

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.01851.H.1.A

Product name(s): see Part A

Chemical active substances:

Ethofumesate, 500 g/L

Clopyralid, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

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

Submission date: July 2024

MS Finalisation date: February 2025 (initial Core Assessment),
June 2025 (final Core assessment)

Version history

When	What
July 2024	Orginal applicant version
February 2025	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.
June 2025	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

Table of Contents

9	Ecotoxicology (KCP 10)	5
9.1	Critical GAP and overall conclusions.....	5
9.1.1	Overall conclusions	7
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).....	7
9.1.1.2	Effects on aquatic organisms (KCP 10.2)	7
9.1.1.3	Effects on bees (KCP 10.3.1)	7
9.1.1.4	Effects on arthropods other than bees (KCP 10.3.2)	7
9.1.1.5	Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)	8
9.1.1.6	Effects on non-target terrestrial plants (KCP 10.6)	8
9.1.1.7	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7).....	8
9.1.2	Grouping of intended uses for risk assessment.....	8
9.1.3	Consideration of metabolites	8
9.2	Effects on birds (KCP 10.1.1)	9
9.2.1	Toxicity data	9
9.2.1.1	Justification for new endpoints.....	10
9.2.2	Risk assessment for spray applications.....	10
9.2.2.1	First-tier assessment (screening/generic focal species)	11
9.2.2.2	Higher-tier risk assessment.....	12
9.2.2.3	Drinking water exposure	12
9.2.2.4	Effects of secondary poisoning.....	13
9.2.2.5	Biomagnification in terrestrial food chains	14
9.2.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	14
9.2.4	Overall conclusions	14
9.3	Effects on terrestrial vertebrates other than birds (KCP 10.1.2).....	14
9.3.1	Toxicity data	14
9.3.1.1	Justification for new endpoints.....	15
9.3.2	Risk assessment for spray applications.....	15
9.3.2.1	First-tier assessment (screening/generic focal species)	16
9.3.2.2	Higher-tier risk assessment.....	17
9.3.2.3	Drinking water exposure	17
9.3.2.4	Effects of secondary poisoning.....	19
9.3.2.5	Biomagnification in terrestrial food chains	19
9.3.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	19
9.3.4	Overall conclusions	19
9.4	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)	19
9.5	Effects on aquatic organisms (KCP 10.2)	19
9.5.1	Toxicity data	19
9.5.1.1	Justification for new endpoints.....	24
9.5.2	Risk assessment	25
9.5.2.1	Individual substance risk assessment	25
9.5.2.2	Mixture toxicity risk assessment	32
9.5.3	Overall conclusions	36
9.6	Effects on bees (KCP 10.3.1)	36
9.6.1	Toxicity data	36
9.6.1.1	Justification for new endpoints.....	38
9.6.2	Risk assessment	38

9.6.2.1	Hazard quotients for bees	38
9.6.2.2	Higher-tier risk assessment for bees (tunnel test, field studies)	40
9.6.3	Effects on bumble bees.....	40
9.6.4	Effects on solitary bees.....	40
9.6.5	Overall conclusions	41
9.7	Effects on arthropods other than bees (KCP 10.3.2)	41
9.7.1	Toxicity data	41
9.7.1.1	Justification for new endpoints.....	42
9.7.2	Risk assessment	42
9.7.2.1	Risk assessment for in-field exposure	42
9.7.2.2	Risk assessment for off-field exposure.....	43
9.7.2.3	Additional higher-tier risk assessment.....	43
9.7.2.4	Risk mitigation measures.....	43
9.7.3	Overall conclusions	43
9.8	Effects on non-target soil meso- and macrofauna (KCP 10.4)	44
9.8.1	Toxicity data	44
9.8.1.1	Justification for new endpoints.....	47
9.8.2	Risk assessment	47
9.8.2.1	First-tier risk assessment	47
9.8.2.2	Higher-tier risk assessment.....	48
9.8.3	Overall conclusions	48
9.9	Effects on soil microbial activity (KCP 10.5)	48
9.9.1	Toxicity data	48
9.9.1.1	Justification for new endpoints.....	49
9.9.2	Risk assessment	49
9.9.3	Overall conclusions	50
9.10	Effects on non-target terrestrial plants (KCP 10.6)	50
9.10.1	Toxicity data	50
9.10.1.1	Justification for new endpoints.....	51
9.10.2	Risk assessment	52
9.10.2.1	Tier-1 risk assessment (based screening data).....	52
9.10.2.2	Tier-2 risk assessment (based on dose-response data)	52
9.10.2.3	Higher-tier risk assessment.....	55
9.10.2.4	Risk mitigation measures.....	58
9.10.3	Overall conclusions	58
9.11	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7).....	58
9.12	Monitoring data (KCP 10.8)	58
9.13	Classification and Labelling	58
Appendix 1	Lists of data considered in support of the evaluation.....	60
Appendix 2	Detailed evaluation of the new studies	64
A 2.1	KCP 10.1 Effects on birds and other terrestrial vertebrates	64
A 2.2	KCP 10.2 Effects on aquatic organisms	64
A 2.3	KCP 10.3 Effects on arthropods	79
A 2.4	KCP 10.4 Effects on non-target soil meso- and macrofauna.....	101
A 2.5	KCP 10.5 Effects on soil nitrogen transformation.....	111
A 2.6	KCP 10.6 Effects on terrestrial non-target higher plants.....	113
A 2.7	KCP 10.7 Effects on other terrestrial organisms (flora and fauna)	127
A 2.8	KCP 10.8 Monitoring data.....	127

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 sit- uation (crop destina- tion / purpose of crop)	F, Fn, Fpn G, Gn, Gp n or I **	Pests or Group of pests controlled (additionally: develop- mental stages of the pest or pest group)	Application				Application rate			PHI (day s)	Re- marks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & sea- son	Max. num- ber a) per use b) per crop/ season	Min. inter- val between applications (days)	L product/ha a) max. rate per appl. b) max. to- tal 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/ma x			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Sugar beet BEAVA Fodder beet BEAVC	F	Annual dicot weeds and annual grasses	Foliar spraying, overall	BBCH 10- 19/March- June	a) 3 b) 3	a) 5 b) 5	a) 0.66 b) 1.98	a) 330 g ethofumesate/h a 49.5 g clopyralid/ha b) 990 g ethofumesate/h a 148.5 g clopyralid/ha	100-400	n.a.	Total rate per season 2 L/ha Max. rate of ethofum esate should not ex- ceed 1.0 kg/ha every 3 years	A	A	R ¹ R3	A	A	A	R ¹
																A				
																Re- main- ing sce- narios				

* 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

¹⁾ Not relevant for Poland

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

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 (<i>e.g.</i> 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 <u>and</u> 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
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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)

The risks to birds from exposure to foodstuffs contaminated with ethofumesate and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to birds from exposure to drinking water contaminated with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to birds from secondary poisoning with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to mammals from exposure to foodstuffs contaminated with ethofumesate and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to mammals from exposure to drinking water contaminated with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to mammals from secondary poisoning with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risks to aquatic organisms from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable with consideration of a 10 m vegetated filter strip in case of **R3 scenario**. It should be indicated that for scenarios relevant for Poland such as: D3, D4 and R1 no risk mitigation measures are required.

The calculated mixture toxicity is similar to the measured mixture toxicity; no unexplainable antagonism or synergism is indicated.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to bees from the use of ethofumesate and clopyralid applied as the formulation ADM.01851.H.1.A to sugar beet was assessed in line with indications of the current guidance document (SANCO/10329/2002 rev 2 final) and is acceptable. The Hazard Quotients for oral (Q_{HO}) and contact exposure (Q_{HC}) are below the trigger of 50. Results of chronic feeding studies show low toxicity to larvae and adults.

In addition, the formulation is applied before the flowering period and sugar beet can be considered as not attractive to bees (harvest before flowering period). In consequence, according to EPPO guideline (EPPO 3/10 (3);09-2010), there is no exposure to bees and so acceptable risk to bees. Therefore, an acceptable risk to bees is expected from the application of ADM.01851.H.1.A.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The in-field and off-field risks to non-target arthropods other than bees from exposure to the formulated product ADM.01851.H.1.A, containing ethofumesate and clopyralid, following application to sugarbeet are acceptable without mitigation.

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 soil meso- and macro-fauna from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable without mitigation.

The risks to soil micro-organisms from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable without mitigation.

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

The risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable without consideration of mitigation measures when probabilistic risk assessment is considered with AF of 1. 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.

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

No further data on effects of ethofumesate, clopyralid or formulation ADM.01851.H.1.A to other terrestrial organisms are available.

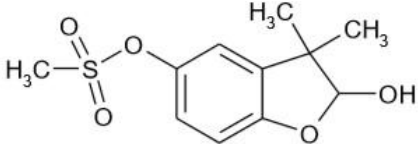
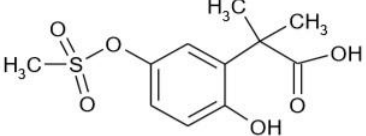
9.1.2 Grouping of intended uses for risk assessment

Not applicable, the only use is on sugar beet.

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.01851.H.1.A is indicated in the table.

Table 9.1-2 Metabolites of ethofumesate

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
NC 8493		258.3	Soil: 24.2% (photolysis)	For soil macro-, meso- and microorganisms as well as aquatic organisms
NC 20645		274.3	Water/Sediment: 18.8%	For aquatic organisms

There are no relevant metabolites of clopyralid.

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-2 are the same as indicated in EFSA Conclusion 4374/2016.

The maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and PEC_{sw/sed} values, considered further in the risk assessment.

As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-2.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with active substances clopyralid and ethofumesate. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of ADM.01851.H.1.A were not evaluated as part of the EU assessments of active substances clopyralid or ethofumesate.

However, the provision of further data on ADM.01851.H.1.A is not considered essential, because the risk assessment can be calculated from the active substance endpoints.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Anas platyrhynchos</i>	Ethofumesate	Oral Acute	LD ₅₀ = >2000 mg/kg bw	EFSA Conclusion 4374/2016
<i>Anas platyrhynchos</i>	Ethofumesate	Oral Acute	LD ₅₀ = >3552 mg/kg bw	EFSA Conclusion 4374/2016
<i>Colinus virginianus</i>	Ethofumesate	Oral Acute	LD ₅₀ = >2000 mg/kg bw	EFSA Conclusion 4374/2016
<i>Colinus virginianus</i>	Ethofumesate	Oral Acute	LD ₅₀ = >8743 mg/kg bw	EFSA Conclusion 4374/2016
Extrapolated endpoint	Ethofumesate	Acute	LD₅₀ = 3776* mg/kg bw	EFSA Conclusion 4374/2016
<i>Anas platyrhynchos</i>	Clopyralid	Oral Acute	LD₅₀ = 1465 mg/kg bw	EFSA Conclusion 5389/2018
<i>Anas platyrhynchos</i>	Ethofumesate	Dietary Reproductive toxicity	NOEL = 406.0 mg/kg bw/d (maximum test concentration, no effect on growth or reproduction)	EFSA Conclusion 4374/2016
<i>Colinus virginianus</i>	Ethofumesate	Dietary Reproductive toxicity	NOEL = 265.0 mg/kg bw/d (maximum test concentration, no effect on growth or reproduction)	EFSA Conclusion 4374/2016
<i>Anas platyrhynchos</i>	Clopyralid	Dietary Reproductive toxicity	NOEC = 118 mg/kg bw/d	EFSA Conclusion 5389/2018

Bold: Endpoints used for risk assessments.

* LD₅₀ extrapolated according to the EFSA Guidance Document on Birds and Mammals (2009), based on the lowest endpoint for mallard duck and bobwhite quail of 2000 mg a.s./kg bw with an extrapolation factor of 1.888.

zRMS comments:

Avian toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Mixture toxicity

Besides the risk assessment for the active substances ethofumesate and clopyralid, risk assessment has been performed for the formulation by calculation of a surrogate LD₅₀ for acute effects of the mixture. This surrogate LD₅₀ was calculated assuming dose or concentration additivity of toxicity of the active substances (based on the worst-case assumption that the substances have the same mode of action). 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.01851.H.1.A (mg/kg bw)
Acute	Ethofumesate	0.870	3776	4342	72.1	3132
	Clopyralid	0.130	1465	11232	27.9	

zRMS comments:

The LD₅₀mix of 3132 mg /kg bw presented in Table 9.2-2 has been validated by the zRMS and is confirmed to be correct.

