}CA}$ calculated with Rel. proportions in PEC_{mix}	Comparison
Aquatic invertebrates	56522	83496	0.677
Algae	830.6	348.2	2.385
Aquatic plants	7.735	1.641	4.712

All comparison values are greater than 1.2 or less than 0.8, therefore the mixtures are not considered to be similar.

The next step is to check if one substance is clearly responsible for the toxic effects (a single driver). This comparison is carried out by calculating TU values using the following equation:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

The calculations are presented in the table below.

Table 9.5.2-40: TU values for comparative toxicity of active substances to aquatic organisms (with safener)

Substance	Concentration in formulation (g/L)	Aquatic endpoint					
		Inverteb. acute EC_{50} (µg/L)	Inverteb. TU values (% of total TU)	Algae E_rC_{50} (µg/L)	Algae TU values (% of total TU)	Aq. Plants E_rC_{50} (µg/L)	Aq. Plants TU values (% of total TU)
Mesosulfuron-methyl	12	100000	0.0001 (1.6)	290	0.0414 (42.3)	1.29	9.3023 (99.9)
Pinoxaden	60	52000	0.0012 (15.1)	1324.4	0.0453 (46.3)	9730	0.0062 (0.07)
Mefenpyr-diethyl	35	5500	0.0064 (83.3)	3120	0.0112 (11.5)	7600	0.0046 (0.05)
		Total	0.0076	Total	0.0979	Total	9.3131

The TU values indicate that mesosulfuron-methyl is clearly the single driver for toxicity to aquatic plants (≥90%). There is no clear single driver for toxicity to aquatic invertebrates or algae. For the risk

assessment for aquatic plants please refer to the risk assessment for mesosulfuron-methyl (Tables 9.5.2-1 to 9.5.2-4). For the risk assessments for aquatic invertebrates and algae please see below.

The TU calculation is presented again below, without mefenpyr-diethyl as this is a safener rather than an active substance.

Table 9.5.2-41: TU values for comparative toxicity of active substances to aquatic organisms (without safener)

Substance	Concentration in formulation (g/L)	Aquatic endpoint					
		Inverteb. acute EC_{50} (µg/L)	Inverteb. TU values (% of total TU)	Algae E_rC_{50} (µg/L)	Algae TU values (% of total TU)	Aq. Plants E_rC_{50} (µg/L)	Aq. Plants TU values (% of total TU)
Mesosulfuron-methyl	12	100000	0.0001 (9.4)	290	0.0414 (47.7)	1.29	9.3023 (99.9)
Pinoxaden	60	52000	0.0012 (90.6)	1324.4	0.0453 (52.3)	9730	0.0062 (0.07)
		Total	0.0013	Total	0.0867	Total	9.3085

The TU values indicate that pinoxaden is the clear driver for aquatic invertebrate toxicity (≥90%) and mesosulfuron-methyl is clearly the single driver for toxicity to aquatic plants (≥90%). There is no clear single driver for toxicity to algae. For the risk assessment for aquatic invertebrates please refer to the risk assessment for pinoxaden (Table 9.5.2-15). For the risk assessment for aquatic plants please refer to the risk assessment for mesosulfuron-methyl (Tables 9.5.2-1 to 9.5.2-4). For the risk assessments for algae please see below.
For the three-way mixture of mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl, there were no clear drivers for toxicity to aquatic invertebrates or algae. Therefore, the risks to aquatic invertebrates and algae have been assessed using the calculated $ECx_{mix\text{-}CA}$ and the calculated PEC_{mix} values, please see table below.

Table 9.5.2-112: Aquatic invertebrates and algae: acceptability of risk (PEC/RAC < 1) for formulation ADM.06001.H.2.B for the use of ADM.06001.H.2.B in winter and spring cereals based on calculated mixture toxicity (1 x 1 L product/ha) (with safener) with updated PECsw values following applications at BBCH 20-39 and BBCH 35-39

Group		Aquatic invertebrates	Algae
Test species		*Calculated*	*Calculated*
Endpoint (µg/L)		$ECx_{mix\text{-}CA}$ 14010	$ECx_{mix\text{-}CA}$ 1093
AF		100	10
RAC (µg/L)		140.1	109.3
Scenario	PEC_{mix} (µg/L)		
No buffer	2.023	0.014	0.019
No buffer (BBCH 20-39)	1.952	0.014	0.018
No buffer (BBCH 35-39)	2.728	0.019	0.025

The risks to aquatic invertebrates and algae from exposure to ADM.06001.H.2.B following application to winter and spring cereals are acceptable without mitigation.

For the two-way mixture of mesosulfuron-methyl and pinoxaden, there was no clear driver for toxicity to algae. Therefore, the risk to algae has been assessed using the calculated $ECx_{mix\text{-}CA}$ and the calculated PEC_{mix} values, please see table below.

~~**Table 9.5.2-43: Algae: acceptability of risk (PEC/RAC < 1) for formulation ADM.06001.H.2.B for the use of ADM.06001.H.2.B in winter and spring cereals based on calculated mixture toxicity (1 x 1 L product/ha) (without safener) with updated PECsw values following applications at BBCH 20-39 and BBCH 35-39**~~

~~Group~~		~~Algae~~
~~Test species~~		~~*Calculated*~~
~~Endpoint (µg/L)~~		~~$ErC_{x\ mix\ CA}$ 830.6~~
~~AF~~		~~10~~
~~RAC (µg/L)~~		~~83.06~~
~~Scenario~~	~~PEC_{mix} (µg/L)~~	
~~No buffer~~	~~1.798~~	~~0.022~~
~~No buffer (BBCH 20-39)~~	~~1.729~~	~~0.021~~
~~No buffer (BBCH 35-39)~~	~~2.484~~	~~0.030~~

~~The risk to algae from exposure to ADM.06001.H.2.B following application to winter and spring cereals is acceptable without mitigation.~~

9.5.3 Overall conclusions

Acceptable risks for aquatic organisms from exposure to ADM.06001.H.2.B following application to winter cereals are indicated with a 10-m no-spray buffer with a 10-m vegetative strip, except for scenarios D1 and D2. The route of exposure is drainage in these scenarios. Mitigation measures provided at Step 4 will not reduce these PEC_{SW} values. Acceptable risks for aquatic organisms from exposure to ADM.06001.H.2.B following application to spring cereals are indicated with a 20-m no-spray buffer with a 20-m vegetative strip, except for scenarios D1 and D2.
Member States should consider drainage mitigation to protect aquatic organisms.
~~**Risk assessments for aquatic organisms following application to winter and spring cereals using updated PECsw values indicate a safe use for all scenarios with 10m VFS,**~~

zRMS comments:

The risk assessment for aquatic organism for pinoxaden and its metabolites as well as for safener mefenpyr diethyl demonstrated an acceptable risk without risk mitigation measures.

Based on the calculations of the risk assessment for aquatic organism for mesosulfuron-methyl the following conclusions has been derived:

1. **Winter cereals at BBCH 20-39:**
   - acceptable risk with no need for risk mitigation measures: D3, D4, D5, R1, R4 scenarios
   - scenario R3: risk acceptable with 10 m VFS
   - scenarios: D1, D2, D6 an unacceptable risk
2. **Winter cereals at BBCH 35-39:**
   - acceptable risk with no need for risk mitigation measures: D1 (stream), D3, D4, D5, D6, R1 scenarios
   - scenario R3: risk acceptable with 10 m VFS
   - scenario R4: risk acceptable with 10 m VFS
3. **Spring cereals at BBCH 13-39:**
   - acceptable risk with no need for risk mitigation measures: D3, D4, R4, D5 scenarios
   - ~~scenario R4: risk acceptable with 20 m VFS~~
   - scenarios D1: risk an unacceptable risk
4. **Spring cereals at BBCH 35-39:**
   - acceptable risk with no need for risk mitigation measures: ~~D1 (ditch),~~ D3, D4, D5 scenarios
   - scenario R4: risk acceptable with 20 m VFS
   - scenario D1 (stream), D1 (ditch): risk an unacceptable risk

It should be noted that the risk from the mixture toxicity assessment is acceptable for fish, aquatic invertebrates and algae at the screening step. For aquatic macrophytes the a.s.- mesosulfuron-methyl is driving the toxicity (contributes to more than 90% of the toxicity).
It can be concluded that the mitigations from the active substance mesosulfuron-methyl are relevant and cover mixture toxicity assessment.

Please note that Additional calculations may be required by cMS that do not accept surface water exposure derived using FOCUS models.
The acceptability and applicability of the indicated risk mitigation measures has to be confirmed at the cMS level.

The following text is added due to agreements during the Central Zone harmonisation meetings.
It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.065001.H.2.B, which was performed in line with the EU agreed methodology.

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

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with the active substances mesosulfuron-methyl and pinoxaden. Full details of these studies are provided in the respective EU DAR and related documents. Furthermore, Syngenta has developed additional data on chronic toxicity and larval toxicity of pinoxaden in honey bees after the EU review of pinoxaden.

Effects on bees of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
Apis mellifera	Mesosulfuron-methyl	Oral	**LD_{50} > 105.6 µg a.s./bee**	EFSA Conclusion 4584/2016
Apis mellifera	Mesosulfuron-methyl	Contact	**LD_{50} > 100 µg a.s./bee**	EFSA Conclusion 4584/2016
Apis mellifera	Mesosulfuron-methyl	Chronic, 10 d	LC_{50} > 120 mg a.s./kg food LDD_{50} > 4.85 µg a.s./bee/d[a]	EFSA Conclusion 4584/2016
Pinoxaden				
Apis mellifera	Pinoxaden	Oral	**LD_{50} > 200 µg a.s./bee**	EFSA Conclusion 3269/2013
Apis mellifera	Pinoxaden	Contact	**LD_{50} > 100 µg a.s./bee**	EFSA Conclusion 3269/2013
Apis mellifera	Pinoxaden	Chronic, 10 d	LDD_{50} > 24 µg a.s./bee/d NOEDD = 24 µg a.s./bee/d	Rathjen K., 2017, 1781.7153 (NOA407855_50594)[c]
Apis mellifera	Pinoxaden	Larval toxicity, 22 d	LD_{50} = 4.6 µg a.s./larva NOED = 2.2 µg a.s./larva	Rathjen K., 2017a, 1781.7152 (NOA407855_50599)[c]
Mefenpyr-diethyl				
No valid data (not a critical data gap for a safener)				
ADM.06001.H.2.B				
Apis mellifera	ADM.06001.H.2.B	Oral	**LD_{50} > 224.0 µg prod./bee**	Sekine T., 2020, 140711035 (000105366)
Apis mellifera	ADM.06001.H.2.B	Contact	**LD_{50} > 200 µg prod./bee**	Sekine T., 2020, 140711035 (000105366)
Apis mellifera	ADM.06001.H.2.B	Chronic, 10 d	LC_{50} > 5000 mg prod./kg LDD_{50} > 105.0 µg prod./bee/d NOEC = 5000 mg	Sekine T. and Kowalczyk F., 2021, 140711136 (000105367)

Species	Substance	Exposure System	Results	Reference
			prod./kg NOEDD = 105.0 µg prod./bee/d	
Apis mellifera	ADM.06001.H.2.B	Larval toxicity, 22 d	EC_{50} = 1408 mg prod./kg ED_{50} = 217 µg prod./larva NOEC = 1033 mg prod./kg NOED = 159 µg prod./larva	Colli M., 2020, BT138/20 (000105368)
Higher-tier studies (tunnel test, field studies)				
Not available				

Endpoints in bold are used in the risk assessment

[a] There was no mortality at the LDD_{50}.

[b] Corresponding to concentration of mesosulfuron-methyl present in the spray tank with a standard water volume of 400 L/ha

[c] Syngenta developed this data after the EU review of pinoxaden.

zRMS comments:

Endpoints presented in Table 9.6-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 for mesosulfuron-methyl and EFSA Journal 2013;11(8):3269 for pinoxaden.
The new chronic studies for adult bees and larvae for a.s. – pinoxaden was not taken into consideration by zRMS in the current dossier.
Instead of these studies, the chronic studies for formulation were used in the risk assessment.
Studies on toxicity of ADM.06001.H.2.B to bees were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.6-1 are confirmed to be correct.

9.6.1.1 Justification for new endpoints

Syngenta has developed additional data on chronic toxicity and larval toxicity of pinoxaden in honey bees after the EU review of pinoxaden. The endpoints are included in Table 9.6-1, but they are not used in the present risk assessment due to the continuing review of EFSA Guidance on Risk Assessment on Bees, EFSA Journal 2013; 11(7): 3295 (see below).

New studies and endpoints are provided on acute oral, acute dermal, chronic and larval toxicity of ADM.06001.H.2.B in the honeybee to address current data requirements.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The EFSA Guidance on Risk Assessment on Bees, EFSA Journal 2013; 11(7): 3295, is not yet noted in the Standing Committee SCoPAFF. According to the EFSA document "Outline of the revision of the Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp.* and solitary bees) (EFSA,2013)" dated July 2019, EFSA Guidance 3295, 2013 continues to be reviewed and revised in a programme of work which continues in 2021.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for bees from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of ADM.06001.H.2.B in winter and spring cereals in accordance with SANCO/10329/2002 rev. 2

Intended use	Winter and spring cereals (field crops), BBCH 13-39		
Active substance	Mesosulfuron-methyl		
Application rate (g/ha)	12 g a.s./ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 105.6	12	0.11
Contact toxicity	> 100		0.12
Intended use	Winter and spring cereals (field crops), BBCH 13-39		
Active substance	Pinoxaden		
Application rate (g/ha)	60 g a.s./ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 200	60	0.30
Contact toxicity	> 100		0.60
Product	ADM.06001.H.2.B		
Application rate (g/ha)	1 × 1 L product/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 224	970[a]	4.33
Contact toxicity	> 200		4.85

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
[a] Calcuated based on the product density of 0.97 g/mL

Based on first-tier assessments, the risk to bees from oral and contact exposure to the active substances mesosulfuron-methyl and pinoxaden and the formulation ADM.06001.H.2.B is acceptable.

zRMS comments:

Acute risk assessment:

The acute risk assessment for bees presented in Table 9.6-2 is agreed by the zRMS. HQoral, contact values for the active substances and the formulated product are below the trigger of 50, indicating a low acute risk for bees.
Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bees may be concluded from the intended uses of ADM.06001.H.2.B.

Chronic risk assessment:

The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final.
Due to the fact that the chronic tests are available for adult bee and larvae, the screening step and Tier 1 risk assessment in line with EFSA (2013) for request of some cMS in Central Zone , has been performed by the zRMS below, using endpoints from submitted studies. All steps for the chronic risk assessment, i.e. the screening step, 1st and 2nd oral tier calculations were performed using the corresponding EFSA Bee calculator Tool (Bee-Tool v.3) provided by EFSA.

Screening step risk assessment

The chronic risks to adult honey bees and honey bee larvae bees from the use of ADM.06001.H.2.B.were assessed using the maximum single application rates and the respective 'hazard quotients' (HQs) and 'exposure toxicity ratios' (ETRs).

Test	Endpoint µg prod./bee	Calculation factor	ETR	Trigger	Risk acceptable?
Cereals, BBCH , 10-39 maximum application dose 0.97 kg product/ha					
Oral route of exposure					
Honey bee, chronic	105	7.6 / 10.6	**0.07**	0.03	**No**
Honey bee, larvae	159	4.4 / 6.1	0.03	0.2	Yes

HQ/ETR values in bold are above the trigger value

Considering the proposed uses of at ADM.06001.H.2.B. a maximum application rate of 0.97 kg product/ha a potential risk of formulation is indicated following the chronic exposure of adult bees at this stage of testing. Therefore, 1st tier oral risk assessments were carried out (see Table below).

1st tier, oral risk assessment

In the screening step, potential risk was indicated for adult honey bees following the chronic exposure as well. In the following, a crop and life stage-specific (adult) risk assessment is carried out, which is a first step of refinement. On the one hand, this takes into account crop dependent exposure factors (Ef), and on the other hand it considers SV values, which depend on default values for pollen and nectar consumption, sugar content in nectar, residues (RUDs) in pollen and nectar as well as crop attractiveness (see table below). It is noted that 1st tier risk assessment scheme in EFSA (2013) allows for distinguishing between particular BBCH stages of the crop in question. Therefore, it was decided by the zRMS to perform separate risk assessment for particular stages at which ADM.06001.H.2.B will be applied to cereals.

1st tier oral risk assessment for honey bees (chronic and larvae)

Crop (Crop group according to EFSA tool)	Endpoint	ETR (oral exposure scenario)					Trigger
		Treated crop	Weeds	Field margin	Adjacent crop	Next crop	
Maximum single application rate: 0.97 kg product/ha, BBCH 10-29							
Cereals	adult, chronic	0.006	0.019	0.00	0.00	0.004	0.03
Maximum single application rate: 0.97 kg product/ha, BBCH 30-39							
Cereals	adult, chronic	0.006	0.019	0.00	0.00	0.004	0.03

Based on provided above calculations for application of ADM.06001.H.2.B. to cereals an acceptable chronic risk could be concluded for adult bees at Tier 1.
Risk assessment based on EFSA (2013) is provided above for informative purposes only.
This issue should be further resolved at the product authorization in Member States considering indications of the not yet noted EFSA guidance in their national assessments.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.6.3 Effects on bumble bees

The EFSA guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* sp. and solitary bees), EFSA Journal 2013; 11(7):3295, has not yet entered into force at the time of preparing this dossier. No studies on bumble bees are available in the active substance dossiers and no studies on bumble bees have been carried out on the product ADM.06001.H.2.B.

9.6.4 Effects on solitary bees

The EFSA guidance document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* sp. and solitary bees), EFSA Journal 2013; 11(7):3295, has not yet entered into force at the time of preparing this dossier. No studies on solitary bees are available in the active substance dossiers and no studies on solitary bees have been carried out on the product ADM.06001.H.2.B.

9.6.5 Overall conclusions

The risks to bees from the use of the active substances mesosulfuron-methyl and pinoxaden applied as the formulation ADM.06001.H.2.B to winter and spring cereals is acceptable.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have not been carried out with the active substances mesosulfuron and pinoxaden. Studies were carried out on formulated products. Full details of these studies are provided in the respective EU DAR and related documents. Endpoints are presented in the following table for information only, we are not relying on the data for the risk assessment for ADM.06001.H.2.B.

Effects on non-target arthropods of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Risk assessments have been performed with the endpoints from the new data on ADM.06001.H.2.B.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Methosulfuron-methyl				
Typhlodromus pyri	Atlantis OD[a]	Standard laboratory test Glass plates (2D)	LR_{50} > 1500 mL prod./ha	EFSA Conclusion 4584/2016
Aphidius rhopalosiphi	Atlantis OD[a]	Standard laboratory test Glass plates (2D)	LR_{50} = 877.3 mL prod./ha	EFSA Conclusion 4584/2016
Chrysoperla carnea	Atlantis OD[a]	Extended laboratory test Maize leaves (2D)	LR_{50} > 1500 mL prod./ha ER_{50} > 1500 mL prod./ha	EFSA Conclusion 4584/2016
Aphidius rhopalosiphi	Atlantis OD[a]	Extended laboratory test Barley seedlings (3D)	LR_{50} > 1500 mL prod./ha ER_{50} > 1500 mL prod./ha	EFSA Conclusion 4584/2016
Pinoxaden				
Typhlodromus pyri	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Laboratory test glass plates (2D)	LR_{50} = 17.7 mL/ha (LR_{50} = 1.81 g a.s./ha)	EFSA Conclusion 3269/2013
Aphidius rhopalosiphi	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Laboratory test glass plates (2D)	LR_{50} = 60.96 mL/ha (LR_{50} = 6.22 g a.s./ha)	EFSA Conclusion 3269/2013
Typhlodromus pyri	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Extended laboratory test Bean leaves (2D)	Mortality: LR_{50} = 764.7 mL/ha Reproduction: ER_{50} > 1200 mL/ha	EFSA Conclusion 3269/2013
Aphidius rhopalosiphi	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Extended laboratory test Barley seedlings (3D)	Mortality: LR_{50} > 600 mL/ha Reproduction: ER_{50} > 600 mL/ha	EFSA Conclusion 3269/2013
Chrysoperla carnea	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Extended laboratory test Bean leaves (2D)	Mortality: LR_{50} > 600 mL/ha Reproduction: ER_{50} > 600 mL/ha	EFSA Conclusion 3269/2013
Aleochara bilineata	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Extended laboratory test Sandy soil (2D)	Mortality: LR_{50} > 1500 mL/ha Reproduction: ER_{50} > 1500 mL/ha	EFSA Conclusion 3269/2013

Species	Substance	Exposure System	Results	Reference
Mefenpyr-diethyl				
Not required for a safener				
ADM.06001.H.2.B				
Typhlodromus pyri	ADM.06001.H.2.B	Standard laboratory test Glass plates (2D)	**LR_{50} > 1000 mL prod./ha** **ER_{50} > 1000 mL prod./ha**	Leopold J., 2020a, 140711063 (000105370)
Aphidius rhopalosiphi	ADM.06001.H.2.B	Standard laboratory test Glass plates (2D)	**LR_{50} = 1327 mL prod./ha** **ER_{50} = 603.6 mL prod./ha**	Leopold J., 2020b, 140711001 (000105369)
Aphidius rhopalosiphi	ADM.06001.H.2.B	Extended laboratory test Barley seedlings (3D)	**LR_{50} > 2000 mL prod./ha** **ER_{50} > 2000 mL prod./ha**	Leopold J., 2020c, 140711002 (000105372)
Field or semi-field tests				
Not available				

Endpoints in bold are used in the risk assessment

[a] Oil dispersion (OD) containing 10 g/L mesosulfuron-methyl in the form of mesosulfuron-methyl sodium (10.4 g/L), 1.9 g/L iodosulfuron-methyl in the form of iodosulfuron-methyl-sodium (2 g/L) and 30 g/L mefenpyr-diethyl as a safener.

[b] Emulsifiable concentrate (EC) containing 100 g/L pinoxaden; application always made with adjuvant A-12127R at 0.5% concentration of spray solution, or in some countries 3:1 ratio with A-12303C dose rate.

zRMS comments:

Endpoints presented in Table 9.7-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 and EFSA Journal 2013;11(8):3269.

Studies on toxicity of ADM.06001.H.2.B to non-target arthropods were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.7-1 are confirmed to be correct.

9.7.1.1 Justification for new endpoints

New standard laboratory (Tier 1) studies testing ADM.06001.H.2.B in *Typhlodromus pyri* and *Aphidius rhopalosiphi* and a new extended laboratory (Tier 2) study testing ADM.06001.H.2.B in *Aphidius rhopalosiphi* and their endpoints are provided to address current data requirements.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for non-target arthropods from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	Winter and spring cereals (field crops), BBCH 13-39		
Product	ADM.06001.H.2.B		
Application rate (L/ha)	1 × 1 L product/ha		
MAF	1.0		
Test species (Standard laboratory test, Tier 1)	**LR_{50} (lab.)/ER50*(L/ha)**	**$PER_{in\text{-}field}$ (L/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 1.0	1.0	1.0
Aphidius rhopalosiphi	1.327 0.6036*		0.75 1.66
Test species (Extended laboratory test, Tier 2)	**ER_{50} (lab.) (L/ha)**	**$PER_{in\text{-}field}$ (L/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Aphidius rhopalosiphi	> 2.0	1.0	0.5

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
*In line with Working document on Risk Assessment of Plant Protection Products in the Central Zone, Version 2.0, August 2023, the ER_{50} value from A.rhopalosiphi in Tier I when is lower than the LR_{50} should be used.

No in-field risk is indicated for both *Typhlodromus pyri* and *Aphidius rhopalosiphi* based on the standard laboratory tests at Tier 1 and based on the extended laboratory test at Tier 2 in the case of *Aphidius rhopalosiphi*.

zRMS comments:

The risk assessment presented in Table 9.7-2 is agreed by the zRMS.

Based on calculations performed with consideration of the Tier I and Tier II data acceptable in-field risk to non-target arthropods from ADM.06001.H.2.B for all intended uses of may be concluded.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	Winter and spring cereals (field crops), BBCH 13-39						
Product	ADM.06001.H.2.B						
Application rate (L/ha)	1 × 1 L product/ha						
MAF	1.0						
vdf	10 (2D) / 1 (3D)						
Test species (Laboratory test, Tier 1)	**LR_{50} (lab.) (L/ha)**	**90^{th} percentile drift (%)**	**Drift rate (L/ha)**	**vdf**	**$PER_{off\text{-}field}$ (L/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 1.0	2.77	0.0277	10	0.00277	10	0.0277
Aphidius rhopalosiphi	1.327						0.0209
Test species (Extended laboratory test, Tier 2)	~~ER_{50} (lab.) (L/ha)~~ **Rate with ≤ 50 % effect***	**90^{th} percentile drift (%)**	**Drift rate (L/ha)**	**vdf**	**$PER_{off\text{-}field}$ (L/ha)**	**CF**	~~$HQ_{off\text{-}field}$ criterion: HQ ≤ 1~~ **PERin-field below rate with ≤ 50 % effect?**

Aphidius rhopalosiphi	> 2.0	2.77	0.0277	1	0.0277	5	Yes 0.07

MAF: Multiple application factor; vdf: Vegetation distribution factor; PER: Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
*If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

$HQ_{off\text{-}field}$ values are all below the Tier 1 ~~and Tier 2~~ trigger~~s~~ of 2 ~~and 1, respectively~~. I
In addition, at Tier-2 the PER in-field was below the rate with ≤ 50 % effect for Aphidius rhopalosiphi. Therefore, unacceptable effects on arthropods are not expected in the off-crop area without the consideration of risk mitigation measures, i.e. for the default distance of 1 m.

zRMS comments:

The risk assessment presented in Table 9.7-3 is validated by the zRMS. The VDF of 10 was used by the Applicant.
It should be noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further.
Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. In line with Bullet Points: Ecotoxicology (CZSC November 2021) as long as adjustment to the guidance document has not been made, a VDF of 10 should be applied in core risk assessment.

Finally, based on calculations performed with consideration of the Tier I and Tier II data acceptable off-field risk to non-target arthropods from ADM.06001.H.2.B for all intended uses may be concluded with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Acceptable in-field risk is indicated based on studies with *Typhlodromus pyri* (standard laboratory test, Tier 1) and *Aphidius rhopalosiphi* (standard and extended laboratory tests, Tier 1 and Tier 2) after application of the formulation ADM.06001.H.2.B. Furthermore, acceptable effects on arthropods are expected in the off-crop area without the consideration of risk mitigation measures, i.e. for the default distance of 1 m.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

Studies on chronic toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with the active substance mesosulfuron-methyl and/or its relevant metabolites as well as with metabolite AE F094270 of the safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU DAR and related documents. Furthermore, Syngenta has developed additional data on chronic toxicity of the pinoxaden metabolite NOA 447204 in *Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer* after the EU review of pinoxaden.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. In the case of the pinoxaden metabolite NOA 447204, chronic endpoints were not available in the EU review process. Therefore, the endpoints of the additional studies developed by Syngenta after the EU review of pinoxaden have been used in the present risk assessment.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
Eisenia fetida	Mesosulfuron-methyl	Mixed into substrate 56 d, chronic 10% peat content	**NOEC = 125 mg a.s./kg dw**	EFSA Conclusion 4584/2016
Eisenia fetida	AE F160459	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 90 mg/kg dw**	EFSA Conclusion 4584/2016
Eisenia fetida	AE F099095	Mixed into substrate 56 d, chronic 10% peat content	**NOEC = 100 mg/kg dw**	EFSA Conclusion 4584/2016
Eisenia fetida	AE F092944	Mixed into substrate 56 d, chronic 10% peat content	**NOEC = 10 mg/kg dw[a]**	EFSA Conclusion 4584/2016
Eisenia fetida	AE F160460	Mixed into substrate 56 d, chronic 10% peat content	**NOEC = 100 mg/kg dw**	EFSA Conclusion 4584/2016
Eisenia fetida	AE F140584	Mixed into substrate 56 d, chronic 10% peat content	**NOEC = 117 mg/kg dw**	EFSA Conclusion 4584/2016
Eisenia fetida	AE F147447	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 90 mg/kg dw**	EFSA Conclusion 4584/2016
Folsomia candida	Mesosulfuron-methyl	Mixed into substrate 28 d, chronic 5% peat content	**NOEC = 1000 mg a.s./kg dw**	EFSA Conclusion 4584/2016
Folsomia candida	Mesosulfuron	Mixed into substrate 28 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	EFSA Conclusion 4584/2016

Species	Substance	Exposure System	Results	Reference
Folsomia candida	AE F160459	Mixed into substrate 28 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	EFSA Conclusion 4584/2016
Folsomia candida	AE F092944	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 100 mg/kg dw	EFSA Conclusion 4584/2016
Folsomia candida	AE F092944	Mixed into substrate 28 d, chronic 5% peat content	**NOEC = 50 mg/kg dw[b]**	EFSA Conclusion 4584/2016
Folsomia candida	AE F147447	Mixed into substrate 28 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	EFSA Conclusion 4584/2016
Hypoaspis aculeifer	Mesosulfuron-methyl	Mixed into substrate 14 d, chronic 5% peat content	**NOEC = 1000 mg a.s./kg dw**	EFSA Conclusion 4584/2016
Hypoaspis aculeifer	AE F092944	Mixed into substrate 14 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	EFSA Conclusion 4584/2016
Pinoxaden				
~~*Eisenia fetida*~~	~~NOA 447204~~	~~Mixed into substrate 56 d, chronic 5% peat content~~	~~**NOEC = 556 mg/kg dw**~~	~~Friedrich S., 2016, 16 10 48 150 S[e]~~
~~*Folsomia candida*~~	~~NOA 447204~~	~~Mixed into substrate 28 d, chronic 5% peat content~~	~~**NOEC = 1000 mg/kg dw**~~	~~Friedrich S., 2016a, 16 10 48 151 S[e]~~
~~*Hypoaspis aculeifer*~~	~~NOA 447204~~	~~Mixed into substrate 14 d, chronic 5% peat content~~	~~**NOEC = 1000 mg/kg dw**~~	~~Schulz L., 2016, 16 10 48 149 S[e]~~
Mefenpyr-diethyl				
Eisenia fetida	AE F094270	No information available	NOEC = 100 mg/kg dw **$NOEC_{corr}$ = 50 mg/kg dw[d]**	Proposed in Monograph (list of endpoints) Oct 2011[g]
ADM.06001.H.2.B				
Eisenia fetida	ADM.06001.H.2.B	Mixed into substrate 56 d, chronic 10% peat content	Reproduction NOEC = 511 mg prod./kg dw $NOEC_{corr}$ = 255.5 mg prod./kg dw[d] EC_{10} = 37.5 mg prod./kg dw **$EC_{10\ corr}$ = 18.75 mg prod./kg dw[d, e]** EC_{20} = 140.2 mg prod./kg dw EC_{50} > 920 mg prod./kg dw	Straube D. and Gourlay V., 2021, 140711022 (000105375)

Species	Substance	Exposure System	Results	Reference
Folsomia candida	ADM.06001.H.2.B	Mixed into substrate 28 d, chronic 5% peat content	Reproduction NOEC = 296 mg prod./kg dw **$NOEC_{corr}$ = 148 mg prod./kg dw[d, f]** EC_{10} = 281.0 mg prod./kg dw[f] EC_{20} = 323.7 mg prod./kg dw EC_{50} = 424.4 mg prod./kg dw	Straube D., 2020a, 140711016 (000105376)
Hypoaspis aculeifer	ADM.06001.H.2.B	Mixed into substrate 14 d, chronic 5% peat content	Reproduction NOEC = 1000 mg prod./kg dw **$NOEC_{corr}$ = 500 mg prod./kg dw[d]** EC_{10} > 1000 mg prod./kg dw EC_{20} > 1000 mg prod./kg dw EC_{50} > 1000 mg prod./kg dw	Straube D., 2020b, 140711089 (000105377)
Field studies				
Not available				
Litter bag test				
Not available				

Endpoints in bold are used in the risk assessment

[a] It is noted that a lower NOEC (0.045 mg/kg dw) was reported in the EFSA conclusion for sulfosulfuron (2014c), however, being the latter obtained in a test with three metabolites, this endpoint was not used in this conclusion.

[b] Refer to the EFSA conclusion on the peer review of the active substance flupyrsulfuron-methyl, EFSA (2014a)

[c] Syngenta developed this data after the EU review of pinoxaden.

[d] Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

[e] Since a reliable median EC_{10} could be calculated, the lower between the EC_{10} and the NOEC was used in the risk assessment, i.e., $EC_{10\ corr}$, in accordance with the "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA 2015).

[f] The calculated EC_{10} value cannot be considered to be a reliable endpoint (EFSA 2019) since the lower 95% confidence interval of the EC_{20} (246.0 mg prod./kg dw) is lower than the median EC_{10} value and a visual check of the reproduction results shows high variability about the lower concentrations.Therefore, the $NOEC_{corr}$ is used for the risk assessment.

[g] Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl.

zRMS comments:

Endpoints presented in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 for mesosulfuron-methyl and its metabolites.

Further, chronic endpoint for pinoxaden was not required in the EFSA 2013 conclusion so it not required for the product authorization. Pinoxaden metabolites were not considered to be ecotoxicologically relevant in soil according to EFSA Conclusion 2013 and based on that for these metabolites chronic risk assessment is not required at the product authorization. Therefore, the new endpoints for metabolite NOA 447204 presented by the Applicant are not considered by zRMS. It is not clear why these points have been submitted as they are not required. In addition, these studies are not presented in this dossier. At the same time, it must be emphasized that new studies for a.s. and its metabolite, if not necessary, they should not be evaluated at the product authorization.

For the safener mefenpyr-diethyl, no chronic endpoints are available for soil organisms either in Monograph (LoEP), 2011 voluntarily prepared by AGES and ANSES in the context of zonal authorization of plant

protection products containing safener mefenpyr-diethyl. An endpoint is available for the metabolite AE F094270 in *Eisenia fetida* which has been used by the Applicant in the risk assessment.

Studies on toxicity of ADM.06001.H.2.B to non-target soil organisms were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.8-1 are confirmed to be correct.

9.8.1.1 Justification for new endpoints

Syngenta has developed additional data on chronic toxicity of the pinoxaden metabolite NOA 447204 in *Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer* after the EU review of pinoxaden. The endpoints are included in Table 9.8-1, and they are not used in the present risk assessment by zRMS.

New studies and endpoints are provided on chronic toxicity of ADM.06001.H.2.B in *Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer* to address current data requirements.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for mesosulfuron-methyl and its metabolites AE F099095 and AE F092944, the pinoxaden metabolite NOA 447204 and the mefenpyr-diethyl metabolite AE F094270.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Table 9.8-2: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	1 × 1 L product/ha in winter and spring cereals, BBCH 13-39		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Mesosulfuron-methyl	125	0.017[a]	7353
Mesosulfuron	12.5[b]	0.003[c]	4167
AE F160459	90	0.001[c]	90000
AE F099095	100	0.002[a]	50000
AE F092944	10	0.001[a]	10000
AE F160460	100	0.001[c]	100000
AE F140584	117	0.001[c]	117000
AE F147447	90	0.001[c]	90000
NOA 447204	556	0.084[a, d]	6619
AE F094270	50	0.032[a]	1563
ADM.06001.H.2.B	18.75	1.293[c]	14.5

Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
F. candida			
Mesosulfuron-methyl	1000	0.017[a]	58824
Mesosulfuron	100	0.003[c]	33333
AE F160459	100	0.001[c]	100000
AE F099095	100[b]	0.002[a]	50000
AE F092944	50	0.001[a]	50000
AE F160460	100[b]	0.001[c]	100000
AE F140584	100[b]	0.001[c]	100000
AE F147447	100	0.001[c]	100000
~~NOA 447204~~	~~1000~~	~~0.084[a, d]~~	~~11905~~
ADM.06001.H.2.B	148	1.293[c]	114
H. aculeifer			
Mesosulfuron-methyl	1000	0.017[a]	58824
Mesosulfuron	100[b]	0.003[c]	33333
AE F160459	100[b]	0.001[c]	100000
AE F099095	100[b]	0.002[a]	50000
AE F092944	100	0.001[a]	100000
AE F160460	100[b]	0.001[c]	100000
AE F140584	100[b]	0.001[c]	100000
AE F147447	100[b]	0.001[c]	100000
~~NOA 447204~~	~~1000~~	~~0.084[a, d]~~	~~11905~~
ADM.06001.H.2.B	500	1.293[c]	387

TER values shown in bold fall below the relevant trigger.

[a] $PEC_{accumulation}$

[b] Since no toxicity endpoint for the metabolite is available, the toxicity endpoint for the parent divided by 10 was used.

[c] $PEC_{soil\ initial}$

[d] PEC for acidic soil, which represents a worst case compared to the PEC for alkaline soil

~~For the active substance pinoxaden, no chronic endpoints are available for soil organisms as pinoxaden is of short persistence in soil and degrades very fast to its major metabolites NOA 407854 and NOA 447204. Endpoints are available for the pinoxaden metabolite NOA 447204 (*Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer*) which have been used in the risk assessment. The risk assessment for NOA 447204 is considered to also cover the risk assessment for pinoxaden and its metabolite NOA 407854, since NOA 447204 is the only substance that shows a potential for accumulation and its $PEC_{accumulation}$ (0.084 mg/kg dw) is comparable to the $PEC_{soil\ initial}$ of pinoxaden (0.080 mg/kg dw) and NOA 407854 (0.063 mg/kg dw). With TER_{lt} values of 6619 (*Eisenia fetida*), 11905 (*Folsomia candida*) and 11905 (*Hypoaspis aculeifer*), a high margin of safety is given covering the risk even for a possibly higher toxicity of pinoxaden and its metabolite NOA 407854.~~

For the safener mefenpyr-diethyl, no chronic endpoints are available for soil organisms either. An endpoint is available for the metabolite AE F094270 in *Eisenia fetida* which has been used in the risk assessment. The risk assessment for AE F094270 is considered to also cover the risk assessment for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046, since AE F094270 is the only substance that shows a potential for accumulation and its $PEC_{accumulation}$ (0.032 mg/kg dw) is comparable to the $PEC_{soil\ initial}$ of mefenpyr-diethyl (0.047 mg/kg dw) and higher than the $PEC_{soil\ initial}$ of AE F113225 (0.019 mg/kg dw) and AE F2211046 (0.005 mg/kg dw). With a TER_{lt} value of 1563 (*Eisenia fetida*), a high margin of safety is given covering the risk even for a possibly higher toxicity of mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046. No chronic endpoints are available for *Folsomia candida* and *Hypoaspis aculeifer*. However, the low toxicity of mefenpyr-diethyl, AE F094270 and AE F113225 in *Daphnia magna* (EC_{50} of 5.5 to > 100 mg/L) suggest a low toxicity also towards soil invertebrates. Furthermore, the risk from the formulation ADM.06001.H.2.B has shown to be

acceptable, indicating that mefenpyr-diethyl and its metabolites pose no specific risk towards non-target soil organisms.

Overall, acceptable risk towards non-target soil organisms is indicated at Tier 1 for the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl and their metabolites as well as for the formulation ADM.06001.H.2.B applied in winter and spring cereals.

zRMS comments:

The calculations of the risk assessment provided in the Table 9.8 for mesosulfuron-methyl and its metabolites is validated by zRMS.

The chronic risk assessment for pinoxaden for soil meso- and macrofauna was not required in the EFSA 2013 conclusion so it not required for the product authorization as they were not considered to be ecotoxicologically relevant in soil.

For the safener mefenpyr-diethyl, no chronic endpoints are available for soil organisms either. An endpoint is available for the metabolite AE F094270 in *Eisenia fetida* which has been used in the risk assessment by the Applicant.
The risk assessment for AE F094270 is considered to also cover the risk assessment for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046, since AE F094270 is the only substance that shows a potential for accumulation and its $PEC_{SOIL,ACCU}$ (0.032 mg/kg dw) is comparable to the PEC_{SOIL} initial of mefenpyr-diethyl (0.047 mg/kg dw) and higher than the PEC_{SOIL} initial of AE F113225 (0.019 mg/kg dw) and AE F2211046 (0.005 mg/kg dw).
With a TER_{LT} value of 1563 (*Eisenia fetida*), a high margin of safety is given covering the risk even for a possibly higher toxicity of mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046.
No chronic endpoints are available for *Folsomia candida* and *Hypoaspis aculeifer*. However, the low toxicity of mefenpyr-diethyl, AE F094270 and AE F113225 in *Daphnia magna* (EC_{50} of 5.5 to > 100 mg/L) suggest a low toxicity also towards soil invertebrates. The considerations provided by applicant cannot be completely verify since information on the toxicity assessment for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046 are missing.
However, as crop safener, mefenpyr-diethy is not considered as an active substance, and consequently has not been subject to review on EU level for inclusion into Annex I of Directive 91/414/EEC or Regulation (EC) No 1107/2009.

In conclusion, since risk assessment for the formulation ADM.06001.H.2.B has been correctly presented, zRMS considers risk assessment for soil organisms finalized.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risks to non-target soil organisms from the use of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B in winter and spring cereals are acceptable.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with the active substances mesosulfuron-methyl and pinoxaden and their relevant metabolites as well as with metabolite AE F094270 of the safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU DAR and related documents. Furthermore, Syngenta has developed additional data on effects of the pinoxaden metabolite NOA 447204 on soil microorganisms (N-mineralisation) after the EU review of pinoxaden.

Effects on soil microorganisms of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. In the case of the pinoxaden metabolite NOA 447204, the endpoint of the additional study generated by Syngenta has been used in the present risk assessment (showing < 25% effect at a higher concentration than in the original study).

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
N-mineralisation	Mesosulfuron-methyl	28 d, aerobic soil type	**< 25% effect at day 28 at 0.1 mg a.s./kg dw**	EFSA Conclusion 4584/2016
N-mineralisation	Mesosulfuron	28 d, aerobic soil type	**< 25% effect at day 28 at 0.1 mg/kg dw**	EFSA Conclusion 4584/2016
N-mineralisation	AE F160459	28 d, aerobic soil type	**< 25% effect at day 42 at 0.1 mg/kg dw**	EFSA Conclusion 4584/2016
N-mineralisation	AE F099095	28 d, aerobic soil type	**< 25% effect at day 28 at 0.1 mg/kg dw**	EFSA Conclusion 4584/2016
N-mineralisation	AE F092944	28 d, aerobic soil type	< 25% effect at day 28 at 0.06 mg/kg dw[a]	EFSA Conclusion 4584/2016
N-mineralisation	AE F092944	28 d, aerobic soil type	**< 25% effect at day 28 at 0.137 mg/kg dw**	EFSA Conclusion 4584/2016
N-mineralisation	AE F147447	28 d, aerobic soil type	**< 25% effect at day 28 at 0.057 mg/kg dw**	EFSA Conclusion 4584/2016
Pinoxaden				
N-mineralisation	Pinoxaden (assumed also NOA 407854)	28 d, aerobic soil type	**< 25% effect at 0.4 mg a.s./kg dw**	EFSA Conclusion 3269/2013
~~N-mineralisation~~	~~NOA 447204~~	~~28 d, aerobic soil type~~	~~< 25% effect at 0.066 mg/kg dw[b]~~	~~EFSA Conclusion 3269/2013~~
~~N-mineralisation~~	~~NOA 447204~~	~~28 d, aerobic soil type~~	~~**< 25% effect at 0.66 mg/kg dw**[c]~~	~~Völkel W., 2006, A39003[d]~~
Mefenpyr-diethyl				
N-mineralisation	AE F094270	28 d, aerobic soil type	**< 25% effect at 0.67 mg/kg dw**	Proposed in Monograph (list of endpoints) Oct 2011[e]
ADM.06001.H.2.B				

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	ADM.06001.H.2.B	28 d, aerobic soil type	**< 25% effect at day 28 at 14.0 mg prod./kg dw**	Hammesfahr U., 2020, 140711080 (000105378)

Endpoints in bold are used in the risk assessment

[a] Refer to the EFSA conclusion on the peer review of the active substance flazasulfuron (EFSA, 2016c)

[b] This endpoint was reported incorrectly as 0.0066 mg/kg dw in the EFSA Conclusion (2013) on pinoxaden due to a typing error in the Draft Assessment Report.

[c] Other than in the EU review process of pinoxaden, this endpoint has been used in the present risk assessment (showing < 25% effect at a higher concentration than in the original study).

[d] ~~Syngenta submitted additional data after the EU review of pinoxaden.~~

[e] Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl.

zRMS comments:

Endpoints presented in Table 9.9-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 for mesosulfuron-methyl and its metabolites and EFSA Journal 2013;11(8):3269 for pinoxaden and its metabolites.

According to the EFSA conclusion there are no ecotoxicologically relevant pinoxaden metabolites in soil. Therefore, these endpoints have been not used in the risk assessment.

For the safener mefenpyr-diethyl, no endpoint is available for N-mineralisation in soil according to Monograph LoEP, 2011 in the context of zonal authorization of plant protection products containing safener mefenpyr-diethyl. An endpoint is available for the metabolite AE F094270 which has been used in the risk assessment.

The study on effects of ADM.06001.H.2.B on soil nitrogen transformation was evaluated by the zRMS and is considered acceptable. For details of evaluation, please refer to Appendix 2. The endpoint reported in Table 9.9-1 is confirmed to be correct.

9.9.1.1 Justification for new endpoints

Syngenta has developed additional data on effects of the pinoxaden metabolite NOA 447204 on soil microorganisms (N-mineralisation). The endpoint is included in Table 9.9-1, and it is used in the present risk assessment.

A new study and endpoint is provided on effects of ADM.06001.H.2.B on soil microorganisms (N-mineralisation) to address current data requirements.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 0). According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for mesosulfuron-methyl and its metabolites AE F099095 and AE F092944, the pinoxaden metabolite NOA 447204 and the mefenpyr-diethyl metabolite AE F094270.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1 L product/ha in winter and spring cereals also covers the risk for soil microorganisms from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	1 × 1 L product/ha in winter and spring cereals, BBCH 13-39		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Mesosulfuron-methyl	< 25% effect at day 28 at 0.1 mg a.s./kg dw	0.017[a]	yes
Mesosulfuron	< 25% effect at day 28 at 0.1 mg/kg dw	0.003[b]	yes
AE F160459	< 25% effect at day 42 at 0.1 mg/kg dw	0.001[b]	yes
AE F099095	< 25% effect at day 28 at 0.1 mg/kg dw	0.002[a]	yes
AE F092944	< 25% effect at day 28 at 0.137 mg/kg dw	0.001[a]	yes
AE F160460	< 25% effect at day 28 at 0.01 mg a.s./kg dw[c]	0.001[b]	yes
AE F140584	< 25% effect at day 28 at 0.01 mg a.s./kg dw[c]	0.001[b]	yes
AE F147447	< 25% effect at day 28 at 0.057 mg/kg dw	0.001[b]	yes
Pinoxaden (assumed also NOA 407854)	< 25% effect at 0.4 mg a.s./kg dw	0.080[b] (pinoxaden) 0.063[b] (NOA 407854)	yes (pinoxaden) yes (NOA 407854)
~~NOA 447204~~	~~< 25% effect at 0.66 mg/kg dw~~	~~0.084[a, d]~~	~~yes~~
AE F094270	< 25% effect at 0.67 mg/kg dw	0.032[a]	yes
ADM.06001.H.2.B	< 25% effect at day 28 at 14.0 mg prod./kg dw	1.293[b]	yes

[a] $PEC_{accumulation}$
[b] $PEC_{soil\ initial}$
[c] Since no toxicity endpoint for the metabolite is available, the toxicity endpoint for the parent divided by 10 was used.
[d] ~~PEC for acidic soil, which represents a worst case compared to the PEC for alkaline soil~~

For the safener mefenpyr-diethyl, no endpoint is available for N-mineralisation in soil. An endpoint is available for the metabolite AE F094270 which has been used in the risk assessment. The risk assessment for AE F094270 is considered to also cover the risk assessment for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046, since AE F094270 is the only substance that shows a potential for accumulation and its $PEC_{accumulation}$ (0.032 mg/kg dw) is comparable to the $PEC_{soil\ initial}$ of mefenpyr-diethyl (0.047 mg/kg dw) and higher than the $PEC_{soil\ initial}$ of AE F113225 (0.019 mg/kg dw) and AE F2211046 (0.005 mg/kg dw). With < 25% effects at 0.67 mg/kg dw and a $PEC_{accumulation}$ of 0.032 mg/kg dw for AE F094270, a sufficient margin of safety is considered to be given to cover also the risk for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046. Furthermore, the risk from the formulation ADM.06001.H.2.B has shown to be acceptable, indicating that mefenpyr-diethyl and its metabolites pose no specific risk towards soil microorganisms.

Overall, acceptable risk for soil microorganisms is indicated for the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl and their metabolites as well as for the formulation ADM.06001.H.2.B applied in winter and spring cereals.

zRMS comments:

The calculations of the risk assessment provided in the Table 9.9-2 for mesosulfuron-methyl and its metabolites is validated by zRMS.

According to the EFSA conclusion there are no ecotoxicologically relevant pinoxaden metabolites in soil.

Therefore, these endpoints have been no used in the risk assessment.

For the safener mefenpyr-diethyl, no chronic endpoints are available for soil organisms either. An endpoint is available for the metabolite AE F094270 in *Eisenia fetida* which has been used in the risk assessment.
The Applicant proposes that ‘’The risk assessment for AE F094270 is considered to also cover the risk assessment for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046, since AE F094270 is the only substance that shows a potential for accumulation and its $PEC_{SOIL,ACCU}$ (0.032 mg/kg dw) is comparable to the PEC_{SOIL} initial of mefenpyr-diethyl (0.047 mg/kg dw) and higher than the PEC_{SOIL} initial of AE F113225 (0.019 mg/kg dw) and AE F2211046 (0.005 mg/kg dw). With < 25% effects at 0.67 mg/kg dw and a $PEC_{SOIL,ACCU}$ of 0.032 mg/kg dw for AE F094270, a sufficient margin of safety is considered to be given to cover also the risk for mefenpyr-diethyl and its metabolites AE F113225 and AE F2211046.
This speculation cannot be completely verified since the lack of data; however, as crop safener, mefenpyr-diethyl is not considered as an active substance, and consequently has not been subject to review on EU level for inclusion into Annex I of Directive 91/414/EEC or Regulation (EC) No 1107/2009; but, since risk assessment for the formulation ADM.06001.H.2.B has been correctly presented, zRMS considers the risk assessment in soil micro-organisms finalized.

Overall, acceptable risk for soil microorganisms is indicated for the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl and their metabolites as well as for the formulation ADM.06001.H.2.B applied in winter and spring cereals.

9.9.3 Overall conclusions

The risks for soil microorganisms from the use of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl applied as the formulation ADM.06001.H.2.B in winter and spring cereals are acceptable.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with formulations of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ADM.06001.H.2.B were not evaluated as part of the EU assessment of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Risk assessments have been performed with the endpoints from the new data on ADM.06001.H.2.B.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
Mesosulfuron-methyl				
Allium cepa m	Atlantis OD[a]	Seedling emergence	ER_{50} = 64 mL prod./ha	EFSA Conclusion 4584/2016
Helianthus annuus d	Atlantis OD[a]	Vegetative vigour	ER_{50} = 27 mL prod./ha	EFSA Conclusion 4584/2016
Species Sensitivity Distribution (SSD)	Atlantis OD[a]	Vegetative vigour	HC_5 = 16 mL prod./ha	EFSA Conclusion 4584/2016
Pinoxaden				
Lolium perenne m	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Seedling emergence	ER_{50} = 42 g a.s./ha	EFSA Conclusion 3269/2013
Avena sativa m	„A-12303C“ (+ adjuvant „A-12127R“)[b]	Vegetative vigour	ER_{50} = 9.16 g a.s./ha	EFSA Conclusion 3269/2013
Mefenpyr-diethyl				
Avena sativa m *Brassica napus* d *Cucumis sativus* d *Glycine max* d *Helianthus annuus* d *Lolium perenne* m *Lycopersicon esculentum* d *Pisum sativum* d *Zea mays* m	Safener formulation (7.81% mefenpyr-diethyl, w/w)	Seedling emergence	ER_{50} > 95.7 g a.s./ha	Proposed in Monograph (list of endpoints) Oct 2011[c]
Allium cepa m *Avena sativa* m *Brassica napus* d *Cucumis sativus* d *Glycine max* d *Helianthus annuus* d *Hordeum vulgare* m *Lolium perenne* m *Lycopersicon esculentum* d	Safener formulation (7.81% mefenpyr-diethyl, w/w)	Vegetative vigour	ER_{50} > 95.7 g a.s./ha	Proposed in Monograph (list of endpoints) Oct 2011[c]

Species	Substance	Exposure System	Results	Reference
Zea mays m				
ADM.06001.H.2.B				
Brassica napus d *Raphanus sativus* d *Glycine max* d *Helianthus annuus* d *Solanum lycopersicum* d *Beta vulgaris* d *Zea mays* m *Lolium perenne* m *Avena sativa* m *Allium cepa* m	ADM.06001.H.2.B	14/21 d Seedling emergence	ER_{50} emergence > 1000 mL prod./ha (all species) **ER_{50} plant dry weight = 351 mL prod./ha (*Raphanus sativus*)** ER_{50} plant height > 1000 mL prod./ha (all species) ER_{50} phytotoxicity > 1000 mL prod./ha	Spatz, B. and Kowalczyk, F., 2021a, 140711086 (000105379)
Brassica napus d *Raphanus sativus* d *Glycine max* d *Helianthus annuus* d *Solanum lycopersicum* d *Beta vulgaris* d *Zea mays* m *Lolium perenne* m *Avena sativa* m *Allium cepa* m	ADM.06001.H.2.B	21 d Vegetative vigour	**ER_{50} plant dry weight = 133 mL prod./ha (*Brassica napus*)** ER_{50} plant height = 185 mL prod./ha (*Solanum lycopersicum*) LR_{50} mortality = 842 mL prod./ha (*Helianthus annuus*) ~~ER_{50} phytotoxicity > 111 mL prod./ha~~ HC_5 and (lower limit) =0.08956 (0.06023)	Spatz, B. and Kowalczyk, F., 2021b, 140711087 (000105380)

m: monocotyledonous; d: dicotyledonous
Endpoints in bold are used in the risk assessment

[a] Oil dispersion (OD) containing 10 g/L mesosulfuron-methyl in the form of mesosulfuron-methyl sodium (10.4 g/L), 1.9 g/L iodosulfuron-methyl in the form of iodosulfuron-methyl-sodium (2 g/L) and 30 g/L mefenpyr-diethyl as a safener.

[b] Emulsifiable concentrate (EC) containing 100 g/L pinoxaden; application always made with adjuvant A-12127R at 0.5% concentration of spray solution, or in some countries 3:1 ratio with A-12303C dose rate.

[c] Monograph has been voluntarily prepared by AGES and ANSES in the context of zonal authorisation of plant protection products containing safener mefenpyr-diethyl.

zRMS comments:

Endpoints presented in Table 9.10-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(10):4584 and EFSA Journal 2013;11(8):3269 for A-12303C. The endpoints for safener formulation are agreed in Monograph and LoEP 2011.

Studies on toxicity of ADM.06001.H.2.B to non-target terrestrial plants were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2.
The endpoints reported in Table 9.10-1 are confirmed to be correct.

9.10.1.1 Justification for new endpoints

New studies on the toxicity of ADM.06001.H.2.B in non-target terrestrial plants (seedling emergence and vegetative vigour) and their endpoints are provided to address current data requirements.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 1 x 1.0 L product/ha in winter and spring cereals also covers the risk for non-target terrestrial plants from the intended use of 0.75 L product/ha in winter cereals (see 9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in winter and spring cereals

Intended use	Winter and spring cereals (field crops), BBCH 13-39
Active substance/product	ADM.06001.H.2.B
Application rate (g/ha)	1 × 1 L product/ha, 0.75 L*
MAF	1.0

Test species	**ER_{50} (L/ha)**	**Drift rate (1 meter)**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**
Raphanus sativus (most sensitive, seedling emergence)	0.351	0.0277	0.0277 0.020*	12.7 17.55*
Brassica napus (most sensitive, vegetative vigour)	0.133	0.0277	0.0277 0.020*	**4.80** 6.65*

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

zRMS comments:

The above deterministic risk assessment for product ADM.06001. H.2.B has been checked and confirmed as correct.
For the highest intended rate, the trigger is met for seedling emergence test however is not reached for vegetative vigour test. For lower rate 0.75 L/ha the risk is considered acceptable without needs to further refinement.
In order to reduce the off-field exposure for max. application rate of 1 L/ha, risk mitigation measures can be implemented.
These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles.
The results of the lowest ER_{50} (vegetative vigour) as well as typical mitigation measures (no-spray buffer zones of 5 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarized in the following table.

Risk mitigation measures based on deterministic assessment of the risk for non-target plants due to the use of ADM.06001. H.2.B in cereals.

Intended use		Cereals			
Application rate (Lha)		1 L/ha			
MAF		1.0			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (L/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (L/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g a.s /ha)**
no buffer	2.77	0.0277	0.0135	0.006925	0.000277
5 m	0.57	0.0057	-	-	-
Toxicity value ER_{50} = 0.133 L/ha		**TER criterion: TER ≥ 5**			
no buffer		**4.8**	9.85	19.20	480.14

5 m	23.33	-	-	-

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

Risk mitigation measures based on deterministic assessment of the risk for non-target plants due to the use of ADM.06001. H.2.B at max. application rate of 1 L/ha in cereals are as follows.

- 5 m buffer zone, or alternatively 50% drift reducing spray nozzles

9.10.2.3 Higher-tier risk assessment

The TER calculated with the lowest plant dry weight EC_{50} value of the vegetative vigour test (133 mL prod./ha for *Brassica napus*) is below the trigger value of 5 for the use representing the GAP (1 x 1.0 L prod./ha). A refined risk assessment is calculated below, using an SSD determination based on EC_{50} values for plant dry weight from the vegetative vigour study by Spatz and Kowalczyk (2021b, 140711087, 000105380).

The SSD was calculated using the software ETX version 2.3 (RIVM, 2020).

A median HC_5 value of 116 mL prod./ha was estimated for 8 plant species based on plant dry weight EC_{50} values in the vegetative vigour test (the 2 plant species resulting in unbound values, i.e., EC_{50} > 1000 mL prod./ha, were not taken into account). Goodness of fit calculations for all three tests (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) indicated that the data set is normally distributed as presented in the table and figure below. Therefore, the HC_5 value derived from the data set for plant dry weight EC_{50} values is used in a probabilistic approach as higher-tier risk assessment.

Table 9.10-3: Results of SSD calculations based on plant dry weight EC_{50} values in the vegetative vigour test

Number of species	8[a]
Endpoints used	*Brassica napus* ER_{50} = 133 mL product/ha *Raphanus sativus* ER_{50} = 232 mL product/ha *Glycine max* ER_{50} = 379 mL product/ha *Helianthus annuus* ER_{50} = 197 mL product/ha *Solanum lycopersicum* ER_{50} = 168 mL product/ha *Beta vulgaris* ER_{50} = 200 mL product/ha *Zea mays* ER_{50} = 447 mL product/ha *Avena sativa* ER_{50} = 339 mL product/ha
Mean of the log toxicity values	2.384
Anderson-Darling test for normality (p = 0.05)	Accepted
Kolmogorov-Smirnov test for normality (p = 0.05)	Accepted
Cramer von Mises test for normality (p = 0.05)	Accepted
HC_5 (median estimate)	116
Lower level 90 % confidence interval of HC_5	62.5
Upper level 90 % confidence interval of HC_5	161

[a] The ER_{50} (plant dry weight) for the test species perennial ryegrass (*Lolium perenne*) and onion (*Allium cepa*) were not used in the SSD since they are unbound values (ER_{50} > 1000 mL prod./ha).

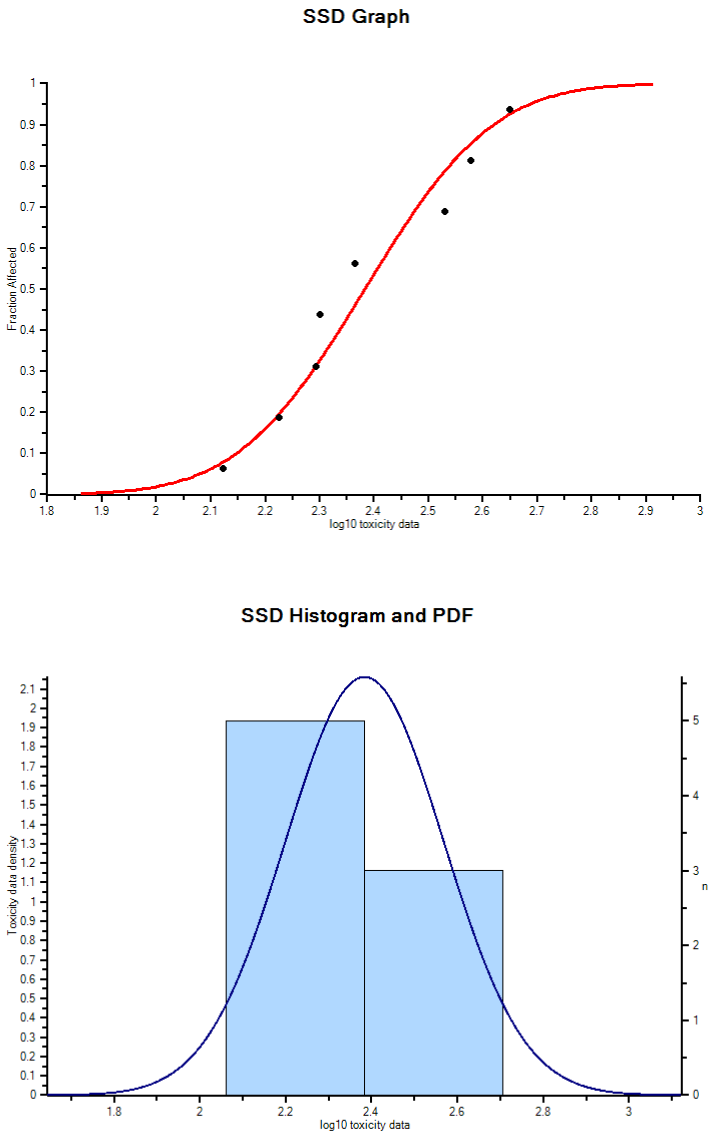


Figure 9.10-1: SSD based on plant dry weight EC_{50} values in the vegetative vigour test

The probabilistic approach as higher-tier risk assessment is presented in the following table.

Table 9.10-4: Assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in winter and spring cereals using refined HC_5 endpoint

Intended use	Winter and spring cereals (field crops), BBCH 13-39
Active substance/product	ADM.06001.H.2.B
Application rate (g/ha)	1 × 1 L product/ha
MAF	1.0

Test species	**HC_5~~ER_{50}~~ (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 1**
Plant dry weight HC_5	0.116	0.0277	0.0277	4.19

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Based on the use of 1 x 1.0 L product/ha in winter and spring cereals, the TER value is above the trigger of 1 using the calculated HC_5 value in this probabilistic approach as higher-tier risk assessment. Risk mitigation measures are therefore not required to ensure acceptable risks to non-target plants.

Based on request from MS's (DE) during commenting period process an additional SSD has been calculated for biomass including unbound values. For this calculation the fitdistrplus package for R, which is implemented in OpenRTox (https://zenodo.org/record/7249239) was used as proposed in the request by DE. For the SSD calculation the lower and upper limit of the confidence intervals have been used where possible.

A HC_5 value of 89.56 mL product/ha was estimated for the 10 plant species based on plant dry weight results in the vegetative vigour test and used in a probabilistic approach as higher-tier risk assessment.

Table 9.10-5: Results of SSD calculations based on plant dry weight endpoint confidence intervals in the vegetative vigour test and including unbound values using OpenRTox

Number of species	10
Endpoints used	Plant species ER_{50} endpoints (Confidence intervals): *Brassica napus* ER_{50} = 133 mL product/ha (94.0 – 189) *Raphanus sativus* ER_{50} = 232 mL product/ha (62.6-4006) *Glycine max* ER_{50} = 379 mL product/ha (223-691) *Helianthus annuus* ER_{50} = 197 mL product/ha (132-279) *Solanum lycopersicum* ER_{50} = 168 mL product/ha (113-251) *Beta vulgaris* ER_{50} = 200 mL product/ha (106-343) *Zea mays* ER_{50} = 447 mL product/ha (392-522) *Avena sativa* ER_{50} = 339 mL product/ha (268-433) *Lolium perenne* ER_{50} >1000 mL product/ha *Allium cepa* ER_{50} >1000 mL product/ha
Model	log-normal (lowest AIC)
Meanlog	5.93 (Std. Error = 0.31)
Sdlog	0.88 (Std. Error = 0.25)
Number of bootstrap iterations	5000
HC_5 (median estimate)	89.56
Lower level 90 % confidence interval of HC_5	60.23
Upper level 90 % confidence interval of HC_5	181.47

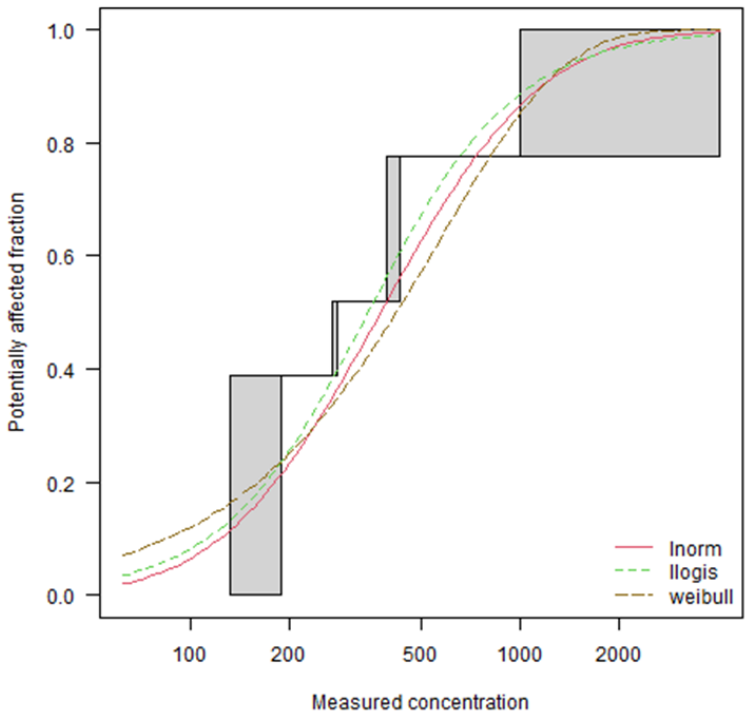


Figure 9.10-2: SSD based on plant dry weight endpoint confidence intervals in the vegetative vigour test and including unbound values

The probabilistic approach as higher-tier risk assessment is presented in the following table. Based on the request from DE the lower limit of the HC_5 has also been calculated. However, to ensure a high level of protection the most sensitive species based on available EU data for pinoxaden and mesosulfuron-methyl were selected for the vegetative vigour test with ADM.06001.H.2.B and are therefore covered by the sensitivity distribution and impact the distribution. For the biomass SSD endpoints from 10 species can be used to calculate the HC_5 value which fulfills the requirements of 6-10 species as outlined in the terrestrial guidance document SANCO/10329/2002 rev 2 final from 17 October 2002. As the lower limit of the HC_5 value for biomass is higher than 1/3 of the median HC_5, the median HC_5 is considered to be sufficient protective for the use in the probabilistic risk assessment.
The terrestrial guidance document further states that "If the ED50 for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable." Thus, a trigger value of 1 is justified and applied for acceptable risk in case of the HC_5. It can be further shown that the HC_5 for biomass of 89.56 mL product/ha is higher than the RAR derived from the lower Tier (26.6 mL product/ha, i.e., lowest ER50/5)).

Table 9.10-6: Assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in winter and spring cereals using refined HC_5 endpoint (and lower limit) based on plant dry weight endpoint confidence intervals and including unbound values

Intended use	Winter and spring cereals (field crops), BBCH 13-39
Active substance/product	ADM.06001.H.2.B
Application rate (g/ha)	1 × 1 L product/ha, 1 x 0.75 L/product/ha
MAF	1.0

Test species	**HC_5 (lower limit) (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 1**
Plant dry weight HC_5 10 species	0.08956 (0.06023)	0.0277	0.0277 0.020	3.23 (2.17) 4.47 (3.01)

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For the use of 1 x 0.75 - 1.0 L product/ha in winter and spring cereals, the TER value is above the trigger of 1 using the calculated HC_5 value (and lower limit of HC_5) based on plant dry weight endpoint confidence intervals and including unbound values.
According to this probabilistic approach as higher-tier risk assessment risk mitigation measures are therefore not required to ensure acceptable risks to non-target plants.

Phytotoxicity:

Based on request from MS's an additional SSD has been calculated for phytotoxicity including unbound values. For this calculation the fitdistrplus package for R, which is implemented in OpenRTox (https://zenodo.org/record/7249239) was used For the SSD calculation the lower and upper limit of the confidence intervals have been used where possible.

A HC_5 value of 49.93 mL prod./ha was estimated for the 8 plant species based on phytotoxicity results in the vegetative vigour test and used in a probabilistic approach as higher-tier risk assessment. For *Avena sativa* and *Glycine max* no reliable ER_{50} could be calculated as no statistically significant concentration/response was found. Therefore, these species were excluded from the SSD.

Table 9.10-7: Results of SSD calculations based on phytotoxicity endpoint confidence intervals in the vegetative vigour test and including unbound values using OpenRTox

Number of species	8
Endpoints used	Plant species ER_{50} endpoints (Confidence intervals): *Brassica napus* ER_{50} = 117.95 mL product/ha (77.50-181.42) *Raphanus sativus* ER_{50} = 60.10 mL product/ha (50.05-72.18) *Helianthus annuus* ER_{50} = 171.23 mL product/ha (162.37-181.80) *Solanum lycopersicum* ER_{50} = 199.70 mL product/ha (162.61-243.82) *Beta vulgaris* ER_{50} = 182.56 mL product/ha (166.95-200.39) *Zea mays* ER_{50} = 590.30 mL product/ha (576.99-603.86) *Lolium perenne* ER_{50} = 913.94 mL product/ha (910.87-917.01) *Allium cepa* ER_{50} >1000 mL product/ha
Model	log-normal (lowest AIC)
Meanlog	5.65 (Std. Error = 0.38)
Sdlog	1.06 (Std. Error = 0.29)
Number of bootstrap iterations	5000
HC_5 (median estimate)	49.93
Lower level 90 % confidence interval of HC_5	25.65
Upper level 90 % confidence interval of HC_5	133.17

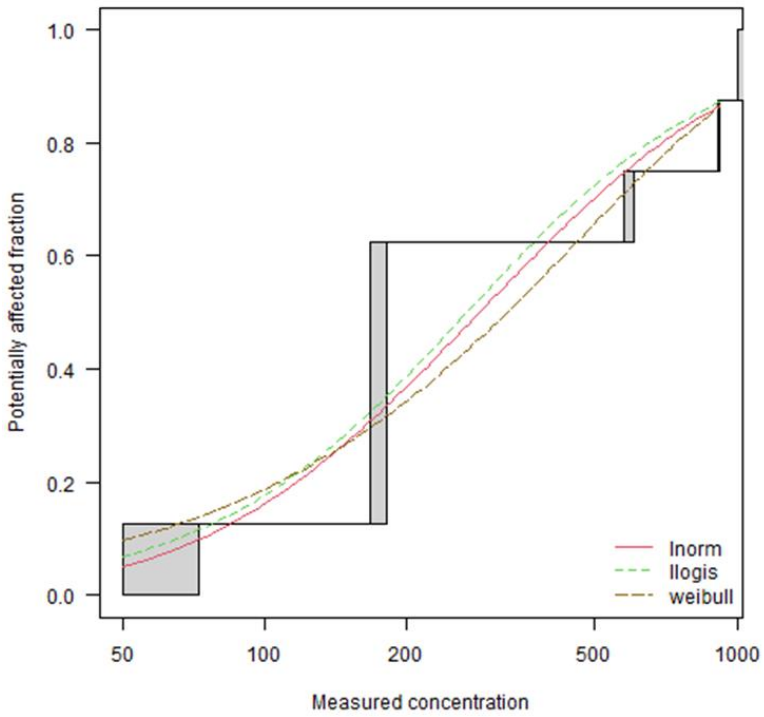


Figure 9.10-3: SSD based on phytotoxicity endpoint confidence intervals in the vegetative vigour test and including unbound values

The probabilistic approach as higher-tier risk assessment is presented in the following tables. The lower limit of the HC_5 has also been calculated. However, to ensure a high level of protection most sensitive species based on available EU data for pinoxaden and mesosulfuron-methyl were selected for the vegetative vigour test with ADM.06001.H.2.B and are therefore covered by the sensitivity distribution. For the phytotoxicity SSD endpoints from 8 species can be used to calculate the HC_5 value which fulfills the requirements of 6-10 species as outlined in the terrestrial guidance document SANCO/10329/2002 rev 2 final from 17 October 2002. As the lower limit of the HC_5 value for phytotoxicity is higher than 1/3 of the median HC_5, the median HC_5 is considered to be sufficient protective for the use in the probabilistic risk assessment. The terrestrial guidance document further states that "If the ED50 for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable." Thus, a trigger value of 1 is justified and applied for acceptable risk in case of the HC_5. It can be further shown that the HC_5 for phytotoxicity of 49.93 mL product/ha is higher than the RAR derived from the lower Tier (12.02 mL product/ha).

Table 9.10-8: Assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in winter and spring cereals using refined HC_5 endpoint (and lower limit) based on phytotoxicity endpoint confidence intervals and including unbound values (1 x 0.75 L product/ha)

Intended use	Winter and spring cereals (field crops), BBCH 13-39
Active substance/product	ADM.06001.H.2.B
Application rate (g/ha)	1 × 0.75 L product/ha
MAF	1.0

Test species	**HC_5 (lower limit) (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 1**
Phytotoxicity HC_5 8 species	0.04993 (0.02565)	0.0277	0.02078	2.40 (1.23)

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.10-9: Assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in winter and spring cereals using refined HC_5 endpoint (and lower limit) based on phytotoxicity endpoint confidence intervals and including unbound values (1 x 1 L product/ha)

Intended use	Winter and spring cereals (field crops), BBCH 13-39			
Active substance/product	ADM.06001.H.2.B			
Application rate (g/ha)	1 × 1 L product/ha			
MAF	1.0			
Test species	**HC_5 (lower limit) (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 1**
Phytotoxicity HC_5 8 species	0.04993 (0.02565)	0.0277	0.0277	1.80 (**0.93**)

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.10-10: Risk mitigation measures based on probabilistic assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in winter and spring cereals considering phytotoxicity (1 x 1 L product/ha)

Intended use		Winter and spring cereals (field crops), BBCH 13-39			
Active substance/product		ADM.06001.H.2.B			
Application rate (L/ha)		1 x 1 L product/ha			
MAF		1.0			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (L/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (L/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (L/ha)**
no buffer	2.77	0.0277	0.0139	0.0069	0.0028
5 m	0.57	0.0057	-	-	-
Toxicity value (phytotox) HC_5 lower limit = 0.02565 L/ha		**TER criterion: TER ≥ 1**			
no buffer		**0.93**	1.85	3.70	9.26
5 m		4.50	-	-	-

Risk mitigation measures based on probabilistic assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in cereals considering phytotoxicity endpoints are as follows:

- 1 x 0.75 L product/ha using HC_5: no mitigations needed
- 1 x 0.75 L product/ha using HC_5 lower limit: no mitigations needed
- 1 x 1 L product/ha using HC_5: no mitigations needed
- 1 x 1 L product/ha using HC_5 lower limit: 5 m buffer zone, or alternatively 50% drift reducing spray nozzles

zRMS comments:

Based on the probabilistic risk assessment the risk for non-target terrestrial plants based on HC_5 of 0.116 mg product/L value is considered acceptable with no buffer zone or drift reducing spraying equipment for all proposed uses in spring and winter cereals.
The additional calculations of the probabilistic risk for non-target plants performed after commenting period process due to the use of ADM.06001.H.2.B in winter and spring cereals using refined HC_5 endpoint (and lower limit) based on plant dry weight endpoint confidence intervals and including unbound values indicated that the risk mitigation measures are still not required to ensure acceptable risks to non-target plants when trigger value of 1 is applied.
It is the position of the zRMS-PL that a trigger value of 1 should be used in the probabilistic risk assessment with a HR5 value; however, it is noted that this is not a Central Zone harmonised position and other member states may consider the use of a different trigger value at National Registration.

The additional probabilistic risk assessment considering phytotoxicity endpoints indicated the following risk mitigation measures for non -target plants:

- 1 x 0.75 L product/ha using HC_5: no mitigations needed
- 1 x 0.75 L product/ha using HC_5 lower limit: no mitigations needed
- 1 x 1 L product/ha using HC_5: no mitigations needed
- 1 x 1 L product/ha using HC_5 lower limit: 5 m buffer zone, or alternatively 50% drift reducing spray nozzles

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

~~The off-field risks to non-target plants from the use of ADM.06001.H.2.B in winter and spring cereals are acceptable without risk mitigation measures.~~
The risk for non-target plants is considered acceptable. The conclusion if/which risk mitigations measures are required depends on MS decisions concerning the relevant metric/trigger used for risk assessment.

Based on the probabilistic risk assessment the risk for non-target terrestrial plants is considered acceptable with no buffer zone or drift reducing spraying equipment for all proposed uses in cereals when trigger value of 1 is applied.
~~It is the position of the zRMS-PL that a trigger value of 1 should be used in the probabilistic risk assessment with a HR5 value; however, it is noted that this is not a Central Zone harmonised position and other member states may consider the use of a different trigger value at National Registration.~~
Risk mitigation measures based on probabilistic assessment of the risk for non-target plants due to the use of ADM.06001.H.2.B in cereals considering phytotoxicity endpoints are as follows:
- 1 x 0.75 L product/ha using HC5: no mitigations needed
- 1 x 0.75 L product/ha using HC5 lower limit: no mitigations needed
- 1 x 1 L product/ha using HC5: no mitigations needed
- 1 x 1 L product/ha using HC5 lower limit: 5 m buffer zone, or alternatively 50% drift reduc-ing spray nozzles

Risk mitigation measures based on deterministic assessment of the risk for non-target plants due to the use of ADM.06001. H.2.B at max. application rate of 1 L/ha in cereals are as follows.
- 5 m buffer zone, or alternatively 50% drift reducing spray nozzles

Risk mitigation measures based on deterministic assessment of the risk for non-target plants due to the use of ADM.06001. H.2.B at rate of 0.75 L/ha in cereals is not required.
~~The final decision of risk mitigation measures is left at MSs level.~~

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No further data on effects of the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl or the formulation ADM.06001.H.2.B on other terrestrial organisms are available.

9.12 Monitoring data (KCP 10.8)

No further monitoring data on the active substances mesosulfuron-methyl and pinoxaden and the safener mefenpyr-diethyl or the formulation ADM.06001.H.2.B are available.

9.13 Classification and Labelling

Formulation ADM.06001.H.2.B is classified as H410 Toxic to aquatic life with long lasting effects.

In accordance with ECHA Guidance on the Application of the CLP Criteria v. 5.0, July 2017, ADM.06001.H.2.B is classified as aquatic environment hazard category chronic 1 because:

Short-term (acute) aquatic hazard

- 48 h EC_{50} (for crustacea) ≤ 1 mg/L - *Daphnia magna* 48 h EC_{50} = 79.5 mg product/L

- 72 h or 96 h E_rC_{50} (for algae or other aquatic plants) ≤ 1 mg/L – *Raphidocelis subcapitata* 72 h E_rC_{50} = 54.2 mg product/L – *Lemna gibba* 7 d E_rC_{50} = 0.074 mg product/L

→ **Acute 1**

Long-term (chronic) aquatic hazard (non-rapidly degradable substances)

- Chronic NOEC or EC_x (for fish)

  - *Pimephales promelas* (ELS) NOEC = 95 mg a.s./L (mesosulfuron-methyl), no category
  - *Pimephales promelas* (ELS) NOEC = 1.0 mg/L (pinoxaden metabolite NOA 407854), category chronic 2
  - *Oncorhynchus mykiss* (28-day chronic) NOEC = 0.1 mg/L (mefenpyr-diethyl), category chronic 1 (M factor: 1)

Classification of a mixture for long-term (chronic) hazards, based on summation of the concentrations of classified components:

- (Chronic 1 x M for mefenpyr-diethyl) ≥ 25%
  (3.608%[a] x 1) = 3.608% → not chronic 1
- (M x 10 x Chronic 1 for mefenpyr-diethyl) + Chronic 2 (pinoxaden metabolite NOA 407854) ≥ 25%
  1 x 10 x 3.608%[a] + 6.186%[a] = 42.27% → **Chronic 2**
  [a] calculated with product density of 0.970 g/cm³

- Chronic NOEC or EC_x (for crustacea)

  - *Daphnia magna* (21 d) NOEC = 1.8 mg a.s./L (mesosulfuron-methyl), no category
  - *Daphnia magna* (21 d) NOEC = 6.25 mg a.s./L (pinoxaden metabolite NOA 407854), no category
  - *Daphnia magna* (21 d) NOEC = 0.32 mg/L (mefenpyr-diethyl), category chronic 2

Classification of a mixture for long-term (chronic) hazards, based on summation of the concentrations of classified components:

- (M x 10 x Chronic 1 for no component) + Chronic 2 (mefenpyr-diethyl) ≥ 25% 0 + 3.608%[a] = 3.608% → not chronic 2
- (M x 100 x Chronic 1 for no component) + (10 x Chronic 2 for mefenpyr-diethyl) + Chronic 3 for no component ≥ 25% 0 + 10 x 3.608%[a] + 0 = 36.08% → **Chronic 3**

  [a] calculated with product density of 0.970 g/cm³

- Chronic NOEC or EC_x (for algae or other aquatic plants), ≤ 0.1 mg/L for category chronic 1 and < 0.1 to ≤ 1 mg/L for chronic category 2

  - *Raphidocelis subcapitata* 72 h NOE_rC = 4.58 mg product/L
  - *Lemna gibba* 7 d NOE_rC = 0.0147 mg product/L, EC_{10}= 0.013 mg product/L

  → **Chronic 1**

Signal word "Warning" is associated with hazard statement H410.

The recommended precautionary statements are:
P273 Avoid release to the environment
P391 Collect spillage
P501 Dispose of contents/container in accordance with local regulations

zRMS comments:

zRMS agrees with the classification of the product H400 (acute) and H410 (chronic) but not with the justification for chronic hazard. The endpoint to be used in the classification procedure should be the EC_{10} for macrophytes (0.013 mg/L).