Due to that none of active substances have more significant impact in the toxicity of mixture, the risk assessment for acute effects is performed for the individual active substances, as well as for the calculated mixture toxicity in relevant Tables below.

9.2.1.1 Justification for new endpoints

No deviation from the EU agreed endpoints.

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).

The recently published guidance Risk Assessment for Birds and Mammals (EFSA Journal 2023;21(2):7790, is not yet noted in the Standing Committee SCoPAFF, hence it has not been used in this submission.

For the formulation ADM.01851.H.1.A, acute risk for birds has been assessed using the surrogate LD₅₀ assuming dose or concentration additivity of toxicity (mixture toxicity; please see above) of the active substances in the virtual compound. The content of the active substances 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.

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

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet				
Active substance/product		Ethofumesate				
Application rate (g/ha)		3 x 335*				
Acute toxicity (mg/kg bw)		3776				
TER criterion						
10						
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Sugar beet (root vegetables) BBCH 10-18	Small omnivorous bird	158.8	1.6	85.12	44.36	
Reprod. toxicity (mg/kg bw/d)		265				
TER criterion						
5						
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Sugar beet (root vegetables) BBCH 10-18	Small omnivorous bird	64.8	1.06	23.01	11.52	

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. * Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

Table 9.2-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet				
Active substance/product		Clopyralid				
Application rate (g/ha)		3 x 50.25*				
Acute toxicity (mg/kg bw)		1465				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species		SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Sugar beet (root vegetables) BBCH 10-18	Small omnivorous bird		158.8	1.6	12.77	114.7
Reprod. toxicity (mg/kg bw/d)		118				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species		SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Sugar beet (root vegetables) BBCH 10-18	Small omnivorous bird		64.8	1.06	3.452	34.19

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. * Different values than the ones presented

in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

Table 9.2-5: Screening assessment of the acute risk for birds due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet				
Active substance/product		ADM.01851.H.1.A				
Application rate (g/ha)		3 x 385.25 *				
Acute toxicity (mg/kg bw)		3132				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Sugar beet (root vegetables) BBCH 10-18	Small omnivorous bird	158.8	1.6	97.88	32.00	

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. * Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

zRMS comments:

Screening risk assessment

The screening risk assessment performed in the Table from 9.2-3 to Table 9.2-5 for active substances and formulation ADM.01851.H.1.A with LD₅₀ mix = 3132 mg sum of active substances/kg bw value has been validated by zRMS.

TER_A and TER_{LT} values are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

Overall, acceptable acute and long-term risk may be concluded for birds exposed to active substances in ADM.01851.H.1.A.

9.2.2.2 Higher-tier risk assessment

Higher tier risk assessments for the uses of ADM.01851.H.1.A in sugar beet 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.01851.H.1.A is not a product for spray applications / 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 118 mL/g, ethofumesate belongs to the group of less sorptive substances.

The worst-case cumulative application rate has been considered (3 x 330 g a.s./ha).

Effective application rate (g/ha)	=	990		
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.26
Reprod. toxicity (mg/kg bw/d)	=	265	quotient =	3.74

The exposure of birds to pertinent soil metabolites must also be considered. With a $K(f)_{oc}$ of 2.1 mL/g, NC8493 belongs to the group of less sorptive substances. The effective application rate has been calculated with consideration of the molar ratio between the active substance and the metabolite (, the worst-case cumulative application rate of the parent and the peak occurrence in soil ((24.2%, soil photolysis study). In absence of the respective toxicity data, 10 times toxicity of the parent was assumed as representing worst case.

Effective application rate (g/ha)	=	216.1		
Acute toxicity (mg/kg bw)	=	377.6	quotient =	0.57
Reprod. toxicity (mg/kg bw/d)	=	26.5	quotient =	8.15

With a $K(f)_{oc}$ of 1.41 mL/g, clopyralid belongs to the group of less sorptive substances.

The worst-case cumulative application rate has been considered (3 x 49.5 g a.s./ha).

Effective application rate (g/ha)	=	148.5		
Acute toxicity (mg/kg bw)	=	1465	quotient =	0.10
Reprod. toxicity (mg/kg bw/d)	=	118	quotient =	1.26

zRMS comments:

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

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of ethofumesate amounts to 2.7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of clopyralid amounts to -2.63 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

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

The risks to birds from exposure to foodstuffs contaminated with ethofumesate and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to birds from exposure to drinking water contaminated with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to birds from secondary poisoning with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

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 active substances clopyralid and ethofumesate. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of ADM.01851.H.1.A were not evaluated as part of the EU assessment of active substances clopyralid or ethofumesate.

However, the provision of further data on ADM.01851.H.1.A is not considered essential, because the risk assessment can be calculated from the active substance endpoints.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Ethofumesate	Oral 1 d Acute	LD ₅₀ = >5000 mg/kg bw	EFSA Conclusion 4374/2016
Rat	Clopyralid	Oral 1 d Acute	LD ₅₀ = >5000 mg/kg bw	EFSA Conclusion 5389/2018
Rat	Ethofumesate	Dietary Reproductive toxicity Two-generation study	NOAEL = 60.9 mg/kg bw/d (offspring survival)	EFSA Conclusion 4374/2016
Rat	Clopyralid	Dietary 2 y	NOAEL = 50 mg/kg bw/d*	EFSA Conclusion 5389/2018

* The NOAEL used in the wild mammal's risk assessment is based on an endpoint derived from a two year rat study. It is noted that this study was not considered acceptable in the Pesticide Peer Review experts' meeting 175 (mammalian toxicology) due to fundamental deviations from the study protocol. The outcome of the risk assessment does not change when the available reproductive NOAEL is considered being the latter higher than the endpoint used in the risk assessment. It is noted that uncertainties still stand on whether the endpoint used in the risk assessment would cover developmental effects in rabbit noting that a developmental LOAEL of 50 mg/kg bw/d (based on mean foetal weight decrease and delayed ossification) was agreed at the Pesticide Peer Review experts' meeting 175. Considering that a clear dose response was not observed in the effects on body

weight and delayed ossification and that the incidence of delayed ossification was relatively low, the ecotoxicological relevance of the effects seen in the rabbit study is not confirmed as a consequence the risk assessment was not changed; the NOEL of 50 mg/kg bw/d was still considered.

zRMS comments:

Mammalian toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Mixture toxicity

Besides the risk assessment for the active substances ethofumesate and clopyralid, risk assessment has been performed for the formulation by calculation of a surrogate LD₅₀ for acute effects of the mixture. This surrogate LD₅₀ was calculated assuming dose or concentration additivity of toxicity of the active substances (based on the worst-case assumption that the substances have the same mode of action). 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 9.3-2: 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.01851.H.1.A (mg/kg bw)
Acute	Ethofumesate	0.870	5000	5750	87.0	5000
	Clopyralid	0.130	5000	38333	13.0	

zRMS comments:

The LD₅₀mix of 5000 mg /kg bw value presented in Table 9.3-2 has been validated by the zRMS and is confirmed to be correct.
Due to that none of active substances have more significant impact in the toxicity of mixture, the risk assessment for acute effects is performed for the individual active substances, as well as for the calculated mixture toxicity in relevant Tables below.

9.3.1.1 Justification for new endpoints

No deviation from the EU agreed endpoints.

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 Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

The recently published guidance Risk Assessment for Birds and Mammals (EFSA Journal 2023;21(2):7790, is not yet noted in the Standing Committee SCoPAFF, hence it has not been used in this submission.

For the formulation ADM.01851.H.1.A, acute risk for mammals has been assessed using the surrogate LD₅₀ assuming dose or concentration additivity of toxicity (mixture toxicity; please see above) of the active substances in the virtual compound. The content of the active substances 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.

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

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet				
Active substance/product		Ethofumesate				
Application rate (g/ha)		3 x 330*				
Acute toxicity (mg/kg bw)		5000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Sugar beet (root vegetables) BBCH 10-18	Small herbivorous mammal	118.4	1.6	63.46	78.79	
Reprod. toxicity (mg/kg bw/d)		60.9				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}	
Sugar beet (root vegetables) BBCH 10-18	Small herbivorous mammal	48.3	1.06	17.15	3.55	
Sugar beet BBCH 10-19	Small insectivorous mammal “shrew” Common shrew <i>Sorex araneus</i>	4.2	1.06	1.491	40.83	
Sugar beet BBCH 10-39	Large herbivorous mammal “lagomorph” Rabbit <i>Oryctolagus cuniculus</i>	14.3	1.06	5.078	11.99	
Sugar beet BBCH 10-39	Small omnivorous mammal “mouse” Wood mouse <i>Apodemus sylvaticus</i>	7.8	1.06	2.770	21.99	

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.* Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet				
Active substance/product		Clopyralid				
Application rate (g/ha)		3 x 50.25*				
Acute toxicity (mg/kg bw)		5000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Sugar beet (root vegetables) BBCH 10-18	Small herbivorous mammal	118.4	1.6	9.519	525.2	
Reprod. toxicity (mg/kg bw/d)		50				

TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Sugar beet (root vegetables) BBCH 10-18	Small herbivorous mammal	48.3	1.06	2.573	19.43

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.* Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.01851.H.1.A in sugar beet

Intended use	Sugar beet				
Active substance/product	ADM.01851.H.1.A				
Application rate (g/ha)	3 x 385.25*				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Sugar beet (root vegetables) BBCH 10-18	Small herbivorous mammal	118.4	1.6	72.98	68.51

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.* Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

zRMS comments:

Screening risk assessment

The screening risk assessment performed in the Table from 9.3-3 to Table 9.3-5 for active substances and formulation ADM.01851.H.1.A with LD₅₀ mix = 5000 mg sum of active substances/kg bw value has been validated by zRMS.

TER_A and TER_{LT} values are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for mammals.

Overall, acceptable acute and long-term risk may be concluded for mammals exposed to active substances in ADM.01851.H.1.A.

9.3.2.2 Higher-tier risk assessment

Higher tier risk assessments for the uses of ADM.01851.H.1.A in sugar beet are not required as the screening step and tier 1 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With a $K(f)_{oc}$ of 118 mL/g, ethofumesate belongs to the group of less sorptive substances.

The worst-case cumulative application rate has been considered (3 x 330 g a.s./ha).

Effective application rate (g/ha)	=	990		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.2
Reprod. toxicity (mg/kg bw/d)	=	60.9	quotient =	16.26

The exposure of birds to pertinent soil metabolites must also be considered. With a $K(f)_{oc}$ of 2.1 mL/g, NC8493 belongs to the group of less sorptive substances. The effective application rate has been calculated with consideration of the molar ratio between the active substance and the metabolite (, the worst-case cumulative application rate of the parent and the peak occurrence in soil ((24.2%, soil photolysis study). In absence of the respective toxicity data, 10 times toxicity of the parent was assumed as representing worst case.

Effective application rate (g/ha)	=	216.1		
Acute toxicity (mg/kg bw)	=	500	quotient =	0.43
Reprod. toxicity (mg/kg bw/d)	=	6.09	quotient =	35.48

With a $K(f)_{oc}$ of 1.41 mL/g, clopyralid belongs to the group of less sorptive substances.

The worst-case cumulative application rate has been considered (3 x 49.5 g a.s./ha).

Effective application rate (g/ha)	=	148.5		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.03
Reprod. toxicity (mg/kg bw/d)	=	50	quotient =	2.97

zRMS comments:

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

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of ethofumesate amounts to 2.7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of clopyralid amounts to -2.63 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

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

The risks to mammals from exposure to foodstuffs contaminated with ethofumesate and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to mammals from exposure to drinking water contaminated with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

The risks to mammals from secondary poisoning with ethofumesate, its metabolite NC8493 and clopyralid following application of ADM.01851.H.1.A to sugar beet are acceptable.

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

No further data available.

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 ethofumesate and its relevant metabolites and clopyralid. Full details of these studies are provided in the respective EU DAR and

related documents.

Effects on aquatic organisms of ADM.01851.H.1.A were not evaluated as part of the EU assessments of ethofumesate or clopyralid. 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 process.

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

Species	Substance	Exposure System	Results	Reference
<i>Cyprinus carpio</i>	Ethofumesate	96h, ss	LC ₅₀ = 10.92 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Oncorhynchus mykiss</i>	Ethofumesate	96h, ss	LC ₅₀ = 11.91 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Lepomis macrochirus</i>	Ethofumesate	96h, ss	LC ₅₀ = 21.2 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Cyprinodon variegatus</i>	Ethofumesate	96h, s	LC ₅₀ = 25.0 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Danio rerio</i>	Ethofumesate	160 d (FLC), f	NOEC = 0.156 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Pimephales promelas</i>	Ethofumesate	28 d (ELC), f	NOEC = 4.17 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Ethofumesate	48 h, s	EC ₅₀ = 13.52 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Americamysis bahia</i>	Ethofumesate	96 h, s	EC ₅₀ = 5.4 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Crassostrea virginica</i>	Ethofumesate	96 h, f	EC ₅₀ = 1.7 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Metabolite NC 8493	48 h, ss	EC ₅₀ = >10 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Metabolite NC 8493	48 h, s	EC ₅₀ = >100 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Metabolite NC 20645	48 h, ss	EC ₅₀ = >10 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Metabolite NC 20645	48 h, s	EC ₅₀ = >100 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Ethofumesate	21 d, ss	NOEC = 0.32 mg a.s./L nom	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Ethofumesate	21 d, ss	NOEC = 0.25 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Daphnia magna</i>	Ethofumesate	21 d, ss	NOEC = 1.06 mg a.s./L mm	EFSA Conclusion 4374/2016
<i>Chironomus riparius</i>	Ethofumesate	28 d, spiked water	NOEC = 3.82 mg a.s./L im	EFSA Conclusion 4374/2016
<i>Chironomus riparius</i>	Ethofumesate	28 d, spiked water	NOEC = 5.33 mg a.s./L im	EFSA Conclusion 4374/2016
<i>Chironomus riparius</i>	Ethofumesate	28 d, spiked water	NOEC = 14.05 mg a.s./L im	EFSA Conclusion 4374/2016

Species	Substance	Exposure System	Results	Reference
<i>Pseudokirchneriella subcapitata</i>	Ethofumesate	72 h, s	ErC ₅₀ = 16.3 mg a.s./L (NOEC = 5.91 mg a.s./L) _{mm} EyC ₅₀ = 9.68 mg a.s./L (NOEC = 5.91 mg a.s./L) _{mm}	EFSA Conclusion 4374/2016
<i>Anabaena flos-aquae</i>	Ethofumesate	96 h, s	ErC ₅₀ = >20 mg a.s./L (NOEC = 20 mg a.s./L) _{nom} EbC ₅₀ = >20 mg a.s./L (NOEC = 20 mg a.s./L) _{nom}	EFSA Conclusion 4374/2016
<i>Skeletonema costatum</i>	Ethofumesate	72 h, s	ErC ₅₀ = >20 mg a.s./L (NOEC = 5 mg a.s./L) _{nom} EbC ₅₀ = 14.5 mg a.s./L (NOEC = 2.5 mg a.s./L) _{nom}	EFSA Conclusion 4374/2016
<i>Pseudokirchneriella subcapitata</i>	Metabolite NC 8493	72 h, s	ErC ₅₀ = 20.7 mg a.s./L (NOEC = 0.367 mg a.s./L) _{nom} EyC ₅₀ = 0.865 mg a.s./L (NOEC = 0.367 mg a.s./L) _{nom}	EFSA Conclusion 4374/2016
<i>Desmodesmus subspicatus</i>	Metabolite NC 8493	72 h, s	ErC ₅₀ = 4.83 mg a.s./L (NOEC = 1.33 mg a.s./L) _{mm} EyC ₅₀ = 1.87 mg a.s./L (NOEC = 1.33 mg a.s./L) _{mm}	EFSA Conclusion 4374/2016
<i>Desmodesmus subspicatus</i>	Metabolite NC 20645	72 h, s	ErC ₅₀ = 52.4 mg a.s./L (NOEC = 1.25 mg a.s./L) _{mm} EyC ₅₀ = 8.83 mg a.s./L (NOEC = 1.25 mg a.s./L) _{mm}	EFSA Conclusion 4374/2016
<i>Pseudokirchneriella subcapitata</i>	Metabolite NC 20645	72 h, s	ErC ₅₀ = >10 mg a.s./L (NOEC = 10 mg a.s./L) _{nom} EyC ₅₀ = >10 mg a.s./L (NOEC = 10 mg a.s./L) _{nom}	EFSA Conclusion 4374/2016
<i>Lemna gibba</i>	Ethofumesate	14 d, ss	ErC ₅₀ = >52.8 mg a.s./L (NOEC = 4.3 mg a.s./L) _{mm} EbC ₅₀ = 50.4 mg a.s./L (NOEC = 4.3 mg a.s./L) _{mm}	EFSA Conclusion 4374/2016

Species	Substance	Exposure System	Results	Reference
<i>Lemna gibba</i>	Ethofumesate	7 d, ss	ErC ₅₀ = >42 mg a.s./L (NOEC = 26 mg a.s./L) mm EbC ₅₀ = 35.0 mg a.s./L (NOEC = 17 mg a.s./L) mm	EFSA Conclusion 4374/2016
<i>Myriophyllum spicatum</i>	Ethofumesate	14 d, s	ErC ₅₀ = 0.479 mg a.s./L (NOEC = 0.036 mg a.s./L) mm EyC ₅₀ = 0.25 mg a.s./L (NOEC = 0.036 mg a.s./L) mm	EFSA Conclusion 4374/2016
Higher-tier studies (micro- or mesocosm studies)				
None				

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

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

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Clopyralid	96h, s	LC ₅₀ >99.9 mg a.s./L mm	EFSA Conclusion 5389/2018
<i>Pimephales promelas</i>	Clopyralid	34d (ELS), f	NOEC = 10.8 mg a.s./L mm	EFSA Conclusion 5389/2018
<i>Daphnia magna</i>	Clopyralid	48h, s	EC ₅₀ >99.0 mg a.s./L mm	EFSA Conclusion 5389/2018
<i>Daphnia magna</i>	Clopyralid	21d, ss	NOEC = 17 mg a.s./L mm	EFSA Conclusion 5389/2018
<i>Chironomus riparius</i>	Clopyralid	28d, s	NOEC = 50 mg a.s./L mm	EFSA Conclusion 5389/2018
<i>Selenastrum capricornutum</i>	Clopyralid	96h, s	72h ErC ₅₀ = 30 mg a.s./L mm 72h EbC ₅₀ = 30.9 mg a.s./L mm 72h NOEC <3.45 ^a mg a.s./L mm 96h ErC ₅₀ = 33.1 mg a.s./L mm 96h EbC ₅₀ = 32.7 mg a.s./L mm 96h NOEC = 24.8 mg a.s./L mm	EFSA Conclusion 5389/2018
<i>Navicula pelliculosa</i>	Clopyralid	96h, s	72h ErC ₅₀ = 31.3 mg a.s./L mm 72h EbC ₅₀ = 32.1 mg a.s./L mm 72h EyC ₅₀ = 31.5 mg a.s./L mm	EFSA Conclusion 5389/2018

Species	Substance	Exposure System	Results	Reference
			72h NOEC = 1.60 mg a.s./L _{mm} 96h E _r C ₅₀ = 30.5 mg a.s./L _{mm} 96h E _b C ₅₀ = 28.7 mg a.s./L _{mm} 96h NOEC = 1.60 mg a.s./L _{mm}	
<i>Lemna gibba</i>	Clopyralid	14d, s	EC ₅₀ = 89.0 mg a.s./L _{mm}	EFSA Conclusion 5389/2018
<i>Myriophyllum spicatum</i>	Clopyralid	14d, s	E _r C ₅₀ > 3.0 mg a.s./L _{mm} LOE _r C = 0.0286 mg a.s./L _{mm} NOE _r C = 0.0089 mg a.s./L _{mm} E _y C ₅₀ = 1.225 mg a.s./L _{mm} LOE _y C = 0.0286 mg a.s./L _{mm} NOE _y C = 0.0089 mg a.s./L _{mm}	EFSA Conclusion 5389/2018

Higher-tier studies (micro- or mesocosm studies)

None

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; ^a no effect at 6.38, 11.8 and 24.8 mg a.s./L

zRMS comments:

Aquatic toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – ADM.01851.H.1.A

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	ADM.01851.H.1.A	96 h, s	LC ₅₀ = 31.8 mg/L _{nom}	██████/ 2024a/ 23 48 AFA 0006
<i>Daphnia magna</i>	ADM.01851.H.1.A	48 h, s	EC ₅₀ = 61.4 mg/L _{mm}	Renner P./ 2024b/ 23 48 ADL 0020
<i>Raphidocelis subcapitata</i>	ADM.01851.H.1.A	72 h, s	E _r C ₁₀ = 11.7 mg/L _{nom} E _r C ₂₀ = 17.8 mg/L _{nom} E _r C ₅₀ = 39.2 mg/L _{nom} E _y C ₁₀ = 14.6 mg/L _{nom} E _y C ₂₀ = 16.2 mg/L _{nom} E _y C ₅₀ = 19.9 mg/L _{nom}	Renner P./ 2024c/ 23 48 AAL 0014
<i>Myriophyllum spicatum</i>	ADM.01851.H.1.A	14d, s	Freshweight E _r C ₅₀ = 2.49 mg/L _{nom} E _y C ₅₀ = 1.08 mg/L _{nom} E _r C ₂₀ = 0.318 mg/L _{nom} E _y C ₂₀ = 0.172 mg/L _{nom}	Warning D./2022/ 2248AMS001

Species	Substance	Exposure System	Results	Reference
			ErC ₁₀ = 0.108 mg/L _{nom} EyC ₁₀ = 0.066 mg/L _{nom} LOEC = 0.143 mg/L _{nom} NOEC = 0.045 mg/L _{nom} Dryweight ErC ₅₀ = 40.6 mg/L _{nom} EyC ₅₀ = 15.6 mg/L _{nom} ErC ₂₀ = 1.50 mg/L _{nom} EyC ₂₀ = 0.710 mg/L _{nom} ErC ₁₀ = 0.267 mg/L _{nom} EyC ₁₀ = 0.141 mg/L _{nom} LOEC = 0.458 mg/L _{nom} NOEC = 0.143 mg/L _{nom} Main shoot length ErC ₅₀ = 1.20 mg/L _{nom} EyC ₅₀ = 0.617 mg/L _{nom} ErC ₂₀ = 0.175 mg/L _{nom} EyC ₂₀ = 0.134 mg/L _{nom} ErC ₁₀ = 0.064 mg/L _{nom} EyC ₁₀ = 0.060 mg/L _{nom} LOEC = 0.143 mg/L _{nom} NOEC = 0.045 mg/L _{nom} Total shoot length ErC ₅₀ = 1.36 mg/L _{nom} EyC ₅₀ = 0.677 mg/L _{nom} ErC ₂₀ = 0.272 mg/L _{nom} EyC ₂₀ = 0.197 mg/L _{nom} ErC ₁₀ = 0.117 mg/L _{nom} EyC ₁₀ = 0.103 mg/L _{nom} LOEC = 0.458 mg/L _{nom} LOEC _{yC} = 0.143 mg/L _{nom} NOEC _{rC} = 0.143 mg/L _{nom} NOEC _{yC} = 0.045 mg/L _{nom}	

Higher-tier studies (micro- or mesocosm studies)

None

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

zRMS comments:

Studies on effects of the formulated product on aquatic organisms listed in Table 9.5-3 were evaluated by the zRMS and considered acceptable.
Summaries of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.5.1.1 Justification for new endpoints

New endpoints are provided for the formulated product as it was not the representative product assessed in either the ethofumesate or clopyralid active substance submissions.

9.5.2 Risk assessment

9.5.2.1 Individual substance 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).

As discussed in Part B8, section 8.9.1 of this dRR PEC_{SW} values for ethofumesate have been calculated considering plant uptake factors (PUF) of 0 and 0.5. The value of 0.5 is in line with the value used at a European level for surface water modelling (with reference to the EFSA Conclusion for ethofumesate, EFSA Journal 2016; 14(1):4374, Page 89). It has recently been observed by some Member State Authorities that a PUF of 0 is more conservative and should be used as a worst-case scenario. PEC_{SW} values for ethofumesate metabolite NC20645 have also been calculated twice considering ethofumesate PUFs of 0 and 0.5. The PUF for NC20645 was always 0.