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 10.2.1/01	Seidel U. and Mollandin G.	2021a	ADM.06001.H.2.B: Acute Toxicity to *Daphnia magna* in a Semi-Static 48-hour Immobilisation Test 140711220 (ADAMA No. 000105363) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.2.1/02	Seidel U. and Mollandin G.	2021b	ADM.06001.H.2.B: Toxicity to *Raphidocelis subcapitata* (=*Pseudokirchneriella subcapitata*) in an Algal Growth Inhibition Test 140711210 (ADAMA No. 000105364) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.2.1/03	Seidel U. and Mollandin G.	2021c	ADM.06001.H.2.B: Toxicity to the Aquatic Plant Lemna gibba in a Semi-Static Growth Inhibition Test 140711240 (ADAMA No. 000105365) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.3.1.1.1/01	Sekine T.	2020	ADM.06001.H.2.B: Acute Contact and Oral Effects on Honey Bees (*Apis mellifera* L.) in the Laboratory 140711035 (ADAMA No. 000105366) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.3.1.1.2/01	Sekine T.	2020	ADM.06001.H.2.B: Acute Contact and Oral Effects on Honey Bees (*Apis mellifera* L.) in the Laboratory 140711035 (ADAMA No. 000105366) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.3.1.2/01	Sekine T. and Kowalczyk F.	2021	ADM.06001.H.2.B: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory 140711136 (ADAMA No. 000105367) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP	N	ADAMA

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**
			Unpublished		
KCP 10.3.1.3/01	Colli M.	2020	Effects of ADM.06001.H.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure BT138/20 (ADAMA No. 000105368) BioTecnologie BT S.r.l., Frazione Pantalla, 06059 Todi (PG), Italy GLP Unpublished	N	ADAMA
KCP 10.3.2/01	Leopold, J.	2020a	ADM.06001.H.2.B: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates 140711063 (ADAMA No. 000105370) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.3.2/02	Leopold, J.	2020b	ADM.06001.H.2.B: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates 140711001 (ADAMA No. 000105369) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.3.2/03	Leopold, J.	2020c	ADM.06001.H.2.B: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), Extended Laboratory Study - Dose Response Test - 140711002 (ADAMA No. 000105372) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.4.1.1/01	Straube D. and Gourlay V.	2021	ADM.06001.H.2.B: Determination of chronic toxicity to the earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae) in an artificial soil substrate 140711022 (ADAMA No. 000105375) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.4.2.1/01	Straube D.	2020a	ADM.06001.H.2.B: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* (Acari: Laelapidae) in Artificial Soil 140711089 (ADAMA No. 000105377)	N	ADAMA

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**
			ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished		
KCP 10.4.2.1/02	Straube D.	2020b	ADM.06001.H.2.B: Effects on Reproduction of *Folsomia candida* (Collembola: Isotomidae) in Artificial Soil 140711016 (ADAMA No. 000105376) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.5/01	Hammesfahr U.	2020	ADM.06001.H.2.B: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation) 140711080 (ADAMA No. 000105378) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.6.2/01	Spatz, B. and Kowalczyk, F.	2021a	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test 140711086 (ADAMA No 000105379) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.6.2/02	Spatz, B. and Kowalczyk, F.	2021b	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test 140711087 (ADAMA No 000105380) ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	ADAMA
KCP 10.6.2/03	Haaf, S	2023	Statistical evaluation of the phytotoxicity results in the study: ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test (ADAMA No 000117985) ADAMA Agan, Israel Non-GLP Unpublished	N	ADAMA
~~KCP 10.2.1/04~~	~~na~~	~~2023~~	~~Aquatic mixture toxicity assessment for winter cereals including safener using previous PECs~~	~~N~~	~~ADAMA~~
KCP 10.2.1/05	na	2023	Aquatic mixture toxicity assessment for winter cereals including safener BBCH 20-39	N	ADAMA
KCP 10.2.1/06	na	2023	Aquatic mixture toxicity assessment for winter cereals including safener BBCH 35-39	N	ADAMA

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/07	na	2023	Aquatic mixture toxicity assessment for spring cereals including safener using previous PECs	N	ADAMA
KCP 10.2.1/08	na	2023	Aquatic mixture toxicity assessment for spring cereals including safener BBCH 13-39	N	ADAMA
KCP 10.2.1/09	na	2023	Aquatic mixture toxicity assessment for spring cereals including safener BBCH 35-39	N	ADAMA
~~KCP 10.2.1/10~~	~~na~~	~~2023~~	~~Aquatic mixture toxicity assessment for winter cereals without safener using previous PECs~~	~~N~~	~~ADAMA~~
KCP 10.2.1/11	na	2023	Aquatic mixture toxicity assessment for winter cereals without safener BBCH 20-39	N	ADAMA
KCP 10.2.1/12	na	2023	Aquatic mixture toxicity assessment for winter cereals without safener BBCH 35-39	N	ADAMA
KCP 10.2.1/13	na	2023	Aquatic mixture toxicity assessment for spring cereals without safener using previous PECs	N	ADAMA
KCP 10.2.1/14	na	2023	Aquatic mixture toxicity assessment for spring cereals without safener BBCH 13-39	N	ADAMA
KCP 10.2.1/15	na	2023	Aquatic mixture toxicity assessment for spring cereals without safener BBCH 35-39	N	ADAMA

List of data referred to by the applicant and relied on, but not evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.2/02	Rathjen K.	2017	Pinoxaden: Chronic (10-Day) Laboratory Feeding Study with the Adult Honey Bee (Apis mellifera) Syngenta File No NOA407855_50594, report num1781.7153 Syngenta Crop Protection, LLC, Greensboro, NC, USA GLP Unpublished	N	Syngenta
KCP 10.3.1.3/02	Rathjen K.	2017a	Pinoxaden: Chronic (22-Day) Larval Toxicity Study with the Honey Bee, Apis mellifera L. Syngenta File No NOA407855_50599, report num1781.7152 Syngenta Crop Protection, LLC, Greensboro, NC, USA GLP Unpublished	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.4.1.1/02	Friedrich S.	2016	NOA447204 - Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5 % Peat Report Number 16 10 48 150 S BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany GLP Unpublished	N	Syngenta
KCP 10.4.2.1/03	Schulz L.	2016	NOA447204 - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer Report Number 16 10 48 149 S BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany GLP Unpublished	N	Syngenta
KCP 10.4.2.1/04	Friedrich S.,	2016a	NOA447204 - Effects on the Reproduction of the Collembolan Folsomia candida Report Number 16 10 48 151 S BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany GLP Unpublished	N	Syngenta
KCP 10.5/02	Völkel	2006	Pinoxaden (NOA407855) metabolite (NOA447204): Determination of effects on soil microflora activity Report Number A39003 RCC, Itingen, Switzerland GLP Unpublished	N	Syngenta

List of product data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review
None

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/04	na	2023	Aquatic mixture toxicity assessment for winter cereals including safener using previous PECs	N	ADAMA
KCP 10.2.1/07	na	2023	Aquatic mixture toxicity assessment for spring cereals including safener using previous PECs	N	ADAMA
-	Meregalli, G et. Al (2023	2023	INTRA-LABORATORY VARIABILITY OF VISUAL PHYTOTOXICITY ASSESSMENTS IN NON-TARGET TERRESTRIAL PLANT STUDIES.	N	-

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 new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1 Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study was conducted in line with OECD 202 with no deviations to the guideline but with minor deviations to the study plan. It was noted in the study report that with regard to the analysis of the test item concentrations / LC-MS/MS conditions the flow rate of 0.65 mL/min instead of 0.6 mL/min was applied due to human error. This deviation is considered to have no negative effect on the outcome of the study since all measurements were perfomed with the same flow rate. It was also noted that during the test the light intensity was in the range of 320 to 560 lux instead of 540 to 1080 lux due to human and/or technical error. This deviation is also considered to have no negative effect on the outcome of the study since the test was valid. The analytical measurements demonstrated that the measured concentrations of the active substances mesosulfuron-methyl and pinoxaden were within ± 20% of the nominal concentrations during the test. Therefore, the endpoint can be based on the nominal concentration. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: EC_{50} = 79.5 mg product/L

Reference:	KCP 10.2.1/01
Report	ADM.06001.H.2.B: Acute Toxicity to *Daphnia magna* in a Semi-Static 48-hour Immobilisation Test, Seidel U. and Mollandin G., 2021a, 140711220 (ADAMA No. 000105363)
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	None to the guideline, minor to the study plan (see the commenting box above) ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022

Reference substance	Potassium dichromate is tested at least twice a year to demonstrate satisfactory test conditions. The most recent reference substance test (performed in September 2020) resulted in a 24-hour EC_{50} of 0.918 mg/L, which is consistent with the level proposed by OECD 202 (24-hour EC_{50} between 0.6 and 2.1 mg potassium dichromate/L).

Test organism:

Test species	*Daphnia magna* (Straus), clone 5
Origin	In-house laboratory culture
Age at test start	0.5-17.5 hours The test organisms were not first brood progeny.
Acclimation	Not necessary. The test organisms were bred in test medium and under similar temperature and light conditions as used in the test.
No. of daphnia per test vessel	5 (loading: 20 mL test solution per *Daphnia*)
No. of daphnia per test substance concentration	20
No. of test vessels (replicates) per test substance concentration	4

Test conditions:

Test substance concentrations	4.3, 9.4, 20.7, 45.5 and 100 mg product/L (nominal) The test concentrations were chosen based on a non-GLP range-finding test. The test solutions of the highest test concentration were prepared by dissolving 111.5 and 97.6 mg test substance in 1115 and 976 mL test medium, respectively. Adequate volumes of these stock solutions were diluted with test medium to prepare the test solutions of the lower test concentrations. The test solutions were prepared just before introduction of the daphnids (= start of the test) and test medium renewal after 24 hours.
Control	Untreated test medium
Test duration	48 h
Test medium	Reconstituted water (Elendt "M4") Main compounds: $CaCl_2 \cdot 2\ H_2O$ 293.80 mg/L $MgSO_4 \cdot 7\ H_2O$ 123.30 mg/L KCl 5.80 mg/L $NaHCO_3$ 64.80 mg/L $Na_2SiO_3 \cdot 9\ H_2O$ 10.00 mg/L $NaNO_3$ 0.27 mg/L KH_2PO_4 0.14 mg/L K_2HPO_4 0.18 mg/L Furthermore, trace elements and vitamins were added. The test water was sterile filtered before use.
Test type	Semi-static
Test water renewal	After 24 hours
Test medium pH	7.6-7.7 at 0 h 7.6-7.7 at 24 h (aged) 7.6-7.8 at 24 h (fresh) 7.7-7.8 at 48 h
Water temperature	18-22°C (nominal, constant within ± 1°C) 19.7-20.8°C (actual) 20.1 to 20.8 °C in the freshly prepared media 19.7 to 20.6°C in the aged test media
Dissolved oxygen	9.3-9.9 mg O_2/L (105-110%) at 0 h

	7.9-8.1 mg O_2/L (88-91%) at 24 h (aged) 8.1-8.6 mg O_2/L (91-97%) at 24 h (fresh) 8.6-8.7 mg O_2/L (95-97%) at 48 h
Hardness	250 mg $CaCO_3$/L
Alkalinity	0.9 mmol/L
Test solution appearance	The test item ~~substance~~ caused turbidity in higher test concentrations and was observed floating at the surface of all except the lowest test concentration.
Test vessel	Glass beakers covered with lids (150 mL)
Test volume	100 mL
Light intensity	320-560 lux
Photoperiod	16 h light : 8 h dark
Feeding	None

Observations:

Daphnia observations	Immobility (including sub-lethal effects): 24 and 48 h
Test substance concentration	0, 24 h (aged), 24 h (fresh), 48 h
Test conditions	Water temperature, pH and dissolved oxygen: in freshly prepared (0 and 24 h) and aged (24 and 48 h) test solutions of all test concentrations Light intensity: once during the test

Analytical method:

Method type	LC-MS/MS
Equipment	Agilent Series 1290 pump and autosampler
Column	Phenomenex Synergi 4 µm Fusion-RP 80A (50 x 2 mm)
Column temperature	40°C
Detector	Mass spectrometer API 5500 Detection: ESI positive MRM mass transitions: Mesosulfuron-methyl: m/z 504.1 → 182.1 (quantifier); 504.1 → 83.0 (qualifier) Pinoxaden: m/z 401.5 → 317.2 (quantifier); 401.5 → 56.9 (qualifier) NOA 407854: m/z 317.8 → 171.2 (quantifier); 317.8 → 131.1 (qualifier) Mefenpyr-diethyl: m/z 389.9 → 327.0 (quantifier); 389.9 → 160.0 (qualifier)
Flow rate	0.65 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid B: Acetonitrile containing 0.1% formic acid 0.0 min 95% A, 5% B 2.0 min 95% A, 5% B 2.5 min 50% A, 50% B 4.5 min 5% A, 95% B 5.2 min 5% A, 95% B 5.3 min 95% A, 5% B 7.0 min 95% A, 5% B

Experimental dates: 28 Oct to 19 Nov 2020

Calculations:

Mean percentage immobility was calculated for all replicates of each test group.

Statistics:

Statistical analyses were performed following the recommendations of OECD Guidance Document 54 (2006) and using the program ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.
The 24-hour and 48-hour EC_{50}, EC_{20} and EC_{10} and their 95% confidence intervals were calculated by Probit analysis.

The NOEC and LOEC after 24 hours were determined by Fisher's exact binominal test, as no trend of contrasts was significant (Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response)). After 48 hours, the NOEC and LOEC were determined by Step-down Cochrane-Armitage test, as a linear trend of contrasts was determined and no signs of extra-binomial variance were found.

Results and discussions

Validity criteria:

- Immobility or other signs of disease or stress in control daphnids ≤ 10%
- Dissolved oxygen concentration at the end of the test in control and test vessels ≥ 3 mg O_2/L

Immobility in control daphnids was 0% and furthermore, no daphnid showed signs of disease or stress. Dissolved oxygen concentration was ≥ 8.6 mg O_2/L in in all treatment groups at the end of the test. Therefore, all validity criteria were met.

Test substance concentrations were determined by analysis of the analytes mesosulfuron-methyl, pinoxaden, NOA 407854 (metabolite of pinoxaden) and mefenpyr-diethyl. Percentage recovery of analytes in test solutions is presented in the table below.

Table A2.2.1.1-1: Percentage recovery of analytes in test solutions

Test substance concentration nominal (mg product/L)	Analyte concentration nominal (mg/L)	Recovery (% of nominal)[a]			
		0 h (fresh test solutions)	24 h (aged test solutions)	24 h (fresh test solutions)	48 h (aged test solutions)
Mesosulfuron-methyl					
Control	Control	n.a.	n.a.	n.a.	n.a.
4.3	0.0516	88	91	92	88
9.4	0.1128	87	87	90	89
20.7	0.2484	91	89	89	85
45.5	0.5460	85	86	86	85
100	1.200	88	87	86	94
Pinoxaden					
Control	Control	n.a.	n.a.	n.a.	n.a.
4.3	0.2709	92	82	93	85
9.4	0.5922	91	81	94	83
20.7	1.3041	99	90	98	88
45.5	2.8665	105	92	105	90
100	6.300	94	87	94	81
NOA 407854 (metabolite of pinoxaden)					
Control	Control	n.a.	n.a.	n.a.	n.a.
4.3	0.214014[b]	n.a.	14	n.a.	16
9.4	0.467845[b]	n.a.	14	n.a.	16
20.7	1.030255[b]	n.a.	16	n.a.	17
45.5	2.264571[b]	3	15	5	17
100	4.977079[b]	4	14	5	16
Mefenpyr-diethyl					
Control	Control	n.a.	n.a.	n.a.	n.a.
4.3	0.1634	106	97	108	109
9.4	0.3572	107	95	106	93
20.7	0.7866	109	117	123	105
45.5	1.729	120	82	116	98
100	3.800	90	81	92	81

n.a. not applicable

Mesosulfuron-methyl: LOD: 0.003 µg mesosulfuron-methyl/L, LOQ: 1.9 µg mesosulfuron-methyl/L
Pinoxaden: LOD: 0.004 µg pinoxaden/L, LOQ: 10.1 µg pinoxaden/L
NOA 407854: LOD: 0.281 µg NOA 407854/L, LOQ: 2 µg NOA 407854/L
Mefenpyr-diethyl: LOD: 0.156 µg mefenpyr-diethyl/L, LOQ: 6.1 µg mefenpyr-diethyl/L

[a] Mean value of duplicate samples

[b] Nominal NOA 407854 concentration was calculated using the nominal content of pinoxaden (6.3%) given in analytical certificate and the molar ratio of pinoxaden (400.5 g/mol) and NOA 407854 (316.4 g/mol) and assumption that 100% of nominal of pinoxaden transformed to NOA 407854.

Recoveries of mesosulfuron-methyl and mefenpyr-diethyl showed that the test substance was dosed correctly and both substances were stable during the test. Recoveries of pinoxaden showed that the test substance was dosed correctly. Concentrations of pinoxaden decreased slightly during the renewal periods concurrently with the slight increase of the concentrations of NOA 407854, the metabolite of pinoxaden. However, concentrations of pinoxaden were sufficiently stable during the test (± 20% of nominal). Therefore, the biological results were based on nominal test substance concentrations.

Observations of immobility of daphnids are presented in the table below.

Table A2.2.1.1-2: Immobility of *Daphnia magna* exposed to ADM.06001.H.2.B

Product concentration nominal (mg product/L)	Immobility of *Daphnia magna* (%)	
	24 hours	48 hours
Control	0	0
4.3	0	0
9.4	0	0
20.7	0	0
45.5	0	5
100	25	75

Based on these results, the following endpoints were obtained.

Table A2.2.1.1-3: Endpoints of the acute toxicity test with ADM.06001.H.2.B in *Daphnia magna*

Endpoint	Nominal concentration (mg product/L)
48 h NOEC	45.5
48 h LOEC	100
48 h EC_{10} (95% confidence interval)	51.5 (32.8 – 63.0)
48 h EC_{20} (95% confidence interval)	59.8 (42.6 – 71.4)
48 h EC_{50} (95% confidence interval)	79.5 (65.6 – 96.6)

After 48 hours of exposure, no immobilisation of the test animals was observed in the control and up to and including the test concentration of 20.7 mg product/L. At the test concentration of 45.5 mg product/L, one animal was immobile and 15 animals were immobile at the highest test concentration of 100 mg product/L. Based on these results, the 48-hour NOEC, LOEC, EC10, EC20 and EC50 were determined as 45.5, 100, 51.5 (32.8 – 63.0, 95% confidence interval), 59.8 (42.6 – 71.4, 95% confidence interval) and 79.5 (65.6 – 96.6, 95% confidence interval) mg product/L, respectively.

Conclusion

In this test on acute toxicity of ADM.06001.H.2.B in *Daphnia magna*, the 48-hour EC_{50} was determined to be 79.5 mg product/L (65.6 – 96.6 mg product/L, 95% confidence interval).

A 2.2.1.2 Effects on aquatic algae

Comments of zRMS:	The study was conducted in line with OECD 201 with a minor deviation to the guideline and with a minor deviation to the study plan.

	It was noted in the study report that with regard to the analysis of test item concentrations / LC-MS/MS conditions the flow rate of 0.65 mL/min instead of 0.6 mL/min was applied due to human error. This deviation is considered to have no negative effect on the outcome of the study since all measurements were perfomed with the same flow rate. The analytical measurements demonstrated that the measured concentrations of the active substance mesosulfuron-methyl were within ± 20% of the nominal concentrations but the measured concentrations of the active substance pinoxaden were not within the ± 20% of the nominal concentrations during the test. The endpoints reported in the study are based on adjusted test item concentrations, i.e. the sum of active substance contents (total active substance load) using nominal concentrations of mesosulfuron-methyl and geometric mean measured concentrations of pinoxaden. In zRMS opinion the sum of the active substance content (total active substance load) should be expressed in the same units i.e. as the sum of geometric mean measured concentrations of both active substances. Consequently, the endpoints were recalculated by the Applicant for zRMS's request. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: Growth rate E_rC_{50} = 54.8 mg product/L (calculated for mesosulfuron-methyl (geometric mean measured) and pinoxaden (geometric mean measured) Yield E_yC_{50}= 27.8 mg product/L (calculated for mesosulfuron-methyl (geometric mean measured) and pinoxaden (geometric mean measured)

Reference:	KCP 10.2.1/02
Report	ADM.06001.H.2.B: Toxicity to *Raphidocelis subcapitata* (=*Pseudokirchneriella subcapitata*) in an Algal Growth Inhibition Test, Seidel U. and Mollandin G., 2021b, 140711210 (ADAMA No. 000105364)
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	Minor (see the commenting box above) ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable (recalculated endpoints are requested) ~~Yes~~
Duplication (if vertebrate study)	Not applicable

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	Potassium dichromate is tested at least twice a year to demonstrate the quality of the algae and the experimental conditions. The most recent reference substance test (performed in September 2020) resulted in 72-hour E_rC_{50} and E_yC_{50} of 0.878 and 0.402 mg/L, respectively.

Test organism:

Test species	*Raphidocelis subcapitata* (KORSHIKOV), Strain No. 61.81 SAG (recently renamed from *Pseudokirchneriella subcapitata* and formerly known as *Selenastrum capricornutum*)
Origin	In-house culture, originally obtained from "Sammlung von Algenkulturen, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen", 37073 Göttingen, Germany
Acclimation	The algae were cultivated under standardised conditions according to test guideline.
Cell concentration at test start	5 x 10^3 cells/mL The cells were taken from an exponentially growing pre-culture, which was set up 4 days prior to test start under the same conditions as in the test.
No. of test vessels (replicates) per test substance concentration	control group: 6 treated group: 3 2 additional test vessels were set up for each test group for analytical dose verification after 24 and 48 hours. 1 additional test vessel was set up for each test group without algae inoculum to serve as a blank for spectrophotometric measurements. All additional test vessels were incubated under the same conditions as the regular test vessels.

Test conditions:

Test substance concentrations	6.25, 12.5, 25, 50 and 100 mg product/L (nominal), corresponding to adjusted test substance concentrations based on sum of active substance contents using nominal concentrations of mesosulfuron-methyl and geometric mean measured concentrations of pinoxaden of 4.58, 9.96, 19.7, 40.9 and 83.9 mg product/L (total active substance load) The test concentrations were chosen based on a non-GLP range-finding test. The test solution of the highest test concentration was prepared by dissolving 100.7 mg test item ~~substance~~ in 1007 test medium. Adequate volumes of this stock solution were diluted with test medium to prepare the test solutions of the lower test concentrations. The test solutions were prepared just before introduction of the algae (= start of the test).
Control	Untreated test medium
Blank	One replicate for each test group was prepared without algae to provide a blank for spectrophotometric measurements. Absorption of these blank samples was subtracted from absorption in the samples with algae.
Test duration	72 h
Test medium	OECD 201 medium: Macronutrients: NH_4Cl 15.0 mg/L $MgCl_2 \cdot 6\ H_2O$ 12.0 mg/L $CaCl_2 \cdot 2\ H_2O$ 18.0 mg/L $MgSO_4 \cdot 7\ H_2O$ 15.0 mg/L KH_2PO_4 1.6 mg/L $NaHCO_3$ 50.0 mg/L Trace elements: H_3BO_3 185.0 µg/L $MnCl_2 \cdot 4\ H_2O$ 415.0 µg/L $ZnCl_2$ 3.0 µg/L $CoCl_2 \cdot 6\ H_2O$ 1.5 µg/L $CuCl_2 \cdot 2\ H_2O$ 0.01 µg/L $Na_2MoO_4 \cdot 2\ H_2O$ 7.0 µg/L

	$FeCl_3 \cdot 6\ H_2O$ 64.0 µg/L $Na_2EDTA \cdot 2\ H_2O$ 100.0 µg/L (reagents of analytical grade) The buffer 2-(N-morpholino)ethanesulfonic acid (MES buffer) was added at 1.95 g/L. The culture medium was prepared 4 days before test start to allow the pH to stabilise and sterile filtered.
Test type	Static
Test medium pH	6.8-6.9 at 0 h 6.8-6.9 at 72 h
Water temperature	21-24°C (nominal, controlled at ± 2°C) 22.5-22.8°C (actual)
Hardness	24 mg $CaCO_3$/L
Shaking	Continuously by magnetic stirrers
Test solution appearance	The test item ~~substance~~ caused turbidity at nominal concentrations of 50 and 100 product/L, and slight turbidity at the concentration of 25 mg product/L. At the highest concentration of nominal 100 mg product/L, the test substance was observed to be on the surface of the medium.
Test vessel	50-mL Erlenmeyer flasks covered with sterile caps
Test volume	30 mL
Sterility	The test was conducted under a sterile bench. Glassware was sterilised before use. The test medium was sterile filtered before use. Algae originated from a sterile culture. The test vessels were covered with semi-permeable sterile caps and opened only for observations under the sterile bench.
Light intensity	Mean 6455 lux (range: 6020 to 7040 lux)
Photoperiod	Continuous illumination

Observations:

Algal cell density	24, 48 and 72 h Algal cell density was determined by spectrophotometric measurement. Based on the counted cell densities and the absorption from an algal suspension and its dilutions, a linear regression was performed for the calculation of the cell densities of the replicates during the test.
Microscopical observations of algal cells	72 h
Test substance concentration	0, 24, 48 and 72 h
Appearance of test substance in test solutions	0, 24, 48 and 72 h
Test conditions	Water temperature: daily in an Erlenmeyer flask filled with water and incubated under the same conditions as the test flasks pH: in each test group at the start and end of the test Light intensity: once during the test (at 6 positions distributed over the experimental area at the surface of the test solutions)

Analytical method:

Method type	LC-MS/MS
Equipment	Agilent Series 1290 pump and autosampler
Column	Phenomenex Synergi 4 µm Fusion-RP 80A (50 x 2 mm)
Column temperature	40°C
Detector	Mass spectrometer API 5500 Detection: ESI positive MRM mass transitions: Mesosulfuron-methyl: m/z 504.1 → 182.1 (quantifier); 504.1 → 83.0 (qualifier) Pinoxaden: m/z 401.5 → 317.2 (quantifier); 401.5 → 56.9 (qualifier) NOA 407854:

	m/z 317.8 → 171.2 (quantifier); 317.8 → 131.1 (qualifier) Mefenpyr-diethyl: m/z 389.9 → 327.0 (quantifier); 389.9 → 160.0 (qualifier)
Flow rate	0.65 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid B: Acetonitrile containing 0.1% formic acid 0.0 min 95% A, 5% B 2.0 min 95% A, 5% B 2.5 min 50% A, 50% B 4.5 min 5% A, 95% B 5.2 min 5% A, 95% B 5.3 min 95% A, 5% B 7.0 min 95% A, 5% B

Experimental dates: 02 to 20 Nov 2020

Calculations:

Based on the cell densities at 0, 24, 48 and 72 hours, the growth rates and yields as well as their percentage inhibition were calculated in accordance with OECD 201 (2011).

Statistics:

Statistical analyses were performed following the recommendations of OECD Guidance Document 54 (2006) and using the program ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Based on the calculated cell densities, the 72-hour ErC_{50} and the 72-hour EyC_{50}, the corresponding EC_{20} and EC_{10} values and, where possible, their 95% confidence intervals were calculated by Weibull analysis.

For determination of the 72-hour LOEC and 72-hour NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams t-test (yield) and Bonferroni-Welch t-test (growth rate), $\alpha = 0.05$, one-sided smaller. For yield, normal distribution and variance homogeneity were confirmed. For growth rate, normal distribution was confirmed, but the check for variance homogeneity failed.

Results and discussions

Validity criteria:

- Minimum 16-fold biomass increase in the control culture during the 72-hour test period
- Mean coefficient of variation for section-by-section specific growth rates in control cultures must not exceed 35%
- Coefficient of variation of average specific growth rates in replicate control cultures must not exceed 7%

A 164.0-fold biomass increase in the control culture during the 72-hour test period was observed. The mean coefficient of variation for section-by-section specific growth rates in control cultures was 13.2% and the coefficient of variation of average specific growth rates in replicate control cultures was 0.7%. Therefore, all validity criteria were met.

The test substance concentrations were determined by analysis of the analytes mesosulfuron-methyl, pinoxaden, NOA 407854 (metabolite of pinoxaden) and mefenpyr-diethyl. Measured test concentrations and percentage recovery of analytes in test solutions is presented in the table below.

Table A2.2.1.2-1: Measured test concentrations and percentage recovery of analytes in test solutions

Test substance nominal concentration (mg product/L)	Analyte concen-tration nominal (µg/L)	0 h (fresh)		24 h (aged)		48 h (aged)		72 h (aged)	
		Analyte conc. actual (µg/L)[a]	Reco-very (% of nominal)	Analyte conc. actual (µg/L)[a]	Reco-very (% of nominal)	Analyte conc. actual (µg/L)[a]	Reco-very (% of nominal)	Analyte conc. actual (µg/L)[a]	Reco-very (% of nominal)
Mesosulfuron-methyl									
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6.25	75.0	76.3	102	73.8	98	79.6	106	78.3	104
12.5	150	153	96	153	102	156	104	156	104
25	300	305	103	307	102	308	103	310	103
50	600	610	106	617	103	646	108	614	102
100	1200	1221	106	1192	99	1326	111	1424	119
Pinoxaden									
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6.25	394	338	86	281	71	249	63	227	58
12.5	788	706	90	661	84	548	70	491	62
25	1575	1380	88	1205	77	1107	70	1086	69
50	3150	2959	94	2449	78	2406	76	2195	70
100	6300	6049	96	4866	77	4890	78	5081	81
NOA 407854 (metabolite of pinoxaden)									
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6.25	311.063[b]	20.126	6	52.567	17	80.731	26	100.561	32
12.5	622.125[b]	47.830	8	117.205	19	166.060	27	206.868	33
25	1244.25[b]	103.247	8	257.400	21	339.017	27	406.840	33
50	2488.5[b]	243.049	10	498.706	20	666.538	27	811.380	33
100	4977.0[b]	540.356	11	988.215	20	1452.63	29	1792.89	36
Mefenpyr-diethyl									
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6.25	238	219	92	219	92	221	93	209	88
12.5	475	439	95	442	93	438	92	399	84
25	950	878	96	887	93	870	92	868	91
50	1900	1755	101	1614	85	1638	86	1640	86
100	3800	3510	117	3061	81	3037	80	3548	93

n.a. not applicable
Mesosulfuron-methyl: LOD: 0.002 µg mesosulfuron-methyl/L, LOQ: 0.6 µg mesosulfuron-methyl/L
Pinoxaden: LOD: 0.008 µg pinoxaden/L, LOQ: 3.2 µg pinoxaden/L
NOA 407854: LOD: 0.08 µg NOA 407854/L, LOQ: 1.2 µg NOA 407854/L
Mefenpyr-diethyl: LOD: 0.06 µg mefenpyr-diethyl/L, LOQ: 1.9 µg mefenpyr-diethyl/L

[a] Mean value of duplicate samples

[b] Nominal NOA 407854 concentration was calculated using the nominal content of pinoxaden (6.3%) given in analytical certificate and the molar ratio of pinoxaden (400.5 g/mol) and NOA 407854 (316.4 g/mol) and assumption that 100% of nominal of pinoxaden transformed to NOA 407854.

Recoveries of mesosulfuron-methyl and mefenpyr-diethyl showed that the test substance was dosed correctly and both substances were stable during the test. Recoveries of pinoxaden showed that the test substance was dosed correctly. Concentrations of pinoxaden decreased during the period of the study (falling below 80% of nominal) concurrently with the increase of the concentrations of NOA 407854, the metabolite of pinoxaden. Therefore, the biological results were based on nominal test substance concentrations as well as on concentrations calculated based on the total active substance load calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (geometric mean measured) as presented in the following table.

Table A2.2.1.2-2: Total active substance load (sum of mesosulfuron-methyl + pinoxaden)

Test substance	Mesosulfuron-methyl	Pinoxaden		Total active substance load (sum of mesosulfuron-methyl + pinoxaden)			
Nominal concentration (mg product/L)	Nominal concentration (µg a.s./L)	Nominal conc. (µg a.s./L)	Geometric mean measured conc. (µg a.s./L)	Nominal sum of a.s. conc.[a] (µg sum of a.s./L)	Calculated sum of a.s. conc.[b] (µg sum of a.s./L)	Calculated % of nominal (%)	Calculated test sub. conc. (mg product/L)

Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6.25	75.0	394	269	469	344	73	4.58
12.5	150	788	597	938	747	80	9.96
25	300	1575	1178	1875	1478	79	19.7
50	600	3150	2467	3750	3067	82	40.9
100	1200	6300	5090	7500	6290	84	83.9

n.a. Not applicable
[a] Sum of nominal concentrations of mesosulfuron-methyl and pinoxaden
[b] Sum of nominal concentration of mesosulfuron-methyl and geometric mean measured concentration of pinoxaden

Mean algal cell densities at each time point and concentration are presented in the table below.

Table A2.2.1.2-3: Algal cell densities during exposure to ADM.06001.H.2.B.

Total active substance load[a] (mg product/L)	**Density of algal cells (mean ± standard deviation) (10000/mL)**			
	0 hours	**24 hours**	**48 hours**	**72 hours**
Control	0.5	2.166 ± 0.227	14.007 ± 1.070	81.985 ± 3.082
4.58		2.359 ± 0.925	14.999 ± 1.144	78.871 ± 4.915
9.96		1.122 ± 0.587	12.939 ± 0.229	76.077 ± 2.888
19.7		15.235 ± 0.353	16.295 ± 0.132	50.447 ± 1.814
40.9		32.969 ± 6.994	18.278 ± 7.069	32.082 ± 4.572
83.9		47.542 ± 1.870	10.575 ± 8.276	22.660 ± 4.969[b]

[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (geometric mean measured)
[b] Values of the concentration of 83.9 mg product/L after 72 hours were set to the initial cell density for further evaluation (yield and growth rate) since the microscopic observation showed no visible cells.

The calculated growth rates and percentage inhibition of growth rates in comparison to the control are presented in the table below.

Table A2.2.1.2-4: Growth rates of algae and their percentage inhibition

Total active substance load[a] (mg product/L)	**0 - 24 h**		**0 - 48 h**		**0 - 72 h**	
	Growth rate μ (day^{-1})	**% Inhibition**	**Growth rate μ (day^{-1})**	**% Inhibition**	**Growth rate μ (day^{-1})**	**% Inhibition**
Control	1.462	n.a.	1.665	n.a.	1.700	n.a.
4.58	1.496	-2.3	1.700	-2.1	1.687	0.8
9.96	0.693	52.6	1.627	2.3	1.675	1.5*
19.7	3.417	-133.7[b]	1.742	-4.6	1.538	9.5*
40.9	4.172	-185.4[b]	1.770	-6.3	1.385	18.5*
83.9	4.554	-211.6[b]	1.263	24.1	0.000	100.0*

Note: Negative "% Inhibition" values indicate an increase in growth relative to the control.
n.a. Not applicable
* Mean value significantly different from the control (Bonferroni-Welch t-test (24 h), Median (2x2 Table) Test after Bonferroni-Holm (48 h) and Bonferroni-Welch t-test (72 h), $\alpha = 0.05$, one-sided smaller)
[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (geometric mean measured)
[b] Results are deemed to be unreliable since turbidity of the test solutions caused a bias in the measurements, which resulted in implausible algal cell densities. The 48- and 72-hour values by spectrophotometric measurement are seemingly more plausible, except for the highest test concentration after 72 hours. Since no algal cells were found in the highest test concentration at 72 hours in the microscopic observation, a more conservative approach was taken by setting the cell density to the initial values.

The calculated yield and percentage inhibition of yield in comparison to the control are presented in the table below.

Table A2.2.1.2-5: Yields of algae and their percentage inhibition

Total active substance load[a] (mg product/L)	0 - 24 h		0 - 48 h		0 - 72 h	
	Yield y [10000 cells/mL]	% Inhibition	Yield y [10000 cells/mL]	% Inhibition	Yield y [10000 cells/mL]	% Inhibition
Control	1.666	n.a.	13.507	n.a.	81.485	n.a.
4.58	1.859	-11.6	14.499	-7.3	78.371	3.8
9.96	0.622	62.6	12.439	7.9	75.577	7.3*
19.7	14.735	-784.4[b]	15.795	-16.9	49.947	38.7*
40.9	32.469	-1848.8[b]	17.778	-31.6	31.582	61.2*
83.9	47.042	-2723.4[b]	10.075	25.4	0.000	100.0*

Note: Negative "% Inhibition" values indicate an increase in yield relative to the control.
n.a. Not applicable
* Mean value significantly different from the control (Median (2x2 Table) Test after Bonferroni-Holm (24 h), Bonferroni-Welch t-test (48 h) and Williams t-test (72 h), $\alpha = 0.05$, one-sided smaller)
[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (geometric mean measured)
[b] Results are deemed to be unreliable since turbidity of the test solutions caused a bias in the measurements, which resulted in implausible algal cell densities. The 48- and 72-hour values by spectrophotometric measurement are seemingly more plausible, except for the highest test concentration after 72 hours. Since no algal cells were found in the highest test concentration at 72 hours in the microscopic observation, a more conservative approach was taken by setting the cell density to the initial values.

Based on these results, the following endpoints were obtained.

Table A2.2.1.2-6: Study endpoints of algae growth inhibition test with ADM.06001.H.2.B.

Parameter	Growth rate	Yield
Endpoints based on total active substance load[a]		
72-h NOEC	4.58	4.58
72-h LOEC	9.96	9.96
72-h EC_{10} (95% confidence interval)	34.3 (30.8 – 38.1)	8.3 (6.1 – 11.2)
72-h EC_{20} (95% confidence interval)	41.1 (38.2 – 44.3)	13.8 (11.2 – 17.1)
72-h EC_{50} (95% confidence interval)	54.2 (49.3 – 59.5)	30.0 (26.8 – 33.7)
Endpoints based on nominal concentrations		
72-h NOEC	6.25	6.25
72-h LOEC	12.5	12.5
72-h EC_{10} (95% confidence interval)	42.1 (38.1 – 46.7)	10.7 (8.0 – 14.3)
72-h EC_{20} (95% confidence interval)	50.2 (46.8 – 54.0)	17.6 (14.4 – 21.4)
72-h EC_{50} (95% confidence interval)	65.6 (59.9 – 71.7)	37.1 (33.4 – 41.4)

[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (geometric mean measured)

At the end of the 72-hour exposure, growth rates of algae as well as yield of algae were statistically significantly inhibited at the test concentration of 9.96 mg product/L and all higher test concentrations. Therefore, the LOEC and NOED were determined as 4.58 and 9.96 mg product/L, respectively, both based on growth rate and yield.

72-hour E_rC_{10}, E_rC_{20} and E_rC_{50} were calculated to be 34.3 (30.8 – 38.1, 95% confidence interval), 41.1 (38.2 – 44.3, 95% confidence interval) and 54.2 (49.3 – 59.5, 95% confidence interval) mg product/L, respectively, and 72-hour E_yC_{10}, E_yC_{20} and E_yC_{50} were calculated to be 10.7 (8.0 – 14.3, 95% confidence interval), 17.6 (14.4 – 21.4, 95% confidence interval) and 37.1 (33.4 – 41.4, 95% confidence interval) mg product/L, respectively.

Microscopic examination of the shape of algal cells after 72 hours of test duration did not show any difference between algae that had been growing up to a test concentration of 40.9 mg product/L and algal cells in the control. At the highest concentration of 83.9 mg product/L, no cells were visible.

Conclusion

In this test to determine the growth inhibition of ADM.06001.H.2.B in the green algae *Raphidocelis*

~~*subcapitata*, the 72-hour NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on growth rate were calculated to be 4.58, 34.3 (30.8 – 38.1, 95% confidence interval), 41.1 (38.2 – 44.3, 95% confidence interval) and 54.2 (49.3 – 59.5, 95% confidence interval) mg product/L, respectively. The 72-hour NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on yield were determined as 4.58, 10.7 (8.0 – 14.3, 95% confidence interval), 17.6 (14.4 – 21.4, 95% confidence interval) and 37.1 (33.4 – 41.4, 95% confidence interval) mg product/L, respectively. These endpoints are based on adjusted test substance concentrations, i.e. the sum of active substance contents (total active substance load) using nominal concentrations of mesosulfuron-methyl and geometric mean measured concentrations of pinoxaden.~~

Applicant's recalculation of endpoints:

Based on a request by Poland the endpoints have been updated in a report amendment (Siche, O. and Mollandin G, 2023) considering the mean measured concentrations of both active substances.

Table A2.2.1.2-7: Study endpoints of algae growth inhibition test with ADM.06001.H.2.B.