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.

In the following table, 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.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ethofumesate (PUF = 0) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.01851.H.1.A in sugar beet (3 x 335 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic Macrophyte
Test species		<i>Cyprinus carpio</i>	<i>Danio rerio</i>	<i>Crassostrea virginica</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		LC ₅₀ 10920	NOEC 156	EC ₅₀ 1700	NOEC 250	EyC ₅₀ 9680	NOEC 3820	EyC ₅₀ 250
AF		100	10	100	10	10	10	10
RAC (µg/L)		109.2	15.6	17.0	25.0	968	382	25.0
FOCUS Scenario	PEC gl-max (µg/L)							
Step 1								
	298.7012	2.7354	19.1475	17.5707	11.9480	0.3086	0.7819	11.9480
Step 2								
N-Europe	42.6302	0.3904	2.7327	2.5077	1.7052	0.0440	0.1116	1.7052
S-Europe	79.3439	0.7266	5.0861	4.6673	3.1738	0.0820	0.2077	3.1738
Step 3								
D3/ditch*	1.758	0.0161	0.1127	0.1034	0.0703	0.0018	0.0046	0.0703
D4/pond	0.6032	0.0055	0.0387	0.0355	0.0241	0.0006	0.0016	0.0241
D4/stream*	1.454	0.0133	0.0932	0.0855	0.0582	0.0015	0.0038	0.0582
R1/pond	0.5146	0.0047	0.0330	0.0303	0.0206	0.0005	0.0013	0.0206
R1/stream	8.639	0.0791	0.5538	0.5082	0.3456	0.0089	0.0226	0.3456
R3/stream	19.99	0.1831	1.2814	1.1759	0.7996	0.0207	0.0523	0.7996

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; * single application resulted in higher PEC_{sw} value than multiple applications

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ethofumesate (PUF = 0.5) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.01851.H.1.A in sugar beet (3 x 335 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Aquatic Macrophyte
Test species		<i>Cyprinus carpio</i>	<i>Danio rerio</i>	<i>Crassostrea virginica</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. sub-capitata</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		LC ₅₀ 10920	NOEC 156	EC ₅₀ 1700	NOEC 250	EyC ₅₀ 9680	NOEC 3820	EyC ₅₀ 250
AF		100	10	100	10	10	10	10
RAC (µg/L)		109.2	15.6	17.0	25.0	968	382	25.0
FOCUS Scenario	PEC gl-max (µg/L)							
Step 1								
	298.7012	2.7354	19.1475	17.5707	11.9480	0.3086	0.7819	11.9480
Step 2								
N-Europe	42.6302	0.3904	2.7327	2.5077	1.7052	0.0440	0.1116	1.7052
S-Europe	79.3439	0.7266	5.0861	4.6673	3.1738	0.0820	0.2077	3.1738
Step 3								
D3/ditch*	1.758	0.0161	0.1127	0.1034	0.0703	0.0018	0.0046	0.0703
D4/pond	0.5516	0.0051	0.0354	0.0324	0.0221	0.0006	0.0014	0.0221
D4/stream*	1.453	0.0133	0.0931	0.0855	0.0581	0.0015	0.0038	0.0581
R1/pond	0.4732	0.0043	0.0303	0.0278	0.0189	0.0005	0.0012	0.0189
R1/stream	7.728	0.0708	0.4954	0.4546	0.3091	0.0080	0.0202	0.3091
R3/stream	18.74	0.1716	1.2013	1.1024	0.7496	0.0194	0.0491	0.7496

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; * single application resulted in higher PEC_{sw} value than multiple applications

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ethofumesate metabolite NC8493 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.01851.H.1.A in sugar beet (3 x 335 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Aquatic Macro-phyte
Test species		<i>Cyprinus carpio</i>	<i>Danio rerio</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Myriophyllum spicatum</i>
End-point (µg/L)		LC ₅₀ 1092*	NOEC 15.6*	EC ₅₀ 1000000	E _y C ₅₀ 86.5	E _y C ₅₀ 25*
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	10000	8.65	2.50
FOCUS Scenario	PEC _{gl-max} (µg/L)					

Step 1

	72.9389	6.6794	46.7557	0.0073	8.4322	29.1756
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Step 2

N-Europe	0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
S-Europe	0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; * studies are not available for the metabolite, therefore x 10 toxicity of the active substance is assumed

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ethofumesate metabolite NC20645 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.01851.H.1.A in sugar beet (3 x 335 g a.s./ha) (Ethofumesate PUF = 0)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Aquatic Macro-phyte
Test species		<i>Cyprinus carpio</i>	<i>Danio rerio</i>	<i>Crassostrea virginica</i>	<i>Pseudokirchn. subcapitata</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		LC ₅₀ 1092*	NOEC 15.6*	EC ₅₀ 1000000	E _y C ₅₀ 8830	E _y C ₅₀ 25*
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	10000	883	2.50
FOCUS Scenario	PEC _{gl-max} (µg/L)					

Step 1

	67.3995	6.1721	43.2048\	0.0067	0.0763	26.9598
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Step 2

N-Europe	8.7816	0.8042	5.6292	0.0009	0.0099	3.5126
S-Europe	16.3832	1.5003	10.5021	0.0016	0.0186	6.5533

Step 3

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Aquatic Macro- phyte
D3/ditch**	0.000391	<0.0001	0.0003	<0.0001	<0.0001	0.0002
D4/pond	0.008967	0.0008	0.0057	<0.0001	<0.0001	0.0036
D4/stream	0.001091	0.0001	0.0007	<0.0001	<0.0001	0.0004
R1/pond	0.01122	0.0010	0.0072	<0.0001	<0.0001	0.0045
R1/stream	0.01221	0.0011	0.0078	<0.0001	<0.0001	0.0049
R3/stream	0.07607	0.0070	0.0488	<0.0001	0.0001	0.0304

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; * studies are not available for the metabolite, therefore x 10 toxicity of the active substance is assumed; ** single application resulted in higher PEC_{sw} value than multiple applications

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ethofumesate metabolite NC20645 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.01851.H.1.A in sugar beet (3 x 335 g a.s./ha) (Ethofumesate PUF = 0.5)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Aquatic Macro- phyte
Test species		<i>Cyprinus carpio</i>	<i>Danio rerio</i>	<i>Crassostrea virginica</i>	<i>Pseudokirchn. subcapitata</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		LC ₅₀ 1092*	NOEC 15.6*	EC ₅₀ 1000000	E _y C ₅₀ 8830	E _y C ₅₀ 25*
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	10000	883	2.50
FOCUS Scenario	PEC _{gl-max} (µg/L)					

Step 1

	67.3995	6.1721	43.2048\	0.0067	0.0763	26.9598
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Step 2

N-Europe	8.7816	0.8042	5.6292	0.0009	0.0099	3.5126
S-Europe	16.3832	1.5003	10.5021	0.0016	0.0186	6.5533

Step 3

D3/ditch**	0.000521	<0.0001	0.0003	<0.0001	<0.0001	0.0002
D4/pond	0.008334	0.0008	0.0053	<0.0001	<0.0001	0.0033
D4/stream	0.00098	0.0001	0.0006	<0.0001	<0.0001	0.0004
R1/pond	0.01032	0.0009	0.0066	<0.0001	<0.0001	0.0041
R1/stream	0.01092	0.0010	0.0070	<0.0001	<0.0001	0.0044
R3/stream	0.07129	0.0065	0.0457	<0.0001	0.0001	0.0285

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; * studies are not available for the metabolite, therefore x 10 toxicity of the active substance is assumed; ** single application resulted in higher PEC_{sw} value than multiple applications

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for clopyralid for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.01851.H.1.A in sugar beet (3 x 50.25 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. pro-longed	Aquatic Macrophyte
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Navicula pelliculosa</i>	<i>Chironomus riparius</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg/L)		LC ₅₀ 99900	NOEC 10800	EC ₅₀ 99000	NOEC 17000	E _b C ₅₀ 28700	NOEC 50000	E _y C ₅₀ 1225
AF		100	10	100	10	10	10	10
RAC (µg/L)		999	1080	990	1700	2870	5000	122.5
FOCUS Scenario	PEC gl-max (µg/L)							
Step 1								
	51.5421	0.0516	0.0477	0.0521	0.0303	0.0180	0.0103	0.4208
Step 2								
N-Europe	4.5942	0.0046	0.0043	0.0046	0.0027	0.0016	0.0009	0.0375
S-Europe	8.1788	0.0082	0.0076	0.0083	0.0048	0.0028	0.0016	0.0668

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, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for prolonged exposure to fish as characterised by a NOEC for *Danio rerio* of 156 µg ethofumesate/L in connection with an assessment factor of 10) in several FOCUS Step 3 scenarios. Therefore, no further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

The higher tier risk assessment has been calculated using a plant uptake factor (PUF) of 0. As discussed in Part B8, section 8.9.2.1 of this dRR, the Step 3 PEC_{SW} calculations indicated that there was not a significant difference between values calculated with a PUF of 0 or a PUF of 0.5, therefore it can reasonably be assumed that the same mitigation factors would be required for Step 4 PEC_{SW} values calculated using either PUF.

Table 9.5-10: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for ethofumesate based on FOCUS Step 4 calculations and toxicity data for prolonged exposure to fish with mitigation of spray drift and run-off for the use of ADM.01851.H.1.A in sugar beet (3 x 335 g a.s./ha)

Intended use		Sugar beet		
Active substance		Ethofemesate		
Application rate (g/ha)		3 x 335		
Nozzle reduction	No-spray buffer (m)	1	10	10
	Vegetated filter strip (m)	0	0	10
None	R3 stream	19.99	19.99	9.12
RAC (µg/L)		PEC/RAC ratio		
15.6				
None	R3 stream	1.2814	1.2814	0.5846

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

zRMS comments:

Based on the performed calculations following conclusions may be derived:

Sugar beet :

- Clopyralid : acceptable risk with no need for risk mitigation measures
- Ethofumeaste:
 - **D** and **R1** scenarios: risk acceptable with no need for risk mitigation measures
 - scenario **R3**: risk acceptable with 10 m VFS.

Based on the performed calculations for the worst-case scenario acceptable risk following application of ADM.01851.H.1.A according to the Central Zone GAP may be concluded.

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

For remaining metabolites of both active compounds, the risk is acceptable in both crops with no need for risk mitigation measures.

9.5.2.2 Mixture toxicity risk assessment

The evaluation of the mixture toxicity risks for aquatic (and sediment-dwelling) organisms was performed in accordance with the “Guidance 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) and in EFSA Journal 2013;11(7):3290 (Aquatic Guidance Document, abbreviated as EFSA, 2013). The whole mixture assessment is based on the model of concentration addition (CA) and particularly the decision scheme as provided by EFSA (2013, section 10.3.11) was applied. The calculations were performed with the tool “AGD_AquaMix”.¹

¹Sometimes also termed “Aquatic MixTox Tool”. The current version can be found on the EFSA Knowledge Junction platform, DOI: [10.5281/zenodo.4593675](https://doi.org/10.5281/zenodo.4593675) ([Aquatic Mixture Toxicity Tool and additional Information | Zenodo](#))

Table 9.5-11: Summary of assessment steps considered for the mixture risk assessment

Steps	Conclusion on the Steps			
	Fish	Invertebrates	Algae	Macrophytes
Screening 1: ETR trigger (optional Step)	Acceptable risk at FOCUS step 3	No acceptable risk at FOCUS step 3	Acceptable risk at FOCUS step 3	No acceptable risk at FOCUS step 3
Screening 2: Driver (optional Step)	Driver detected	Driver detected	Driver detected	Driver detected
Step 1: data available?	Yes	Yes	Yes	Yes
Step 2: apparent synergism or antagonism?	No	Antagonism	No	No
Step 3: mixture similar or not?	Yes	Yes	Yes	Yes
Step 4: ETRmix assessment (ECxPPP)	Acceptable risk at FOCUS step 3	Acceptable risk at FOCUS step 3	Acceptable risk at FOCUS step 3	Acceptable risk at FOCUS step 3
Step 5: driver available?	Not required	Not required	Not required	Not required
Step 6: driver assessment	Not required	Not required	Not required	Not required
Step 7: synergism assessment (few data)	Not required	Not required	Not required	Not required
Step 8a: ETRmix assessment	Not required	Not required	Not required	Not required
Step 8b: RQmix assessment	Not required	Not required	Not required	Not required
Step 9: antagonism assessment	Not required	Apparent antagonism caused by tests run with different species.	Not required	Not required
Step 10: synergism assessment	Not required	Not required	Not required	Not required

Overview of the assessment steps: Table 9.5-11 gives a summary of the decisions made for each organism group during the mixture risk assessment with respect to the decision scheme as provided by EFSA (2013, section 10.3.11).

Screening Assessment: In the screening 1 assessment some $ETR_i > ETR \text{ trigger}/n$ at FOCUS Step 3 (where ETR_i is the exposure toxicity ratio for an individual substance) for invertebrates and macrophytes. All $ETR_i \leq ETR \text{ trigger}/n$ at FOCUS Step 3 for fish and algae. The assessment cannot be concluded at this stage. The screening 2 assessment was carried out. The results of the screening 1 assessment are summarised in the table below.

Table 9.5-12: Summary of Screening 1 Assessment of Ethofumesate and Clopyralid

Scenario	ETR_i of ethofumesate	ETR_i of clopyralid	Any a.s. above $ETR \text{ trigger}$?	$ETR \text{ trigger}/n$	All $ETR_i \leq ETR \text{ trigger}/n$?
Fish					
Step 1	0.027	0.001	Yes	-	-
Step 2 NEU	0.004	0.000	No	0.005	Yes
Step 2 SEU	0.007	0.000	No	0.005	No
Step 3					
D3 ditch	0.000	0.000	No	0.005	Yes
D4 pond	0.000	0.000	No	0.005	Yes
D4 stream	0.000	0.000	No	0.005	Yes
R1 pond	0.000	0.000	No	0.005	Yes
R1 stream	0.001	0.000	No	0.005	Yes
R3 stream	0.002	0.000	No	0.005	Yes
Invertebrates					
Step 1	0.176	0.001	Yes	-	-
Step 2 NEU	0.025	0.000	Yes	-	-
Step 2 SEU	0.047	0.000	Yes	-	-
Step 3					
D3 ditch	0.001	0.000	No	0.005	Yes
D4 pond	0.000	0.000	No	0.005	Yes
D4 stream	0.001	0.000	No	0.005	Yes
R1 pond	0.000	0.000	No	0.005	Yes
R1 stream	0.005	0.000	No	0.005	No
R3 stream	0.012	0.000	Yes	-	-
Algae					
Step 1	0.031	0.002	No	0.05	Yes
Step 2 NEU	0.004	0.000	No	0.05	Yes
Step 2 SEU	0.008	0.000	No	0.05	Yes
Macrophytes					
Step 1	1.195	0.042	Yes	-	-
Step 2 NEU	0.171	0.004	Yes	-	-
Step 2 SEU	0.317	0.007	Yes	-	-
Step 3					
D3 ditch	0.007	0.000	No	0.005	Yes
D4 pond	0.002	0.000	No	0.005	Yes
D4 stream	0.006	0.000	No	0.005	Yes
R1 pond	0.002	0.000	No	0.005	Yes
R1 stream	0.035	0.000	No	0.005	Yes
R3 stream	0.080	0.001	No	0.005	No

ETR default trigger values ≤ 0.01 (fish, invertebrates) and ≤ 0.1 (algae, macrophytes)

In the screening 2 assessments drivers were detected for all trophic levels for all scenarios. No synergism between the two active substances was detected.

The full step-wise assessment of mixture toxicity is presented below.

Step 1: Measured toxicity data are available for both formulation and active substances. Go to Step 2.

Step 2: The plausibility of the measure formulation toxicity against the calculated mixture toxicity has been checked. MDR values were calculated according to the following equation:

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

Where the calculated mixture toxicity was determine using the following equation:

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

where:

- n: number of mixture components
- i: index from 1...n mixture components
- p_i: the ith component as a relative fraction of the mixture composition (note: $\sum p_i$ must be 1)
- ECx_i: concentration of component i provoking x % effect (pragmatically, NOEC_i may be inserted, too).

The results are summarised in the table below.

Table 9.5-13: Step 2 - MDR values

Trophic level	MDR value	Next step
Fish	0.79	3
Invertebrates	0.06	9
Algae	0.55	3
Macrophytes	0.47	3

The MDR values indicated that the calculated toxicity is similar to the measured toxicity for fish, algae and macrophytes. For invertebrates the MDR value indicates that the calculated mixture is less toxic than the measured toxicity (i.e. antagonistic).

Step 3: Mixture toxicity was calculated as described in Step 2 and compared to the mixture composition at the PEC_{mix} for each FOCUS Step PEC calculation. The results are summarised in the table below.

Table 9.5-14: Step 3 - EC_{xmix-CA} (a.s. in PPP)/EC_{xmix-CA} (a.s. in PEC_{mix})

FOCUS scenario	Fish	Invertebrates	Algae	Macrophytes
Step 1	0.98	0.98	0.99	0.99
Step 2 NEU	1.03	1.04	1.02	1.03
Step 2 SEU	1.04	1.04	1.03	1.03
Step 3				
D3 ditch	1.00	1.00	1.00	1.00
D4 pond	1.06	1.06	1.04	1.05
D4 stream	1.00	1.00	1.00	1.00
R1 pond	1.09	1.10	1.06	1.08
R1 stream	1.09	1.10	1.06	1.08
R3 stream	1.06	1.06	1.04	1.05

All values are between 0.8 to 1.2, therefore the mixture toxicity is similar to the measured composition at the PEC_{mix} for each FOCUS Step PEC calculation. Go to step 4.

Step 4: A mixture risk assessment was conducted based on measure mixture toxicity. The ETR_{mix-PPP} values (i.e. the PEC/RAC values) are summarised in the table below. Values in bold indicate unacceptable risks.

Table 9.5-15: Step 4 - ETR_{mix}-PPP

FOCUS scenario	Fish	Invertebrates	Algae	Macrophytes
Step 1	0.02	0.01	0.02	0.59
Step 2 NEU	0.00	0.00	0.00	0.08
Step 2 SEU	0.01	0.00	0.00	0.15
Step 3				
D3 ditch	0.00	0.00	0.00	0.00
D4 pond	0.00	0.00	0.00	0.00
D4 stream	0.00	0.00	0.00	0.00
R1 pond	0.00	0.00	0.00	0.00
R1 stream	0.00	0.00	0.00	0.02
R3 stream	0.00	0.00	0.00	0.04

Acceptable risks are indicated for all trophic levels at FOCUS step 3. No further assessment is required.

Step 5: Not required.

Step 6: Not required.

Step 7: Not required.

Step 8: Not required.

Step 9: Antagonism was indicated for invertebrate toxicity of measure mixture toxicity compared to calculated toxicity. The reason for this is because the endpoint for ethofumesate is based on a study with *Crassostrea virginica*, whereas the endpoints for clopyralid and for the mixture ADM.01851.H.1.A are based on studies with *Daphnia magna*. When the MDR value for invertebrates is calculated only using *Daphnia magna* endpoints no antagonism is indicated as the MDR value is 0.50.

zRMS comments:

zRMS agrees with mixture toxicity assessment for aquatic organism.

The calculated mixture toxicity is similar to the measured mixture toxicity, when the same species for formulation and active substances is considered, no unexplainable antagonism or synergism is indicated.

The mixture risk assessment based on measure mixture toxicity and PEC_{sw} at STEP 3 values, indicated an acceptable risk.

9.5.3 Overall conclusions

The risks to aquatic organisms from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable with consideration of a 10m vegetated filter strip.

The calculated mixture toxicity is similar to the measured mixture toxicity, no unexplainable antagonism or synergism is indicated.

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 ethofumesate and clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of ADM.01851.H.1.A were not evaluated as part of the EU assessments of ethofumesate or clopyralid. 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. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Ethofumesate	Oral	LD ₅₀ = >106.3 µg a.s./bee	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Ethofumesate	Oral	LD ₅₀ = >50 µg a.s./bee	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Ethofumesate	Oral	LD ₅₀ = >100 µg a.s./bee	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Clopyralid	Oral	LD ₅₀ = >100 µg a.s./bee	EFSA Conclusion 5389/2018
<i>Apis mellifera</i>	ADM.01851.H.1.A	Oral	LD ₅₀ = >199 µg product/bee (equivalent to >12.80 µg clopyralid/bee and >89.95 µg ethofumesate/bee)	Poráčzki K./2022/22 48 BAA 0079
<i>Apis mellifera</i>	Ethofumesate	Contact	LD ₅₀ = >100 µg a.s./bee	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Ethofumesate	Contact	LD ₅₀ = >50 µg a.s./bee	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Ethofumesate	Contact	LD ₅₀ = >100 µg a.s./bee	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Clopyralid	Contact	LD ₅₀ = >98.1 µg a.s./bee	EFSA Conclusion 5389/2018
<i>Apis mellifera</i>	ADM.01851.H.1.A	Contact	LD ₅₀ = >200 µg product/bee (equivalent to >12.86 µg clopyralid/bee and >90.40 µg ethofumesate/bee)	Poráčzki K./2022/22 48 BAA 0079
<i>Apis mellifera</i>	Ethofumesate	Chronic, 10d	LC ₅₀ = >120 mg a.s./kg LDD ₅₀ = >4.4 µg a.s./bee/d	EFSA Conclusion 4374/2016
<i>Apis mellifera</i>	Clopyralid	Chronic, 10d	LDD ₅₀ = >71.2 µg a.s./bee/d	EFSA Conclusion 5389/2018
<i>Apis mellifera</i>	ADM.01851.H.1.A	Chronic, 10d	LDD ₅₀ = >476.1 µg product/bee/d (equivalent to >30.61 µg clopyralid/bee and >215.2 µg ethofumesate/bee) LC ₅₀ = >20370 mg product/kg food (equivalent to >1310 mg clopyralid/ kg food and >9207 mg ethofumesate/ kg food)	Ruhland S./2023/22 48 BAC 0024
<i>Apis mellifera</i>	Clopyralid	Larval toxicity, 22d	LD ₁₀ = 12.5 µg	EFSA Conclusion

Species	Substance	Exposure System	Results	Reference
			a.s./larva	5389/2018
<i>Apis mellifera</i>	ADM.01851.H.1.A	Larval toxicity, 22d	ED ₅₀ = 257.0 µg product/larva ED ₂₀ = 166.4 µg product/larva ED ₁₀ = 132.5 µg product/larva NOED = 100.3 µg product/larva EC ₅₀ = 1624.5 mg product/kg food EC ₂₀ = 1051.6 mg product/kg food EC ₁₀ = 837.7 mg product/kg food NOEC = 634.1 mg product/kg food	Kleebaum K./2022/2248 BLC 0025
Higher-tier studies (tunnel test, field studies)				
None				

zRMS comments:

Bee toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Studies on effects of the formulated product on aquatic organisms listed in Table 9.6-1 were evaluated by the zRMS and considered acceptable.

Summaries of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.6.1.1 Justification for new endpoints

New endpoints are provided for the formulated product as it was not the representative product assessed in either the ethofumesate or clopyralid active substance submissions.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of ADM.01851.H.1.A in sugar beet

Intended use	Sugar beet		
Active substance	Ethofumesate		
Application rate (g/ha)	1 x 330 g a.s./ha*		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>50	330	6.60

Contact toxicity	>50		6.60
Active substance		Clopyralid	
Application rate (g/ha)		1 x 49.5 g a.s./ha*	
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>100	49.5	0.50
Contact toxicity	>98.1		0.50
Product		ADM.01851.H.1.A	
Application rate (g/ha)		1 x 767.1 g product/ha**	
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	>199	767.1	3.85
Contact toxicity	>200		3.84

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger. * This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application. ** Based on a single application rate of 0.66 L product/ha and a density of 1.1622 g/mL. Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

Both the Hazard Quotients for the oral (Q_{HO}) and the contact exposure (Q_{HC}) are below the relevant trigger of 50. Thus, according to SANCO/10329/2002 rev.2, an overall acceptable risk to honeybees can be concluded from the application of ADM.01851.H.1.A.