Parameter	Growth rate	Yield
Endpoints based on total active substance load[a]		
72 h NOEC	4.61	4.61
72 h LOEC	10.0	10.0
72 h EC_{10} (95% confidence interval)	34.6 (30.0 - 37.8)	9.96 (7.16 - 12.4)
72 h EC_{20} (95% confidence interval)	41.5 (38.1 - 44.6)	14.2 (11.1 - 16.8)
72 h EC_{50} (95% confidence interval)	54.8 (50.5 - 61.9)	27.8 (24.4 - 31.7)

[a] Calculated from the observed concentrations for mesosulfuron-methyl (geometric mean measured) and pinoxaden (geometric mean measured)

A 2.2.1.3 Effects on aquatic macrophytes

Comments of zRMS:	The study was conducted in line with OECD 221 with minor deviations to the guideline and the study plan. It was noted that the pH in the control medium increased by more than 1.5 units during the last test medium renewal period. However, with an increase of 1.6 units it was only 0.1 units above the required 1.5 units and this does not invalidate the test. Also, the guideline recommends the use of $NaHCO_3$ as buffer in the test medium for *Lemna gibba* instead of MOPS. However, this deviation is not considered to affect the quality and integrity of the study. It was noted in the study report that with regard to the analysis of the test item concentrations / LC-MS/MS conditions the flow rate of 0.65 mL/min instead of 0.6 mL/min was applied due to human error. This deviation is considered to have no negative effect on the outcome of the study since all measurements were perfomed with the same flow rate. For preparation of the stability samples a mixture of the test water at pH 4/ acetonitrile (9/1, v/v) instead of at pH 4/ acetonitrile (4/1, v/v) was used by mistake. But no analysis of the stability samples was necessary, since all test samples were analysed within 30 days of the test start and this deviation had no effect on the outcome of the study. The analytical measurements demonstrated that the measured concentrations of the active substance mesosulfuron-methyl were within ± 20% of the nominal concentrations but the measured concentrations of the active substance pinoxaden were not within the ± 20% of the nominal concentrations during the test. The endpoints reported in the study are based on adjusted test item concentrations, i.e. the sum of active substance contents (total active substance load) using nominal concentrations of mesosulfuron-methyl and time-weighted average concentrations of pinoxaden. In zRMS opinion the sum of the active substance content (total active substance load) should be expressed in the same units i.e. as the sum of time-weighted average concentrations of both active substances. Consequently, the endpoints were recalculated by the Applicant for zRMS's request.

	All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: Frond number: 7 d E_rC_{50} = 0.074 mg product/L (calculated for mesosulfuron-methyl (time-weighted average) and pinoxaden (time-weighted average)) 7 d E_yC_{50} = 0.035 mg product/L (calculated for mesosulfuron-methyl (time-weighted average) and pinoxaden (time-weighted average)) Dry weight: 7 d E_rC_{50} >0.495 mg product/L (calculated for mesosulfuron-methyl (time-weighted average) and pinoxaden (time-weighted average)) 7 d E_yC_{50} =0.073 mg product/L (calculated for mesosulfuron-methyl (time-weighted average) and pinoxaden (time-weighted average))

Reference:	KCP 10.2.1/03
Report	ADM.06001.H.2.B: Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test, Seidel U. and Mollandin G., 2021c, 140711240 (ADAMA No. 000105365)
Guideline(s):	Yes, OECD 221 (2006), EPA Guideline 712-C-008: OCSPP 850.4400 (2012)
Deviations:	Minor (see the commenting box above) ~~The pH in the control medium increased by more than 1.5 units during the last test medium renewal period. However, with an increase of 1.6 units it was only 0.1 unit above the required 1.5 units and this does not invalidate the test since the validity criteria were met.~~ ~~OECD 221 (2006) recommends the use of $NaHCO_3$ as buffer in the test medium for *Lemna gibba* instead of MOPS.~~ ~~These deviations are not considered to affect the quality and integrity of the study.~~
GLP:	Yes
Acceptability:	Acceptable (recalculated endpoints are requested) ~~Yes~~
Duplication (if vertebrate study)	Not applicable

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	3,5-dichlorophenol is tested at least twice a year to demonstrate the quality of *Lemna gibba* and the experimental conditions. The most recent reference substance test (performed in October/November 2020) resulted in 7-day E_rC_{50} of 5.75 (frond number) and 5.16 (dry weight) mg/L and 7-day E_yC_{50} of

	3.76 (frond number) and 4.34 (dry weight) mg/L, respectively.

Test organism:

Test species	*Lemna gibba* G 3
Origin	In-house culture
Acclimation	The plants were cultivated under standardised conditions according to the test guidelines.
Introduction of plants	Colonies consisting of 4 fronds were transferred from the pre-culture. A pre-culture was set up for at least 7 days under test conditions with weekly test medium exchange.
No. of colonies per test vessel	3 colonies consisting of 4 fronds each (12 fronds per test vessel)
No. of test vessels (replicates) per test substance concentration	4

Test conditions:

Test substance concentrations	0.01, 0.03, 0.09, 0.27 and 0.81 mg product/L (nominal), corresponding to adjusted test substance concentrations based on sum of active substance contents using nominal concentrations of mesosulfuron-methyl and time-weighted average measured concentrations of pinoxaden of 0.00479, 0.0147, 0.0488, 0.163 and 0.510 mg product/L (total active substance load) The test concentrations were chosen based on a non-GLP range-finding test. A stock solution of 10 mg product/L was prepared by dissolving 7.4, 8.9 and 9.8 mg product into 740, 890 and 980 mL test medium, respectively. Adequate volumes of this stock solution were diluted with test medium to prepare the test solutions of the desired test concentrations. The test solutions were prepared just before introduction of the *Lemna* (= start of the test) and each test solution renewal.
Control	Untreated test medium
Test duration	7 d
Test medium	20x AAP-Growth Medium: Macro-nutrients: $NaHCO_3$ 300 mg/L $K_2HPO_4 \cdot 3\ H_2O$ 30 mg/L $MgSO_4 \cdot 7\ H_2O$ 290 mg/L $NaNO_3$ 510 mg/L $MgCl_2 \cdot 6\ H_2O$ 240 mg/L $CaCl_2 \cdot 2\ H_2O$ 90 mg/L Micro-nutrients: H_3BO 3.7 mg/L $MnCl_2 \cdot 4\ H_2O$ 8.3 mg/L $ZnCl_2$ 0.066 mg/L $CoCl_2 \cdot 6\ H_2O$ 0.029 mg/L $CuCl_2 \cdot 2\ H_2O$ 0.00024 mg/L $Na_2MoO_4 \cdot 2\ H_2O$ 0.145 mg/L $FeCl_3 \cdot 6\ H_2O$ 3.2 mg/L $Na_2EDTA \cdot 2\ H_2O$ 6.0 mg/L The buffer 4-morpholinepropane sulphonic acid (MOPS buffer) was added at 700 mg/L. The test medium was prepared 4 - 25 days before test start or test solution renewal to allow the pH to stabilise.
Test type	Semi-static, test solution renewal on days 2 and 4
Test medium pH	7.5-7.7 in freshly prepared test solutions at test start and each renewal 8.3-9.1 in aged test solutions at each renewal and test end
Water temperature	24 ± 2°C (nominal) 23.1-24.5°C (actual)

Test solution appearance	No remarkable observations
Test vessel	250 mL glass vessels covered with watch glasses
Test volume	Approximately 200 mL
Light intensity	Mean 7472 lux (range: 6830 to 8020 lux)
Photoperiod	Continuous illumination

Observations:

Frond number and appearance of colonies	2, 4 and 7 d
Observations of phytotoxic symptoms	7 d
Dry weight	0 d: The initial dry weight of a sample of fronds identical to that used to inoculate the test vessels was determined. 7 d: The dry weight of all plants from each vessel was determined. The plants were dried at 60°C to a constant weight.
Test substance concentration	Freshly prepared test solutions: 0 d and 2 and 4 d at renewal Aged test solutions: 2 and 4 d at renewal and 7 d Furthermore, pinoxaden and its metabolite NOA 407854 were taken for analysis at additional sampling points with no renewal, i.e. at 5 and 6 d.
Test conditions	Water temperature: daily in a test vessel filled with water and incubated under the same conditions as the test vessels. pH: in freshly prepared test solutions at test start and each renewal and in aged test solutions at each renewal and test end. Light intensity: once during the test (at 9 places distributed over the experimental area at the surface of the test solutions).

Analytical method:

Method type	LC-MS/MS
Equipment	Agilent Series 1290 pump and autosampler
Column	Phenomenex Synergi 4 µm Fusion-RP 80A (50 x 2 mm)
Column temperature	40°C
Detector	Mass spectrometer API 5500 Detection: ESI positive MRM mass transitions: Mesosulfuron-methyl: m/z 504.1 → 182.1 (quantifier); 504.1 → 83.0 (qualifier) Pinoxaden: m/z 401.5 → 317.2 (quantifier); 401.5 → 56.9 (qualifier) NOA 407854: m/z 317.8 → 171.2 (quantifier); 317.8 → 131.1 (qualifier) Mefenpyr-diethyl: m/z 389.9 → 327.0 (quantifier); 389.9 → 160.0 (qualifier)
Flow rate	0.65 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid B: Acetonitrile containing 0.1% formic acid 0.0 min 95% A, 5% B 2.0 min 95% A, 5% B 2.5 min 50% A, 50% B 4.5 min 5% A, 95% B 5.2 min 5% A, 95% B 5.3 min 95% A, 5% B 7.0 min 95% A, 5% B

Experimental dates: 09 to 18 Nov 2020

Calculations:

Based on frond numbers at 0, 2, 4 and 7 days and dry weights at 0 and 7 days, the growth rates and yields as well as their percentage inhibition were calculated in accordance with the guidelines.

Statistics:
Statistical analyses were performed following the recommendations of OECD Guidance Document 54 (2006) and using the program ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

The E_rC_{50} and the E_yC_{50}, the corresponding EC_{20} and EC_{10} values and where possible their 95% confidence intervals were calculated by Probit analysis.

For yield based on frond number, the check for normal distribution and variance homogeneity failed, due to a high coefficient of variation in the concentration of nominal 0.01 mg product/L. By leaving out this concentration in the statistical evaluation, ToxRat automatically chose the Williams t-test as normal distribution and variance homogeneity check was passed and the trend analysis showed a linear trend. Therefore, the Williams t-test was still applied using all five concentrations. For growth rate based on frond number and yield and growth rate based on dry weight, the check for normal distribution and variance homogeneity was passed and the trend analysis revealed a linear trend.

For determination of the 7-day LOE_rC and NOE_rC values and the 7-day LOE_yC and NOE_yC values, significant differences at the test concentrations compared to the control values were tested by the Williams t-test (frond number and dry weight, $\alpha = 0.05$, one-sided smaller).

Results and discussions

Validity criterion:

- The doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}.

The doubling time of frond number in the control was 1.4 days. Therefore, the validity criterion was met.

The test substance concentrations were determined by analysis of the analytes mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and NOA 407854 (metabolite of pinoxaden). Percentage recovery of analytes in test solutions is presented in the table below.

Table A2.2.1.3-1: Measured test concentrations and percentage recovery of analytes in test solutions

Test substance nominal conc. (mg product/L)	Analyte concen-tration nominal (µg/L)	Recovery[a] (% of nominal)					
		0 d (fresh)	2 d (aged)	2 d (fresh)	4 d (aged)	4 d (fresh)	7 d (old)
Mesosulfuron-methyl							
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.01	0.120	99	102	96	101	92	88
0.03	0.360	93	96	95	101	84	89
0.09	1.080	98	95	101	94	88	90
0.27	3.240	102	104	106	107	96	98
0.81	9.720	101	87	100	100	97	80
Mefenpyr-diethyl							
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.01	0.38	105	83	109	77	104	n.a.
0.03	1.14	92	66	90	56	75	n.a.
0.09	3.42	103	63	96	46	85	20
0.27	10.26	99	55	96	55	86	27
0.81	30.78	83	58	82	53	87	30

		0 d (fresh)	2 d (aged)	2 d (fresh)	4 d (aged)	4 d (fresh)	5 d (old) no renewal	6 d (old) no renewal	7 d (old)
Pinoxaden									

Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.01	0.630	96	n.a.	96	n.a.	85	n.a.	n.a.	n.a.
0.03	1.890	88	30	89	25	81	27	n.a.	n.a.
0.09	5.670	99	33	102	19	88	45	16	n.a.
0.27	17.010	95	41	93	30	88	57	30	10
0.81	51.030	100	44	96	32	93	60	33	11
NOA 407854 (metabolite of pinoxaden)									
Control	Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.01	0.498[b]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.03	1.493[b]	n.a.	48	n.a.	49	n.a.	53	80	55
0.09	4.479[b]	185	50	20	58	n.a.	37	65	50
0.27	13.438[b]	20	47	21	55	n.a.	22	52	52
0.81	40.314[b]	21	49	23	55	n.a.	25	52	56

n.a. not applicable
Mesosulfuron-methyl: LOD: 0.002 µg mesosulfuron-methyl/L, LOQ: 0.05 µg mesosulfuron-methyl/L
Mefenpyr-diethyl: LOD: 0.036 µg mefenpyr-diethyl/L, LOQ: 0.15 µg mefenpyr-diethyl/L
Pinoxaden: LOD: 0.002 µg pinoxaden/L, LOQ: 0.25 µg pinoxaden/L
NOA 407854: LOD: 0.13 µg NOA 407854/L, LOQ: 0.58 µg NOA 407854/L
[a] Mean value of duplicate samples
[b] Nominal NOA 407854 concentration was calculated using the nominal content of pinoxaden (6.3%) given in analytical certificate and the molar ratio of pinoxaden (400.5 g/mol) and NOA 407854 (316.4 g/mol) and assumption that 100% of nominal of pinoxaden transformed to NOA 407854.

Recoveries of mesosulfuron-methyl showed that the test substance was dosed correctly, and mesosulfuron-methyl was stable during the test. Recoveries of pinoxaden and mefenpyr-diethyl showed that the test substance was dosed correctly. Concentrations of pinoxaden decreased during the test solution renewal periods (falling below 80% of nominal) concurrently with the increase of the concentrations of NOA 407854, the metabolite of pinoxaden. Therefore, the biological results were based on nominal test substance concentrations as well as on concentrations calculated based on the total active substance load calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (time-weighted average) as presented in the following table.

Table A2.2.1.3-2: Total active substance load (sum of mesosulfuron-methyl + pinoxaden)

Test substance	**Mesosulfuron-methyl**	**Pinoxaden**		**Total active substance load (sum of mesosulfuron-methyl + pinoxaden)**			
Nominal concentration (mg product/L)	**Nominal concentration (µg a.s./L)**	**Nominal conc. (µg a.s./L)**	**Time-weighted average conc. (µg a.s./L)**	**Nominal sum of a.s. conc.[a] (µg sum of a.s./L)**	**Calculated sum of a.s. conc.[b] (µg sum of a.s./L)**	**Calculated % of nominal[c] (%)**	**Calculated test item ~~sub.~~ conc.[d] (mg product/L)**
Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.01	0.120	0.63	0.239	0.750	0.359	48	0.00479
0.03	0.360	1.89	0.745	2.25	1.105	49	0.0147
0.09	1.08	5.67	2.58	6.75	3.659	54	0.0488
0.27	3.24	17.01	9.01	20.3	12.253	61	0.163
0.81	9.72	51.03	28.5	60.8	38.264	63	0.510

n.a. Not applicable
[a] Sum of nominal concentrations of mesosulfuron-methyl and pinoxaden
[b] Sum of nominal concentration of mesosulfuron-methyl and time-weighted average concentration of pinoxaden
[c] % of nominal calculated using nominal concentration of sum of a.s. and calculated concentration of sum of the nominal concentration of mesosulfuron-methyl and TWA concentration of pinoxaden
[d] test item concentration calculated using calculated % of nominal

The calculated growth rates and percentage inhibition of growth rates based on frond number in comparison to the control are presented in the table below.

Table A2.2.1.3-3: Growth rates of *Lemna gibba* and their percentage inhibition based on frond number

Total active substance load[a] (mg product/L)	**0 - 2 d**		**0 - 4 d**		**0 - 7 d**	
	Growth rate µ (day^{-1})	**% Inhibition**	**Growth rate µ (day^{-1})**	**% Inhibition**	**Growth rate µ (day^{-1})**	**% Inhibition**

Control	0.445	n.a.	0.472	n.a.	0.482	n.a.
0.00479	0.485	-8.9	0.495	-4.7	0.498	-3.2
0.0147	0.458	-2.8	0.461	2.5	0.488	-1.3
0.0488	0.336	24.6*	0.279	40.9*	0.261	45.9*
0.163	0.280	37.2*	0.151	68.0*	0.125	74.2*
0.510	0.216	51.6*	0.134	71.7*	0.088	81.7*

Note: Negative "% Inhibition" values indicate an increase in growth rate relative to the control.
n.a. Not applicable
* Mean value significantly different from the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)
[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (time-weighted average)

The calculated yield and percentage inhibition of yield in comparison to the control are presented in the table below.

Table A2.2.1.2-4: Yields of *Lemna gibba* and their percentage inhibition based on frond number

Total active substance load[a] (mg product/L)	0 - 2 d		0 - 4 d		0 - 7 d	
	Yield y	% Inhibition	Yield y	% Inhibition	Yield y	% Inhibition
Control	17.3	n.a.	67.5	n.a.	338.8	n.a.
0.00479	19.8	-14.5	75.0	-11.1	381.0	-12.5
0.0147	18.0	-4.3	64.3	4.8	354.3	-4.6
0.0488	11.5	33.3*	24.8	63.3*	62.8	81.5*
0.163	9.0	47.8*	10.0	85.2*	16.8	95.1*
0.510	6.5	62.3*	8.5	87.4*	10.3	97.0*

Note: Negative "% Inhibition" values indicate an increase in yield relative to the control.
n.a. Not applicable
* Mean value significantly different from the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)
[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (time-weighted average)

Table A2.2.1.2-5: Growth rate and yields of *Lemna gibba* and their percentage inhibition based on dry weight after 7 days of exposure

Total active substance load[a] (mg product/L)	Growth rate after 7 d		Yield after 7 d	
	μ (day^{-1})	% Inhibition	Yield y	% Inhibition
Control	0.532	n.a.	44.5	n.a.
0.00479	0.544	-2.3	48.8	-9.7
0.0147	0.533	-0.2	44.8	-0.7
0.0488	0.434	18.4*	21.8	50.9*
0.163	0.357	32.8*	12.3	72.2*
0.510	0.327	38.4*	9.8	78.0*

Note: Negative "% Inhibition" values indicate an increase in growth rate/yield relative to the control.
n.a. Not applicable
* Mean value significantly different from the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)
[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (time-weighted average)

Based on these results, the following endpoints were obtained.

Table A2.2.1.2-6: Study endpoints of *Lemna gibba* growth inhibition test with ADM.06001.H.2.B.

Parameter	Based on frond number		Based on dry weight	
	Growth rate	Yield	Growth rate	Yield
Endpoints based on total active substance load[a]				
7 d NOEC	0.0147	0.0147	0.0147	0.0147
7 d LOEC	0.0488	0.0488	0.0488	0.0488
7 d EC_{10} (95% confidence interval)	0.013 (0.008-0.019)	0.022 (0.009-0.029)	0.025 (0.013-0.038)	0.010 (0.005-0.016)
7 d EC_{20} (95% confidence interval)	0.024 (0.016-0.032)	0.026 (0.013-0.033)	0.084 (0.058-0.111)	0.020 (0.011-0.029)
7 d EC_{50} (95% confidence interval)	0.074 (0.060 – 0.092)	0.035 (0.024-0.040)	> 0.510 (n.d.)	0.073 (0.055-0.098)
Endpoints based on nominal concentrations				

7 d NOEC	0.030	0.030	0.030	0.030
7 d LOEC	0.090	0.090	0.090	0.090
7 d EC_{10} (95% confidence interval)	0.027 (0.016-0.038)	0.044 (0.019-0.057)	0.047 (0.025-0.071)	0.021 (0.010-0.033)
7 d EC_{20} (95% confidence interval)	0.047 (0.032-0.061)	0.051 (0.026-0.062)	0.148 (0.105-0.193)	0.039 (0.023-0.055)
7 d EC_{50} (95% confidence interval)	0.132 (0.107-0.162)	0.067 (0.048-0.075)	> 0.81 (n.d.)	0.131 (0.100-0.172)

n.d. Not determinable

[a] Calculated from the observed concentrations for mesosulfuron-methyl (nominal) and pinoxaden (time-weighted average)

At the end of the 7-day exposure, growth rates and yields based on frond number and dry weight were all statistically significantly inhibited at the three highest test concentrations of 0.0488, 0.163 and 0.510 mg product/L. Therefore, the LOEC and NOEC for growth rates and yields based on frond number and dry weight were determined as 0.0488 and 0.0147 mg product/L, respectively.

E_rC_{10}, E_rC_{20}, E_rC_{50}, E_yC_{10}, E_yC_{20} and E_yC_{50} values based on frond number were calculated as 0.013 (0.008-0.019, 95% confidence interval), 0.024 (0.016-0.032, 95% confidence interval), 0.074 (0.060 – 0.092, 95% confidence interval), 0.022 (0.009-0.029, 95% confidence interval), 0.026 (0.013-0.033, 95% confidence interval) and 0.035 (0.024-0.040, 95% confidence interval) mg product/L, respectively. E_rC_{10}, E_rC_{20}, E_rC_{50}, E_yC_{10}, E_yC_{20} and E_yC_{50} values based on dry weight were determined to be 0.025 (0.013-0.038, 95% confidence interval), 0.084 (0.058-0.111, 95% confidence interval), > 0.510 (95% confidence interval not determinable), 0.010 (0.005-0.016, 95% confidence interval), 0.020 (0.011-0.029, 95% confidence interval) and 0.073 (0.055-0.098, 95% confidence interval) mg product/L, respectively.

The shape of fronds and colonies after the test period of 7 days was not different to those in the control up to and including the test concentration of 0.0147 mg productc/L. At higher test substance concentrations, the fronds showed deviations from the control replicates after 7 days, *i.e.* gibbous growth (0.0488, 0.163 and 0.510 mg product/L), and necrosis (0.163 and 0.510 mg product/L).

Conclusion

In this test to determine the effects of ADM.06001.H.2.B in the aquatic plant *Lemna gibba*, the 7-day NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on frond number were calculated to be 0.0147, 0.013, 0.024 and 0.074 mg product/L, respectively. The 7-day NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on frond number were determined as 0.0147, 0.022, 0.026 and 0.035 mg product/L, respectively. The 7-day NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on dry weight were calculated to be 0.0147, 0.025, 0.084 and > 0.510 mg product/L, respectively. The 7-day NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on dry weight were determined as 0.0147, 0.010, 0.020 and 0.073 mg product/L, respectively.

The Applicant's recalculation of endpoints:

Based on a request by Poland the endpoints have been updated in a report amendment (Siche, O. and Mollandin G, 2023) considering the time-weighted mean measured concentrations of both active substances.

Table A2.2.1.2-7: Study endpoints of *Lemna gibba* growth inhibition test with ADM.06001.H.2.B.

Parameter	Based on frond number		Based on dry weight	
	Growth rate	Yield	Growth rate	Yield
Endpoints based on total active substance load[a] (mg/L)				
7 d NOEC	0.0143	0.0143	0.0143	0.0143
7 d LOEC	0.0479	0.0479	0.0479	0.0479
7 d EC_{10} (95% confidence interval)	0.013 (0.008-0.020)	0.022 (0.014-0.033)	0.024 (0.015-0.041)	0.010 (0.005-0.017)

7 d EC_{20} (95% confidence interval)	0.023 (0.017-0.033)	0.026 (0.018-0.036)	0.083 (0.061-0.112)	0.019 (0.013-0.030)
7 d EC_{50} (95% confidence interval)	**0.074** (0.060-0.091)	*0.035* (0.029-0.041)	> 0.495 (n.d.)	0.073 (0.055-0.096)

n.d. Not determinable
[a] Calculated from the observed concentrations for mesosulfuron-methyl (time-weighted average) and pinoxaden (time-weighted average)

A 2.2 1.4 Conclusions of aquatic mixture toxicity assessments

Reference: KCP 10.2.1/04

Report: Aquatic mixture toxicity assessment for winter cereals including safener using previous PECs

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	-	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	-	-	-
Step 2: apparent synergism or antagonism?	-	-	-
Step 3: mixture similar or not?	-	-	-

Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-
Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/05

Report: Aquatic mixture toxicity assessment for winter cereals including safener BBCH 20-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes

Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	-	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	-	-	-
Step 2: apparent synergism or antagonism?	-	-	-
Step 3: mixture similar or not?	-	-	-
Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-

Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/06

Report: Aquatic mixture toxicity assessment for winter cereals including safener BBCH 35-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	-	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	-	-	-
Step 2: apparent synergism or antagonism?	-	-	-
Step 3: mixture similar or not?	-	-	-

Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-
Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/07

Report: Aquatic mixture toxicity assessment for spring cereals including safener using previous PECs

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes

Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	-	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	-	-	-
Step 2: apparent synergism or antagonism?	-	-	-
Step 3: mixture similar or not?	-	-	-
Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-

Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/08

Report: Aquatic mixture toxicity assessment for spring cereals including safener BBCH 13-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	-	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	-	-	-
Step 2: apparent synergism or antagonism?	-	-	-
Step 3: mixture similar or not?	-	-	-

Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios in FOCUS step 1-3.
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-
Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/09

Report: Aquatic mixture toxicity assessment for spring cereals including safener BBCH 35-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes

Screening 1: ETR trigger (optional Step)	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	-	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	-	-	-
Step 2: apparent synergism or antagonism?	-	-	-
Step 3: mixture similar or not?	-	-	-
Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-

Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/10

Report: Aquatic mixture toxicity assessment for winter cereals without safener using previous PECs

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	No driver detected, go to Step 1.	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	Endpoints available for a.s. and the ppp, go to Step 2.	-	-
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to Step 10.	-	-
Step 3: mixture similar or not?	-	-	-

Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-
Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference:	KCP 10.2.1/11
Report	Aquatic mixture toxicity assessment for winter cereals without safener BBCH 20-39
Guideline(s):	n.a.
Deviations:	n.a.
GLP:	n.a.
Acceptability:	n.a.
Duplication (if vertebrate study)	n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes

Screening 1: ETR trigger (optional Step)	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	No driver detected, go to Step 1.	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	Endpoints available for a.s. and the ppp, go to Step 2.	-	-
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to Step 10.	-	-
Step 3: mixture similar or not?	-	-	-
Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-

Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/12

Report: Aquatic mixture toxicity assessment for winter cereals without safener BBCH 35-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	No driver detected, go to Step 1.	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	Endpoints available for a.s. and the ppp, go to Step 2.	-	-
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to Step 10.	-	-
Step 3: mixture similar or not?	-	-	-

Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-
Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/13

Report: Aquatic mixture toxicity assessment for spring cereals without safener using previous PECs

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes

Screening 1: ETR trigger (optional Step)	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	No driver detected, go to Step 1.	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	Endpoints available for a.s. and the ppp, go to Step 2.	-	-
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to Step 10.	-	-
Step 3: mixture similar or not?	-	-	-
Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-

Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/14

Report: Aquatic mixture toxicity assessment for spring cereals without safener BBCH 13-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	No driver detected, go to Step 1.	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	Endpoints available for a.s. and the ppp, go to Step 2.	-	-
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to Step 10.	-	-
Step 3: mixture similar or not?	-	-	-

Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios in FOCUS step 1-3.
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-
Step 9: antagonism assessment	-	-	-
Step 10: synergism assessment	-	-	-

Reference: KCP 10.2.1/15

Report: Aquatic mixture toxicity assessment for spring cereals without safener BBCH 35-39

Guideline(s): n.a.

Deviations: n.a.

GLP: n.a.

Acceptability: n.a.

Duplication (if vertebrate study): n.a.

Steps	Conclusion on the Steps		
	Invertebrates	Algae	Macrophytes

Screening 1: ETR trigger (optional Step)	Synergism, detected, go to Step 1.	All ETRi ≤ ETR trigger/n, acceptable risk can be concluded on screening level.	ETRi ≤ ETR trigger/n not fullfilled, go to Screening 2.
Screening 2: Driver (optional Step)	No driver detected, go to Step 1.	-	Driver detected (and no synergism). Check for which FOCUS scenarios (Step 5). Assess driver for the respective scenarios (Step 6). For the others start at Step 1.
Step 1: data available?	Endpoints available for a.s. and the ppp, go to Step 2.	-	-
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to Step 10.	-	-
Step 3: mixture similar or not?	-	-	-
Step 4: ETRmix assessment (ECxPPP)	-	-	-
Step 5: driver available?	-	-	There is a driver for macrophytes in all scenarios. Assess driver, go to Step 6.
Step 6: driver assessment	-	-	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).
Step 7: synergism assessment (few data)	-	-	-
Step 8a: ETRmix assessment	-	-	-
Step 8b: RQmix assessment	-	-	-

~~Step 9: antagonism assessment~~	-	-	-
~~Step 10: synergism assessment~~	-	-	-

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted in line with OECD 213 with no deviations. All the validity criteria were met and the study is considered acceptable with the following enpoint relevant for the risk assessment: oral LD_{50} >224 µg product/bee (corresponding to > 2.79 µg mesosulfuron-methyl/bee, > 14.2 µg pinoxaden/bee)

Reference:	KCP 10.3.1.1.1/01
Report	ADM.06001.H.2.B: Acute Contact and Oral Effects on Honey Bees (*Apis mellifera* L.) in the Laboratory, Sekine T., 2020, 140711035 (ADAMA No. 000105366)
Guideline(s):	OECD 213 (1998)
Deviations:	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) 400 g dimethoate/L (nominal), 408 g dimethoate/L (actual) Batch No. 10214034

Test organism:

Test species	Honey bee – *Apis mellifera* L. (Hymenoptera, Apoidea)
Origin	Honey bee colonies maintained in accordance to good beekeeping practice. The colonies were disease-free, queen-right and were bred at the test facility.
Collection	With plastic tubes, from the outer honeycombs (away from the brood) of a single hive without the use of smoke and without anaesthetics, collected in the morning of use.

Age at test start	Female, adult worker bees
No. of bees per replicate	10
No. of replicates per test substance, reference substance or control	5

Test conditions:

Test substance dosage	Nominal dose rate: 200.0 µg product/bee Actually consumed dose rate: 224.0 µg product/bee, equivalent to 2.79 µg mesosulfuron-methyl/bee, 14.2 µg pinoxaden/bee and 8.45 µg mefenpyr-diethyl/bee, based on analysed contents and product density The test solutions were prepared with 50% (w/v) sucrose solution.
Reference substance dosage	Nominal dose rates: 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee, Actually consumed dose rates: 0.06, 0.08, 0.16 and 0.33 µg dimethoate/bee The test solutions were prepared with 50% (w/v) sucrose solution.
Control	50% (w/v) sucrose solution
Administration	Groups of 10 bees per cage were provided with the test solutions in syringes, which were weighed before and after introduction into the cages (duration of the uptake was 2 hours). The mean target dose level (200.0 µg product/bee, nominal) would have been obtained if exactly 20 mg product/bee of the treated food had been ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg product/bee and results are given based on measured consumption.
Test duration	48 hours after application
Starvation	15 minutes prior to application
Test units	Stainless steel chambers (8.2 cm x 5.9 cm x 4.2 cm, length x height x width) with a removable glass sheet at the front side, perforated bottom with 98 ventilation holes (1 mm in diameter), inner walls lined with filter paper
Temperature	25 ± 2°C (nominal), 24 - 25°C (actual, short-term deviations ≤ 2 hours not reported)
Relative humidity	Approximately 50% - 70% (nominal), 59% - 62% (actual, short-term deviations ≤ 2 hours not reported)
Illumination	Darkness (except during observations)
Ventilation	Ventilation in the controlled environmental room
Feeding	50% w/v sucrose solution (500 g/L tap water) *ad libitum* was given directly after treatment. This was done with syringes that were inserted into the cages *via* an opening in the top of the test units.

Observations:

Mortality	4, 24 and 48 h after application
Behaviour	4, 24 and 48 h after application Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded.
Test conditions	For the duration of the study

Experimental dates: 04 to 07 May 2020

Calculations:
Mean percentage mortality and mean percentage bees showing behavioural abnormalities were calculated for all replicates of each test group.

Statistics:

The oral LD_{50} value of the reference substance was determined with Probit Analysis (according to Finney 1971).

The NOED (oral) of the test substance was evaluated using Fisher's Exact Binomial test (pairwise comparison, one-sided greater, $\alpha = 0.05$).0

The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

Results and discussions

Validity criteria:
- Control mortality ≤ 10% at the end of the test
- Reference mortality 0.10 – 0.35 µg a.s./bee after 24 hours

Control mortality was 2.0% at the end of the test. Reference mortality was 0.14 µg dimethoate/bee after 24 hours. Therefore, all validity criteria were met.

Observations of mortality and behaviour are presented in the table below.

Table A2.3.1.1.1-1: Mortality and behaviour of bees following oral exposure to ADM.06001.H.2.B

Treatment (dosage unit)	Dosage (nominal)	Dosage (consumed)	Mean mortality (%)			Behavioural abnormalities (%)		
			4 h	24 h	48 h	4 h	24 h	48 h
Control	-	-	0.0	2.0	2.0	2.0	0.0	0.0
ADM.06001.H.2.B (µg product/bee)	200.0	224.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference substance (dimethoate) (µg a.s./bee)	0.05	0.06	0.0	0.0	2.0	0.0	0.0	0.0
	0.08	0.08	0.0	4.0	4.0	0.0	14.0	0.0
	0.15	0.16	0.0	66.0	76.0	6.0	14.0	0.0
	0.30	0.33	14.0	100.0	100.0	18.0	0.0	0.0

Note: No statistically significant effect on mortality was determined in the test substance treatment group when compared to the control by Fisher's Exact Binomial test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Based on these results, the following endpoints were obtained.

Table A2.3.1.1.1-2: Endpoints of the acute oral toxicity test with ADM.06001.H.2.B in the honey bee

Endpoint	(µg product/bee) / (µg mesosulfuron-methyl/bee) / (µg pinoxaden/bee) / (µg mefenpyr-diethyl/bee)
48-hour LD_{50}	> 224.0 / > 2.79 / > 14.2 / > 8.45
48-hour NOED	224.0 / 2.79 / 14.2 / 8.45

After 48 hours, 2.0% mortality was observed in the control group. In the test substance treatment of 224.0 µg product/bee, mortality was 0.0% after 48 hours. No statistically significant effect on mortality was determined in the test substance treatment group when compared to the control by Fisher's Exact Binomial test (pairwise comparison, one-sided greater, $\alpha = 0.05$). Therefore, the 48-hour oral LD_{50} was estimated to be > 224.0 µg product/bee and the NOED was 224.0 µg product/bee. No effects on behaviour compared to the control were observed in the test substance treatment group.

In the reference substance treatment groups, mortality was between 0.0% and 100.0% after 24 hours. The 24-hour oral LD_{50} was calculated as 0.14 µg a.s./bee.

Conclusion

In this test on acute oral toxicity of ADM.06001.H.2.B in the honey bee *Apis mellifera* L., the 48-hour oral LD_{50} was > 224.0 µg product/bee which corresponds to > 2.79 µg mesosulfuron-methyl/bee, > 14.2 µg pinoxaden/bee and > 8.45 µg mefenpyr-diethyl/bee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was conducted in line with OECD 214 with a minor deviation. It was noted that a single 5 µL droplet of product in an appropriate carrier (tap water + 0.5 % Adhäsit) was placed on the dorsal bee thorax. For the control one 5 µL droplet of tap water containing 0.5 % Adhäsit was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5 % Adhäsit). A 5 µL droplet was chosen in deviation from the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; the testing facility experience has shown that higher volumes are suitable and no adverse effects on the outcome of the study are expected. The wetting agent Adhäsit was used to improve the spreading of the test item droplet on the bee body. Adhäsit is non-toxic to honey bees. This deviation is considered to have no effect on the outcome of the study. All the validity criteria were met and the study is considered acceptable with the following enpoint relevant for the risk assessment: contact LD_{50} > 200µg product/bee (corresponding to > 2.49 µg mesosulfuron-methyl/bee, > 12.7 µg pinoxaden/bee)

Reference:	KCP 10.3.1.1.2/01
Report	ADM.06001.H.2.B: Acute Contact and Oral Effects on Honey Bees (*Apis mellifera* L.) in the Laboratory, Sekine T., 2020, 140711035 (ADAMA No. 000105366)
Guideline(s):	OECD 213 (1998)
Deviations:	Minor (see the commenting box above) ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate)

	400 g dimethoate/L (nominal), 408 g dimethoate/L (actual) Batch No. 10214034

Test organism:

Test species	Honey bee – *Apis mellifera* L. (Hymenoptera, Apoidea)
Origin	Honey bee colonies maintained in accordance to good beekeeping practice. The colonies were disease-free, queen-right and were bred at the test facility.
Collection	With plastic tubes, from the outer honeycombs (away from the brood) of a single hive without the use of smoke and without anaesthetics, collected in the morning of use.
Age at test start	Female, adult worker bees
No. of bees per replicate	10
No. of replicates per test substance, reference substance or control	5

Test conditions:

Test substance dosage	Nominal dose rate: 200.0 µg product/bee equivalent to 2.49 µg mesosulfuron-methyl/bee, 12.7 µg pinoxaden/bee and 7.55 µg mefenpyr-diethyl/bee, based on analysed contents and product density The test substance solution was prepared in tap water + 0.5% Adhäsit.
Reference substance dosage	Nominal dose rates: 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee, The reference substance solutions were prepared in tap water + 0.5% Adhäsit.
Control	Tap water + 0.5% Adhäsit
Wetting agent	Adhäsit (100 g/L Marlopon, nominal)
Administration	A single 5 µL droplet of the test or reference substance solution or control solution was placed on the dorsal bee thorax using a calibrated pipette (Multipette©, Eppendorf). A 5 µL droplet was chosen in deviation from the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test/reference substance. Experience of the testing facility has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected (Schmitzer *et al*. 2002).
Test duration	48 hours after application
Anaesthetization	Bees were anaesthetised for *ca*. 20 seconds with CO_2 until they were completely immobilised immediately before application.
Test units	Stainless steel chambers (8.2 cm x 5.9 cm x 4.2 cm, length x height x width) with a removable glass sheet at the front side, perforated bottom with 98 ventilation holes (1 mm in diameter), inner walls lined with filter paper
Temperature	25 ± 2°C (nominal), 24 - 25°C (actual, short-term deviations ≤ 2 hours not reported)
Relative humidity	Approximately 50% - 70% (nominal), 59% - 62% (actual, short-term deviations ≤ 2 hours not reported)
Illumination	Darkness (except during observations)
Ventilation	Ventilation in the controlled environmental room
Feeding	50% w/v sucrose solution (500 g/L tap water) *ad libitum* was given directly after treatment. This was done with syringes that were inserted into the cages *via* an opening in the top of the test units.

Observations:

Mortality	4, 24 and 48 h after application
Behaviour	4, 24 and 48 h after application Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded.
Test conditions	For the duration of the study

Experimental dates: 04 to 07 May 2020

Calculations:

Mean percentage mortality and mean percentage bees showing behavioural abnormalities were calculated for all replicates of each test group.

Statistics:

The contact LD_{50} value of the reference substance was determined with Probit Analysis (according to Finney 1971).

The NOED (contact) of the test substance was evaluated using Fisher's Exact Binomial test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

Results and discussions

Validity criteria:

- Control mortality ≤ 10% at the end of the test
- Reference mortality 0.10 – 0.30 µg a.s./bee after 24 hours

Control mortality was 4.0% at the end of the test. Reference mortality was 0.22 µg dimethoate/bee after 24 hours. Therefore, all validity criteria were met.

Observations of mortality and behaviour are presented in the table below.

Table A2.3.1.1.2-1: Mortality and behaviour of bees following contact exposure to ADM.06001.H.2.B

Treatment (dosage unit)	Dosage (nominal)	Mean mortality (%)			Behavioural abnormalities (%)		
		4 h	24 h	48 h	4 h	24 h	48 h
Control	-	0.0	0.0	4.0	0.0	0.0	0.0
ADM.06001.H.2.B (µg product/bee)	200.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference substance (dimethoate) (µg a.s./bee)	0.10	0.0	4.0	4.0	0.0	0.0	0.0
	0.15	10.0	26.0	36.0	4.0	0.0	0.0
	0.20	10.0	42.0	50.0	12.0	0.0	0.0
	0.30	24.0	72.0	78.0	30.0	0.0	4.0

Note: No statistically significant effect on mortality was determined in the test substance treatment group when compared to the control by Fisher's Exact Binomial test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Based on these results, the following endpoints were obtained.

Table A2.3.1.1.2-2: Endpoints of the acute contact toxicity test with ADM.06001.H.2.B in the honey bee

Endpoint	(µg product/bee) / (µg mesosulfuron-methyl/bee) / (µg pinoxaden/bee) / (µg mefenpyr-diethyl/bee)
48-hour LD_{50}	> 200.0 / > 2.49 / > 12.7 / > 7.55
48-hour NOED	200.0 / 2.49 / 12.7 / 7.55

After 48 hours, 4.0% mortality was observed in the control group. In the test substance treatment of 200.0 µg product/bee, mortality was 0.0% after 48 hours. No statistically significant effect on mortality was determined in the test substance treatment group when compared to the control by Fisher's Exact Binomial test (pairwise comparison, one-sided greater, $\alpha = 0.05$). Therefore, the 48-hour oral LD_{50} was estimated to be > 200.0 µg product/bee and the NOED was 200.0 µg product/bee. No effects on behaviour compared to the control were observed in the test substance treatment group.

In the reference substance treatment groups, mortality was between 4.0% and 72.0% after 24 hours. The 24-hour oral LD_{50} was calculated as 0.22 µg a.s./bee.