The chronic effects were evaluated by feeding tests with adult and juvenile honeybees. The results show low toxicity of ADM.01851.H.1.A for adult honeybees (LLD₅₀ = >476 µg product a.s./bee/day) and for juvenile bees (ED₅₀ = 257.0 µg product a.s./larva/developmental period). For the details of the studies, please refer to data points KCP 10.3.1.2 and KCP 10.3.1.3 in Appendix 2.

As stated in the RAR (2015) of ethofumesate, the risk for honeybees to get in contact with contaminated nectar and pollen is negligible as sugar beets do not build flowers within the first year. Sugar beets are harvested by the end of the first year at BBCH 49 which is before flowering. In the rare case that shoots with flowers are produced in the first year or beets are flowering in the second year (if beets are grown for seed production) no risk for honeybees is expected as beet flowers are wind pollinated.

Sugar beet flowers are not mentioned in any standard or handbook on honeybee foraging plants (e.g. Maurizio & Schaper, 1994² ; Pritsch, 2007³).

Moreover, bees are not likely to be exposed to the whole formulation for the same reason, particularly as application is distinctly before flowering.

In conclusion, it can be reasoned that the acute risk for bees can be considered as acceptable, both from the toxicity and the exposure point of view.

zRMS comments:

In general, according to conclusions of the Central Zone Steering Committee (CZSC) at the Central Zone level the risk assessment for bees should be performed in line with recommendations of SANCO/10329/2002 rev 2 final, while recommendations of EFSA (2013) should not be considered until the guidance is noted at the EU level.

As SANCO/10329/2002 rev 2 final does not provide any indications on how to perform the chronic and larvae risk assessment, consideration of indications of EFSA (2013) to perform dietary oral risk assessment seems to be reasonable approach, even if the guidance itself is not noted yet.

² Maurizio, A., Schaper, F., 1994, Das Trachtpflanzenbuch

³ Pritsch, G., 2007, Bienenweide

This was confirmed by among of experts during Harmonisation Meeting in Warsaw in December 2023 where it was concluded that the chronic and larvae RA based on EFSA (2013) should be presented in the Core Dossier for ppp.

The situation with the bee guidance document is complicated since the only GD in force is the old terrestrial ecotoxicology guidance (SANCO/10329/2002 rev. 2 final of October 2002), which is, however, not aligned with the current data requirements and does not cover critical issues related e.g. with chronic risk to adult bees and larvae. Although in 2013 the new EFSA bee guidance was issued, it was never noted at the EU level, in the meantime new GD of 2023 became available, still has not been noted yet. Only the EFSA (2013) Bee Guidance document contains a risk assessment method for chronic and larval exposure for all types of uses. For EFSA 2023, this is pending the publication of a calculator tool: the methodology is extremely complicated, and therefore a simple calculation by hand is not possible.

Taking this into account the risk assessment performed using both guidance documents (EFSA 2023) has been performed by the zRMS and presented below:

Chronic risk assessment to bees according to EFSA GD 2013:

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 acute and chronic risks to adult honey bees and honey bee larvae bees from the use of ADM.03502.F.1.A 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?
Sugar beet, BBCH 10-19, maximum application dose 0.7671 kg product/ha					
Oral route of exposure					
Honey bee, chronic 10-39	476.1	7.6 / 10.6	0.012	0.03	Yes
Honey bee, Larvae 10-39	100.3	4.4 / 6.1	0.03	0.2	Yes

Considering the proposed uses of at a maximum application rate of 0.07671 kg product/ha an acceptable risk of formulation is indicated following the chronic exposure of adults and for honey bee larvae at this stage of testing. Therefore, 1st tier oral risk assessments is not required.

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 dossier and no studies on bumble bees have been carried out on the product ADM.01851.H.1.A.

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

dossier and no studies on solitary bees have been carried out on the product ADM.01851.H.1.A.

9.6.5 Overall conclusions

The risk to bees from the use of ethofumesate and clopyralid applied as the formulation ADM.01851.H.1.A to sugar beet was assessed in line with indications of the current guidance document (SANCO/10329/2002 rev 2 final) and is acceptable. The Hazard Quotients for oral (Q_{HO}) and contact exposure (Q_{HC}) are below the trigger of 50. Results of chronic feeding studies show low toxicity to larvae and adults.

In addition, the formulation is applied before the flowering period and sugar beet can be considered as not attractive to bees (harvest before flowering period). In consequence, according to EPPO guideline (EPPO 3/10 (3);09-2010), there is no exposure to bees and so acceptable risk to bees. Therefore, an acceptable risk to bees is expected from the application of ADM.01851.H.1.A.

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 been carried out with separate products containing ethofumesate and clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of ADM.01851.H.1.A were not evaluated as part of the EU assessment of ethofumesate or clopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	Ethofumesate 500 SC	Laboratory test glass plates (2D)	LR ₅₀ >1000 g a.s./ha ER ₅₀ >1000 g a.s./ha	EFSA Conclusion 4374/2016
<i>Typhlodromus pyri</i> (protonymphs)	EF-1136 (clopyralid 100 g/L SC)	Laboratory test glass plates (2D)	LR ₅₀ >200 g a.s./ha	EFSA Conclusion 5389/2018
<i>Typhlodromus pyri</i> (protonymphs)	ADM.01851.H.1.A	Laboratory test glass plates (2D)	LR ₅₀ >3000 mL product/ha ER ₅₀ >3000 mL product/ha	Röhlig U./2024a/ 23 48 NTL 0010
<i>Aphidius rhopalosiphi</i> (adults)	Ethofumesate 500 SC	Laboratory test glass plates (2D)	LR ₅₀ >1000 g a.s./ha ER ₅₀ >1000 g a.s./ha	EFSA Conclusion 4374/2016
<i>Aphidius rhopalosiphi</i> (adults)	EF-1136 (clopyralid 100 g/L SC)	Laboratory test glass plates (2D)	LR ₅₀ >200 g a.s./ha	EFSA Conclusion 5389/2018
<i>Aphidius rhopalosiphi</i> (adults)	EF-1136 (clopyralid 100 g/L SC)	Laboratory test glass plates (2D)	LR ₅₀ >300 g a.s./ha	EFSA Conclusion 5389/2018
<i>Aphidius rhopalosiphi</i> (adults)	ADM.01851.H.1.A	Laboratory test glass plates (2D)	LR ₅₀ >3000 mL product/ha ER ₅₀ >3000 mL product/ha	Röhlig U./2024b/ 23 48 NAL 0010
<i>Aleochara bilineata</i>	Tramat 500 (ethofumesate)	Laboratory test Quartz sand (2D)	LR ₅₀ = >1252.5 g a.s./ha ER ₅₀ = >1252.5 g a.s./ha	EFSA Conclusion 4374/2016
<i>Chrysoperla carnea</i>	Tramat 500	Laboratory test	LR ₅₀ = >2000 g a.s./ha	EFSA Conclusion

Species	Substance	Exposure System	Results	Reference
	(ethofumesate)	Glass plates (2D)	ER ₅₀ = >2000 g a.s./ha	4374/2016
<i>Chrysoperla carnea</i>	EF-1136 (clopyralid 100 g/L SC)	Laboratory test Glass plates (2D)	LR ₅₀ = >200 g a.s./ha	EFSA Conclusion 5389/2018
<i>Poecilus cupreus</i>	Tramat 500 (ethofumesate)	Laboratory test Quartz sand (2D)	LR ₅₀ = >2000 g a.s./ha	EFSA Conclusion 4374/2016
<i>Poecilus cupreus</i>	EF-1136 (clopyralid 100 g/L SC)	Laboratory test	LR ₅₀ = >200 g a.s./ha	EFSA Conclusion 5389/2018
<i>Poecilus cupreus</i>	EF-1136 (clopyralid 100 g/L SC)	Quartz sand (2D)	LR ₅₀ = >120 g a.s./ha	EFSA Conclusion 5389/2018
Field or semi-field tests				
None				

zRMS comments:

NTA toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Studies on toxicity of ADM.01851.H.1.A 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 endpoints are provided for the formulated product as it was not the representative product assessed in either the ethofumesate or clopyralid active substance submissions.

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.

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.01851.H.1.A in sugarbeet

Intended use	Sugarbeet			
Active substance/product	Ethofumesate and clopyralid/ADM.01851.H.1.A			
Application rate (g/ha)	3 x 0.67 L product/ha*			
MAF	2.3			
Test species Tier I	LR₅₀ (lab.) (L/ha)	PER_{in-field} (L/ha)	HQ_{in-field} criterion: HQ ≤ 2	
<i>Typhlodromus pyri</i>	>3.0	1.541	<0.514	
<i>Aphidius rhopalosiphi</i>	>3.0		<0.514	

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger. * Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable.

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.01851.H.1.A in sugarbeet

Intended use	Sugarbeet				
Active substance/product	Ethofumesate and clopyralid/ADM.01851.H.1.A				
Application rate (g/ha)	3 x 0.67 L product/ha*				
MAF	2.3				
vdf	5 (Tier 1) **				
Test species Tier I	LR₅₀ (lab.) (L/ha)	Drift rate	PER_{off-field} (L/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>3.0	0.0201	0.006	10	<0.021
<i>Aphidius rhopalosiphi</i>	>3.0				<0.021

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger. * Different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable. ** Refer to EFSA supporting publication 2019:EN-1673, Outcome of pesticides peer review meeting on recurring issues on ecotoxicology

zRMS comments:

The risk assessment presented in Table 9.7-2 and 9.7-3 is validated by the zRMS.

As a worst case the VDF of 5 has been considered, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure.

It should be, however, 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. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by the zRMS.

Based on calculations performed with consideration of the Tier I laboratory data acceptable in-field or off-field risk to non-target arthropods from all intended uses of ADM.01851.H.1.A 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

The in-field and off-field risks to non-target arthropods other than bees from exposure to the formulated product ADM.01851.H.1.A, containing ethofumesate and clopyralid, following application to sugarbeet are acceptable without mitigation.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with the relevant metabolites of ethofumesate and clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ADM.01851.H.1.A were not evaluated as part of the EU assessments of ethofumesate or clopyralid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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
<i>Eisenia fetida</i>	Ethofol 500 SC	Overspray, 56d chronic, 10% organic material	EC ₁₀ = 8.84 mg a.s./kg soil dw EC ₁₀ corr. = 4.42 mg a.s./kg soil*	EFSA Conclusion 4374/2016
<i>Eisenia fetida</i>	Ethofumesate 500 SC	Overspray, 56d chronic, 10% organic material	NOEC = 25 mg a.s./kg dw NOEC _{corr.} = 12.5 mg a.s./kg dw	EFSA Conclusion 4374/2016
<i>Eisenia fetida</i>	Metabolite NC 8493	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 16 mg/kg dw (max. concentration tested)	EFSA Conclusion 4374/2016
<i>Eisenia fetida</i>	Metabolite NC 8493	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100 mg/kg dw (limit test)	EFSA Conclusion 4374/2016
<i>Eisenia fetida</i>	Metabolite NC 20645	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion 4374/2016
<i>Eisenia fetida</i>	Clopyralid	Overspray, 56d chronic, 10% organic material	NOEC = 1.97 mg a.s./kg soil dw	EFSA Conclusion 5289/2018
<i>Folsomia candida</i>	Ethofol 500 SC	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 26.7 mg a.s./kg soil dw NOEC _{corr.} = 13.35 mg a.s./kg soil	EFSA Conclusion 4374/2016
<i>Folsomia candida</i>	Ethofumesate 500 SC	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 44.1 mg a.s./kg soil dw NOEC _{corr.} = 22.05 mg a.s./kg soil dw	EFSA Conclusion 4374/2016
<i>Folsomia candida</i>	Metabolite NC 8493	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 556 mg/kg dw	EFSA Conclusion 4374/2016
<i>Folsomia candida</i>	Metabolite NC 20645	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100 mg/kg dw (max. dose tested)	EFSA Conclusion 4374/2016
<i>Hypoaspis aculeifer</i>	Ethofumesate 500 SC	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 44.2 mg a.s./kg soil dw NOEC _{corr.} = 22.1 mg	EFSA Conclusion 4374/2016

Species	Substance	Exposure System	Results	Reference
			a.s./kg soil dw	
<i>Hypoaspis aculeifer</i>	Ethofumesate 500 SC	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 441 mg a.s./kg soil dw NOEC _{corr.} = 220.5 mg a.s./kg soil dw	EFSA Conclusion 4374/2016
<i>Hypoaspis aculeifer</i>	Metabolite NC 8493	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 309 mg/kg dw	EFSA Conclusion 4374/2016
<i>Eisenia fetida</i>	ADM.01851.H.1.A	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 37.04 mg product/kg dw (equivalent to NOEC = 2.38 mg clopyralid/ kg dw NOEC _{corr} = 8.37 mg ethofumesate/kg dw*)	Friedrich S./2022/ 22 48 TEC 0019
<i>Folsomia candida</i>	ADM.01851.H.1.A	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 32.7 mg product/kg dw (equivalent to NOEC = 2.10 mg clopyralid/ kg dw NOEC _{corr} = 7.39 mg ethofumesate/kg dw*)	Ruhland S./2024g/ 24 48 TCC 0020
<i>Hypoaspis aculeifer</i>	ADM.01851.H.1.A	Mixed into substrate 14 d, chronic 5 % peat content	NOEC >1000 mg product/kg dw (equivalent to NOEC > 64.3 mg clopyralid/ kg dw NOEC _{corr} > 226 mg ethofumesate/kg dw*)	Schulz L./2024a/ 24 48 THC 0023
Field studies				
None				
Litter bag test				
None				

* The EC₁₀ corr. value for ethofumesate of 4.42 mg /kg soil dw was not used in the risk assessment for earthworms because it was derived from a study where the test substance was oversprayed rather than incorporated into the soil. Application by overspray is no longer recommended.

** Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 and confirmed in EFSA supporting publication 2015:EN-924

zRMS comments:

Soil organism toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Studies on toxicity of ADM.01851.H.1.A to soil meso and macro-fauna 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.

The applicant has considered the C-EU request to include analytical measurements in studies with soil macroorganisms however, this information is not available or provided for the following reasons:

- a) The C-EU request to include analytical measurements in studies with soil organisms represents a new data requirement which is not listed in the uniform principles in the EU. The uniform principles in the EU and the EU agreed endpoints especially for active substance data cannot be changed by single Member States, nor newly generated on request from a single MS. The notifier therefore sticks to the previously agreed endpoints from the EFSA list of endpoints.
- b) It is not specified which soil DT90 should be considered from the efate section. Dissipation in environmental fate studies is usually conducted in natural soils, thus the observed soil degradation is, partly at least, bio transformation mediated by microorganisms. The observed dissipation in these soils does not necessarily indicate a similar dissipation in artificial soil which is used in the ecological laboratory studies (OECD test guidelines 222, 226 and 232) where microorganisms are not present at soil preparation.
- c) It is not clearly specified if the requirement for analysis in soil organism studies is only necessary for those substances considered unstable, based on the CZSC DT90 criteria, or if it will apply to all active substances of a tested product. Considering the degradation of active substances in soil studies where products (containing one or more active substances) are tested and expressing ecotoxicological endpoints based on mean measured or timeweighted- average (twa) active substance concentrations can lead to misleading results and wrong risk assessment outcomes. In case the toxicity is e.g., driven by formulation components, or in case of mixture products the toxicity is driven by the more persistent active substance, but the endpoint is expressed based on analytical measurements of the less persistent and less toxic active substance, can lead to wrong endpoints and a misleading risk assessment. Therefore, endpoints from studies where products are tested should not be expressed as measured active substance endpoints.
- d) Further, there are many ways in which soil analysis can be conducted e.g. harsh extraction could be used for dose verification, whereas other extraction methods could be used to determine the bioavailable component. It is not clear what type of analysis is required.
- e) Existing OECD test guidelines for soil organisms do not include a requirement to include analytical measurements so existing studies are not designed in this way. Analytical measurements in soil laboratory ecotoxicity studies are more complex compared to other areas, e.g., aquatics, as it is not possible to control and maintain constant exposure over time, and soil cannot be simply re-spiked during a test. It is not possible to take soil samples from replicates in which biological assessments will be done. Destructive samplings from additional replicates would be necessary for analytics, and transfer of test organisms to newly spiked beakers may be required in order to keep them under constant test item exposure. A transfer of juveniles or eggs in newly spiked beakers is not possible for soil organisms, due to the minor size or possible damage on the mostly fragile juvenile stages. Also, a transfer of adult mesofauna individuals is very challenging, because all extraction procedures cause high stress and disturbance for the individuals possibly leading to scientifically unreasonable results. Therefore, the relevant guidelines should be updated first to clarify open points on test designs before analytical sampling as requested by C-EU can be considered.
- f) It remains unclear how the measured concentrations are processed to generate endpoints and it is further unclear how the newly generated endpoints shall be used in the risk assessment. Existing risk assessment guidance considers effect endpoints (NOEC or EC10 values) based on nominal (initial) concentrations which are compared to an initial predicted environmental concentration in soil (PECsoil). It is unclear which PECsoil values (mean measured or time-weighted average) shall be applied when using effect endpoints based on mean measured concentrations. Using e.g. a TWA concentration for endpoint expression but using maximum (initial) PECsoil values will lead to an overly conservative risk assessment. TWA PECsoil values were so far not used and precise guidance is needed on how the risk assessment should be conducted.

zRMS comments:

The new requirements of analytical measurements in studies with soil organisms will be discussed further during the next harmonization meeting in CZ in April 2025. For this reason, the risk assessment performed by the Applicant at this stage of evaluation is considered acceptable by zRMS.

9.8.1.1 Justification for new endpoints

New endpoints are provided for the formulated product as it was not the representative product assessed in either the ethofumesate or clopyralid active substance submissions.

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, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for ethofumesate only.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet	
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Ethofumesate	8.37	1.117 1.049 ^a	7.5 7.98
NC8493	100	0.008 ^b	12500
Clopyralid	1.97	0.140 ^b	14.07
ADM.01851.H.1.A	37.04 (10.75)*	0.818 ^b (1.257)*	45.28 (9.62)*
Chronic effects on <i>Folsomia candida</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Ethofumesate	7.39	1.117 1.049 ^a	6.61 7.04
NC8493	556	0.008 ^b	69500
Clopyralid	2.10	0.140 ^b	15.00
ADM.01851.H.1.A	32.70 (9.49)*	0.818 ^b (1.257)*	39.98 (7.54)*
Chronic effects on <i>Hypoaspis aculeifer</i>			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Ethofumesate	22.1	1.117 1.049 ^a	19.8 21.07
NC8493	309	0.008 ^b	38625
Clopyralid	64.3	0.140 ^b	459.3
ADM.01851.H.1.A	1000 (290.3)*	0.818 ^b (1.257)*	1223 230.94*

TER values shown in bold fall below the relevant trigger. ^aPEC_{accumulation}, ^bPEC_{soil} initial,

*TER_{LT} calculated as sum of the PEC_{soil}, accumulation for Ethofumesate of 1.117 mg/kg dw and the initial PEC_{soil} for clopyralid of 0.140 mg/kg dw after multiple applications and endpoint of the formulation expressed as the sum of the amount of the individual active substances

zRMS comments:

The risk assessment for soil macro- and meso-fauna presented in the Table 9.8-2 has been validated by the zRMS with some corrections of PECs_{accumulation} for the a.s. ethofumesate agreed by e - fate expert in Section 8.

In addition, TER_{LT} values for the formulated product were also calculated by zRMS as the ratio of the measured toxicity endpoint of the formulation expressed as the sum of the amount of the individual active substances to the PEC of the formulation expressed as the sum of the PEC_s of the individual active substances (PEC_{acc} for ethofumesate+PEC_{ini}).

All TER_{LT} values for soil meso- and macrofauna (other than earthworms) are greater than the trigger of 5, indicating an overall acceptable risk.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risks to soil meso- and macro-fauna from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable without mitigation.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with the relevant metabolites of ethofumesate and clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of ADM.01851.H.1.A were not evaluated as part of the EU assessments of ethofumesate or clopyralid. 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 process.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Ethofumesate 500 SC	42 d, aerobic loam soil	-16.0% effect at 1.3 mg a.s./kg soil dw -14.3% effect at day 42 at 13.0 mg a.s./kg soil dw	EFSA Conclusion 4374/2016
N-mineralisation	Metabolite NC 8493	28 d, aerobic loamy sand soil	-1.4% effect at 1.20 mg/kg soil dw -15.2% effect at 12 mg/kg soil dw	EFSA Conclusion 4374/2016

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Metabolite NC 20645	28 d, aerobic loamy sand soil	+6.9% effect at 1.38 mg/kg soil dw +6.7% effect at 13.8 mg/kg soil dw	EFSA Conclusion 4374/2016
N-mineralisation	Clopyralid	56 d, aerobic silty sand soil	-2.88% effect at 0.417 mg/kg soil dw +2.88 effect at 209 mg/kg soil dw	EFSA Conclusion 5289/2018
N-mineralisation	ADM.01851.H.1.A	28 d, aerobic soil type	+2.7% effect at 1.3 mg/kg soil dw +21.8% effect at 13.0 mg/kg soil dw	Schulz L/ 2024b/ 23 48 SMN 0023

zRMS comments:

Soil micro-organism toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Studies on toxicity of ADM.01851.H.1.A to soil micro-organism were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.9-1 are confirmed to be correct.

9.9.1.1 Justification for new endpoints

New endpoints are provided for the formulated product as it was not the representative product assessed in either the ethofumesate or clopyralid active substance submissions.

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, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.01851.H.1.A in sugarbeet

Intended use	Sugar beet		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Ethofumesate	13.0 (at 42 d)	1.117 ~~1.049~~	yes
NC8493	12.0 (at 28 d)	0.008 ^b	yes
Clopyralid	209 (at 56 d)	0.140 ^b	yes
ADM.01851.H.1.A	13.0 (at 28 d)	0.818 ^b	yes

^a $PEC_{accumulation}$ (worst-case), ^b PEC_{soil} initial (worst-case), Note that different values than the ones presented in B0 are used due to rounding of numbers, but as the calculations were done with the higher number, they should be considered acceptable

zRMS comments:

The risk assessment presented in Tables 9.9-2 above is in general agreed by the zRMS with some corrections of PECs accum. agreed by e- fate expert in Section 8.

Overall, no unacceptable effects on soil microbial activity are expected following application of ADM.01851.H.1.A.

9.9.3 Overall conclusions

The risks to soil micro-organisms from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable without mitigation.

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 the relevant metabolites of ethofumesate and clopyralid. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ADM.01851.H.1.A were not evaluated as part of the EU assessments of ethofumesate or clopyralid. 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 process.