Conclusion

In this test on acute contact toxicity of ADM.06001.H.2.B in the honey bee *Apis mellifera* L., the 48-hour oral LD_{50} was > 200.0 µg product/bee which corresponds to > 2.49 µg mesosulfuron-methyl/bee, > 12.7 µg pinoxaden/bee and > 7.55 µg mefenpyr-diethyl/bee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted in line with OECD 245 with no deviations. The analytical measurements confirmed that the concentrations of both active substances were maintained at 80-120 % of the nominal concentration. Therefore, the endpoints can be expressed as nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD_{50} > 105 µg product/bee/day NOEDD = 105 µg product/bee/day LC_{50} > 5000 mg product/kg food NOEC = 5000 mg product/kg food

Reference:	KCP 10.3.1.2/01
Report	ADM.06001.H.2.B: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory, Sekine T. and Kowalczyk F., 2021, 140711136 (ADAMA No. 000105367)
Guideline(s):	OECD 245 (2017)
Deviations:	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) 400 g dimethoate/L (nominal), 414 g dimethoate/L (actual) Batch No. 10214034

Test organism:

Test species	Honey bee – *Apis mellifera* L. (Hymenoptera, Apoidea)
Origin	Honey bee colonies maintained in accordance to good beekeeping practice. The colonies were not treated with chemical within the last 5 months before test start, were disease-free, queen-right and were bred at the test facility.
Collection and acclimation	One brood comb each with sealed brood from five different hives were used in the test in which bees were visibly starting to emerge. These combs contained pollen which was used as a first feeding source for the freshly hatched bees. The combs were taken out from the hive and placed in a hatching box in an incubator. The freshly hatched bees remained in the hatching box. After the hatching period of one day, the bees were transferred into the test units, where they were acclimatised under test conditions for one day.
Age at test start	2 days
No. of bees per replicate	10
No. of replicates per test and reference substance dose and control group	5

Test conditions:

Test substance dosage	Nominal dose: 5000 mg product/kg food Corresponding to nominal 100 µg product/bee/day and actual 105.0 µg product/bee/day (based on actually consumed food)

	The limit-test design was chosen based on preliminary dose-response tests using different test concentrations, non-toxic solvents and additives. An appropriate amount of the test substance was dissolved in 50% (w/v) sucrose solution + 0.1% (w/w) xanthan to receive the targeted final concentration of the test substance feeding solution. Fresh test substance feeding solution was prepared daily.
Reference substance dosage	Nominal dose: 1 mg dimethoate/kg food Corresponding to nominal 0.02 µg dimethoate/bee/day and actual 0.015 µg dimethoate/bee/day (based on actually consumed food) A stock solution was prepared once by diluting the appropriate amount of reference substance in deionised water. It was stored in the refrigerator at 4±4°C. From this stock solution, the reference substance feeding solution was prepared by dilution with 50% (w/w) sucrose solution at least every four days.
Control	Control: 50% (w/v) aqueous sucrose solution Solvent control: 50% (w/v) aqueous sucrose solution + 0.1% (w/w) xanthan The control feeding solutions were prepared at least every four days.
Administration	The treated and untreated food was offered using syringes (feeder). Every day, the feeder was replaced by a new one with fresh treated or untreated food. The syringes were weighed before introduction into the cages and after the feeding interval (before replacement with fresh food).
Test duration	10 days
Test units	Bees were kept in stainless steel chambers (8.2 cm x 5.9 cm x 4.2 cm, length x height x width) with a removable glass sheet at the front side. The bottom was perforated with 98 ventilation holes (1 mm in diameter) and the inner walls were lined with filter paper.
Temperature	33±2°C (nominal), 32-33°C (actual, short-term deviations ≤ 2 hours not reported)
Relative humidity	50-70% (nominal), 56-68% (actual, average: 67%)
Illumination	Constant darkness throughout the test (except during observations)
Ventilation	Yes, to avoid possible accumulation of pesticide vapour
Feeding	Continuous feeding *ad libitum* with 50% (w/v) sucrose solution containing either - the test substance, - the reference substance, - pure 50% (w/v) sucrose solution (control) or - 50% (w/v) aqueous sucrose solution + 0.1% (w/w) xanthan (solvent control)

Observations:

Mortality	Daily at about the same time of the day (± 2 h to first application)
Behavioural abnormalities	Daily
Food consumption	Daily by weighing feeders at the beginning and end of the feeding period
Evaporation	Daily in 3 additional replicate cages without bees but with pre-weighed feeders containing control diet. At the daily feeder exchange, the feeders were re-weighed and replaced with new feeders.
Test conditions (temperature, relative	Continuously

humidity)	

Analytical method:

Samples	The test substance concentration in the feeding solutions of the control, the solvent control and the test substance treatment group was determined by analysis of the analytes mesosulfuron-methyl, pinoxaden, NOA 407854 (metabolite of pinoxaden) and mefenpyr-diethyl in one sample of the feeding solution from days 0 and 9.
Method type	LC-MS/MS
Equipment	Agilent Series 1290 pump and autosampler
Column	Luna Omega 3µmPolar C18 (50 x 3 mm)
Column temperature	40°C
Detection	Mass spectrometer API 5500 Detection: MSD, positive mode MRM mass transitions: Mesosulfuron-methyl: m/z 504 → 182 (quantifier); 504 → 83 (qualifier) Pinoxaden: m/z 401 → 317 (quantifier); 401 → 115 (qualifier); 401 → 57 (qualifier) NOA 407854: m/z 318 → 171 (quantifier); 318 → 131 (qualifier); 318 → 115 (qualifier) Mefenpyr-diethyl: m/z 390 → 327 (quantifier); 390 → 160 (qualifier)
Flow rate	0.65 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid B: Acetonitrile containing 0.1% formic acid 0.0 min 95% A, 5% B 2.0 min 95% A, 5% B 2.5 min 50% A, 50% B 4.5 min 5% A, 95% B 5.2 min 5% A, 95% B 5.3 min 95% A, 5% B 7.5 min 95% A, 5% B
Sample preparation	An aliquot of each sample was diluted with acetonitrile/pure water (50:50, v/v) + 0.1% HCOOH

Experimental dates: 23 Jun to 11 Sep 2020

Calculations:

The difference in weight at the start and end of each feeding period represented the food consumed by the bees in one cage for 24 hours. The evaporation figure was then subtracted from the calculated uptake to give the real uptake accounting for the loss by evaporation. This amount of food was divided by the number of living bees at the start of the corresponding exposure interval to obtain the individual food consumption of the bees. Negative food consumption values, due to low or no consumption of feeding solutions by the bees and occurrence of evaporation, were corrected to be 0.0 mg food uptake. The mean food consumption per bee per day over the whole testing period was calculated by averaging the food consumption per replicate over the testing period (only when bees survived). The overall mean uptake of test substance per bee per day (daily dose) was calculated based on the single replicates.

The percentage of cumulative mortality was calculated for each test group and assessment time from the number of dead individuals in relation to the number of introduced test organisms.

Statistics:

Statistical calculations were made with the statistical program ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

The LC_{50}/LDD_{50} values were considered to be greater than the tested concentration since the test substance treatment group did not exceed 50% mortality.

The NOEDD and NOEC of the test substance was estimated using Fisher's Exact Binomial Test (α = 0.05, one-sided greater).

Results and discussions

Validity criteria:

- Average mortality across replicates for the control and solvent control groups ≤ 15% at the end of the test
- Average mortality in the reference substance treated group ≥ 50% at the end of the test

The average mortality across replicates for the control and solvent control was 8.0% and 8.0%, respectively, at the end of the test. The average mortality in the reference substance treated group was 100.0% at the end of the test. Therefore, all validity criteria were met.

The test substance concentrations in the feeding solutions were determined by analysis of the analytes mesosulfuron-methyl, pinoxaden, mefenpyr-diethyl and NOA 407854 (metabolite of pinoxaden). The results are presented in the table below.

Table A2.3.1.2-1: Measured test concentrations and percentage recovery of analytes in honey bee feeding solutions

Treatment	Day	Mesosulfuron-methyl			Pinoxaden			NOA 407854		Mefenpyr-diethyl		
		Concentration (mg/L)		Recovery % of nominal	Concentration (mg/L)		Recovery % of nominal	Concentration (mg/L)	Recovery % of nominal	Concentration (mg/L)		Recovery % of nominal
		nominal	actual		nominal	actual		actual		nominal	actual	
Test substance	0	71.40	68.498	96	374.85	380.71	102	n.d.	n.a.	226.1	206.90	92
	9		72.770	102		385.64	103	n.d.	n.a.		208.51	92
Control	0	0.0	n.a.	n.a.	0.0	n.a.	n.a.	n.a.	n.a.	0.0	n.a.	n.a.
	9		n.a.	n.a.		n.a.	n.a.	n.a.	n.a.		n.a.	n.a.
Solvent control	0	0.0	n.a.	n.a.	0.0	n.a.	n.a.	n.a.	n.a.	0.0	n.a.	n.a.
	9		n.a.	n.a.		n.a.	n.a.	n.a.	n.a.		n.a.	n.a.

n.a. not applicable
n.d. no significant amounts detected
Mesosulfuron-methyl: LOD: 0.02 µg mesosulfuron-methyl/L, LOQ: 0.02 µg mesosulfuron-methyl/L
Pinoxaden: LOD: 0.03 µg pinoxaden/L, LOQ: 0.11 µg pinoxaden/L
NOA 407854: LOD: 0.21 µg NOA 407854/L, LOQ not stated
Mefenpyr-diethyl: LOD: 0.2 µg mefenpyr-diethyl/L, LOQ: 0.14 µg mefenpyr-diethyl/L

Recoveries in the test substance feeding solutions of days 0 and 9 were 96% and 102% for mesosulfuron-methyl, 102% and 103% for pinoxaden and 92% and 92% for mefenpyr-diethyl, respectively. No significant amounts of the pinoxaden metabolite NOA 407854 were detected. Therefore, correct dosing of the test substance was confirmed, and the test results were based on nominal concentrations.

Mean cumulative mortality and behavioural abnormalities of honey bees in the chronic toxicity feeding test during 10 days are presented in the table below.

Table A2.3.1.2-2: Cumulative mortality and behavioural abnormalities of honey bees

Treatment group		Control	Solvent control	Test substance	Reference substance
Nominal concentration (mg product/kg)[a] or (mg a.s./kg)[b]		0.0	0.0	5000	1.0
Nominal dose (µg product/bee/day)[a] or (µg a.s./bee/day)[b]		0.0	0.0	100.0	0.02
Actual dose (µg product/bee/day)[a] or (µg a.s./bee/day)[b]		0.0	0.0	105.0	0.015
Mean cumulative mortality (%)	Day 1	0.0	0.0	0.0	0.0
	Day 2	0.0	0.0	0.0	0.0
	Day 3	0.0	0.0	0.0	20.0
	Day 4	0.0	0.0	0.0	66.0
	Day 5	6.0	2.0	0.0	92.0
	Day 6	6.0	2.0	0.0	98.0
	Day 7	6.0	6.0	0.0	100.0
	Day 8	6.0	6.0	2.0	100.0
	Day 9	6.0	6.0	2.0	100.0
	Day 10	8.0	8.0	2.0	100.0
Behavioural abnormalities (%)	Day 1	0.0	0.0	0.0	0.0
	Day 2	0.0	0.0	0.0	0.0
	Day 3	0.0	0.0	0.0	0.0
	Day 4	0.0	0.0	0.0	6.0
	Day 5	0.0	0.0	0.0	6.0
	Day 6	0.0	0.0	0.0	2.0
	Day 7	0.0	0.0	0.0	0.0
	Day 8	0.0	0.0	0.0	0.0
	Day 9	0.0	0.0	0.0	0.0
	Day 10	0.0	0.0	0.0	0.0

Note: There were no statistically significant differencens in cumulative mortality at day 10 between the test substance treatment group and both control groups (Fisher's Exact Binomial Test, $\alpha = 0.05$, one-sided greater)

[a] test substance

[b] reference substance

Based on the results of the study, the following endpoints were obtained.

Table A2.3.1.2-3: Endpoints of the honey bee chronic test on day 10

Endpoint	(µg product/bee/day)
10 d LDD_{50}[a]	> 105.0
10 d NOEDD[b]	105.0
Endpoint	**(mg product/kg)**
10 d LC_{50}[a]	> 5000
10 d NOEC[b]	5000

After 10 days, 8.0% cumulative mortality was observed in both the control and the solvent control group. In the test substance treatment group of actual 105.0 µg product/bee/day, 2.0% cumulative mortality occurred by the end of the study which was not statistically significantly different from the control (Fisher's Exact Binomial Test, $\alpha = 0.05$, one sided greater). Therefore, the 10-day NOEDD and LDD_{50} were determined to be 105.0 and > 105.0 µg product/bee/day, respectively. This corresponds to 10-day NOEC and LC_{50} values of 5000 and > 5000 mg product/kg, respectively.

No test substance related behavioural abnormalities were observed throughout the study period.

The actual reference substance dosage in the study was 0.015 µg a.s./bee/day, which caused a cumulative mortality of 100.0% after 10 days of exposure.

Conclusion

In this chronic oral test with ADM.06001.H.2.B in the honey bee *Apis mellifera* L., the 10-day NOEDD and LDD_{50} were determined to be 105.0 and > 105.0 µg product/bee/day, respectively. This corresponds to 10-day NOEC and LC_{50} values of 5000 and > 5000 mg product/kg, respectively.

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Comments of zRMS:	The study was conducted in line with OECD 239 with minor deviations. During grafting at day 1 (D1), relative humidity was lower than 90% with an initial value of 67.2%. At D3, temperature was lower than 34°C for about 3 hours (minimum value: 33.2°C). From D8 to D9, relative humidity was higher than 85% (maximum value: 88.1%). The reported deviations are considered to have no impact on the outcome of the study since all the validity criteria were met. The analytical measurements confirmed that the concentrations of active substances were within 80 – 120 % of nominal, therefore the endpoints can be expressed as nominal concentrations. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: EC_{50} = 1408 mg test item/kg food NOEC = 1033 mg test item/kg food ED_{50} = 217 µg test item/larva NOED = 159 µg test item/larva

Reference:	KCP 10.3.1.3/01
Report	Effects of ADM.06001.H.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Colli M., 2020, BT138/20 (ADAMA No. 000105368)
Guideline(s):	OECD 239 (2016)
Deviations:	Minor (see the commenting box above) ~~During grafting at day 1 (D1), relative humidity was lower than 90% with an initial value of 67.2%. At D3, temperature was lower than 34°C for about 3 hours (minimum value: 33.2°C). From D8 to D9, relative humidity was higher than 85% (maximum value: 88.1%).~~ ~~The reported deviations to the guideline are considered not to have impacted the outcome of the study since validity criteria for the control were met.~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (1.2% w/w, actual)

	60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (6.3% w/w, actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (3.8% w/w, actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	Dimethoate Batch No. G941646 purity 99.37%

Test organism:

Test species	Honey bee (*Apis mellifera ligustica*)
Origin	The larvae originated from three different colonies maintained at the test facility. Each colony represented a replicate. The colonies were adequately fed, healthy, diseases-free and with known history and physiological status. No treatment with chemicals, such as antibiotics, anti-varroa etc., had been carried out within the four weeks preceding the start of test.
Collection	On day 1 (D1) of the study, the larvae were taken from the comb of the three colonies and individually placed into 48 well-plates, where they were fed with a standardised amount of artificial diet.
Age at test start	Synchronized first instar (L1) larvae, 3 days old Three days before start of the test (D-3), to ensure the production of synchronized larvae of at least three replicate colonies, the queens of four colonies were confined in their own colony in an excluder cage. The excluder cage was placed close to combs containing brood. At D-2, maximum 30 hours after encaging, the queens were released from their cages. The combs containing eggs were left in the cages, near the brood, during the incubation stage and until hatching (D1).
No. of bees per replicate	12
No. of replicates per test substance, reference substance or control group	3

Test conditions:

Test substance dosage	Test concentrations: 97, 213, 470, 1033 and 2273 mg product/kg diet Corresponding cumulative test doses: 15, 33, 72, 159 and 350 µg product/larva The test substance was dissolved in ultrapure water to get the highest concentration stock solution (S5). The other stock solutions (from S4 to S1) were obtained by sequential dilution. The stock solutions were prepared daily and were mixed into the diet just prior to administration to the larvae on D3, D4, D5 and D6. The volume of the test substance stock solution mixed into the diet did not exceeded 10% of the final diet volume.
Reference substance dosage	Test concentration: 48 mg a.s./kg diet Corresponding cumulative test dose: 7.39 µg a.s./larva The reference substance stock solution was prepared in ultrapure water. The stock solution was prepared once for the test and mixed into the diet just prior to administration to the larvae on D3, D4, D5 and D6.
Control	Diet B and C to which ultrapure water was added
Administration	All larvae were fed once per day from D1 to D6 (except D2),

	and food was added even if the previous food was not totally consumed. From D3 to D6, the test or reference substance stock solutions were mixed into the diet at the targeted concentration, just prior to its administration. The feeding solution was warmed before use and the larvae were fed on a warming plate at about 34-35°C with a multi-stepper pipette. During the feeding procedure, care was taken to avoid touching and drowning the larvae, and the food was placed close to the larva along the wall of the grafting cell. During the treatment phase, no uneaten food was observed in cells where the larvae were alive.
Test duration	22 days
Test units	The larvae were reared in crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm. The cells were sterilised and dried. Each cell was placed into a well of a 48-well plate. The top of the grafting cell was maintained at the level of the plate by placing a piece of dental roll wetted with 500 µL of the sterilising solution enhanced with 15% (w/v) glycerol at the bottom of the wells. From D1 to D8, these plates were placed into a hermetic Plexiglas desiccator and kept at a relative humidity of 95±5% using a dish filled with saturated potassium sulphate solution. The desiccator was placed in an incubator with a forced air circulation system at 34-35°C. At D8, the dental rolls were removed, and the relative humidity was reduced to 80±5% by replacing the potassium sulphate solution with a saturated sodium chloride solution. The desiccator was placed in a ventilated incubator at 34-35°C. From D15 to D22, the test system was maintained at 50-80% relative humidity.
Grafting of larvae	At day 1 (D1), the comb containing first instar larvae were transferred from the hive to the laboratory. A volume of 20 µL of diet A was dropped into each cell, then one larva was grafted from the comb to the cell, onto the surface of the diet, using a grafting tool. On D3, twelve well-fed larvae from each of the three replicates were selected per plate: the grafting cells containing an alive larva were transferred from the plates prepared on D1 to new plates (one plate per treatment) and arranged, so that a clear assignment to the replicates (colonies) was possible.
Temperature	Nominal: 34-35°C, deviations remaining within 23-40°C allowed for ≤ 0.5 h once daily Actual: 33.2-34.7°C (average 34.4°C)
Relative humidity	Nominal: 95±5% at D1-D8, 80±5% at D8-D15, 50-80% at D15-D22 Actual: D1-D8: 65.3-97.9% (average: 95.4%) D8-D15: 78.8-88.1% (average: 82.4%) D15-D22: 57.2-63.4% (average: 58.9%)
Illumination	During the entire test period, the bee larvae were kept under constant darkness except during observations.
Feeding	The diet was prepared using the following ingredients: - Diet A (D1, volume administered: 20 µL/larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose - Diet B (D3, volume administered: 20 µL/larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose - Diet C (from D4 to D6, volume administered:

	30 µL/larva, 40 µL/larva and 50 µL/larva, respectively): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose

Observations:

Mortality	Daily from D4 to D8 and on D15 On D15, larvae that had not transformed into pupae were recorded as dead and removed.
Other observations, e.g. larval appearance and size, behaviour, morphological differences and any other adverse effects	Recorded qualitatively
Emergence rate	Hatched adults were recorded on D22.
Test conditions (temperature, relative humidity)	Continuously

Analytical method:

Samples	Each day from D3 to D6, samples of the test substance stock solutions (lowest and highest concentration, S1 and S5) and samples of the ultrapure water used to treat the controls, were frozen at ≤ -20°C until analysis of the analytes mesosulfuron-methyl, pinoxaden, mefenpyr-diethyl and the pinoxaden metabolite NOA 407854 (analysed separately).
Method type	LC-MS/MS
Equipment	Agilent LC-MS/MS 1290
Column	Agilent Zorbax Eclipse Plus C18 RRHD, 3 x 50 mm, 1.8 µm
Detector	6495a Triple Quadrupole Spectrometer Polarity: Positive Ion mass transition: Mesosulfuron-methyl: m/z 504.1 → 182.1 (quantifier); 504.1 → 83 (qualifier) Pinoxaden: m/z 401 → 317.2 (quantifier); 401 → 56.9 (qualifier) Mefenpyr-diethyl: m/z 373 → 327 (quantifier); 373 → 159.6 (qualifier) NOA 407854: m/z 317.1 → 171.2 (quantifier); 317.1 → 130.9 (qualifier)
Flow rate	0.6 mL/min
Mobile phase	A: Acidified ultra-pure water containing 0.1% formic acid B: Acidified acetonitrile containing 0.1% formic acid 4.5 min 10% A, 90% B 5.5 min 10% A, 90% B 5.6 min 80% A, 20% B
Retention time	Mesosulfuron-methyl: 2.3 min Pinoxaden: 3.4 min Mefenpyr-diethyl: 3.9 min NOA 407854: 2.3 min
Sample preparation	The samples were diluted with acetonitrile at appropriate ratios.

Experimental dates: 22 Jun to 16 Jul 2020

Calculations:

Larval mortality was calculated as a percentage by comparing the number of larvae that died during larval stages (from D3 to D8) to the number of larvae on D3 when dosing started.

The percentage of pupal mortality on D15 was determined by comparing the number of dead pupae from

D8 to D15 to the number of larvae entering in pre-pupa stage on D8. The percentage of pupal mortality on D22 was determined by comparing the number of pupae that failed to emerge, including those bees without emergence on D22 and dead pupae removed during pupa stage (from D8 to D22) to the number of larvae entering in pre-pupa stage on D8.

Adult emergence rate was calculated as a percentage by comparing the number of bees emerged on D22 to the number of larvae on D3 when dosing started.

Statistics:

Statistical calculations were made with the software ToxRatPro Version 3.3.0.

To determine the statistical significance of the data and the NOED/NOEC, the Chi2 2x2 table test with Bonferroni correction (for D8 data) and the step-down Cochran-Armitage test (for D22 data) were used.

For determination of the ED_x/EC_x values, a Probit analysis using linear maximum likelihood regression was performed (this regression showed to be most robust).

Results and discussions

Validity criteria:

- Control cumulative larval mortality (D4-D8): ≤ 15% across all replicates
- Control adult emergence rate: ≥ 70% across all replicates
- Reference cumulative larval mortality: ≥ 50% across all replicates on D8

Control cumulative larval mortality (D4-D8) was 11.11% and control adult emergence rate was 83.33%. In the reference substance group, cumulative larval mortality was 100%. Therefore, all validity criteria were met.

The test substance concentrations in the stock solutions were determined by analysis of the analytes mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl as well as the pinoxaden metabolite NOA 407854 (analysed separately). The results are presented in the table below.

Table A2.3.1.3-1: Measured test concentrations and percentage recovery of analytes in stock solutions

Treatment	Day	Replicate	Mesosulfuron-methyl		Pinoxaden		Mefenpyr-diethyl	
			Recovery		Recovery		Recovery	
			% of nominal	mean % of nominal	% of nominal	mean % of nominal	% of nominal	mean % of nominal
Test substance stock solution S1 (lowest concentration)	D3	1	107.36	105.08	99.34	104.41	94.47	95.45
		2	102.80		109.47		96.43	
	D4	1	101.19	103.56	97.78	103.04	100.01	97.05
		2	105.92		108.29		94.08	
	D5	1	105.48	103.96	103.97	105.45	96.30	96.26
		2	102.43		106.92		96.22	
	D6	1	102.70	105.37	102.47	101.97	96.46	98.65
		2	108.03		101.48		100.84	
Test substance stock solution S5 (highest concentration)	D3	1	98.74	99.90	105.04	103.07	92.65	91.50
		2	101.05		101.09		90.35	
	D4	1	102.07	101.25	104.76	99.70	90.71	93.63
		2	100.42		94.63		96.54	
	D5	1	101.84	100.20	96.71	96.52	100.26	100.94
		2	98.55		96.32		101.61	
	D6	1	97.98	97.91	89.52	93.30	88.58	91.24
		2	98.01		97.08		93.89	

Mesosulfuron-methyl: LOD: 12.37 µg mesosulfuron-methyl/L

Pinoxaden: LOD: 63.57 µg pinoxaden/L
Mefenpyr-diethyl: LOD: 37.02 µg mefenpyr-diethyl/L
NOA 407854: LOD: 10.30 µg NOA 407854/L

Recoveries in the test substance stock solutions of D3-D6 were 103.6-105.4%, 102.0-105.5% and 95.5-98.7% in stock solution S1 (lowest concentration) and 97.9-101.3%, 93.3-103.1% and 91.2-100.9% in stock solution S5 (highest concentration) for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl, respectively. In all samples, concentrations of the pinoxaden metabolite NOA 407854 were below the LOD. In the control samples, concentrations of all analytes were lower than the LOD. Therefore, correct dosing of the test substance was confirmed, and the test results were based on nominal concentrations.

Mortality of larvae, pupae and larvae + pupae as well as adult emergence in the control and the test and reference substance treatment groups are presented in the tables below.

Table A2.3.1.3-2: Larval and pupal mortality of honey bees following exposure to ADM.06001.H.2.B

Treatment	Dose (µg product/larva)[a] (µg a.s./larva)[b]	Concentration (mg product/kg)[a] (mg a.s./kg)[b]	Larval mortality on D8		Mean pupal mortality D8-D15 (%)	Mean pupal mortality D8-D22 (%)
			Mean (%)	Mean corrected[c] (%)		
Control	-	-	11.11	n.a.	0	6.25
Test substance	15	97	0	0	8.33	11.11
	33	213	8.33	0	3.03	3.03
	72	470	8.33	0	6.06	6.06
	159	1033	8.33	0	9.09	24.24
	350	2273	33.33	25	91.67	91.67
Reference substance	7.39	48.0	100	-	-	-

Note: There were no statistically significant differences in larval mortality on D8 between the test substance treatments and the control (Chi2 2x2 table test with Bonferroni correction, α = 0.05, one-sided).
n.a. not applicable
[a] Test substance
[b] Reference substance
[c] Corrected for control mortality

Table A2.3.1.3-3: Combined larval and pupal mortality and adult emergence of honey bees on D22 following exposure to ADM.06001.H.2.B

Treatment	Dose (µg product/larva)[a] (µg a.s./larva)[b]	Concentration (mg product/kg)[a] (mg a.s./kg)[b]	Mortality (larvae + pupae) on D22		Mean adult emergence on D22 (%)
			Mean (%)	Mean corrected (%)	
Control	-	-	16.67	n.a.	83.33
Test substance	15	97	11.11	0	88.89
	33	213	11.11	0	88.89
	72	470	13.89	0	86.11
	159	1033	30.56	16.67	69.44
	350	2273	94.44	93.33*	5.56*

n.a. not applicable
[a] Test substance
[b] Reference substance
[c] Corrected for control mortality
* Statistically significantly different from control (Step-down Cochran-Armitage test, α = 0.05, one-sided greater)

Based on the results of the study, the following endpoints were obtained.

Table A2.3.1.3-4: Endpoints of the honey bee chronic larvae test

Endpoint	Dose (µg product/larva)	
	Larval mortality on D8	Adult emergence on D22
NOED	350	159
ED_{10} (95% confidence interval)	n.d.	144 (114-167)
ED_{20} (95% confidence interval)	n.d.	166 (138-188)
ED_{50} (95% confidence interval)	> 350	217 (191-247)
Endpoint	**Concentration (mg product/kg)**	
	Larval mortality on D8	**Adult emergence on D22**
NOEC	2273	1033
EC_{10} (95% confidence interval)	n.d.	936 (744-1082)
EC_{20} (95% confidence interval)	n.d.	1077 (897-1124)
EC_{50} (95% confidence interval)	> 2273	1408 (1240-1606)

Note: The ED_{10}/EC_{10} values for adult emergence are above the lower limit of the ED_{20}/EC_{20} and therefore the ED_{10}/EC_{10} are not considered to be reliable according to Appendix E of EFSA Technical report 2019: EN-1673 (Outcome of pesticides peer review meeting on general recurring issues in ecotoxicology)

n.d. not determined either due to mathematical reasons or value is beyond the tested concentrations by more than factor 1000.

[a] Test substance

In the control group, mean larval mortality on D8 was 11.11%. Mean corrected larval mortality in the test substance doses of 15, 33, 72, 159 and 350 µg product/larva, was 0%, 0%, 0%, 0% and 25%, respectively, on D8 with no statistically significant difference from the control (Chi2 2x2 table test with Bonferroni correction, $\alpha = 0.05$, one-sided). Therefore, the NOED and ED_{50} for larval mortality on D8 were determined as 350 and > 350 µg product/larvae, respectively, corresponding with a NOEC of 2273 mg product/kg and a EC_{50} of > 2273 mg product/kg. In the reference substance group, mean larval mortality was 100% by D8.

Combined larval and pupal mortality on D22 was 16.67% in the control group and corrected combined larval and pupal mortality on D22 was 0%, 0%, 0%, 16.67% and 93.33% in the test substance doses of 15, 33, 72, 159 and 350 µg product/larva, respectively. At the highest dose of 350 µg product/larva, combined larval and pupal mortality on D22 was statistically significantly different from the control (Step-down Cochran-Armitage test, $\alpha = 0.05$, one-sided greater).

Adult emergence on D22 was 83.33% in the control group compared to 88.89%, 88.89%, 86.11%, 69.44% and 5.56% in the test substance doses of 15, 33, 72, 159 and 350 µg product/larva, respectively. At the highest dose of 350 µg product/larva, adult emergence on D22 was statistically significantly different from the control (Step-down Cochran-Armitage test, $\alpha = 0.05$, one-sided greater). The NOED, ED_{10}, ED_{20} and ED_{50} for adult emergence on D22 were determined as 159, 144 (114-167, 95% confidence interval), 166 (138-188, 95% confidence interval) and 217 (191-247, 95% confidence interval) µg product/larvae, respectively, corresponding with NOEC, EC_{10}, EC_{20} and EC_{50} for adult emergence on D22 of 1033, 936 (744-1082, 95% confidence interval), 1077 (897-1124, 95% confidence interval) and 1408 (1240-1606, 95% confidence interval) mg product/kg. Note: The ED_{10}/EC_{10} values for adult emergence are above the lower limit of the ED_{20}/EC_{20} and therefore the ED_{10}/EC_{10} are not considered to be reliable according to Appendix E of EFSA Technical report 2019: EN-1673 (Outcome of pesticides peer review meeting on general recurring issues in ecotoxicology).

The qualitative observations carried out during the test (e.g. larval and pupal behaviour and morphological differences) did not show any abnormalities in the surviving treated bees.

Conclusion

In this chronic larvae test with ADM.06001.H.2.B in the honey bee *Apis mellifera ligustica*, the 22-day NOED, ED_{10}, ED_{20} and ED_{50} for adult emergence were determined as 159, 144 (114-167, 95% confidence interval), 166 (138-188, 95% confidence interval) and 217 (191-247, 95% confidence interval) µg product/larvae, respectively, corresponding with NOEC, EC_{10}, EC_{20} and EC_{50} of 1033, 936 (744-1082, 95% confidence interval), 1077 (897-1124, 95% confidence interval) and 1408 (1240-1606,

95% confidence interval) mg product/kg.

Note: The ED_{10}/EC_{10} values for adult emergence are above the lower limit of the ED_{20}/EC_{20} and therefore the ED_{10}/EC_{10} are not considered to be reliable according to Appendix E of EFSA Technical report 2019: EN-1673 (Outcome of pesticides peer review meeting on general recurring issues in ecotoxicology).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honey bees

A 2.4 KCP 10.3.2 Effects on arthropods other than bees

A 2.4.1 Acute Effects on *Typhlodromus pyri* – standard laboratory study

Comments of zRMS:	The study was conducted in line with the respective guideline with no deviations. It was noted that three replicates per treatment group were used instead of the typical five replicates per treatment recommended by the guideline. However, it is not considered a deviation and has no impact on the validity or the outcome of the study. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} > 1000 mL product/ha EC_{50} > 1000 mL product/ha

Reference:	KCP 10.3.2/01
Report	ADM.06001.H.2.B: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2020a, 140711063 (ADAMA No. 000105370)
Guideline(s):	Blümel, S., Bakker, F.M., Baier, B., Brown, K., Candolfi, M.P., Goßmann, A., Grimm, C., Jäckel, B., Nienstedt, K., Schirra, K.J., Ufer, A. and Waltersdorfer, A.: Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Candolfi, M. P., Blümel, S., Forster, R., Bakker, F. M., Grimm, C., Hassan, S. A., Heimbach, U., Mead-Briggs, M. A., Reber, B., Schmuck, R., Vogt, H. (eds): Guidelines to evaluate side-effects of plant protection products to non-target arthropods, IOBC, BART and EPPO Joint Initiative, IOBC/WPRS publication 2000, 121-143.
Deviations:	None ~~Three replicate units per treatment group were used instead of five. This is not considered a major deviation since three replicate units per treatment group were analysed in all cases as required by the guideline.~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) 400 g dimethoate/L (nominal), 408 g dimethoate/L (actual) Batch No. 10214034

Test organism:

Test species	*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Origin	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany
Age at test start	Protonymphs (≤ 24 hours)
No. of protonymphs per replicate	20
No. of replicates per test substance, reference substance or control	3
Sex ratio	The numbers of male and female mites in each replicate were recorded at day 7 (beginning of reproduction assessment). The sex-ratio for reproduction testing was 1 male:5 females at a minimum on day 7 (1:1.17, 1:2.00, 1:1.05, 1:1.94, 1:2.07 and 1:2.60 for the control and the 62.5, 125, 250, 500 and 1000 mL product/ha treatment groups, respectively).

Test conditions:

Test substance concentration	62.5, 125, 250, 500 and 1000 mL product/ha The test concentrations were chosen based on the results of a range-finding test. Application in 200 L water/ha Appropriate amounts of the test substance were diluted in deionised water to obtain the application solutions at the required concentrations.
Reference substance concentration	9.0 mL product/ha (3.6 g dimethoate/ha) Application in 200 L water/ha An appropriate amount of the reference substance was diluted in deionised water to obtain the application solution at the required concentration.
Control	Deionised water at 200 L/ha
Application method	Single application according to agricultural practice using laboratory-spraying equipment: - SprayLab 2100 SPS (Gerätetechnik C. Schachtner, D-71640 Ludwigsburg, Germany) - equipped with broadcast spray nozzle TeeJet EVS 80015 (TeeJetTechnologies, Glendale Heights, IL, USA) Application was calibrated to deliver 2 mg/cm² ± 10% (corresponding to 200 L spray liquid/ha). For the calibration procedure, a glass plate of known surface area was sprayed with deionised water. The weight of the glass plate was determined immediately before and after application and the amount of spray deposit per cm² was calculated as the difference between the weight before and after spraying. The procedure was repeated 5 times in a row without changing the adjustment and every time the application rate was within 200 L/ha ± 10%. The uniformity of the deposit distribution was checked visually.
Test duration	Day 0 to 7: mortality test Day 7 to 14: reproduction test
Test arena	Formed by two cover slides (glass, 24 mm x 60 mm) fixed by gluing small cover slides (glass, 20 mm x 20 mm) to both side-ends. A glue barrier was placed on the test unit to keep the mites on this test arena. The test units were placed in plastic trays (11 cm x 11 cm x 6 cm) half-filled with water, with a foam rubber and a glass-plate on top covered by tissue paper. The tissue paper was in contact with the water and therefore provided a water supply to the mites via the narrow gap between the two cover slides by capillary forces.
Procedure	The bioassays were initiated within 25-50 minutes of

	treatments being applied i.e., once residues had dried on the glass plates. Using a fine brush, impartially selected mites were placed on each treated glass plate.
Temperature	25 ± 2°C (nominal), 23-26°C (actual, short-term deviations ≤ 2 hours not reported)
Relative humidity	60-90% (nominal), 67-72% (actual, short-term deviations ≤ 2 hours not reported)
Light intensity	330-400 lux
Photoperiod	16 hours light, 8 hours dark
Food and feeding regime	A mixture of pine (*Pinus sp.)* and birch *(Betula sp.)* pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one; at least every four days.

Observations:

No. of living, dead and escaped mites	3 and 7 d
No. of female and male adults	7, 10, 13 and 14 d
No. of eggs laid, and no. of juvenile stages (larvae) developed	7, 10, 13 and 14 d The reproduction assessment was performed for the control and the test substance treatment groups where corrected mortality was ≤ 50% (i.e., all test substance treatment groups). No reproduction assessment was performed for the reference substance group.
Test conditions (temperature, relative humidity, light intensity)	Recorded during the study

Experimental dates: 24 Mar to 28 Apr 2020

Calculations:

Mortality (dead and escaped mites) was determined at day 7 after start of exposure. Mean mortality in the test substance and reference substance treatment groups was corrected for mean control mortality according to the formula by Abbott (1925) with improvements by Schneider-Orelli (1947).

Reproduction per replicate of the mites from day 7 to day 14 of the test was calculated as the ratio of eggs per female for each test unit. The number of eggs per female was determined by counting the number of females and eggs and juvenile stages at the 3 assessment days. The number of eggs per female during the reproduction period until day 14 (inclusive) was summed. The values obtained for each replicate were used to calculate the mean egg production per female (± standard deviation). Furthermore, the effect on reproduction (reduction compared to control) was calculated for the test substance treatment groups.

Statistics:

The LR_{50} was not calculated as no mortality above 50% was observed in the test substance treatment groups. Furthermore, the ER_{50} (reproduction) was not calculated as no reduction in reproduction above 50% was observed in the test substance treatment groups.

Mortality data for the control and the test substance treatment groups were analysed for significance using the Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts ($\alpha = 0.05$) and the Tarone´s test ($\alpha = 0.01$) had to be carried out previously to check for the presence of linear or quadratic trends and extra-binomial variance. The two-sample comparison between the control and the reference substance group was analysed using Fisher´s Exact Binomial Test ($\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution, homogeneity of variance and linear or quadratic trends using the Shapiro-Wilk's test (α = 0.01), the Levene's test (α = 0.01) and a trend analysis of contrasts (α = 0.05). Because reproduction data were normally distributed, homogenous and no significant linear or quadratic trend was found, Dunnett's t-test (multiple comparison, α = 0.05, one-sided smaller) was used to compare the test substance treatment groups with the control.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and discussions

Validity criteria:

- Mean mortality in the control ≤ 20% (dead and escaped mites) on day 7
- Cumulative mean mortality (control corrected) in the reference substance treatment 50-100% on day 7
- Mean cumulative number of eggs in the control ≥ 4 per female between 7 and 14 days

Mean mortality in the control was 13.3% on day 7. Corrected mean mortality in the reference substance group was 94.2% on day 7. Mean cumulative number of eggs in the control was 6.9 per female between 7 and 14 days. Therefore, all validity criteria were met.

Observations of mortality and reproduction are presented in the table below.

Table A2.4.1-1: Observations of mortality and reproduction of *T. pyri* following exposure to ADM.06001.H.2.B

Treatment	Mortality			Reproduction	
	Mean[a] ± SD (%)	Corrected[b] (%)	Mean escapees ± SD (%)	Mean no. of eggs per female ± SD	Reduction of reproduction (%)
Control	13.3 ± 2.9	-	0.0 ± 0.0	6.9 ± 1.2	-
ADM.06001.H.2.B 62.5 mL product/ha	15.0 ± 10.0	1.9	3.3 ± 2.9	6.1 ± 1.7	11.6
ADM.06001.H.2.B 125 mL product/ha	35.0 ± 5.0	25.0	1.7 ± 2.9	5.5 ± 1.4	20.8
ADM.06001.H.2.B 250 mL product/ha	16.7 ± 5.8	3.8	1.7 ± 2.9	5.9 ± 1.3	15.3
ADM.06001.H.2.B 500 mL product/ha	28.3* ± 2.9	17.3	0.0 ± 0.0	7.0 ± 0.6	-1.1
ADM.06001.H.2.B 1000 mL product/ha	40.0* ± 15.0	30.8	1.7 ± 2.9	7.0 ± 1.0	-0.8
Reference substance 3.6 g dimethoate/ha	95.0* ± 5.0	94.2	5.0 ± 8.7	n.p.	-

Note: The values for mortality, escapees and no. of eggs per female are means and standard deviations from 3 replicates with 20 mites.
There were no statistically significant differences in reproduction between all test substance treatment groups and the control (Dunnett's t-test, multiple comparison, α = 0.05, one-sided smaller)
SD Standard deviation
n.p. not performed
* Mortality statistically significantly different from control (test substance treatment groups: Step-down Cochran-Armitage Test, α = 0.05, one-sided greater; reference substance treatment group: Fisher´s Exact Binomial Test, α = 0.05, one-sided greater)
[a] Including dead and escaped mites
[b] Corrected for control mortality according to the formula by Abbott (1925) with improvements by Schneider-Orelli (1947)

Based on the study results, the following endpoints were obtained.

Table A2.4.1-2: Endpoints for ADM.06001.H.2.B in the standard laboratory test with *T. pyri*

Endpoint	(mL product/ha)
LR_{50}	> 1000
NOER (mortality)	250
ER_{50} (reproduction)	> 1000
NOER (reproduction)	1000

At day 7, there was 13.3% mortality in the control group, compared with 15.0%, 35.0%, 16.7%, 28.3% and 40.0% mortality in the 62.5, 125, 250, 500 and 1000 mL product/ha treatment groups of ADM.06001.H.2.B, respectively. When adjusted for control mortality, the corrected mortalities were 1.9%, 25.0%, 3.8%, 17.3% and 30.8% in the respective test substance treatment groups. When compared statistically, mortality in the 500 and 1000 mL product/ha treatment groups differed significantly from the control (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). The NOER with respect to mite mortality was therefore determined as 250 mL product/ha. The LR_{50} could not be determined but was > 1000 mL product/ha.

In the reference substance group, 95.0% mortality (94.2% corrected) was recorded at day 7, which differed significantly from the control (Fisher´s Exact Binomial Test, $\alpha = 0.05$, one-sided greater).

Reproduction assessments were carried out for the control and for all test substance treatment groups. The mean number of eggs produced per female was calculated to be 6.9 in the control, compared with 6.1, 5.5, 5.9, 7.0 and 7.0 in the 62.5, 125, 250, 500 and 1000 mL product/ha treatment groups of ADM.06001.H.2.B, respectively. There were no statistically significant differences in reproduction between all test substance treatment groups and the control (Dunnett's t-test, multiple comparison, $\alpha = 0.05$, one-sided smaller). The NOER with respect to reproduction was therefore determined as 1000 mL product/ha. The ER_{50} for reproduction could not be determined but was > 1000 mL product/ha.

Conclusion

In this standard laboratory test (use of glass plates as substrate) to determine the effects of exposure to ADM.06001.H.2.B on mortality and reproduction of *Typhlodromus pyri*, the LR_{50} and ER_{50} (reproduction) could not be determined but were both > 1000 mL product/ha. The NOER with respect to mortality and reproduction were determined as 250 and 1000 mL product/ha, respectively.

A 2.4.2 Acute Effects on *Aphidius rhopalosiphi* – standard laboratory study

Comments of zRMS:	The study was conducted in line with the respective guideline with a minor deviation. It was noted that a solution of fructose (10%) was provided as a source of food during exposure instead of a 1:3 v/v solution of honey and water. This deviation is considered to have no impact on the outcome of the study since all the validity criteria were met. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} = 1327 mL product/ha ER_{50} = 603.6 mL product/ha

Reference:	KCP 10.3.2/02
Report	ADM.06001.H.2.B: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold J., 2020b, 140711001 (ADAMA No. 000105369)

Guideline(s):	Mead-Briggs, M. et al.: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae). In: Candolfi, M. P., Blümel, S., Forster, R., Bakker, F. M., Grimm, C., Hassan, S. A., Heimbach, U., Mead-Briggs, M. A., Reber, B., Schmuck, R., Vogt, H. (eds): Guidelines to evaluate side-effects of plant protection products to non-target arthropods, IOBC, BART and EPPO Joint Initiative, IOBC/WPRS publication 2000, 121-143. Mead-Briggs, M. et al. (2010): An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). BioControl 55:329-338
Deviations:	Minor (see the commenting box above) ~~A solution of fructose (10%) was provided as a source of food during exposure instead of a 1:3 v/v solution of honey and water. This is not considered a relevant deviation from guideline.~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) 400 g dimethoate/L (nominal), 408 g dimethoate/L (actual) Batch No. 10214034

Test organism:

Test species	*Aphidius rhopalosiphi* (DeStefani-Perez) (Hymenoptera, Braconidae)
Origin	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Age at test start	Adults (< 48 hours)
No. of parasitoids per replicate	10
No. of replicates per test substance, reference substance or control	4 For the subsequent reproduction assessments, the performance of 20 individually confined female wasps was evaluated per treatment.
Sex ratio	During exposure: 0.7 females per total number of males and females
Acclimatisation	Approximately 1 - 2 days under test conditions in hatching chambers

Test conditions:

Test substance concentration	125, 250, 500, 1000 and 2000 mL product/ha The test concentrations were chosen based on the results of a range-finding test.

	Application in 200 L water/ha Appropriate amounts of the test substance were diluted in deionised water to prepare the application solutions.
Reference substance concentration	0.3 mL product/ha (0.12 g dimethoate/ha) Application in 200 L water/ha The reference substance was diluted in deionised water to prepare the application solution.
Control	Deionised water at 200 L/ha
Application method	Spray by a laboratory sprayer (SprayLab 2100 SPS, Gerätetechnik C. Schachtner, D-71640 Ludwigsburg, Germany) fitted with a TeeJet EVS 80015 spray nozzle (TeeJetTechnologies, Glendale Heights, IL, USA), 2.5 bar spray pressure, 2.75 km/h spraying speed, calibrated with deionised water to provide a deposition rate at target level equivalent to 200 L/ha ± 10%, uniformity of the deposit distribution checked visually.
Test duration	Day 0 to 2: exposure phase (48-hour mortality test) Day 2 to 3: parasitisation phase (24-hour parasitisation of cereal aphids by the wasps) Day 3 to 14-15: reproduction phase (11- to 12-day reproduction test after removal of the wasps)
Test arena	<u>Mortality phase:</u> Comprising two treated glass plates (13 cm x 13 cm) which were held apart by an untreated aluminium frame (13 cm x 1.5 cm x 1 cm per side). Three sides of the frame had 6 ventilation holes (approximately 1 cm in diameter) covered with a cloth. The 4th side of the frame had one small hole (approximately 1 cm in diameter) for inserting and feeding the test organisms. <u>Reproduction phase (including parasitisation):</u> Untreated pots (13 cm in diameter) with barley seedlings (*Hordeum vulgare* 'Sunshine'; 15 - 25 seedlings, 10 days old) infested with 100 - 200 host aphids of all developmental stages (*Rhopalosiphum padi*; number of aphids estimated) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). The cylinder had two holes (70 x 195 mm) which were closed with a fine gauze to improve ventilation and another hole (approximately 2 cm in diameter) closed with cotton wool for the introduction of the parasitoids. The top of the cylinder was closed with a fine gauze. The soil surface was covered with a thin layer of quartz sand.
Procedure	The parasitoids were exposed to dried residues on treated glass plates for 48 hours. Thereafter, for treatment groups where the corrected mortality was ≤ 50%, the reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids. The females were removed after 24 hours and the aphid-infested plants left for a further 11 - 12 days for development of aphid mummies. Transfer of the parasitoids was performed using an aspirator.
Temperature	18-22°C
Relative humidity	69-71% (acclimatisation, exposure period) 75-78% (post-exposure period; within the test units)
Light intensity	1270-1700 lux (acclimatisation, exposure, parasitisation period) 7920-10420 lux (post-parasitisation period)
Photoperiod	16 hours light, 8 hours dark
Ventilation	The exposure units were ventilated with a small pump (sucking air).

Food and feeding regime	A solution of fructose (10%) was provided *ad libitum* in small test tubes (approximately 1 cm in diameter) which were connected to the exposure units at the beginning of the experiment.

Observations:

Mortality and behavioural abnormalities	2, 24 and 48 hours after start of exposure
No. of parasitized aphids (mummies)	Day 14 or 15 (end of reproduction phase)
Test conditions (temperature, relative humidity)	Not specified, but either continuously or regularly

Experimental dates: 24 Mar to 29 Apr 2020

Calculations:

Mortality (moribund and dead parasitoids) was determined 48 hours after exposure to the test and reference substance and was corrected for the 48-hour control mortality according to the formula by Abbott (1925) with improvements by Schneider-Orelli (1947).

For reproduction, the number of aphid mummies obtained from the maximum 20 replicates per treatment group was used to calculate the mean aphid mummies production per female (± standard deviation) within the 24 hours parasitisation period (post-exposure period). Furthermore, the percentage reduction of reproduction in the test substance treatments in comparison to the control was calculated.

Statistics:

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

The LR_{50} was calculated by applying Weibull Analysis.

Mortality data obtained from the control and test substance treatments was analysed for significance using the Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts ($\alpha = 0.05$) and the Tarone's test ($\alpha = 0.01$) had to be carried out previously to check for the presence of linear or quadratic trends and extra-binomial variance. The two-sample comparison between the reference substance and the control was analysed using the Fisher´s Exact Binomial Test ($\alpha =$
0.05, one-sided greater).

The ER_{50} for reproduction was calculated by Probit-Analysis. Since the mean values of the different test substance treatment groups were close to each other, calculation of the ER_{50} based on a statistically significant dose-response relationship was impeded. Therefore, it was necessary to enlarge the data base for analysis by treating each individual female parasitoid as replicate.

Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). Because reproduction data were normally distributed and inhomogeneous, the Bonferroni-Holm Welch t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used.

Results and discussions

Validity criteria:

- Control mortality ≤ 13% (48 hours)
- Corrected reference mortality > 50% (based on study protocol)

- Control reproduction ≥ 5 mummies per female and no more than two wasps producing zero values

Control mortality was 2.5% and corrected mortality in the reference substance control was 100.0%. Reproduction in the control was 54.3 mummies per female and only one wasp produced zero values. Therefore, all validity criteria were met.

Observations of mortality and reproduction are presented in the table below.

Table A2.4.2-1: Observations of mortality and reproduction of *A. rhopalosiphi* following exposure to ADM.06001.H.2.B

Treatment	Mortality		Reproduction		
	Mean ± SD (%)	Corrected[a] (%)	No. of females successfully assessed for reproduction assessment	Mummies per female Mean ± SD	Reduction of reproduction (%)
Control	2.5 ± 5.0	-	20	54.3 ± 25.5	-
ADM.06001.H.2.B 125 mL product/ha	0.0 ± 0.0	-2.6	20	45.3 ± 15.1	16.7
ADM.06001.H.2.B 250 mL product/ha	5.0 ± 5.8	2.6	18	28.5** ± 15.5	47.5
ADM.06001.H.2.B 500 mL product/ha	5.0 ± 10.0	2.6	20	26.2** ± 12.2	51.8
ADM.06001.H.2.B 1000 mL product/ha	20.0* ± 16.3	17.9	20	26.5** ± 13.2	51.3
ADM.06001.H.2.B 2000 mL product/ha	95.0* ± 5.8	94.9	n.p.	n.p.	n.p.
Reference substance 0.3 mL product/ha	100.0* ± 0.0	100.0	n.p.	n.p.	n.p.

n.p. not performed (reproduction assessment performed for treatment groups where corrected mortality was ≤ 50%)
SD standard deviation
* Mortality statistically significantly different from control (Test substance treatments: Step-down Cochran-Armitage Test, α = 0.0 5, one-sided greater; Reference substance treatment: Fisher's Exact Test, α = 0.05, one-sided greater)
** Reproduction statistically significantly different from control (Bonferroni-Holm Welch t-test, α = 0.05, one-sided smaller)
[a] Corrected for control mortality according to the formula by Abbott (1925) with improvements by Schneider-Orelli (1947)

Based on the study results, the following endpoints were obtained.

Table A2.4.2-2: Endpoints for ADM.06001.H.2.B in the standard laboratory test with *A. rhopalosiphi*

Endpoint	(mL product/ha)
LR_{50} (95% confidence interval)	1327 (685.5 – 1819.5)
NOER (mortality)	500
ER_{50} (reproduction) (95% confidence interval)	603.6 (396.2 – 1363.4)
NOER (reproduction)	125

At 48 hours, there was 2.5% mortality in the control, compared with 0.0%, 5.0%, 5.0%, 20.0% and 95.0% mortality in the 125, 250, 500, 1000 and 2000 mL product/ha treatment groups of ADM.06001.H.2.B, respectively. When adjusted for the deaths in the control, the corrected mortality was -2.6%, 2.6%, 2.6%, 17.9% and 94.9% in the respective test substance treatments. The results for the 1000 and 2000 mL product/ha treatment groups differed significantly from the control (Step-down Cochran-Armitage Test, α = 0.0 5, one-sided greater). The NOER with respect to wasp survival was therefore considered to be 500 mL product/ha. In the reference substance treatment, 100.0% mortality (100.0% corrected) was recorded at 48 hours.

The mean number of mummies produced per surviving female was 54.3 in the control, compared with 45.3, 28.5, 26.2 and 26.5 in the 125, 250, 500 and 1000 mL product/ha treatment groups of ADM.06001.H.2.B, respectively. Therefore, relative to the control, there was a reduction of reproduction of 16.7%, 47.5%, 51.8% and 51.3% in the 125, 250, 500 and 1000 mL product/ha treatment groups of ADM.06001.H.2.B, respectively. The ER_{50} for reproduction was calculated as 603.6 mL product/ha. When the test substance treatments were compared to the control, the results for the 250, 500, 1000 and 2000 mL product/ha treatment groups differed significantly from the control (Bonferroni-Holm Welch t-test, $\alpha = 0.05$, one-sided smaller) and therefore the NOER with respect to reproduction was 125 mL product/ha.

Conclusion

In this standard laboratory test (use of glass plates as substrate) to determine the effects of exposure to ADM.06001.H.2.B on mortality and reproduction of *Aphidius rhopalosiphi*, the LR_{50} and ER_{50} were determined to be 1327 and 603.6 mL product/ha, respectively. The NOER with respect to mortality was 500 mL product/ha and the NOER with respect to reproduction was 125 mL product/ha.

A 2.4.3 Acute Effects on *Aphidius rhopalosiphi* – extended laboratory study

Comments of zRMS:	The study was conducted in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following enpoints relevant for the risk assessment: LR_{50} > 2000 mL product/ha ER_{50} > 2000 mL product/ha

Reference:	KCP 10.3.2/03
Report	ADM.06001.H.2.B: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), Extended Laboratory Study - Dose Response Test -, Leopold, J., 2020c, 140711002 (ADAMA No. 000105372)
Guideline(s):	~~Mead-Briggs, M. et al.: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani Perez) (Hymenoptera, Braconidae). In: Candolfi, M. P., Blümel, S., Forster, R., Bakker, F. M., Grimm, C., Hassan, S. A., Heimbach, U., Mead Briggs, M. A., Reber, B., Schmuck, R., Vogt, H. (eds): Guidelines to evaluate side effects of plant protection products to non target arthropods, IOBC, BART and EPPO Joint Initiative, IOBC/WPRS publication 2000, 121-143.~~ Mead-Briggs, M. et al. (2010): An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). BioControl 55:329-338
Deviations:	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001

Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) 400 g dimethoate/L (nominal), 408 g dimethoate/L (actual) Batch No. 10214034

Test organism:

Test species	*Aphidius rhopalosiphi* (DeStefani-Perez) (Hymenoptera, Braconidae)
Origin	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Age at test start	Adults (< 48 hours)
No. of parasitoids per replicate	5 females
No. of replicates per test substance, reference substance or control	6 For the subsequent reproduction assessments, the performance of 20 individually confined female wasps was evaluated per treatment.
Sex ratio	Not applicable; only females were used in the test.
Acclimatisation	Approximately 1 - 2 days under test conditions in hatching chambers

Test substance concentration	125, 250, 500, 1000 and 2000 mL product/ha The test concentrations were chosen based on the results of a range-finding test. Application in 400 L water/ha Appropriate amounts of the test substance were diluted in deionised water to prepare the application solutions.
Reference substance concentration	10.0 mL product/ha (4 g dimethoate/ha) Application in 400 L water/ha The reference substance was diluted in deionised water to prepare the application solution.
Control	Deionised water at 400 L/ha
Application method	Spray by a laboratory sprayer (SprayLab 2100 SPS, Gerätetechnik C. Schachtner, D-71640 Ludwigsburg, Germany) fitted with a TeeJet 8003 EVS spray nozzle, 2.5 bar spray pressure, 2.25 km/h spraying speed, calibrated with deionised water to provide a deposition rate at target level equivalent to 400 L/ha ± 10%, uniformity of the deposit distribution checked visually.
Test duration	Day 0 to 2: exposure phase (48-hour mortality test) Day 2 to 3: parasitisation phase (24-hour parasitisation of cereal aphids by the wasps) Day 3 to 14-15: reproduction phase (11- to 12-day reproduction test after removal of the wasps)
Test arena	<u>Mortality phase:</u> Treated pots (13 cm in diameter) with 8 - 10 barley seedlings (*Hordeum vulgare* 'Sunshine') per pot. The plants were used for the bioassay when at the 2nd leaf growth stage, *i.e.* BBCH Growth Stage 12. The plants were trimmed to a uniform height of 12 cm prior to test start. The plants were enclosed within a clear polyacrylic cylinder (20 cm high and 10 cm in diameter) with a hole (approximately 2 cm in diameter) for the

	introduction of the parasitoids. After introduction, the hole was closed by a stopper with a hole where the ventilation tube was inserted. The opening of the ventilation tube and the top of the cylinder were closed with a fine mesh gauze. The soil surface was covered with a thin layer of quartz sand before treatment. Reproduction phase (including parasitisation): Untreated pots (13 cm in diameter) with barley seedlings (*Hordeum vulgare* 'Sunshine'; 15 - 25 seedlings, 12 days old) infested with 100 - 200 host aphids of all developmental stages (*Rhopalosiphum padi*; number of aphids estimated) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). The cylinder had two holes (70 x 195 mm) which were closed with a fine gauze to improve ventilation and another hole (approximately 2 cm in diameter) closed with cotton wool for the introduction of the parasitoids. The top of the cylinder was closed with a fine gauze. The soil surface was covered with a thin layer of quartz sand.
Procedure	The parasitoids were exposed to dried residues on treated barley seedlings for 48 hours. Thereafter, for treatment groups where the corrected mortality was ≤ 50%, the reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids. The females were removed after 24 hours and the aphid-infested plants left for a further 11 - 12 days for development of aphid mummies. Transfer of the parasitoids was performed using an aspirator.
Temperature	18-22°C
Relative humidity	68-82% (acclimatisation, exposure period) 78-80% (post-exposure period; within the test units)
Light intensity	860-1070 lux (acclimatisation, exposure period) 1110-1610 lux (parasitisation period) 12710-15670 lux (post-parasitisation period)
Photoperiod	16 hours light, 8 hours dark
Ventilation	The exposure units were ventilated with a small pump (sucking air).
Food and feeding regime	55 minutes to 1 hour and 25 minutes before application, the seedlings were lightly sprayed with a solution of fructose (10%) and were left to dry, *ad libitum.*

Observations:

Mortality and behavioural abnormalities	2, 24 and 48 hours after start of exposure
Settling rate of the parasitoids (repellent effect)	During the initial 3 hours Five separate observations were made at approximately 30-minute intervals starting approximately 30 minutes after the introduction of all wasps.
No. of parasitized aphids (mummies)	Day 14 or 15 (end of reproduction phase)
Test conditions (temperature, relative humidity)	Not specified, but either continuously or regularly

Experimental dates: 25 May to 14 Jul 2020

Calculations:

Mortality (moribund and dead parasitoids) was determined 48 hours after exposure to the test and reference substance and was corrected for the 48-hour control mortality according to the formula by Abbott (1925) with improvements by Schneider-Orelli (1947).

The settling of the parasitoids was assessed over the initial 3 hours of the test. The percentage of wasps settled on the plants in each replicate was calculated from the total of those observed on plant or cylinder. This calculation was performed for each assessment occasion and then a mean value obtained for each replicate. Mean settling rate over all replicates was calculated for each treatment group.

For reproduction, the number of aphid mummies obtained from the maximum 20 replicates per treatment group was used to calculate the mean aphid mummies production per female within the 24 hours parasitisation period (post-exposure period). Furthermore, the percentage reduction of reproduction in the test substance treatments in comparison to the control was calculated.

Statistics:

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

The LR_{50} and ER_{50} were not calculated as no effects on mortality or reproduction above 50% were noted.

Statistical analysis of the mortality data was not performed because no mortality was observed for both the control and any test substance treatment. The two-sample comparison between the reference substance and control was analysed using the Fisher´s Exact Binomial Test ($\alpha = 0.05$, one-sided greater).

The percent values of wasps settled on the plants were angularly transformed (square root arcsine) prior to analysis. The transformed data were tested for normal distribution and homogeneity using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$), respectively. Additionally, test substance data were checked for the presence of linear trends using a trend analysis by contrasts ($\alpha = 0.05$). Because settling data for the test substance treatments were normally distributed and homogenous and a linear trend was revealed, the Williams t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used. For the reference substance treatment, settling data were normally distributed and homogeneous. Therefore, the Student t-test (pair wise comparison, one-sided smaller, $\alpha = 0.05$) was used.

Reproduction data were tested for normal distribution, homogeneity of variance and the presence of linear or quadratic trends using the Shapiro-Wilk's test ($\alpha = 0.01$), the Levene's test ($\alpha = 0.01$) and a trend analysis by contrasts ($\alpha = 0.05$), respectively. Because reproduction data were normally distributed and homogeneous and no linear trend was detected, the Dunnett's t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used.

Results and discussions

Validity criteria:

- Control mortality ≤ 10% (48 hours)
- Corrected reference mortality > 50% (based on study protocol)
- Control reproduction ≥ 5 mummies per female and no more than two wasps producing zero values

Control mortality was 0.0% and corrected mortality in the reference substance control was 100.0%. Reproduction in the control was 52.7 mummies per female and only one wasp produced zero values. Therefore, all validity criteria were met.

Observations of mortality and reproduction are presented in the table below.

Table A2.4.3-1: Observations of mortality, settling rate and reproduction of *A. rhopalosiphi* following exposure to ADM.06001.H.2.B

Treatment	Mortality		Settling rate	Reproduction		
	Mean ± SD (%)	Corrected[a] (%)	Mean ± SD (%)	No. of females successfully assessed for reproduction assessment	Mummies per female Mean ± SD	Reduction of reproduction (%)
Control	0.0 ± 0.0	-	80.7 ± 12.2	20	52.7 ± 20.0	-
ADM.06001.H.2.B 125 mL product/ha	0.0 ± 0.0	0.0	84.0 ± 12.1	20	56.3 ± 15.1	-6.9
ADM.06001.H.2.B 250 mL product/ha	0.0 ± 0.0	0.0	84.0 ± 9.8	20	48.8 ± 21.7	7.4
ADM.06001.H.2.B 500 mL product/ha	0.0 ± 0.0	0.0	85.3 ± 3.3	20	50.7 ± 12.2	3.7
ADM.06001.H.2.B 1000 mL product/ha	0.0 ± 0.0	0.0	84.0 ± 13.1	19	49.4 ± 18.6	6.1
ADM.06001.H.2.B 2000 mL product/ha	0.0 ± 0.0	0.0	63.8** ± 9.9	20	45.2 ± 19.2	14.2
Reference substance 10.0 mL product/ha	100* ± 0.0	100.0	57.8** ± 13.3	n.p.	n.p.	n.p.

Note: For reproduction, there were no statistically significantly differences in the test substance treatments in comparison to the control (Dunnett's t-test, one-sided smaller, $\alpha = 0.05$)
n.p. not performed (reproduction assessment performed for treatment groups where corrected mortality was ≤ 50%)
SD standard deviation
* Mortality statistically significantly different from control (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$)
** Settling rate statistically significantly different from control (test substance: Williams t-test, one-sided smaller, $\alpha = 0.05$; reference substance: Student t-test, one-sided smaller, $\alpha = 0.05$)
[a] Corrected for control mortality according to the formula by Abbott (1925) with improvements by Schneider-Orelli (1947)

Based on the study results, the following endpoints were obtained.

Table A2.4.3-2: Endpoints for ADM.06001.H.2.B in the extended laboratory test with *A. rhopalosiphi*

Endpoint	(mL product/ha)
LR_{50}	> 2000
NOER (mortality)	2000
ER_{50}	> 2000
NOER (reproduction)	2000

At 48 hours, there was 0.0% mortality in the control and all test substance treatments from 125 to 2000 mL product/ha ADM.06001.H.2.B. Therefore, the NOER with respect to wasp survival was 2000 mL product/ha. The LR_{50} could not be determined but was > 2000 mL product/ha. In the reference substance treatment, 100.0% mortality (100.0% corrected) was recorded at 48 hours.

Behavioural abnormalities (affected and/or moribund parasitoids) were not observed in any test substance treatment group.

The settling rate after the initial 3 hours ranged from 63.8% (2000 mL product/ha) to 85.3% (500 mL product/ha) in the control and test substance treatments. Since the settling rate of the parasitoids on the plants was > 30% for all test substance treatments, no repellent effect of the test substance was observed compared to the control.

The mean number of mummies produced per surviving female was 52.7 in the control, compared with 56.3, 48.8, 50.7, 49.4 and 45.2 in the 125, 250, 500, 1000 and 2000 mL product/ha treatment groups of

ADM.06001.H.2.B, respectively. There were no statistically significant differences in reproduction between all test substance treatment groups and the control (Dunnett's t-test, one-sided smaller, α = 0.05). The NOER with respect to reproduction was therefore determined as 2000 mL product/ha. The ER_{50} for reproduction could not be determined but was > 2000 mL product/ha.

Conclusion

In this extended laboratory test (use of barley seedlings as substrate) to determine the effects of exposure to ADM.06001.H.2.B on mortality and reproduction of *Aphidius rhopalosiphi*, the LR_{50} and ER_{50} could not be determined but were > 2000 mL product/ha. The NOER with respect to mortality and reproduction were both determined as 2000 mL product/ha.

A 2.5 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.5.1 KCP 10.4.1 Earthworms

A 2.5.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted in line with OECD 222 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). The reliability of the EC_{10} value was evaluated in line with the recommendations of EFSA Supporting publication 2019:EN-1673: - NW (normalised width) of 1.97 was calculated, which results with rating "poor" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, - median EC_{10} (37.5 mg product/kg soil dw) is lower than $EC_{20,low}$ (58.4 mg product/kg soil dw), - the dose-response curve is shallow with steepness of 0.04 (i.e. <0.33). Based on above indications the calculated EC_{10} is considered to be sufficiently reliable. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 56d NOEC = 511 mg product/kg soil dw 56d EC_{10} = 37.5 mg product/kg soil dw

Reference:	KCP 10.4.1.1/01
Report	ADM.06001.H.2.B: Determination of chronic toxicity to the earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae) in an artificial soil substrate, Straube D. and Gourlay V., 2021, 140711022 (ADAMA No. 000105375)
Guideline(s):	OECD 222 (2016)
Deviations:	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	The reference substance carbendazim was evaluated in a separate test according to OECD 222 (2016). In this test run from May to July 2020, an EC_{50} of 0.88 mg a.s./kg dw (95%

	confidence interval: 0.81-0.94 mg a.s./kg dw) and a NOEC and LOEC for reproduction of 0.482 and 0.694 mg a.s./kg dw, respectively, were obtained. These results demonstrate that the test organisms in the test system responded within the normal level (significant effects between 1 and 5 mg a.s./kg dw).

Test organism:

Test species	Earthworm *Eisenia andrei*
Origin	In-house culture bred at the test facility under standardised conditions
Age at test start	Adult worms (approximately 9 months old with well-developed clitellum, age range not differing by more than 4 weeks)
Weight at test start	302 – 599 mg
No. of test organisms per replicate (test vessel)	10
No. of replicates per treatment group	8 for the control, 4 for the test substance treatments
Acclimatisation	1 day, in artificial soil, under test conditions

Test conditions:

Test substance concentration	15.0, 27.0, 48.7, 87.6, 158, 284, 511 and 920 mg product/kg dw The test concentrations were chosen based on the results of a range-finding test. A stock solution of the test substance was prepared by diluting 4755.0 mg to 650 g with deionised water. Further dilution of this stock solution was performed with deionised water and the artificial soil was either treated with the appropriate amount of stock solution or dilution. To verify homogeneous distribution and correct dosing of the test substance in the soil, samples were taken for analysis after mixing the test substance into the soil. The active substance mesosulfuron-methyl was analysed in soil samples of the lowest and highest test concentration of 15.0 and 920 mg product/kg dw, respectively, and in the control.
Reference substance concentration	The reference substance was tested in a separate study at 0.482, 0.694, 1.00, 1.44 and 2.07 mg a.s./kg dw.
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 10% *Sphagnum* peat (air-dried, finely ground and with no visible plant remains) - 20% kaolinite clay (kaolinite content > 30%) - 0.4% calcium carbonate (for adjustment of pH to 6.0±0.5) - 69.6% fine quartz-sand (air-dried, > 50% of particles between 50 and 200 µm) The artificial soil was moistened to approximately half of the final water content 1 day before application. The additional water required to achieve the final water content was added when applying the test substance.
Application method	For each test substance concentration and the control, the amount of soil needed was split in half, so that each treatment group and the control were treated in two batches. To each test substance treated batch, the corresponding amount of stock solution or dilution was added. Each batch was mixed, and the two batches of each treatment group and the control were combined to one batch and additionally mixed. During test substance application, the soil was moistened with deionised water to obtain the required water content (40-60% of the maximum water holding capacity (WHC)). The soil was

	then split into the replicates. Earthworms were randomly assigned to batches of 10. The different batches were sorted into four classes based on total weight and one batch of each weight class was assigned to each treatment group (two batches for the control) to ensure weights were homogeneous. The earthworms were placed on the surface of the artificial soil after application.
Test duration	56 days After 28 days, adult worms were removed from the test vessels and the soil was returned to the original test vessels for reproduction assessment during the second 28 days of the test.
Test vessels	Plastic boxes (18.3 cm x 13.6 cm x 6 cm, tapered towards the bottom, with a soil surface of approximately 16.5 cm x 11.5 cm = 189.75 cm^2) with perforated transparent lids. Each container was filled with 626.6 g ± 1 g of the prepared soil (500 g dry weight equivalents). The height of the soil layer in the containers was approximately 5 cm.
Temperature	18-22°C
Light intensity	400-800 lux
Photoperiod	16 hours light, 8 hours dark
Water content of artificial soil	Test start: 24.8-26.6% (50.7-54.2% WHC) Test end: 25.3-27.9% (51.7-57.0% WHC)
pH of artificial soil	Test start: 5.7 – 6.0 Test end: 6.5
Food and feeding regime	Air dried and finely ground cattle manure One day after application, 5 g of food were added to the test vessels and moistened with 5 mL of deionised water. The feeding procedure was repeated on a weekly basis until day 28 (with reduced amount in case the food of the previous week had not been fully consumed). At day 28, the food was mixed into the substrate following removal of the adult earthworms.

Measurements:

Adult mortality	At day 28
Behavioural and morphological abnormalities	At day 28
Body weight change	Body fresh weights were determined at test start (day 0) and 28 days after application.
Food consumption	Cumulative amount of food added to each test container during the test period
Reproduction	At day 56 Juveniles were removed by placing the test vessels in a water bath at 50-60°C and counting all emerging earthworms. In addition, the soil of each container was emptied out onto a tray and checked visually for any remaining juvenile earthworms.
Temperature	Not specified, but either continuously or regularly
pH of the soil samples	At test start and end
Water content of the artificial soil	At test start and end During the test, the water content of the soil was checked by weighing the test vessels and evaporated water was replenished, as necessary, to ensure that the difference in water content between experimental start and end was less than 10%.

Analytical method:

Samples	Concentrations of the active substance mesosuflfuron-methyl were analysed in soil samples of the lowest and highest test concentration of 15.0 and 920 mg product/kg dw, respectively, and in the control. About 2 g aliquots (dry weight equivalents) of each soil sample

	were extracted trice with acetonitrile/pure water (80:20, v/v). The three extraction solutions were combined and diluted with acetonitrile/pure water (50:50, v/v).
Method type	LC-MS/MS
Equipment	1200, Agilent
Column	PerfectSil 120 ODS-2 (125 * 3 mm; 5 µm)
Column temperature	20°C
Detector	Mass spectrometer API 3200, Sciex Detection: ESI positive MRM mass transitions: Mesosulfuron-methyl: m/z 504 → 182 (quantifier); 504 → 83 (qualifier)
Flow rate	0.5 mL/min
Mobile phase	A: 30% HPLC water + 5 mM NH_4CH_3COO B: 70% Acetonitrile + 5 mM NH_4CH_3COO isocratic

Experimental dates: 09 Jun 2020 to 20 Jan 2021

Calculations:

The endpoints of the test were mortality, body weight change (difference in fresh weight of surviving worms between test start and 28 days after treatment) and reproduction (number of juveniles present). Furthermore, the percentage reproductive performance of the treated groups based on the control treatment was calculated. The arithmetic means (± standard deviation) per treatment for each endpoint were calculated.

Statistics:

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Mortality data were analysed for significance by using the Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater).

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.01$) using the Shapiro-Wilk´s test and the Levene´s test, respectively.

Since the body weight change data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend), the Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values.

Since the reproduction data were normally distributed and heterogeneous, the Welch t-test after Bonferroni-Holm was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The EC_x values and their 95% confidence limits for reproduction were calculated by applying Weibull Analysis.

Results and discussions

Validity criteria:

- Each control replicate produces ≥ 30 juveniles by the end of the test
- The coefficient of variation of reproduction in the control is ≤ 30%
- Mean mortality of adults in the control is ≤ 10% over the initial 4 weeks of the test

The control replicates produced between 107 and 129 juveniles by the end of the test. The coefficient of variation of reproduction in the control was 6.7%. Mean mortality of adults in the control was 0.0% over the initial 4 weeks of the test. Therefore, all validity criteria were met.

The test substance concentrations in the artificial soil were determined by analysis of the active substance mesosulfuron-methyl. The results are presented in the table below.

Table A2.5.1.1-1: Measured test concentrations and percentage recovery of mesosulfuron-methyl in artificial soil

Test substance nominal concentration (mg product/kg dw)	Sample	Mesosulfuron-methyl			
		Concentration (µg/L)		Recovery	
		nominal	actual	% of nominal	mean % of nominal
15	1	15.181	16.520	109	116
	2	14.805	18.147	123	
920	1	910.771	835.834	92	92
	2	912.237	839.709	92	
Control I-IV	1	0	< LOD	n.a.	n.a.
	2	0	< LOD	n.a.	
Control V-VIII	1	0	< LOD	n.a.	n.a.
	2	0	< LOD	n.a.	

n.a. not applicable
LOD: 0.3 µg mesosulfuron-methyl/L

The recoveries of mesosulfuron-methyl were 116% and 92% of nominal for the lowest (15 mg product/kg dw) and highest (920 mg product/kg dw) test substance concentration, respectively. Therefore, homogeneous distribution and correct dosing of the test substance was demonstrated.

Observations of mortality, body weight change and reproduction are presented in the table below.

Table A2.5.1.1-2: Effects of ADM.06001.H.2.B on mortality, body weight change and reproduction of *Eisenia andrei*

Treatment (mg product/kg dw)	Mean mortality after 28 days of exposure (% ± SD)	Mean body weight change (day 0-28) (% ± SD)	Reproduction after 56 days	
			(mean number of juveniles/replicate ± SD)	Percentage of control (%)
0 (control)	0.0 ± 0.0	30.3 ± 6.2	120 ± 8[a]	-
15.0	0.0 ± 0.0	29.6 ± 9.7	110 ± 26	91.1
27.0	0.0 ± 0.0	35.7 ± 10.3	100 ± 10	83.2
48.7	0.0 ± 0.0	38.0 ± 6.1	107 ± 10	88.6
87.6	0.0 ± 0.0	35.2 ± 11.6	103 ± 10	85.9
158	0.0 ± 0.0	37.7 ± 5.1	103 ± 14	85.7
284	0.0 ± 0.0	39.4 ± 11.0	84 ± 22	69.9
511	0.0 ± 0.0	32.7 ± 12.2	81 ± 17	67.2
920	0.0 ± 0.0	40.9 ± 5.8	57* ± 9	47.6

Note: There were no statistically significant differences in mortality and body weight change between test substance treatments and control (mortality: Step-down Cochran-Armitage Test, α = 0.05, one-sided greater; body weight change: Williams t-test, α = 0.05, one-sided smaller)
SD Standard deviation
[a] Coefficient of variation: 6.7%
* Statistically significantly different compared to control (Welsh-t test after Bonferroni Holm, α = 0.05, one-sided smaller)

Based on the study results, the following endpoints were obtained.

Table A2.5.1.1-3: Endpoints for effects of ADM.06001.H.2.B on mortality, body weight change and reproduction of *Eisenia andrei*

Endpoint	(mg product/kg dw)
EC_{10} (reproduction) (95% confidence limits)	37.5 (6.59 – 80.3)
EC_{20} (reproduction) (95% confidence limits)	140.2 (58.4 – 221.4)
EC_{50} (reproduction) (95% confidence limits)	> 920 (n.d.)
NOEC (reproduction)	511
LOEC (reproduction)	920
NOEC (mortality, body weight change)	920
LOEC (mortality, body weight change)	> 920
LC_{50} (mortality)	> 920

n.d. not determinable

After 28 days, there was 0.0% mortality in the control and all test substance treatments from 15.0 to 920 mg product/kg dw. Therefore, the NOEC with respect to mortality was 920 mg product/kg dw. The LC_{50} could not be determined but was > 920 mg product/kg dw. No behavioural abnormalities were observed in any of the test substance treatment groups.

The feeding activity in all test substance treated groups was comparable to the control.

The changes in adult worm body weight were 30.3% in the control and between 29.6% and 39.4% in all test substance treatments from 15.0 to 920 mg product/kg dw. There were no statistically significant differences in body weight change between the control and all test substance treatment groups (Williams t-test, $\alpha = 0.05$, one-sided smaller). Therefore, the NOEC with respect to body weight change was 920 mg product/kg dw.

At the end of the test after 56 days, the mean number of juveniles per replicate was 120 in the control. In the test substance treatment groups of 15.0 to 511 mg product/kg dw, the mean number of juveniles per replicate ranged between 81 and 110, which was not statistically significantly different from the control. In the highest test substance treatment group of 920 mg product/kg dw, the mean number of juveniles per replicate was 57 which differed statistically significantly from the control (Welsh-t test after Bonferroni Holm, $\alpha = 0.05$, one-sided smaller). Therefore, with respect to reproduction, the LOEC was 920 mg product/kg dw and the NOEC was 511 mg product/kg dw. The EC_{10}, EC_{20} and EC_{50} were determined as 37.5 (95% confidence interval: 6.59 – 80.3), 140.2 (95% confidence interval: 58.4 – 221.4) and > 920 (95% confidence interval: not determinable) mg product/kg dw.

Conclusion

In this test on sub-lethal effects of ADM.06001.H.2.B on the earthworm *Eisenia andrei*, the EC_{10}, EC_{20}, and EC_{50} for reproduction were determined to be 37.5, 140.2 and > 920 mg product/kg dw, respectively. The NOEC for reproduction was determined as 511 mg product/kg dw and the NOEC for mortality and body weight change was 920 mg product/kg dw.

A 2.5.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.5.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.5.2.1 KCP 10.4.2.1 Species level testing

A 2.5.2.1.1 Effects on *Hypoaspis aculeifer*

Comments of zRMS:	The study was conducted in line with OECD 226 with no deviations.

	The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). The EC_x values could not be determined by statistical analysis due to no concentration response. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: NOEC (reproduction) > 1000 mg product/kg soil dw

Reference:	KCP 10.4.2.1/01
Report	ADM.06001.H.2.B: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* (Acari: Laelapidae) in Artificial Soil, Straube D., 2020a, 140711089 (ADAMA No. 000105377)
Guideline(s):	OECD 226 (2016)
Deviations:	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	The reference substance DANADIM PROGRESS (containing the active substance dimethoate at 38.5% w/w) was evaluated in a separate test according to OECD 226 (2016). In this test performed in April/May 2020, an EC_{50} of 3.18 mg a.s./kg dw (95% confidence interval: 3.05 – 3.28 mg a.s./kg dw) was obtained. This result demonstrates that the test organisms in the test system responded within the normal level (EC_{50} between 3.0 and 7.0 mg a.s./kg dw).

Test organism:

Test species	Predatory mite *Hypoaspis aculeifer*
Origin	Culture maintained at the test facility
Age at test start	Adult females from a synchronised cohort, approximately 9 days after reaching the adult stage (30 days after placing adult females in clean rearing vessels over a period of 3 days)
No. of test organisms per replicate (test vessel)	10
No. of replicates per treatment group	8 for the control, 4 for the test substance treatments 1 additional container per treatment to determine the pH and

	water content of the artificial soil after 14 days (and weight at test start and again on day 7)

Test conditions:

Test substance concentration	16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg product/kg dw The test concentrations were chosen based on the results of a range-finding test. A stock solution of the test substance (= application solution of the highest test concentration) was prepared by diluting 700.2 mg to 85.1 g with deionised water. The application solutions of the lower test concentrations were prepared as a dilution series of the stock solution.
Reference substance concentration	The reference substance was tested in a separate study at 1.54, 2.23, 3.23, 4.69 and 6.80 mg a.s./kg dw.
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 5% *Sphagnum* peat (air-dried, finely ground and with no visible plant remains) - 20% kaolinite clay (kaolinite content > 30%) - 0.2% calcium carbonate (for adjustment of pH to 6.0±0.5) - 74.8% fine quartz-sand (> 50% of particles between 50 and 200 µm) The artificial soil was moistened to approximately half of the final water content 2 days before application. The additional water required to achieve the final water content was added when applying the test substance.
Application method	24.3 g of the stock solution or the corresponding dilutions were added to artificial soil equivalent to 200 g dry weight to obtain the targeted concentrations. The soil for each treatment group was mixed with a laboratory mixer to ensure homogeneous distribution. Each group was treated in one batch and then split into the replicates. The test organisms were collected with a fine brush, put into a small glass tube, counted to ensure that 10 adult females were introduced and placed onto the surface of the treated artificial soil within two hours after preparation of the final test substrate.
Test duration	14 days
Test vessels	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil (dry weight equivalent). The height of the soil layer in the containers was 1.5 to 2 cm.
Temperature	18-22°C
Light intensity	400-800 lux
Photoperiod	16 hours light, 8 hours dark
Water content of artificial soil	Test start: 19.4-22.6% (51.1-59.4% of the maximum water holding capacity (WHC)) Test end: 17.8-18.8% (46.9-49.5% WHC)
pH of artificial soil	Test start: 6.4 Test end: 6.3-6.5
Ventilation	All vessels including the additional containers were ventilated on days 2, 5, 7, 9 and 12 by opening the lids for a short period.
Food and feeding regime	One spatula of cheese mites (*Tyrophagus putrescentiae*, cultured by the test facility) at experimental start and on days 2, 5, 7, 9 and 12

Measurements:

Adult mortality and reproduction	After 14 days of exposure, the soil was filled into Millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a heat extractor. The soil including the mites was exposed to a temperature of approximately 25°C to 30°C for about 2 days. Escaping mites were collected in a fixing liquid and cooled at a temperature of approximately 16°C. The fixing liquid contained glycol and a detergent. Adult animals were counted once visually, juvenile animals were counted twice under binocular microscopes. None of the replicate counts deviated by more than 10% from their mean value. In a separate test performed in July 2020, the efficiency of the extraction method was determined to be 96.2%.
Morphological and behavioural abnormalities	At test end
Temperature	Not specified, but either continuously or regularly
pH of the soil samples	At test start and end
Water content of the artificial soil	At test start and end On day 7, the water content of the soil was checked by re-weighing the additional test vessels. Loss of water was not compensated for as it did not deviate by more than 2% from the initial water content.

Experimental dates: 05 to 21 Aug 2020

Calculations:

The endpoints of the test were mortality and reproduction (number of offspring). Furthermore, the percentage reproductive performance of the treated groups based on the control treatment was calculated. The arithmetic means (± standard deviation) per treatment for each endpoint were calculated.

Statistics:

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Mortality data were statistically analysed using Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). The LC_{50} at day 14 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$). Since the reproduction data were normally distributed and homogeneous but did not follow a monotonicity trend (contrast trend), the Dunnett's t-test was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. Due to the lack of a concentration-response relationship, no reliable EC_x calculation was possible and EC_{10}, EC_{20} and EC_{50} could not be reported.

Results and discussions

Validity criteria:

- Mean adult mortality in the control ≤ 20% at the end of the test
- Mean number of juveniles per replicate in the control ≥ 50 at the end of the test
- Coefficient of variation of reproduction per replicate in the control ≤ 30% at the end of the test

Mean adult mortality in the control was 4% at the end of the test. The mean number of juveniles per replicate in the control was 215 at the end of the test (range between 195 and 247). The coefficient of variation of reproduction per replicate in the control was 9.3% at the end of the test. Therefore, all validity criteria were met.

Observations of adult mortality and reproduction are presented in the table below.

Table A2.5.2.1.1-1: Effects of ADM.06001.H.2.B on mortality and reproduction of *Hypoaspis aculeifer*

Treatment (mg product/kg dw)	Mean mortality after 14 days of exposure (% ± SD)	Reproduction after 14 days (mean number of juveniles/replicate ± SD)	Reproduction after 14 days Percentage of control (%)
0 (control)	4 ± 7	215 ± 20[a]	-
16.3	8 ± 10	221 ± 12	103
29.4	5 ± 6	218 ± 18	101
52.9	5 ± 10	223 ± 24	103
95.3	3 ± 5	226 ± 24	105
171	0 ± 0	216 ± 30	100
309	10 ± 14	231 ± 30	107
556	5 ± 6	232 ± 22	108
1000	5 ± 6	233 ± 13	108

Note: There were no statistically significant differences in mortality and reproduction between test substance treatments and control (mortality: Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater; reproduction: Dunnett's t-test, $\alpha = 0.05$, one-sided smaller)
SD Standard deviation
[a] Coefficient of variation: 9.3%

Based on the study results, the following endpoints were obtained.

Table A2.5.2.1.1-2: Endpoints for effects of ADM.06001.H.2.B on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	(mg product/kg dw)
EC_{10} (reproduction)	> 1000
EC_{20} (reproduction)	> 1000
EC_{50} (reproduction)	> 1000
NOEC (reproduction)	1000
LOEC (reproduction)	> 1000
NOEC (mortality)	1000
LOEC (mortality)	> 1000
LC_{50} (mortality)	> 1000

After 14 days of exposure, there was 4% mortality in the control and 0-10% mortality in all test substance treatments from 16.3 to 1000 mg product/kg dw. There were no statistically significant differences in mortality between the control and all test substance treatment groups (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$). Therefore, the NOEC with respect to mortality was 1000 mg product/kg dw. The LC_{50} could not be determined but was > 1000 mg product/kg dw. No differences in morphology or behaviour of the mites between the test substance treatment groups and the control were observed.

After 14 days of exposure, the mean number of juveniles per replicate was 215 in the control and 216 to 233 in all test substance treatments from 16.3 to 1000 mg product/kg dw. There were no statistically significant differences in reproduction between the control and all test substance treatment groups (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller). Therefore, the NOEC with respect to reproduction was 1000 mg product/kg dw. The EC_x values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10}, EC_{20} and EC_{50} were estimated to be > 1000 mg product/kg dw.

Conclusion

In this test on sub-lethal effects of ADM.06001.H.2.B on the predatory mite *Hypoaspis aculeifer*, the EC_{10}, EC_{20}, and EC_{50} for reproduction were estimated to be all > 1000 mg product/kg dw. The NOEC for reproduction and the NOEC for mortality were both determined as 1000 mg product/kg dw.

A 2.5.2.1.2 Effects on *Folsomia candida*

Comments of zRMS:	The study was conducted in line with OECD 232 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). The reliability of the EC_{10} value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: - NW (normalised width) of 0.49 was calculated, which results in rating "good" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, - median EC_{10} (281 mg/kg soil dw) is higher than $EC_{20,low}$ (246 mg/kg dw), but lower than $EC_{50,low}$ (377.3 mg/kg soil dw), - the dose-response curve is medium with steepness of 0.66. Taking the above results into account, the overall certainity of the protection level is medium and the calculated EC_{10} is considered to be sufficiently reliable. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC = 296 mg product/kg soil dw EC_{10} = 281 mg product/kg soil dw

Reference:	KCP 10.4.2.1/02
Report	ADM.06001.H.2.B: Effects on Reproduction of *Folsomia candida* (Collembola: Isotomidae) in Artificial Soil, Straube D., 2020b, 140711016 (ADAMA No. 000105376)
Guideline(s):	OECD 232 (2016), ISO 11267 (2014)
Deviations to OECD 232 (2016):	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022

Reference substance	The reference substance boric acid was evaluated in a separate test according to OECD 232 (2016). In this test performed in October/November 2019, an EC_{50} of 104.6 mg/kg dw (95% confidence interval: 98.5 – 110.1 mg/kg dw) was obtained. This result demonstrates that the test organisms in the test system responded within the normal level (EC_{50} at about 100 mg/kg dw).

Test organism:

Test species	Collembolan *Folsomia candida* (Willem 1902)
Origin	Bred at the test facility
Age at test start	Juvenile collembolans, 9-12 days old
Acclimatisation	The synchronised test organisms were fed with granulated dry yeast and were kept under breeding conditions until test start.
No. of test organisms per replicate (test vessel)	10
No. of replicates per treatment group	8 for the control, 4 for the test substance treatments 1 additional container per treatment to determine the pH and water content of the artificial soil after 28 days (and weight at test start and again on day 14)

Test conditions:

Test substance concentration	58.5, 87.8, 132, 198, 296, 444, 667 and 1000 mg product/kg dw The test concentrations were chosen based on the results of a range-finding test. A stock solution of the test substance (= application solution of the highest test concentration) was prepared by diluting 850.3 mg to 103.2 g with deionised water. The application solutions of the lower test concentrations were prepared as a dilution series of the stock solution.
Reference substance concentration	The reference substance was tested in a separate study at 30.5, 48.8, 78.1, 125 and 200 mg/kg dw.
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 5% *Sphagnum* peat (air-dried, finely ground and with no visible plant remains) - 20% kaolinite clay (kaolinite content > 30%) - 0.2% calcium carbonate (for adjustment of pH to 6.0±0.5) - 74.8% fine quartz-sand (> 50% of particles between 50 and 200 µm) The artificial soil was moistened to approximately half of the final water content 2 days before application. The additional water required to achieve the final water content was added when applying the test substance.
Application method	25.5 g of the stock solution or the corresponding dilutions were added to artificial soil equivalent to 210 g dry weight to obtain the targeted concentrations. The soil for each treatment group was mixed with a laboratory mixer to ensure homogeneous distribution. Each group was treated in one batch and then split into the replicates. The test organisms were collected with an aspirator, put into a small glass tube, counted to ensure that 10 individuals were introduced and placed onto the surface of the treated artificial soil within two hours after preparation of the final test substrate.
Test duration	28 days
Test vessels	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g ± 1.0 g

	artificial soil (dry weight equivalent). The height of the soil layer in the containers was 2 to 3 cm.
Temperature	20 ± 1°C (nominal mean, with temperature range of 20 ± 2°C), 20.2°C (actual mean, with temperature range of 19.9-20.7°C)
Light intensity	400-800 lux
Photoperiod	16 hours light, 8 hours dark
Water content of artificial soil	Test start: 19.9-20.1% (52.4-53.0% of the maximum water holding capacity (WHC)) Test end: 17.5-19.3% (46.0-50.7% WHC)
pH of artificial soil	Test start: 6.3-6.4 Test end: 6.0
Ventilation	All vessels including the additional containers were ventilated on days 2, 5, 7, 9, 12, 14, 16, 19, 21, 23 and 26 by opening the lids for a short period.
Food and feeding regime	After the introduction of the test organisms (day 0), and after 14 days, approximately 2 mg (one spoon spatula) of granulated dried yeast were spread over the soil surface.

Measurements:

Adult mortality and reproduction	At day 28 The content of the test containers was suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. The collembola drifted to the surface. Adult animals were counted once visually, juvenile animals were counted using FolsomiaCounter, a photo-based evaluation software, which automatically determines the number of juvenile animals from a digital photograph (validated counting system, FolsomiaCounter Version 1.23, © 2020 Visionalytics). In a separate test performed in December 2019, the efficiency of the extraction method was determined to be 98.3%.
Behaviour of surviving collembola	At day 28
Temperature	Not specified, but either continuously or regularly
pH of the soil samples	At test start and end
Water content of the soil samples	At test start and end On day 14, the water content of the soil was checked by re-weighing the additional test vessels. Loss of water was not compensated for as it did not deviate by more than 2% from the initial water content.

Experimental dates: 01 to 31 Jul 2020

Calculations:

The endpoints of the test were mortality and reproduction (number of juveniles). Furthermore, the percentage reproductive performance of the treated groups based on the control treatment was calculated. The arithmetic means (± standard deviation) per treatment for each endpoint were calculated.

Statistics:

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Mortality data were statistically analysed using Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). An LC_{50} value and its 95% confidence limits at day 28 was calculated by applying Weibull Analysis.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$). Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend), the Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values for reproduction were calculated by Probit Analysis.

Results and discussions

Validity criteria:

- Mean adult mortality in the control is ≤ 20% at the end of the test
- Mean number of juveniles per replicate in the control is ≥ 100 at the end of the test
- Coefficient of variation of reproduction in the control is ≤ 30% at the end of the test

Mean adult mortality in the control was 5% at the end of the test. The mean number of juveniles per replicate in the control was 1005 (range between 747 and 1370) and the coefficient of variation of reproduction in the control was 22.4% at the end of the test. Therefore, all validity criteria were met.

Observations of adult mortality and reproduction are presented in the table below.

Table A2.5.2.1.2-1: Effects of ADM.06001.H.2.B on mortality and reproduction of *Folsomia candida*

Treatment (mg product/kg dw)	Mean mortality after 28 days of exposure (% ± SD)	Reproduction after 28 days (mean number of juveniles/replicate ± SD)	Reproduction after 28 days Percentage of control (%)
0 (control)	5 ± 8	1005 ± 225[a]	-
58.5	3 ± 0	940 ± 235	93.5
87.8	5 ± 10	893 ± 147	88.9
132	5 ± 6	976 ± 81	97.1
198	0 ± 0	991 ± 247	98.6
296	8 ± 5	836 ± 216	83.2
444	33* ± 5	487** ± 233	48.5
667	75* ± 29	27** ± 44	2.6
1000	100* ± 0	0** ± 0	0.0

SD Standard deviation
* Statistically significantly different from the control (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater)
** Statistically significantly different from the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)
[a] Coefficient of variation: 22.4%

Based on the study results, the following endpoints were obtained.

Table A2.5.2.1.2-2: Endpoints for effects of ADM.06001.H.2.B on mortality and reproduction of *Folsomia candida*

Endpoint	(mg product/kg dw)
EC_{10} (reproduction) (95% confidence interval)	281.0[a] (191.0 – 329.1)
EC_{20} (reproduction) (95% confidence interval)	323.7 (246.0 – 366.6)
EC_{50} (reproduction) (95% confidence interval)	424.4 (377.3 – 477.0)
NOEC (reproduction)	296
LOEC (reproduction)	444
NOEC (mortality)	296
LOEC (mortality)	444
LC_{50} (mortality) (95% confidence interval)	562.0 (518.0 – 606.5)

[a] The calculated EC_{10} value cannot be considered to be a reliable endpoint (EFSA 2019) since the lower 95% confidence interval of the EC_{20} is lower than the median EC_{10} value and a visual check of the reproduction results shows high variability about the lower concentrations.

After 28 days of exposure, there was 5% mortality in the control treatment and 0-8% mortality in the

test substance treatments from 58.5 to 296 mg product/kg dw. Mortality in the test substance treatments of 444, 667 and 1000 mg product/kg dw was 33%, 75% and 100%, respectively, which was statistically significantly different from the control (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). Thus, the NOEC and LOEC with respect to springtail mortality were 296 and 444 mg product/kg dw, respectively. The LC_{50} was calculated as 562.0 (95% confidence interval: 518.0 – 606.5) mg product/kg dw based on Weibull Analysis. No abnormal behaviour was observed for the surviving collembola.

The mean number of juveniles produced per replicate was 1005 in the control and 836-991 in the test substance treatments from 58.5 to 296 mg product/kg dw. In the test substance treatments of 444, 667 and 1000 mg product/kg dw, the mean number of juveniles produced per replicate was 487, 27 and 0, respectively, which was statistically significantly different from the control (Williams t-test, $\alpha = 0.05$, one-sided smaller). Thus, the NOEC and LOEC with respect to springtail reproduction were 296 and 444 mg product/kg dw, respectively. The EC_{10}, EC_{20} and EC_{50} were determined as 281.0 (95% confidence interval: 191.0 – 329.1), 323.7 (95% confidence interval: 246.0 – 366.6) and 424.4 (95% confidence interval: 377.3 – 477.0) mg product/kg dw.

Conclusion

In this test on sub-lethal effects of ADM.06001.H.2.B on the collembolan *Folsomia candida*, the EC_{10}, EC_{20}, and EC_{50} for reproduction were determined to be 281.0, 323.7 and 424.4 mg product/kg dw, respectively. The NOEC for both reproduction and mortality was 296 mg product/kg dw and the LC_{50} was determined to be 562.0 mg product/kg dw.

A 2.5.2.2 KCP 10.4.2.2 Higher tier testing

A 2.6 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted in line with OECD 216 with no deviations. All the validity criteria were met and the study is considered acceptable. It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (28 days) up to 14 mg product/kg soil dw

Reference:	KCP 10.5/01
Report	ADM.06001.H.2.B: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation), Hammesfahr U., 2020, 140711080 (ADAMA No. 000105378)
Guideline(s):	OECD 216 (2000)
Deviations:	None ~~No major deviations~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	Sodium chloride was tested at 16 g/kg dw in a separate study within one year before start of the experimental phase of this study.

Test soil:

Name	F 5M
Origin	"In der Speyerer Hohl ", No. 977 Mechtersheim, Rhineland Palatinate, Germany
Cultivation	Fallow grassland
History	No pesticides or organic or mineral fertilizer had been used on the soil for at least four years prior to test initiation.
Batch	F 5M 1320
Soil sampling	From the top 20 cm 25 Mar 2020
Soil preparation	After arrival at the sampling laboratory, the soil was air dried, pre-sieved (mesh 10 mm) and sieved (mesh 2 mm) at room temperature.
Soil storage	The soil was stored at 20°C ± 2°C with appropriate ventilation and periodical moisture adjustment for 4 days (time between sieving and receipt at the testing laboratory, where pre-

	incubation started directly).
Soil pre-incubation	At 20±2°C for 9 days
Physico-chemical properties	Parameters determined by the supplier for the same soil (different batch): pH: 7.4 C_{org}: 0.89% Total N: 0.11% NH_4^+-N: 0.317 mg/kg dw NO_2^--N: 0.010 mg/kg dw NO_3^--N: 25.742 mg/kg dw N_{min}-N: 26.069 mg/kg dw Cation exchange capacity: 12.7 meq/kg dw Max. water holding capacity: 40.8% Particle size distribution: Clay: 11.4% Silt: 36.0% Sand: 52.6% Soil texture: Sandy loam Parameters determined by the testing laboratory on the batch used in the test: Dry weight: 89.31% Microbial biomass: 291.01 mg C/kg dw= 3.27% of C_{org} NO_3^--N: 10.842 mg/kg dw

Test conditions:

Test substance concentration	1.40 (low dose) and 14.0 (high dose) mg product/kg dry soil, corresponding with 1 and 10 L product/ha assuming a soil density of 1.5 g/cm³ and a soil depth of 5 cm A stock solution was prepared by dissolving 45 mg ADM.06001.H.2.B in 50 mL ultrapure water.
Reference substance concentration	16 g/kg dw (separate study within one year before start of the experimental phase of this study)
Control	Ultrapure water
No. of replicates per treatment	3
Application method	Appropriate amounts of the test substance stock solution and additionally 0.5% lucerne meal (related to soil dry weight) were mixed into the soil using a laboratory mixer. Throughout application, the soil was ventilated and the soil water content was adjusted to 50% of the maximum water holding capacity. For the control, ultrapure water and additionally 0.5% lucerne meal (related to soil dry weight) were mixed into the soil. Throughout application, the soil was ventilated and the soil water content was adjusted to 53% of the maximum water holding capacity. The lucerne meal had a carbon to nitrogen ratio of 15.0:1.
Test duration	28 days
Test vessels	Disposable plastic boxes of approximately 0.5 L (dimensions: 0.10 m width x 0.10 m depth x 0.065 m height) An amount of 300 g soil (dry weight equivalent) was filled loosely into the boxes, which were covered by perforated lids to allow air exchange to ensure aerobic incubation conditions.
Temperature	20±2°C Short-term deviations (< 2 hours) from the recommended temperature range were not regarded to result in major disturbances of the test performance and were not reported.
Illumination	Constant darkness
Water content of the soil	Soil dry weight: 82.1-83.3%, corresponding with a water content of 16.7-17.9% (equivalent to 49-53% of the maximum water holding capacity)

pH of soil	7.2-7.3

Measurements:

NH_4^+-N, NO_2^--N and NO_3^--N contents of the soil samples	Soil samples were taken within 6 hours (day 0) and on days 7, 14 and 28 after application. An amount of 24 g to 25 g soil was suspended in 100 mL 0.1 M KCl solution and agitated for one hour. After centrifugation, the extracts were stored deep frozen. Frozen extract samples were thawed and NH_4^+-N, NO_3^--N and NO_2^--N contents were determined photometrically using a AA3 Continuous Flow Analyser (wavelengths: 550 nm for NO_3^--N and NO_3^--N, 660 nm for NH_4^+-N). Quantification was performed with solutions of ammonium sulphate (0.5-3.0 mg/L), sodium nitrite (0.5-3.0 mg/L) and potassium nitrate (1.0-12.0 mg/L) in 0.1 M KCl.
Test conditions	Temperature: Continuously Soil dry weight and water content: 0, 7, 14 and 28 d The soil water content was checked once a week until test end by re-weighing each test container and adding ultrapure water as needed to compensate water losses. pH: 0 and 28 d

Experimental dates: 07 Apr to 08 May 2020

Calculations:

Amounts of NH_4^+-N, NO_2^--N and NO_3^--N were calculated based on concentrations determined in soil extracts and the amount of extracted soil. The mineral nitrogen content (N_{min}) was calculated as sum of the three nitrogen species (N_{min} = NH_4^+-N + NO_2^--N + NO_3^--N). For each test group, the mean NH_4^+-N, NO_2^--N, NO_3^--N and N_{min} were calculated for the three replicates, including the coefficient of variance for the control group.

The nitrate formation rate was calculated using an incremental approach i.e., the difference between soil nitrate contents from two consecutive sampling times; i.e., NO_3^--N per day = [NO_3^--N (d 7) - NO_3^--N (d 0)]/7 days or [NO_3^--N (d 14) - NO_3^--N (d 7)]/7 days or [NO_3^--N (d 28) - NO_3^--N (d 14)]/14 days. Furthermore, the % deviations in nitrate formation rate between the control and the test substance treatment groups were calculated.

Statistics:

The nitrate formation rates were tested for normality and homogeneity of variance using the R/S-Test (α = 0.01) and Levene´s test (α = 0.01), respectively. The Student t-test (pair wise comparison, two-sided, α = 0.05) was used for comparison of test substance treatment groups and control.

The software used to conduct the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and discussions

Validity criterion:

- Variation between replicate control samples: ≤ ± 15%

The variation between replicate control samples at day 28 was 6.0%, 0.0%, 0.88% and 0.91% for NH_4^+-N, NO_2^--N, NO_3^--N and N_{min} contents, respectively. Furthermore, the variation in nitrate formation rates between replicate control samples was 2.77% for the interval between the last two sampling times (days 14-28). Therefore, the validity criterion was met.

NH_4^+-N, NO_2^--N, NO_3^--N and N_{min} contents and nitrate formation rates are presented in the following two tables.

Table A2.6-1: NH_4^+-N, NO_2^--N, NO_3^--N and N_{min} contents in the nitrogen transformation test

Test group	Control		1.40 mg product/kg dw	14.0 mg product/kg dw
Sampling (days)	NH_4^+-N content (mg/kg dw)	Coefficient of variation (%)	NH_4^+-N content (mg/kg dw)	NH_4^+-N content (mg/kg dw)
0	17.594	0.880	17.082	16.794
7	11.959	1.750	11.368	11.253
14	0.866	1.040	0.799	0.801
28	0.650	6.000	0.622	0.609
Sampling (days)	NO_2^--N content (mg/kg dw)	Coefficient of variation (%)	NO_2^--N content (mg/kg dw)	NO_2^--N content (mg/kg dw)
0	0.732	2.32	0.689	0.683
7	0.513	1.17	0.493	0.499
14	0.126	0.00	0.126	0.126
28	0.126	0.00	0.126	0.126
Sampling (days)	NO_3^--N content (mg/kg dw)	Coefficient of variation (%)	NO_3^--N content (mg/kg dw)	NO_3^--N content (mg/kg dw)
0	11.682	0.21	11.469	11.486
7	10.908	3.90	9.874	10.145
14	21.804	1.47	20.687	21.451
28	37.501	0.88	36.040	36.687
Sampling (days)	N_{min} content (mg/kg dw)	Coefficient of variation (%)	N_{min} content (mg/kg dw)	N_{min} content (mg/kg dw)
0	30.008	0.50	29.241	28.963
7	23.380	2.09	21.735	21.896
14	22.795	1.43	21.612	22.378
28	38.277	0.91	36.788	37.422

Note: The values are means of triplicate samples.
Measured values below LOQ were set at LOQ.
LOQ: 0.383 mg NH_4^+-N/kg dw
LOQ: 0.126 mg NO_2^--N/kg dw
LOQ: 0.159 mg NO_3^--N/kg dw

Table A2.6-2: Nitrate formation rates in the nitrogen transformation test

Test group	Control		1.40 mg product/kg dw		14.0 mg product/kg dw	
Time interval (days)	NO_3^--N formation rate		NO_3^--N formation rate		NO_3^--N formation rate	
	Mean ± SD (mg/kg dw/day)	CV (%)	Mean ± SD (mg/kg dw/day)	Dev. from control (%)	Mean ± SD (mg/kg dw/day)	Dev. from control (%)
0-7	-0.110 ± 0.058	-52.73	-0.228 ± 0.127	107.27	-0.191 ± 0.091	73.64
7-14	1.557 ± 0.064	4.11	1.544 ± 0.056	-0.83	1.615 ± 0.134	3.73
14-28	1.121 ± 0.031	2.77	1.097 ± 0.021	-2.14	1.089 ± 0.085	-2.85

Note: The values are means of triplicate samples.
There were no statistically significant differences in nitrate formation rates between the test substance treatment groups and the control at the time interval of 14-28 days (Student t-test, pair wise comparison, two-sided, $\alpha = 0.05$).
SD: Standard deviation
CV: Coefficient of variation

At the end of the 28-day exposure, NH_4^+-N contents were 0.650, 0.622 and 0.609 mg/kg dw, NO_2^--N contents were 0.126, 0.126 and 0.126 mg/kg dw (= LOQ), NO_3^--N contents were 37.501, 36.040 and 36.687 mg/kg dw and N_{min} contents were 38.277, 36.788 and 37.422 mg/kg dw in the control and the test soil treated at 1.40 and 14.0 mg product/kg dw, respectively.

For the interval between the last two sampling times (days 14-28), nitrate formation rates were 1.121, 1.097 and 1.089 mg/kg dw/day in the control and the test soil treated at 1.40 and 14.0 mg product/kg dw, respectively. The deviations in nitrate formation rate between the test substance treatments at 1.40 and 14.0 mg product/kg dw and the control were -2.14% and -2.85%, respectively, and were thus below the trigger of 25%. Furthermore, there were no statistically significant differences in nitrate formation rates between the test substance treatment groups and the control at the time interval of 14-28 days

(Student t-test, pair wise comparison, two-sided, $\alpha = 0.05$). Therefore, no adverse effects of the test substance on nitrogen transformation in the test soil were observed up to 14.0 mg product/kg dw.

In a separate study, the reference substance sodium chloride was tested within one year before start of the experimental phase of this study. The reference substance had a retarding effect of more than ± 25% compared to the control at days 28 (-42.60% NO_3^--N content, -98.51% nitrate formation rate) and 98 (-68.42% NO_3^--N content, -117.39% nitrate formation rate) after application. Therefore, the sensitivity of the test system and adequate laboratory test conditions were demonstrated.

Conclusion

In this nitrogen transformation test, the test substance ADM.06001.H.2.B caused no adverse effects (deviation from control < 25%) on soil nitrogen transformation (measured as nitrate formation rate per day) at the end of the 28-day incubation period when tested up to 14.0 mg product/kg dw.

A 2.7 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.7.1 KCP 10.6.1 Summary of screening data

A 2.7.2 KCP 10.6.2 Testing on non-target plants

A 2.7.2.1 Seedling Emergence

Comments of zRMS:	The study was conducted in line with OECD 208 with minor deviations to the guideline and minor deviations to the study plan. It was noted that the salt content as electronic conductivity of the soil used to grow the seedlings was not reported. For plant dry weight determination, the plants were dried to constant weight at ≥ 70°C instead of 60°C. These deviations are considered to have no impact on the quality and integrity of the study. It was also noted that mesosulfuron-methyl expired during the course of the study but a certified reference material was used for determination of mesosulfuron-methyl concentrations. Therefore, this deviation is considered to have no effect on the outcome of the study. The analytical measurements showed that the concentrations of both active substances were within 80 – 120 % of nominal; therefore, the endpoint can be expressed as nominal concentration. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The most sensitive species was radish *Raphanus sativus* $ER_{50,\ dry\ weight}$ = 351 mL product/ha $ER_{20,\ dry\ weight}$ = 48.4 mL product/ha $ER_{10,\ dry\ weight}$ = 17.2 mL product/ha Phytotoxic effects higher than 50% were observed at 1000 mL product/ha for oilseed rape and radish (54.0% and 65.0%, respectively). Sugar beet and perennial ryegrass showed phytotoxicity values of 43% and 18% at 1000 mL prod./ha, respectively. For all other species, phytotoxic effects remained low (≤ 7%) at 1000 mL prod./ha. Therefore, the ER_{50} for phytotoxicity is > 1000 ml product/ha.

Reference:	KCP 10.6.2/01
Report	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test, Spatz, B. and Kowalczyk, F., 2021a, 140711086 (ADAMA No 000105379)
Guideline(s):	OECD 208 (2006)
Deviations:	Minor (see the commenting box above) ~~The texture and salt content of the soil used to grow the seedlings is not reported. For plant dry weight determination, the plants were dried to constant weight at ≥ 70°C instead of 60°C.~~ ~~These deviations are considered minor, not having an impact on the study quality and integrity.~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	No reference substance was used.

Test organism:

Test species	Dicotyledons: Oilseed rape, *Brassica napus* (Brassicaceae) Radish, *Raphanus sativus* (Brassicaceae) Soybean, *Glycine max* (Fabaceae) Sunflower, *Helianthus annuus* (Asteraceae) Tomato, *Solanum lycopersicum* (Solanaceae) Sugar beet, *Beta vulgaris* (Amaranthaceae) Monocotyledons: Corn, *Zea mays* (Poaceae) Perennial ryegrass, *Lolium perenne* (Poaceae) Oat, *Avena sativa* (Poaceae) Onion, *Allium cepa* (Amaryllidaceae)
Origin of test species	For a given test species, all seeds used in the test were from the same source and lot number.
No. of seeds per pot	2 (soybean, tomato, sugar beet and corn) 3 (oilseed rape, radish and sunflower) 5 (perennial ryegrass, oat and onion)
No. of replicates (pots) per treatment group	6 (perennial ryegrass, oat and onion) 10 (oilseed rape, radish and sunflower) 15 (soybean, tomato, sugar beet and corn)
No. of seeds per treatment group	30 3 (Brassica napus, Raphanus sativus, Helianthus annuus) 2 (Glycine max, Beta vulgaris, Solanum lycopersicum) 5 (Lolium perenne, Avena sativa, Allium cepa)
Bioassay pots	Commercial plastic flowerpots (15 cm in diameter)
Preparation	The seeds were introduced manually into the soil within 24 hours.
Fertilisation	After development of the first true leaves, Ferty® 9 “Hydro” (Planta-Düngemittel GmbH) at 3 g/L and Terraflor®-AZ (Terraflor GmbH) at 0.4 g/L were added to the water up to two times a week, depending on the development of the plants. Pots of one treatment obtained the same level of fertilizer.
Watering	After sowing, the pots were placed on saucers and watered. Bottom watering (through saucers) was done where necessary after a daily check.

Test soil:

Soil batch	LUFA 2.3
Soil type (USDA)	Sandy loam
Physico-chemical properties Soil particle size (mm): Organic carbon (%):	 ≤ 2 0.65±0.08

pH:	6.1±0.4
Pesticide treatments:	None in the year of sampling and for at least the two previous years
Soil pre-treatment	The soil was steam sterilised.

Test conditions:

Test substance concentration	All test species: 12.3, 37.0, 111, 333 and 1000 mL prod./ha Additionally, sunflower and sugar beet were tested at 1.37 and 4.12 mL prod./ha and tomato was tested at 0.457, 1.37 and 4.12 mL prod./ha. The test rates were chosen based on the results of a range-finding test. Application in 200 L water/ha A stock solution (= application solution of the highest test rate) was prepared by weighing 9.70 g of homogenised ADM.06001.H.2.B into a glass beaker, transferring it to a 2000-mL volumetric flask and filling it up to the mark with deionised water. This corresponded with 4.85 g prod./L or with 1000 mL prod./ha in 200 L/ha. After sampling for analysis and thorough stirring, the further application solutions were prepared by serial dilution with a geometric factor of 3, i.e., 600 g were filled up to 1800 g with deionised water. Before dilution and before application, the application solutions were stirred intensively.
Control	Deionised water
Application time	Application was performed pre-emergence, i.e., one day after sowing.
Application method	Application was conducted using freshly prepared control and test substance application solutions and calibrated laboratory spraying equipment. Applications were made using a laboratory-spraying equipment (Fa. Schachtner, 71640 Ludwigsburg, Germany) with a TeeJet 8002 EVS spray nozzle. For all applications, the pressure was 2.00 bar and the nozzles were 40.0 cm above the soil surface. The sprayer speed was 2.50 km/h for test rates up to and including 111 mL prod./ha but was 2.25 km/h for the two highest test rates of 333 and 1000 mL prod./ha. The sprayer was calibrated using a glass plate of known surface area with filter paper in the same size by spraying with deionised water and weighing immediately before and after application to deliver 200 L/ha ± 10%. Verification of the applied amount was thereafter performed for each test rate applied by the same method. Deviation in the spray deposit applied did not exceed ± 10% of nominal for any application rate. The uniformity of the deposit distribution was checked visually.
Test duration	14 or 21 days after 50% emergence of seedlings in the control
Test facility type	The test was performed in a growth chamber under controlled conditions.
Temperature	22 ± 10ºC (nominal), 16.7-23.3°C (mean: 20.3°C, actual)
Relative humidity	70% ± 25% (nominal), 47% – 74% (mean: 57%, actual)
Light intensity	350 ± 50 µE/m^2/s (nominal), 300-400 µmol/m^2/s (mean: 353 µmol/m^2/s, actual)
Photoperiod	16 hours light : 8 hours dark

Observations:

Observation period	14 or 21 days following 50% emergence in the control plants
Emergence, mortality and visual phytotoxicity (e.g., chlorosis, necrosis and	Day 7 and 14, and day 21 if species were exposed longer

deformation)	
Plant fresh weight	Day 14 or 21 (final assessment)
Plant height	Day 14 or 21 (final assessment)
Growth stages	Day 7 and 14, and day 21 if species were exposed longer
Test conditions	Temperature and relative humidity: every 15 minutes Light intensity: once a week with 5 different measuring points for each species at the top of the canopy

Analytical method:

Samples	The concentration of the test substance in the stock solution and the control was verified by analysis of mesosulfuron-methyl, pinoxaden, NOA 407854 (M2, metabolite of pinoxaden) and mefenpyr-diethyl. Three samples (5 mL each) from the continuously stirred, homogeneous stock solution (= application solution of the highest test rate) and the control were taken before application. One sample was analysed directly after sampling. The others were stored deep frozen (≤ -20°C) as retained samples.
Method type	LC-MS/MS
Equipment	API 5500 mass spectrometer with Agilent Series 1290 pump and autosampler
Column	Luna Omega Polar C18 (50 x 3 mm, 3 µm)
Column temperature	40ºC
Flow rate	0.65 mL/min
Mobile phase	A: HPLC-water + 0.1% formic acid B: acetonitrile + 0.1% formic acid Gradient: 2.0 min at 95% A/5% B, in 0.5 min to 50% A/50% B, in 2.0 min to 5% A/95% B, 0.7 min at 5% A/95% B, in 0.1 min to 95% A/5% B, 1.7 min at 95% A/5% B
Injection volume	5 µL
Detector	MSD, positive mode Ion source: 5500 V Temperature: 350°C Scan type: MRM
Mass transitions	Mefenpyr-diethyl: 390 m/z → 327 m/z (quantifier) 390 m/z → 160 m/z (qualifier) Mesosulfuron-methyl: 504 m/z → 182 m/z (quantifier) 504 m/z → 83 m/z (qualifier) Pinoxaden: 401 m/z → 317 m/z (quantifier) 401 m/z → 115 m/z (qualifier) NOA 407854 (M2): 318 m/z → 171 m/z (quantifier) 318 m/z → 131 m/z (qualifier)
Sample preparation	Samples were made up to 25 mL with acetone. These solutions were then diluted with acetonitrile/pure water (50/50, v/v) + 0.1% HCOOH to match the calibration range.

Experimental dates: 24 Nov 2020 to 23 Aug 2021

Calculations:

Mean emergence was calculated as percentage of seeds sown, mean mortality and mean visual phytotoxicity were calculated as percentage of emerged seedlings.

The final mean dry weight per pot and the final mean height per pot (including standard deviations) were calculated for each plant species and treatment group as well as the percentage in comparison to the control and the percentage effect.

Statistics:

Plant dry weight data and plant height data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test (α = 0.01) and the Levene's test (α = 0.01), respectively. In case the data were normally distributed and homogeneous, the Dunnett's t-test (multiple comparison, one-sided smaller, α = 0.05), or in case the data showed a monotonic dose response, the Williams t-test (multiple comparison, one-sided smaller, α = 0.05) were used for comparing treatment groups and control. When the data were normally distributed but not homogeneous, the Bonferroni-Welch t-test (multiple comparison, one-sided smaller, α = 0.05) was used. In case the data were not normally distributed but homogeneous, the Bonferroni-Holm U-test (multiple comparison, one-sided smaller, α = 0.05), or in case the data showed a monotonic dose response, the Step-down-Jonckheere Terpstra test (one-sided smaller, α = 0.05) were used for comparing treatment groups and control.

In order to determine ER_{10}, ER_{20} and ER_{50} values (dry weight and height), a regression analysis was performed (Probit-analysis).

For emergence and mortality data, Fisher's Exact Binomial Test (with Bonferroni Correction, multiple comparison, one-sided greater, α = 0.05) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH. Statistical analyses were conducted in compliance with the recommendations provided by OECD 54 (2006).

Results and discussions

Validity criteria:

- Seedling emergence in controls is at least 70%
- Control seedlings do not exhibit visible signs of phytotoxicity and show normal variation in growth and morphology
- Mean survival of emerged control seedlings is at least 90% for the duration of the study
- Environmental conditions per species are identical and growing media are the same

Seedling emergence in controls ranged from 80% to 100%. The control seedlings did not exhibit visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for the particular species. Mean survival of emerged control seedlings was 96% to 100% for the duration of the study. Environmental conditions per species were identical and growing media were the same. All seeds for a given test species were from the same source and lot number. Therefore, the validity criteria were met.

Analytical verification of the test substance concentration (measured as concentrations of mesosulfuron-methyl, pinoxaden, NOA 407854 (M2, metabolite of pinoxaden) and mefenpyr-diethyl) in the stock solution (= application solution of the highest test rate) and the control is summarised in the table below.

Table A2.7.2.1-1: Verification of the test substance concentration in the stock solution of ADM.06001.H.2.B and in the control

Sample	Analyte	Nominal concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
Stock solution	Mesosulfuron-methyl	58.20	70.277	121
	Pinoxaden	305.55	391.683	128
	NOA 407854 (M2)	n.a.	n.d.	n.a.
	Mefenpyr-diethyl	184.30	213.174	116
Control	Mesosulfuron-methyl	0.00	n.d.	n.a.
	Pinoxaden	0.00	n.d.	n.a.
	NOA 407854 (M2)	n.a.	n.d.	n.a.
	Mefenpyr-diethyl	0.00	n.d.	n.a.

n.a. not applicable

n.d. not detectable

Analytical recoveries of mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl in the stock solution (= application solution of the highest test rate) were 121%, 128% and 116% of nominal, respectively. NOA 407854 (M2), the metabolite of pinoxaden, could not be detected in the stock solution. Thus, study endpoints were calculated and reported in terms of nominal application rates of ADM.06001.H.2.B. In the control application solution (deionised water), none of the analytes were detected.

Effects of ADM.06001.H.2.B on seedling emergence, seedling mortality, plant dry weight, plant height and visual phytotoxicity are summarised in the tables below.

Table A2.7.2.1-2: Seedling emergence (final assessment on day 14 or 21) of plants exposed to ADM.06001.H.2.B

Application rate (mL prod./ha)	Emergence (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	83	100	97	83	90
0.457	-	-	-	-	93
1.37	-	-	-	80	93
4.12	-	-	-	83	93
12.3	87	97	100	70	93
37.0	90	90	100	87	97
111	97	93	100	80	90
333	73	97	100	73	93
1000	97	100	100	83	93
Application rate (mL prod./ha)	**Emergence (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	93	87	80	87	97
0.457	-	-	-	-	-
1.37	97	-	-	-	-
4.12	93	-	-	-	-
12.3	97	97	83	93	100
37.0	97	93	83	97	97
111	93	80	87	80	100
333	93	97	77	87	100
1000	97	100	83	90	97

Note: None of the emergence in the treatment groups is statistically significantly different from the control (multiple sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$)

Table A2.7.2.1-3: Seedling mortality (final assessment on day 14 or 21) of plants exposed to ADM.06001.H.2.B

Application rate (mL prod./ha)	Mortality (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	4	0	0	0	0
0.457	-	-	-	-	0
1.37	-	-	-	0	0
4.12	-	-	-	0	0
12.3	0	0	0	0	0
37.0	0	0	3	0	0
111	0	0	0	0	0
333	0	0	0	0	0
1000	0	0	0	0	0
Application rate (mL prod./ha)	**Mortality (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	0	0	0	0	3
0.457	-	-	-	-	-
1.37	0	-	-	-	-
4.12	0	-	-	-	-
12.3	0	0	0	0	0
37.0	0	7	0	0	0

111	0	0	0	0	0
333	0	0	0	0	0
1000	0	0	4	0	3

Note: None of the mortality in the treatment groups is statistically significantly different from the control (multiple sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05)

Table A2.7.2.1-4: Effects on plant dry weight (final assessment on day 14 or 21) from exposure to ADM.06001.H.2.B

Application rate (mL prod./ha)	Plant dry weight (g) / Effect (%)				
	Oilseed rape	**Radish**	**Soybean**	**Sunflower**	**Tomato**
Control	1.20 / -	1.11 / -	1.08 / -	2.38 / -	1.24 / -
0.457	-	-	-	-	1.30 / 5.2
1.37	-	-	-	2.40 / 1.0	1.27 / 2.1
4.12	-	-	-	2.60 / 9.4	1.29 / 3.8
12.3	1.27 / 5.6	1.18 / 6.6	1.10 / 1.8	2.03 / -14.6	1.14 / -8.2
37.0	1.50 / 24.6	0.79* / -28.5	1.14 / 5.5	2.40 / 0.8	1.43 / 15.5
111	1.15 / -4.3	0.84* / -23.9	1.04 / -3.5	2.15 / -9.8	1.20 / -3.1
333	0.74* / -38.7	0.54* / -51.4	1.15 / 6.6	1.53* / -35.8	1.19 / -4.4
1000	0.38* / -68.1	0.37* / -66.8	1.30 / 20.5	1.31* / -45	1.00 / -19.3
Application rate (mL prod./ha)	**Plant dry weight (g) / Effect (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	0.55 / -	0.96 / -	1.15 / -	0.91 / -	0.19 / -
0.457	-	-	-	-	-
1.37	0.65 / 19.1	-	-	-	-
4.12	0.70 / 28.5	-	-	-	-
12.3	0.70 / 27.9	1.08 / 13.0	1.31 / 14.3	1.01 / 11.1	0.23 / 21.3
37.0	0.71 /28.7	1.23 / 27.9	1.37 / 19.4	1.10 / 21.2	0.22 / 16.9
111	0.63 / 14.3	0+.96 / 0.03	1.28 / 11.5	0.91 / 0.7	0.22 / 20
333	0.44* / -19.8	1.21 / 25.8	1.37 / 18.8	1.00 / 10.2	0.23 / 20.7
1000	0.17* / -68.5	1.32 / 37.4	0.73* / -36.5	1.07 / 18.4	0.19 / 4.3

* Statistically significantly different from the control (multiple comparison Williams t-test, α = 0.05, or multiple comparison Dunnett's t-test, α = 0.05)

Table A2.7.2.1-5: Effects on plant height (final assessment on day 14 or 21) from exposure to ADM.06001.H.2.B

Application rate (mL prod./ha)	Plant height (mm) / Effect (%)				
	Oilseed rape	**Radish**	**Soybean**	**Sunflower**	**Tomato**
Control	94 / -	107 / -	83.83 / -	184 / -	86 / -
0.457	-	-	-	-	85 / -1.6
1.37	-	-	-	122* / -33.8	87 / 0.6
4.12	-	-	-	115* / -37.5	75 / -13.2
12.3	91 / -3.5	109 / 1.9	82.17 / -2.0	110* / -40.1	81 / -5.8
37.0	88 / -6.5	88* / -17.3	79.17 / -5.6	106* / -42.3	79 / -8.7
111	89 / -5.5	81* / -24.1	80.17 / -4.4	107* / -41.9	77* / -10.9
333	86 / -8.8	83* / -22.5	80.17 / -4.4	106* / -42.4	76* / -11.8
1000	55* / -41.8	61* / -43.2	81.33 / -3.0	89* / -52.0	74* / -14.0
Application rate (mL prod./ha)	**Plant height (mm) / Effect (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	89 / -	413 / -	299 / -	456 / -	166 / -
0.457	-	-	-	-	-
1.37	91 / 2.4	-	-	-	-
4.12	94 / 6.4	-	-	-	-
12.3	92 / 4.1	409 / -0.9	306 / 2.2	457 / 0.3	151 / -9.0
37.0	91 / 2.6	437 / 5.8	292 / -2.5	443 / -3.0	164 / -1.1
111	89 / 0.8	432 / 4.5	294 / -1.7	426 / -6.7	159 / -4.1
333	77 / -13.4	420 / 1.7	310 / 3.7	431 / -5.4	165 / -0.8
1000	48* / -45.4	451 / 9.2	205* / -31.3	437 / -4.1	163 / -2.0

* Statistically significantly different from the control (multiple comparison Williams t-test, α = 0.05, multiple comparison Dunnett's t-test, α = 0.05, Step-down Jonckheere-Terpstra Test, α = 0.05, multiple comparison Bonferroni-Welsh t-test, α = 0.05, or multiple sequentially-rejective U-Test after Bonferroni-Holm, α = 0.05)

Table A2.7.2.1-6: Phytotoxicity (final assessment on day 14 or 21) of plants exposed to ADM.06001.H.2.B

Application rate (mL prod./ha)	Phytotoxicity (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	5	0	0	0	0
0.457	-	-	-	-	0
1.37	-	-	-	0	0
4.12	-	-	-	0	0
12.3	2	0	0	0	0
37.0	8	0	3	0	0
111	14	0	3	3	0
333	15	35	0	0	0
1000	54	65	0	7	0
Application rate (mL prod./ha)	**Phytotoxicity (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	0	0	0	0	0
0.457	-	-	-	-	-
1.37	0	-	-	-	-
4.12	0	-	-	-	-
12.3	0	1	0	0	0
37.0	0	0	0	0	0
111	0	0	0	4	0
333	11	1	0	4	0
1000	43	2	18	5	4

Calculated ER_{10}, ER_{20}, ER_{50} and NOER values for the different parameters for each plant species are presented in the table below.

Table A2.7.2.1-7: ER_{10}, ER_{20}, ER_{50} and NOER for various endpoints for seedlings exposed to ADM.06001.H.2.B

Endpoint (mL prod./ha)	Oilseed rape	Radish	Soybean	Sun-flower	Tomato	Sugar beet	Corn	Perennial ryegrass	Oat	Onion
Seedling emergence										
ER_{10}	> 1000									
ER_{20}	> 1000									
ER_{50}	> 1000									
NOER	1000									
Mortality										
LR_{10}	> 1000									
LR_{20}	> 1000									
LR_{50}	> 1000									
NOER	1000									
Plant dry weight										
ER_{10}	119	17.2	n.d.	63.1	313	56.2	n.d.	n.d.	n.d.	n.d.
ER_{20}	199	48.4	n.d.	172	n.d.	147	n.d.	n.d.	n.d.	n.d.
ER_{50}	534	351	> 1000	> 1000	> 1000	931	> 1000	> 1000	> 1000	> 1000
NOER	111	12.3	1000	111	1000	111	1000	333	1000	1000
Plant height										
ER_{10}	328	26.1	n.d.	n.d.	94.7	74.3	n.d.	208	n.d.	n.d.
ER_{20}	520	119	n.d.	n.d.	n.d.	293	n.d.	785	n.d.	n.d.
ER_{50}	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
NOER	333	12.3	1000	< 1.37	37.0	333	1000	333	1000	1000

n.d. not determined due to mathematical reasons

Emergence:

There were no statistically significant effects on seedling emergence when compared to the control for all test species and all tested rates up to and including the highest test rate of 1000 mL prod./ha (multiple sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).

Mortality:

There were no statistically significant effects on seedling mortality when compared to the control for all test species and all tested rates up to and including the highest test rate of 1000 mL prod./ha (multiple sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).

Plant dry weight:

No effects on plant dry weight were observed for the test species soybean, tomato, corn, oat and onion, i.e., the NOER for these species was 1000 mg prod./ha. For perennial ryegrass, the NOER was 333 mL prod./ha, for oilseed rape, sunflower and sugar beet, the NOER was 111 mL prod./ha and for radish, the NOER was 12.3 mL prod./ha. Statistical significance for obtaining the NOER was determined by multiple comparison Williams t-test ($\alpha = 0.05$) or multiple comparison Dunnett's t-test ($\alpha = 0.05$). For effects on plant dry weight, radish was the most sensitive species with an ER_{50} value of 351 mL prod./ha.

Plant height:

No effects on plant height were observed for the test species soybean, corn, oat and onion, i.e., the NOER for these species was 1000 mg prod./ha. For oilseed rape, sugar beet and perennial ryegrass, the NOER was 333 mL prod./ha, for tomato, the NOER was 37.0 mL prod./ha, for radish, the NOER was 12.3 mL prod./ha and for sunflower, the NOER was < 1.37 mL prod./ha. Statistical significance for obtaining the NOER was determined by multiple comparison Williams t-test ($\alpha = 0.05$), multiple comparison Dunnett's t-test ($\alpha = 0.05$), Step-down Jonckheere-Terpstra Test ($\alpha = 0.05$), multiple comparison Bonferroni-Welsh t-test ($\alpha = 0.05$) or multiple sequentially-rejective U-Test after Bonferroni-Holm ($\alpha = 0.05$). For effects on plant height, ER_{50} values were > 1000 mL prod./ha for all plant species. Based on ER_{10} and ER_{20}, radish was the most sensitive species with ER_{10} and ER_{20} values of 26.1 and 119 mL prod./ha, respectively.

Phytotoxicity:

Phytotoxic effects were chlorosis or other discolouration, necrosis and deformation. Oilseed rape and radish showed effects higher than 50% at 1000 mL prod./ha (54.0% and 65.0%, respectively). Sugar beet and perennial ryegrass showed phytotoxicity values of 43% and 18% at 1000 mL prod./ha, respectively. For all other species, phytotoxic effects remained low (≤ 7%) at 1000 mL prod./ha.

Conclusion

In this seedling emergence test with ADM.06001.H.2.B at various application rates on ten plant species, the lowest ER_{50} was determined to be 351 mL prod./ha for effects on plant dry weight in radish (*Raphanus sativus*). The lowest NOER was < 1.37 mL prod./ha for effects on plant height in sunflower (*Helianthus annuus*).

A 2.7.2.2 Vegetative Vigour

Comments of zRMS:	The study was conducted in line with OECD 227 with minor deviations. It was noted that the salt content as electronic conductivity of the soil used to grow the seedlings was not reported. For plant dry weight determination, the plants were dried to constant weight at ≥ 70°C instead of 60°C. These deviations are considered to have no impact on the quality and integrity of the study. It was also noted that mesosulfuron-methyl expired during the course of the study but a certified reference material was used for determination of mesosulfuron-methyl concentrations. Therefore, this deviation is considered to have no effect on the outcome of the study.

The analytical measurements showed that the concentrations of both active substances were within 80 – 120 % of nominal; therefore, the endpoint can be expressed as nominal concentration.

All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:

The most sensitive species was oilseed rape *Brassica napus*
$ER_{50,\ dry\ weight}$ = 133 mL product/ha
$ER_{20,\ dry\ weight}$ = 59.4 mL product/ha
$ER_{10,\ dry\ weight}$ = 39 mL product/ha

The calculations of ER values based on phytotoxicity parameters have been accepted by zRMS.
The most sensitive species in terms of phytotoxicity was Raphanus sativus with a ER_{50} value of 60.10 mL product/ha.
~~Phytotoxic effects higher than 50% were observed at 111 mL product/ha for oilseed rape and radish, at 333 mL product/ha for soybean, sunflower, tomato, sugar beet and oat and at 1000 mL product/ha for corn and perennial ryegrass. For onion, phytotoxic effects remained low (≤ 5%) up to 1000 mL product/ha.~~
~~Therefore, the ER_{50} for phytotoxicity is estimated to be > 111 mL product/ha.~~

ER_{50}=117.95 (oilseed rape)
ER_{50}=60.10 (Radish)
ER_{50}=273.09* (Soybean)
ER_{50}=171.23 (Sunflower)
ER_{50}=199.70 (Tomato)
ER_{50}=182.56 (Sugar beet)
ER_{50}=590.30 (Corn)
ER_{50}=913.94 (Peremtial ryegrass)
ER50=380.67* (oat)
ER_{50}>1000 (onion)
* endpoints not reliable as no statistically significant concentration/response was found

Endpoint (mL prod./ha)	**Oilseed rape** *Brassica napus*	**Radish** *Raphanus sativus*	**Soybean** *Glycine max*	**Sunflower** *Helianthus annuus*	**Tomato** *Solanum lycopersicum*	**Sugar beet** *Beta vulgaris*	**Corn** *Zea mays*	**Perennial ryegrass** *Lolium perenne*	**Oat** *Avena sativa*	**Onion** *Allium cepa*
Phytotoxicity (21d)										
ER_{10} (CI)	**43.23** (7.09-69.08)	**28.23** (20.90-38.14)	**126.00*** (n.d.)	**102.39** (96.27-108.09)	**89.78** (54.97-117.49)	**95.13** (81.75-107.08)	**290.77** (279.61-301.61)	**404.45** (400.36-408.49)	**97.95*** (n.d.)	**>1000** (n.d.)
ER_{20} (CI)	**61.01** (17.50-88.61)	**36.59** (28.86-46.39)	**164.32*** (n.d.)	**122.15** (116.09-128.21)	**118.13** (81.74-147.31)	**118.99** (105.55-131.41)	**370.78** (359.37-381.91)	**535.06** (531.24-538.83)	**156.09*** (n.d.)	**>1000** (n.d.)
ER_{50} (CI)	**117.95** (77.50-181.42)	**60.10** (50.05-72.18)	**273.09*** (n.d.)	**171.23** (162.37-181.80)	**199.70** (162.61-243.82)	**182.56** (166.95-200.39)	**590.30** (576.99-603.86)	**913.94** (910.87-917.01)	**380.67*** (n.d.)	**>1000** (n.d.)

Reference:	KCP 10.6.2/02
Report	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test, Spatz, B. and Kowalczyk, F., 2021b, 140711087 (ADAMA No 000105380)
Guideline(s):	Yes, OECD 227 (July 2006)
Deviations:	Minor (see the commenting box above) ~~The texture and salt content of the soil used to grow the seedlings is not reported. For plant dry weight determination, the plants were dried to constant weight at ≥ 70°C instead of 60°C.~~

	~~These deviations are considered minor, not having an impact on the study quality and integrity.~~
GLP:	Yes
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.06001.H.2.B
Batch No.	A8001
Active ingredient content	12 g mesosulfuron-methyl/L (nominal), 12.1 g mesosulfuron-methyl/L (actual) 60 g pinoxaden/L (nominal), 61.6 g pinoxaden/L (actual) 35 g/L mefenpyr-diethyl/L (nominal), 36.6 g mefenpyr-diethyl/L (actual)
Appearance	Yellow to beige liquid
Density	0.97 g/mL
Expiry date	19 Jan 2022
Reference substance	No reference substance was used.

Test organism:

Test species	Dicotyledons: Oilseed rape, *Brassica napus* (Brassicaceae) Radish, *Raphanus sativus* (Brassicaceae) Soybean, *Glycine max* (Fabaceae) Sunflower, *Helianthus annuus* (Asteraceae) Tomato, *Solanum lycopersicum* (Solanaceae) Sugar beet, *Beta vulgaris* (Amaranthaceae) Monocotyledons: Corn, *Zea mays* (Poaceae) Perennial ryegrass, *Lolium perenne* (Poaceae) Oat, *Avena sativa* (Poaceae) Onion, *Allium cepa* (Amaryllidaceae)
Origin of test species	For a given test species, all uncoated seeds used in the test were from the same source and lot number.
No. of plants per pot	2 (soybean, sunflower, tomato, sugar beet and corn) 3 (oilseed rape and radish) 4 (perennial ryegrass, oat and onion)
No. of replicates (pots) per treatment group	8 (perennial ryegrass, oat and onion) 10 (oilseed rape and radish) 15 (soybean, sunflower, tomato, sugar beet and corn)
No. of plants per treatment group	30 (oilseed rape, radish, soybean, sunflower, tomato, sugar beet and corn) 32 (perennial ryegrass, oat and onion)
Bioassay pots	Commercial plastic flowerpots (15 cm in diameter)
Preparation	The seeds were introduced manually into the soil. To account for the different development speed of the species, the sowing was done on different dates, to ensure that all species were in the 2- to 4- true leaf stage at the application day.
Fertilisation	After development of the first true leaves, Ferty® 9 “Hydro” (Planta-Düngemittel GmbH) at 3 g/L and Terraflor®-AZ (Terraflor GmbH) at 0.4 g/L were added to the water up to two times a week, depending on the development of the plants. Pots of one treatment obtained the same level of fertilizer.
Watering	After sowing, the pots were placed on saucers and watered.

	Bottom watering (through saucers) was done where necessary after a daily check. Water was given individually in order to assure optimal water supply of the plants.

Test soil:

Soil batch	LUFA 2.3
Soil type (USDA)	Sandy loam
Physico-chemical properties Soil particle size (mm): Organic carbon (%): pH:	 ≤ 2 0.66±0.09 (for radish and corn), 0.65±0.08 (for all other test species) 6.2±0.3 (for radish and corn), 6.1±0.4 (for all other test species)
Pesticide treatments:	None in the year of sampling and for at least the two previous years
Soil pre-treatment	The soil was steam sterilised.

Test conditions:

Test substance concentration	All test species: 12.3, 37.0, 111, 333 and 1000 mL prod./ha Additionally, radish was tested at 4.12 mL prod./ha. The test rates were chosen based on the results of a range-finding test. Application in 200 L water/ha Preparation of application solutions for 1st application (all species except tomato): A stock solution (= application solution of the highest test rate) was prepared by diluting 9.70 g ADM.06001.H.2.B to 2000 mL with deionised water. This corresponded with 4.85 g prod./L or with 1000 mL prod./ha in 200 L/ha. After sampling for analysis, the further application solutions were prepared by serial dilution with a geometric factor of 3, i.e., 600 g were filled up to 1800 g with deionised water. Preparation of application solutions for 2nd application (tomato; repeated due to insufficient analytical recovery of pinoxaden and mefenpyr-diethyl in the first run): A stock solution (= application solution of the highest test rate) was prepared by weighing 9.70 g of homogenised ADM.06001.H.2.B into a glass beaker, transferring it to a 2000-mL volumetric flask and filling it up to the mark with deionised water. This corresponded with 4.85 g prod./L or with 1000 mL prod./ha in 200 L/ha. After sampling for analysis and thorough stirring, the further application solutions were prepared by serial dilution with a geometric factor of 3, i.e., 600 g were filled up to 1800 g with deionised water. Before dilution and before application, the application solutions were stirred intensively.
Control	Deionised water
Application time	Post-emergence when the plants reached the 2- to 4- true leaf stage
Application method	Application was conducted using freshly prepared control and test substance application solutions and calibrated laboratory spraying equipment. Applications were made using a laboratory-spraying equipment (Fa. Schachtner, 71640 Ludwigsburg, Germany) with a TeeJet 8002 EVS spray nozzle. For all applications, the pressure was 2.00 bar and the nozzles were 40.0 cm above the top leaves of the plants. The sprayer speed was 2.50 km/h for test rates up to and including 37.0 mL prod./ha. For all species except tomato, the sprayer speed was 2.25 km/h for the three highest test rates of 111, 333 and 1000 mL prod./ha. For

	tomato, the sprayer speed was 2.50 km/h when applying the 111 mL prod./ha rate but was 2.25 km/h for the two highest rates of 333 and 1000 prod./ha. The sprayer was calibrated using a glass plate of known surface area with filter paper in the same size by spraying with deionised water and weighing immediately before and after application to deliver 200 L/ha ± 10%. Verification of the applied amount was thereafter performed for each test rate applied by the same method. Deviation in the spray deposit applied did not exceed ± 10% of nominal for any application rate. The uniformity of the deposit distribution was checked visually.
Test duration	21 days after application
Test facility type	The test was performed in a growth chamber under controlled conditions.
Temperature (during exposure)	22 ± 10ºC (nominal), 16.7-22.6°C (mean: 20.1°C, actual for all species except tomato), 16.8-22.6°C (mean: 20.2°C, actual for tomato)
Relative humidity (during exposure)	70% ± 25% (nominal), 50% – 84% (mean: 60%, actual for all species except tomato), 49% – 74% (mean: 58%, actual for tomato)
Light intensity (during exposure)	350 ± 50 µE/m²/s (nominal), 300-400 µmol/m²/s (mean: 344 µmol/m²/s, actual for all species except tomato), 310-400 µmol/m²/s (mean: 377 µmol/m²/s, actual for tomato)
Photoperiod	16 hours light : 8 hours dark

Observations:

Observation period	21 days following application
Mortality and visual phytotoxicity (e.g., discolouration, necrosis and deformation)	Days 7, 14 and 21
Plant dry weight	Day 21
Plant height	Day 21
Growth stages	Days 7, 14 and 21
Test conditions	Temperature and relative humidity: every 15 minutes Light intensity: once a week with 5 different measuring points for each species at the top of the canopy

Analytical method:

Samples	The concentration of the test substance in the stock solution and the control was verified by analysis of mesosulfuron-methyl, pinoxaden, NOA 407854 (M2, metabolite of pinoxaden) and mefenpyr-diethyl. 1st application (all species except tomato): Duplicate samples from the homogeneous stock solution (= application solution of the highest test rate) and the control were taken before application. The samples were stored deep frozen (≤ -20°C) until analysis. Afterwards, the samples were again stored deep frozen (≤ -20°C). 2nd application (tomato): Three samples (5 mL each) from the continuously stirred, homogeneous stock solution (= application solution of the highest test rate) and the control were taken before application. One sample was analysed directly after sampling. The others were stored deep frozen (≤ -20°C) as retained samples.
Method type	LC-MS/MS
Equipment	API 5500 mass spectrometer with Agilent Series 1290 pump and autosampler

Column	Luna Omega Polar C18 (50 x 3 mm, 3 µm)
Column temperature	40ºC
Flow rate	0.65 mL/min
Mobile phase	A: HPLC-water + 0.1% formic acid B: acetonitrile + 0.1% formic acid Gradient: 2.0 min at 95% A/5% B, in 0.5 min to 50% A/50% B, in 2.0 min to 5% A/95% B, 0.7 min at 5% A/95% B, in 0.1 min to 95% A/5% B, 1.7 min at 95% A/5% B
Injection volume	5 µL
Detector	MSD, positive mode Ion source: 5500 V Temperature: 350°C Scan type: MRM
Mass transitions	Mefenpyr-diethyl: 390 m/z → 327 m/z (quantifier) 390 m/z → 160 m/z (qualifier) Mesosulfuron-methyl: 504 m/z → 182 m/z (quantifier) 504 m/z → 83 m/z (qualifier) Pinoxaden: 401 m/z → 317 m/z (quantifier) 401 m/z → 115 m/z (qualifier) NOA 407854 (M2): 318 m/z → 171 m/z (quantifier) 318 m/z → 131 m/z (qualifier)
Sample preparation	1st application (all species except tomato): An aliquot of each sample was diluted with acetonitrile/pure water (50/50, v/v) + 0.1% HCOOH 2nd application (tomato): Samples were made up to 25 mL with acetone. These solutions were then diluted with acetonitrile/pure water (50/50, v/v) to match the calibration range.

Experimental dates: 29 Oct 2020 to 13 Apr 2021

Calculations:
Mean mortality and mean visual phytotoxicity were calculated as percentage of total plants.

The final mean dry weight per pot and the final mean height per pot (including standard deviations) were calculated for each plant species and treatment group as well as the percentage in comparison to the control and the percentage effect.

Statistics:

Dry weight data and plant height data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$), respectively. In case the data were normally distributed and homogeneous, the Dunnett's t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$), or in case the data showed a monotonic dose response, the Williams t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) were used for comparing treatment groups and control. When the data were normally distributed but not homogeneous, the Bonferroni-Welch t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used. In case the data were not normally distributed independent of the homogeneity, the Bonferroni-Holm U-test (multiple comparison, one-sided smaller, $\alpha = 0.05$), or in case the data showed a monotonic dose response, the Step-down-Jonckheere Terpstra test (one-sided smaller, $\alpha = 0.05$) were used for comparing treatment groups and control.

In order to determine ER_{10}, ER_{20} and ER_{50} values (dry weight and height), a regression analysis was performed (Probit-analysis). For dry weight of perennial ryegrass and oat and for height of sunflower, sugar beet, perennial ryegrass and oat, no significant dose response relation of the mean values for each treatment group was found ($p(F) > 0.05$). Therefore, the regression analysis was performed using all replicates for fitting.

For mortality data, Fisher's Exact Binomial Test (with Bonferroni Correction, multiple comparison, one-sided greater, $\alpha = 0.05$) was used, or in case a linear trend was shown, the Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH. Statistical analyses were conducted in compliance with the recommendations provided by OECD 54 (2006).

Results and discussions

Validity criteria:

- Seedling emergence is at least 70%
- Control plants do not exhibit visible signs of phytotoxicity and show normal variation in growth and morphology
- Mean survival of control plants is at least 90% for the duration of the study
- Environmental conditions per species are identical and growing media are the same

Seedling emergence ranged from 84% to 97%. The control plants did not exhibit visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for the particular species. Mean survival of control plants was 100% for the duration of the study. Environmental conditions and growing media per species were identical. All seeds for a given test species were from the same source and lot number. Therefore, the validity criteria were met.

Analytical verification of the test substance concentration (measured as concentrations of mesosulfuron-methyl, pinoxaden, NOA 407854 (M2, metabolite of pinoxaden) and mefenpyr-diethyl) in the stock solutions (= application solutions of the highest test rate) and the controls is summarised in the table below.

Table A2.7.2.2-1: Verification of test substance concentrations in the stock solutions of ADM.06001.H.2.B and in the controls

Application No	Sample	Analyte	Nominal concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
1	Stock solution	Mesosulfuron-methyl	58.20	52.878	91
		Pinoxaden	305.55	255.088	83
		NOA 407854 (M2)	n.a.	n.d.	n.a.
		Mefenpyr-diethyl	184.30	150.343	82
	Control	Mesosulfuron-methyl	0.00	n.d.	n.a.
		Pinoxaden	0.00	n.d.	n.a.
		NOA 407854 (M2)	n.a.	n.d.	n.a.
		Mefenpyr-diethyl	0.00	n.d.	n.a.
2	Stock solution	Mesosulfuron-methyl	58.20	70.277	121
		Pinoxaden	305.55	391.683	128
		NOA 407854 (M2)	n.a.	n.d.	n.a.
		Mefenpyr-diethyl	184.30	213.174	116
	Control	Mesosulfuron-methyl	0.00	n.d.	n.a.
		Pinoxaden	0.00	n.d.	n.a.
		NOA 407854 (M2)	n.a.	n.d.	n.a.
		Mefenpyr-diethyl	0.00	n.d.	n.a.

n.a. not applicable
n.d. not detectable

For the first application, analytical recoveries of mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl in the stock solution (= application solution of the highest test rate) were 91%, 83% and 82% of nominal, respectively. For the second application, analytical recoveries of mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl in the stock solution (= application solution of the highest test rate) were 121%, 128% and 116% of nominal, respectively. NOA 407854 (M2), the metabolite of pinoxaden, could not be detected in the stock solutions of both the first and second application. Thus, study endpoints were calculated and reported in terms of nominal application rates of ADM.06001.H.2.B. In the control application solutions (deionised water), none of the analytes were detected.

Effects of ADM.06001.H.2.B on plant mortality, plant dry weight, plant height and visual phytotoxicity are summarised in the tables below.

Table A2.7.2.2-2: Mortality (final assessment on day 21) of plants exposed to ADM.06001.H.2.B

Application rate (mL prod./ha)	Mortality (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	0	0	0	0	0
4.12	-	0	-	-	-
12.3	0	0	0	0	0
37.0	0	0	0	0	0
111	0	7	0	0	0
333	0	10	0	3	0
1000	3	7	0	63*	0
Application rate (mL prod./ha)	**Mortality (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	0	0	0	0	0
4.12	-	-	-	-	-
12.3	0	0	0	0	0
37.0	0	0	0	0	0
111	0	0	0	0	0
333	0	0	0	0	0
1000	13	3	0	0	0

* Statistically significantly different from the control (Step-down Cochran-Armitage Test, $\alpha = 0.05$)

Table A2.7.2.2-3: Effects on plant dry weight (final assessment on day 21) from exposure to ADM.06001.H.2.B

Application rate (mL prod./ha)	Plant dry weight (g) / Effect (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	12.52 / -	2.04 / -	4.24 / -	7.43 / -	4.44 / -
4.12	-	1.90 / -6.9	-	-	-
12.3	13.43 / 7.3	2.30 / 12.6	4.62 / 9.0	7.66 / 3.1	4.45 / 0.0
37.0	11.69 / -6.7	1.39* / -31.8	4.56 / 7.8	7.49 / 0.7	4.20 / -5.4
111	7.05* / -43.7	0.97* / -52.5	3.76* / -11.2	6.76 / -9.0	3.12* / -29.7
333	1.98* / -84.2	0.89* / -56.2	1.97* / -53.6	0.81* / -89.1	0.81* / -81.8
1000	1.25* / -90.1	0.82* / -60.0	1.16* / -72.6	0.72* / -90.3	0.49* / -89.0
Application rate (mL prod./ha)	**Plant dry weight (g) / Effect (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	2.90 / -	5.77 / -	3.13 / -	4.98 / -	1.83 / -
4.12	-	-	-	-	-
12.3	2.83 / -2.4	5.91 / 2.4	2.98 / -4.8	5.55 / 11.4	1.77 / -3.6
37.0	2.84 / -2.2	5.51 / -4.5	3.04 / -2.9	4.70 / -5.7	1.52 / -17.2
111	2.51* / -13.5	5.65 / -2.0	3.30 / 5.7	4.93 / -1.1	1.63 / -11.2
333	0.48* / -83.4	3.99* / -30.8	3.24 / 3.5	1.84* / -63.0	1.77 / -3.2
1000	0.39* / -86.7	0.49* / -91.5	1.75* / -43.9	1.51* / -69.7	1.74 / -4.8

* Statistically significantly different from the control (multiple comparison Bonferroni-Holm U-test, α = 0.05, or multiple comparison Williams t-test, α = 0.05, or Step-down Jonckheere-Terpstra Test, α = 0.05, or multiple comparison Bonferroni-Welch t-test, α = 0.05, or multiple comparison Dunnett's t-test, α = 0.05)

Table A2.7.2.2-4: Effects on plant height (final assessment on day 21) from exposure to ADM.06001.H.2.B

Application rate (mL prod./ha)	Plant height (mm) / Effect (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	197 / -	102 / -	279 / -	228 / -	204 / -
4.12	-	94 / -7.7	-	-	-
12.3	195 / -0.9	102 / 0.3	270 / -3.3	244 / 6.9	214 / 4.7
37.0	200 / 1.3	85* / -16.3	283 / 1.6	241 / 5.6	209 / 2.6
111	160* / -18.7	67* / -33.7	240* / -13.9	251 / 9.9	148* / -27.6
333	102* / -48.4	59* / -41.7	117* / -58.0	58* / -74.7	43* / -79.1
1000	85* / -56.9	56* / -45.1	94* / -66.2	54* / -76.5	33* / -83.8
Application rate (mL prod./ha)	**Plant height (mm) / Effect (%)**				
	Sugar beet	**Corn**	**Perennial ryegrass**	**Oat**	**Onion**
Control	133 / -	746 / -	343 / -	536 / -	241 / -
4.12	-	-	-	-	-
12.3	137 / 3.4	730 / -2.1	349 / 1.7	563 / 5.0	232 / -3.9
37.0	135 / 1.9	736 / -1.3	347 / 1.3	533 / -0.5	215* / -10.8
111	121* / -9.0	729 / -2.2	356 / 3.8	546 / 1.9	219* / -9.1
333	57* / -57.2	700* / -6.1	339 / -1.1	388* / -27.6	228* / -5.3
1000	51* / -61.3	215* / -71.2	253* / -26.3	355* / -33.8	213* / -11.7

* Statistically significantly different from the control (multiple comparison Bonferroni-Holm U-test, α = 0.05, or multiple comparison Williams t-test, α = 0.05, or Step-down Jonckheere-Terpstra Test, α = 0.05, or multiple comparison Bonferroni-Welch t-test, α = 0.05)

Table A2.7.2.2-5: Phytotoxicity (final assessment on day 21) of plants exposed to ADM.06001.H.2.B

Application rate (mL prod./ha)	Phytotoxicity (%)				
	Oilseed rape	Radish	Soybean	Sunflower	Tomato
Control	0	0	0	0	0
4.12	-	0	-	-	-
12.3	0	2	0	0	0
37.0	0	20	0	0	0
111	52	86	1	14	17
333	86	93	67	95	80
1000	97	96	80	98	93

Application rate (mL prod./ha)	Phytotoxicity (%)				
	Sugar beet	Corn	Perennial ryegrass	Oat	Onion
Control	0	0	0	0	0
4.12	-	-	-	-	-
12.3	0	0	0	0	0
37.0	2	1	0	0	0
111	16	0	0	1	0
333	88	15	6	58	0
1000	98	83	56	73	5

Calculated ER_{10}, ER_{20}, ER_{50} and NOER values for the different parameters for each plant species are presented in the table below.

Table A2.7.2.2-6: ER_{10}, ER_{20}, ER_{50} and NOER for various endpoints for plants exposed to ADM.06001.H.2.B

Endpoint (mL prod./ha)	Oilseed rape	Radish	Soy-bean	Sun-flower	Tomato	Sugar beet	Corn	Peren-nial ryegrass	Oat	Onion
Mortality										
LR_{10}	> 1000	> 1000	> 1000	441	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
LR_{20}	> 1000	> 1000	> 1000	550	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
LR_{50}	> 1000	> 1000	> 1000	842	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
NOER	1000	1000	1000	333	1000	1000	1000	1000	1000	1000
Plant dry weight										
ER_{10}	39.0	5.75	77.0	114	55.3	100	209	440	59.9	n.d.
ER_{20}	59.4	20.4	133	137	81.0	127	272	606	109	n.d.
ER_{50} (CI)	133 (94.0-189)	232 (62.6-4006)	379 (223-691)	197 (132-279)	168 (113-251)	200 (106-343)	447 (392-522)	> 1000	339 (268-433)	> 1000
NOER	37.0	12.3	37.0	111	37.0	37.0	111	333	111	1000
Plant height										
ER_{10}	51.7	12.3	59.3	137	58.5	62.4	382	212	135	n.d.
ER_{20}	116	54.8	113	168	86.8	122	481	998	343	n.d.
ER_{50}	547	952	385	246	185	440	747	> 1000	> 1000	> 1000
NOER	37.0	12.3	37.0	111	37.0	37.0	111	333	111	12.3

n.d. not determined due to mathematical reasons

Mortality:

There were no statistically significant effects on plant mortality when compared to the control for all test species except sunflower for which effects were observed at the highest test rate of 1000 mL prod./ha. Therefore, the NOER for mortality was 333 mL prod./ha for sunflower (Step-down Cochran-Armitage Test, $\alpha = 0.05$) and 1000 mL prod./ha for all other test species (pair-wise comparison Fisher's Exact Test, $\alpha = 0.05$). The LR_{50} for sunflower was 842 mL prod./ha, while it was > 1000 mL prod./ha for all other test species.

Plant dry weight:

No effects on plant dry weight were observed for the test species onion, i.e., the NOER for this species was 1000 mg prod./ha. For perennial ryegrass, the NOER was 333 mL prod./ha, for sunflower, corn and oat, the NOER was 111 mL prod./ha, for oilseed rape, soybean, tomato and sugar beet, the NOER was 37.0 mL prod./ha and for radish, the NOER was 12.3 mL prod./ha. Statistical significance for obtaining the NOER was determined by multiple comparison Bonferroni-Holm U-test ($\alpha = 0.05$), multiple comparison Williams t-test ($\alpha = 0.05$), Step-down Jonckheere-Terpstra Test ($\alpha = 0.05$), multiple comparison Bonferroni-Welch t-test ($\alpha = 0.05$) or multiple comparison Dunnett's t-test ($\alpha = 0.05$). For effects on plant dry weight, oilseed rape was the most sensitive species with an ER_{50} value of 133 mL prod./ha.

Plant height:

Effects on plant height were observed for all test species. For perennial ryegrass, the NOER was 333 mL prod./ha, for sunflower, corn and oat, the NOER was 111 mL prod./ha, for oilseed rape, soybean, tomato and sugar beet, the NOER was 37.0 mL prod./ha and for radish and onion, the NOER was 12.3 mL prod./ha. Statistical significance for obtaining the NOER was determined by multiple comparison Bonferroni-Holm U-test ($\alpha = 0.05$), multiple comparison Williams t-test ($\alpha = 0.05$), Step-down Jonckheere-Terpstra Test ($\alpha = 0.05$) or multiple comparison Bonferroni-Welch t-test ($\alpha = 0.05$). For effects on plant height, tomato was the most sensitive species with an ER_{50} value of 185 mL prod./ha.

Phytotoxicity:

Phytotoxic effects observed were discolouration, chlorosis, necrosis and deformation. Oilseed rape and radish showed effects higher than 50% at 111, 333 and 1000 mL prod./ha, soybean, sunflower, tomato, sugar beet and oat showed effects higher than 50% at 333 and 1000 mL prod./ha and corn and perennial ryegrass showed effects higher than 50% at 1000 mL prod./ha. For onion, phytotoxic effects remained low (≤ 5%) up to 1000 mL prod./ha.

Reference:	KCP 10.6.2/03
Report	Statistical evaluation of the phytotoxicity results in the study: ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Haaf, S, 2023 (ADAMA No 000117985)
Guideline(s):	not applicable
Deviations:	not applicable
GLP:	No
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Introduction

The applicant (ADAMA) has conducted a vegetative vigour study with the product ADM.06001.H.2.B (Spatz and Kowalczyk, 2021) in order to derive an ER_{50} value for the use in the non-target terrestrial plant risk assessment. In this study 6 dicotyledonous species (*Brassica napus*, *Raphanus sativus*, *Glycine max*, *Helianthus annuus*, *Solanum lycopersicum* and *Beta vulgaris*) and 4 monocotyledonous species (*Zea mays*, *Lolium perenne*, *Avena sativa* and *Allium cepa*) have been exposed to concentrations ranging between 4.12 and 1000 mL product/ha. Effects based on plant dry weight, plant height and mortality were measured and have been statistically evaluated in the study report. Observations on phytotoxicity (e.g. discolouration, necrosis, deformation) have been visually assessed 7, 14 and 21 days after application according to EPPO PP 1/135(4).
However, no statistical evaluation has been done for phytotoxicity in the study report as, at present, there is no ring-tested methodology available for assessing visual injury in any non-target terrestrial plant guideline. While a suggestion for assessment methodology is found in the appendix of the OECD guidelines, this was not ring-tested and there is no suggested approach for the robust statistical evaluation of visual injury. Therefore, any assessment of visual injury lacks the scientific rigor afforded to the EC_{50} values determined for the growth parameters in each of the studies. Even if the performing laboratory conducted the visual assessment according to the suggested guideline methodology, there is no way to know if that assessment is scientifically robust as the ring test has not been performed. Furthermore, by measuring phytotoxicity via visual assessments the scoring is subjective and can vary between different assessors. High variability in scores assigned by assessors was observed especially for intermediate effects as shown in a study presented at SETAC 2023 investigating intra-laboratory variability of visual phytotoxicity assessments in non-target terrestrial plant studies (Meregalli et al., 2023). In sum,

the ER_{50} values obtained from growth endpoints should be considered sufficiently protective for environmental risk assessment.
Although the applicant does not see the statistical evaluation of the visual assessment of phytotoxicity as scientifically justified, a statistical evaluation of the phytotoxicity results based on the vegetative vigour study with the product ADM.06001.H.2.B (Spatz and Kowalczyk, 2021) is provided below.

Results

Statistical analyses were performed with the program ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH. The ER_{10}, ER_{20} and ER_{50} values and their 95% confidence intervals were calculated using Probit analysis. The detailed statistical output files can be found in the Appendix (A1-A10).

A summary of the phytotoxicity results is presented in the table below.

Table 1: ER_{10}, ER_{20} and ER_{50} for phytotoxicity endpoints of the vegetative vigour study with ADM.06001.H.2.B

Endpoint (mL prod./ha)	**Oilseed rape** *Brassica napus*	**Radish** *Raphanus sativus*	**Soybean** *Glycine max*	**Sunflower** *Helianthus annuus*	**Tomato** *Solanum lycopersicum*	**Sugar beet** *Beta vulgaris*	**Corn** *Zea mays*	**Perennial ryegrass** *Lolium perenne*	**Oat** *Avena sativa*	**Onion** *Allium cepa*
Phytotoxicity (21d)										
ER_{10} (CI)	**43.23** (7.09-69.08)	**28.23** (20.90-38.14)	**126.00*** (n.d.)	**102.39** (96.27-108.09)	**89.78** (54.97-117.49)	**95.13** (81.75-107.08)	**290.77** (279.61-301.61)	**404.45** (400.36-408.49)	**97.95*** (n.d.)	**>1000** (n.d.)
ER_{20} (CI)	**61.01** (17.50-88.61)	**36.59** (28.86-46.39)	**164.32*** (n.d.)	**122.15** (116.09-128.21)	**118.13** (81.74-147.31)	**118.99** (105.55-131.41)	**370.78** (359.37-381.91)	**535.06** (531.24-538.83)	**156.09*** (n.d.)	**>1000** (n.d.)
ER_{50} (CI)	**117.95** (77.50-181.42)	**60.10** (50.05-72.18)	**273.09*** (n.d.)	**171.23** (162.37-181.80)	**199.70** (162.61-243.82)	**182.56** (166.95-200.39)	**590.30** (576.99-603.86)	**913.94** (910.87-917.01)	**380.67*** (n.d.)	**>1000** (n.d.)

n.d. not determined due to mathematical reasons
CI Confidence interval (lower 95% and upper 95% confidence interval)
* endpoints not reliable as no statistically significant concentration/response was found

The most sensitive species in terms of phytotoxicity was *Raphanus sativus* with a ER_{50} value of 60.10 mL product/ha.
The endpoints of *Glycine max* and *Avena sativa* are not reliable as no statistically significant concentration/response was found.
The maximum effects of *Allium cepa* were 4.8% and therefore the ER_{10}, ER_{20} and ER_{50} values can be considered >1000 mL product/ha.

Conclusion

Although the applicant does not see the statistical evaluation of the visual assessment of phytotoxicity as scientifically justified, ER_{10}, ER_{20} and ER_{50} values including confidence intervals have been calculated for phytotoxicity based on the results of the vegetative vigour study with the product ADM.06001.H.2.B (Spatz and Kowalczyk, 2021). The most sensitive species in terms of phytotoxicity was *Raphanus sativus* with a ER_{50} value of 60.10 mL product/ha.

Conclusion

In this vegetative vigour test with ADM.06001.H.2.B at various application rates on ten plant species, the lowest ER_{50} was determined to be 133 mL prod./ha for effects on plant dry weight in oilseed rape (*Brassica napus*). The lowest NOER was 12.3 mL prod./ha for effects on plant dry weight in radish

(*Raphanus sativus*) and for effects on plant height in radish (*Raphanus sativus*) and onion (*Allium cepa*).

A 2.7.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.8 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.9 KCP 10.8 Monitoring data