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

Species	Substance	Exposure System	Results	Reference
<i>Lolium perenne</i> ^{m 1)} <i>Triticum aestivum</i> ^{m 2)} <i>Allium cepa</i> ^{m 3)} <i>Zea mays</i> ^{m 4)} <i>Brassica napus</i> ^{d 5)} <i>Glycine max</i> ^{d 6)} <i>Brassica rapa</i> ^{d 7)} <i>Fagopyrum esculentum</i> ^{d 8)} <i>Helianthis annuus</i> ^{d 9)} <i>Pisum sativum</i> ^{d 10)}	Ethofumesate SC 500	14 d Seedling emergence	²⁾ ER ₅₀ survival = 0.142 L prod./ha (71.50 g a.s./ha) ²⁾ ER ₅₀ plant weight = 0.0654 L prod./ha (32.93 g a.s./ha) ³⁾ ER ₅₀ emergence = 1.847 L prod./ha (930.0 g a.s./ha)	EFSA Conclusion 5289/2018
<i>Lolium perenne</i> ^{m 1)} <i>Avena sativa</i> ^{m 2)} <i>Allium cepa</i> ^{m 3)} <i>Brassica napus</i> ^{d 4)} <i>Glycine max</i> ^{d 5)} <i>Cucumis sativa</i> ^{d 6)} <i>Beta vulgaris</i> ^{d 7)} <i>Lactuca sativa</i> ^{d 8)} <i>Lycopersicon esculentum</i> ^{d 9)} <i>Daucus carota</i> ^{d 10)}	EF-797 (clopyralid 750 g/kg SG)	28 d Seedling emergence	⁵⁾ ER ₅₀ survival = 48.80 g a.s./ha ⁵⁾ ER ₅₀ plant weight = 21.47 g a.s./ha ⁵⁾ ER ₅₀ plant height = 162.03 g a.s./ha	EFSA Conclusion 5289/2018
<i>Avena sativa</i> ^{m 1)} <i>Hordeum vulgare</i> ^{m 2)} <i>Triticum aestivum</i> ^{m 3)}	ADM.01851.H.1.A	21 d Seedling emergence	^{3, 6, 8, 9)} ER ₅₀ emergence > 0.413 L prod./ha ^{3, 6, 8, 9)} ER ₅₀ survival >	Friedemann A./ 2024a / 23 46 PSE 0012

Species	Substance	Exposure System	Results	Reference
<i>Allium cepa</i> m ⁴⁾ <i>Lepidium sativum</i> d ⁵⁾ <i>Glycine max</i> d ⁶⁾ <i>Lycopersicon esculentum</i> d ⁷⁾ <i>Vicia faba</i> d ⁸⁾ <i>Lactuca sativa</i> d ⁹⁾ <i>Fagopyrum esculentum</i> d ¹⁰⁾			0.413 L prod./ha 6, 8) NOER phytotoxicity = 0.0176 L prod./ha 3) ER ₅₀ plant height = 0.329 L prod./ha 1) ER ₅₀ shoot dry weight = 0.239 L prod./ha	
<i>Lolium perenne</i> m ¹⁾ <i>Avena sativa</i> m ²⁾ <i>Allium cepa</i> m ³⁾ <i>Brassica napus</i> d ⁴⁾ <i>Glycine max</i> d ⁵⁾ <i>Cucumis sativa</i> d ⁶⁾ <i>Beta vulgaris</i> d ⁷⁾ <i>Lactuca sativa</i> d ⁸⁾ <i>Lycopersicon esculentum</i> d ⁹⁾ <i>Daucus carota</i> d ¹⁰⁾	EF-797 (clopyralid 750 g/kg SG)	21 d Vegetative vigour	8) ER ₅₀ plant weight = 33.78 g a.s./ha 8) ER ₅₀ plant height = 61.66 g a.s./ha (<i>Lactuca sativa</i>)	EFSA Conclusion 5289/2018
<i>Avena sativa</i> m ¹⁾ <i>Hordeum vulgare</i> m ²⁾ <i>Triticum aestivum</i> m ³⁾ <i>Allium cepa</i> m ⁴⁾ <i>Lepidium sativum</i> d ⁵⁾ <i>Glycine max</i> d ⁶⁾ <i>Lycopersicon esculentum</i> d ⁷⁾ <i>Vicia faba</i> d ⁸⁾ <i>Lactuca sativa</i> d ⁹⁾ <i>Fagopyrum esculentum</i> d ¹⁰⁾	ADM.01851.H.1.A	21 d Vegetative vigour	5, 6, 7, 8, 9) ER ₅₀ survival > 0.188 L prod./ha 6) NOER phytotoxicity = 0.00802 L prod./ha 6) ER ₅₀ plant height = 0.0712 L prod./ha 8) ER₅₀ shoot dry weight = 0.0537 L prod./ha EC ₅₀ phytotoxicity around rate 0.0176 L prod.(based on 55% phytotoxicity visual effects at this rate) (estimated by zRMS)	Friedemann A./ 2024b / 23 46 PVV 0011

m: monocotyledonous; d: dicotyledonous

zRMS comments:

NTTP toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374 and for clopyralid in EFSA Journal 5389/2018, respectively.

Studies on toxicity of ADM.01851.H.1.A to NTTP were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2.

Endpoints reported in Table 9.10-1 are confirmed to be correct.

9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product as it was not the representative product assessed in either the ethofumesate or clopyralid active substance submissions.

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.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet		
Active substance/product		Clopyralid and Ethofumesate/ADM.01851.H.1.A		
Application rate (g/ha)		3 x 330 g ethofumesate/ha, 3 x 49.5 g clopyralid/ha, 3 x 0.66 L product/ha		
MAF		2.7		
Test species	ER₅₀ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
<i>Ethofumesate</i>				
<i>Triticum aestivum</i> Seedling emergence	32.93	2.77	24.68	1.33
<i>Clopyralid</i>				
<i>Glycine max</i> Seedling emergence	21.47	2.77	3.702	5.80
ADM.01851.H.1.A				
<i>Vicia faba</i> Vegetative vigour	0.0537 (L prod./ha)	2.77	0.049 (L prod./ha)	1.09

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 risk assessment performed in the Table 9.10-2 has been amended by zRMS.

TER_{LT} values for vegetative vigour for formulation ADM.01851.H.1.A was calculated by the Applicant by comparing the most sensitive ER₅₀ values with the highest predicted exposure level (PER_{off-field}) using the 90th percentile estimates derived by BBA (2006)⁴ from the spray-drift predictions of Ganzelmeier & Rautmann (2000)⁵ as a worst-case scenario.

It should be noted that when 3 applications are used the drift value of 2.01% should be considered

For this reason, the relevant zRMS's calculations are presented below:

4 Spray drift: according to BBA spray drift data from 27. March 2006 (www.jki.bund.de)
5 Ganzelmeier H., Rautmann D. (2000). Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

Table 9.10-3-1: Assessment of the risk for non-target plants due to the use of ADM.01851.H.1.A in sugar beet

Intended use		Sugar beet		
Active substance/product		Clopyralid and Ethofumesate/ADM.01851.H.1.A		
Application rate (g/ha)		3 x 330 g ethofumesate/ha, 3 x 49.5 g clopyralid/ha, 3 x 0.66 L product/ha		
MAF		2.7		
Test species	ER₅₀ L prod./ha	Drift rate	PER_{off-field} L prod./ha	TER criterion: TER ≥ 5
ADM.01851.H.1.A				
<i>Vicia faba</i> Vegetative vigour	0.0537	2.01	0.0358	1.5

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

The results of the deterministic risk assessment for terrestrial plants on vegetative vigour show a TER_{LT} based on the most sensitive species with ER₅₀ value of 0.0537 L/ha is below the trigger of 5. Thus, a refined risk assessment is required.

Further refinement based on the lowest value of ER₅₀ = 0.0537 L/ha and application rate of 3 x 0.66 L/ha is presented by zRMS in the Table below:

Table 9.10-2-2: Deterministic assessment of the risk for non-target plants due to the use of formulation ADM.01851.H.1.A in crop sugar beet for 3 x 0.66 L/ha.

Application Rate L prod./ha	Distance m	Drift value	Drift reduction	MAF	PER _{off-field}	Endpoint L prod./ha	TER _{LT}
0.66	1	0.0201	0	2.7	0.0133	0.0537	1.5
			50%		0.0066		3.07
			75%		0.0033		6.0
			90%		0.0013		15.0
	5	0.0041	0		0.0027		7.35

values in bold are below the trigger of 5

The risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A are acceptable when:

- 75 % drift reduction nozzles (when MAF of 2.7 is considered) or
- 5-meter unsprayed buffer zone to non-crop land is applied.

In addition, for MSs which prefer used the MAF of 1 the following risk assessment is presented:

Table 9.10-2-3: Deterministic assessment of the risk for non-target plants due to the use of formulation ADM.01851.H.1.A in crop sugar beet for 3 x 0.66 L/ha.

Application Rate L prod./ha	Distance m	Drift value	Drift reduction	MAF	PER _{off-field}	Endpoint L prod./ha	TER
0.66	1	0.0201	0	~~2.7~~ 1	0.0133	0.0537	4.05
			50%	1	0.0066		8.10
			75%		0.0033		16.19
			90%		0.0013		40.48
	5	0.0041	0		0.0027		19.84

values in bold are below the trigger of 5

The risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A when MAF of 1 is considered are acceptable when:

- 50 % drift reduction nozzles or
- 5-meter unsprayed buffer zone to non-crop land is applied.

The risk assessment based on phytotoxicity effects:

Table 9.10-4: Assessment of the risk for non-target plants due to the use of ADM.01851.H.1.A in sugar beet

Intended use	Sugar beet			
Active substance/product	Clopyralid and Ethofumesate/ADM.01851.H.1.A			
Application rate (g/ha)	3 x 330 g ethofumesate/ha, 3 x 49.5 g clopyralid/ha, 3 x 0.66 L product/ha			
MAF	2.7			
Test species	ER₅₀ L prod./ha	Drift rate	PER_{off-field} Lprod.ha	TER criterion: TER ≥ 5
ADM.01851.H.1.A				
Field bean Vegetative vigour	~0.0176 (L prod./ha)*	2.01	0.0358	0.49

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

- Based only on visual estimation where 55% effects are observed in the study at rate 0.0176 L/ha.

Further refinement based on the lowest value of ~0.0176 (L prod./ha) and application rate of 3 x 0.66 L/ha is presented by zRMS in the Table below:

Table 9.10-3-1: Deterministic assessment of the risk for non-target plants due to the use of formulation ADM.01851.H.1.A in crop sugar beet for 3 x 0.66 L/ha.

Application Rate L prod./ha	Distance m	Drift value	Drift reduction	MAF	PER _{off-field}	Endpoint L prod./ha	TER _{LT}
0.66	1	0.0201	0	2.7	0.0133	~0.0176	0.49
			50%		0.0066		0.98
			75%		0.0033		1.97
			90%		0.0013		4.98
	5	0.0041	0		0.0027		2.41
			50%		0.0014		4.81
			75%		0.0007		9.63
	10	0.002	0		0.0013		4.93
			50		0.0007		9.87

values in bold are below the trigger of 5

The risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A when MAF of 2.7 is considered are acceptable when:

- 5-meter unsprayed buffer zone with 75% drift reduction nozzles or
- 10-meter unsprayed buffer zone with 50 % drift reduction nozzles to non-crop land is applied.

In addition, for MSs which prefer used the MAF of 1 the following risk assessment is presented:

Table 9.10-3-2: Deterministic assessment of the risk for non-target plants due to the use of formulation ADM.01851.H.1.A in crop sugar beet for 3 x 0.66 L/ha.

Application Rate L prod./ha	Distance m	Drift value	Drift reduction	MAF	PERoff-field	Endpoint L prod./ha	TER
0.66	1	0.0201	0	1	0.0133	~0.0176	1.32
			50%		0.0066		2.66
			75%		0.0033		5.33
			90%		0.0013		13.45
	5	0.0041	0		0.0027		6.51

values in bold are below the trigger of 5

The risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A are acceptable when:

- 75 % drift reduction nozzles or
- 5-meter unsprayed buffer zone to non-crop land is applied.

The risk assessment based on phytotoxicity visual effects are left at national level.

9.10.2.3 Higher-tier risk assessment

A probabilistic approach was taken to improve the risk assessment. The species sensitivity distribution (SSD) was established for the vegetative vigour EC₅₀ value for shoot dry weight of the ADM.01851.H.1.A study because this was the most critical endpoint of the risk assessment. The hazardous concentration for 5% of species (HC₅) was calculated using ETX 2.3.1. Only unbound values were included. The goodness-of-fit of the data was assessed using three tests for normality, the Anderson-Darling test, the Kolmogorov-Smirnov test and the Cramer von Mises test.

The input values from the study are listed below.

Table 9.10-5: EC₅₀ shoot dry weight endpoints (mL ADM.01851.H.1.A/ha) from vegetative vigour study

Test species	EC ₅₀ shoot dry weight (mL prod./ha)
<i>Avena sativa</i> _m ¹⁾	>2000*
<i>Hordeum vulgare</i> _m ²⁾	1806
<i>Triticum aestivum</i> _m ³⁾	468
<i>Allium cepa</i> _m ⁴⁾	>2000*
<i>Lepidium sativum</i> _d ⁵⁾	189
<i>Glycine max</i> _d ⁶⁾	96.8
<i>Lycopersicon esculentum</i> _d ⁷⁾	94.9
<i>Vicia faba</i> _d ⁸⁾	53.7
<i>Lactuca sativa</i> _d ⁹⁾	106
<i>Fagopyrum esculentum</i> _d ¹⁰⁾	>188*

m: monocotyledonous; d: dicotyledonous; * unbound values not included in HC₅ calculation

The SSD is presented in the graph below.

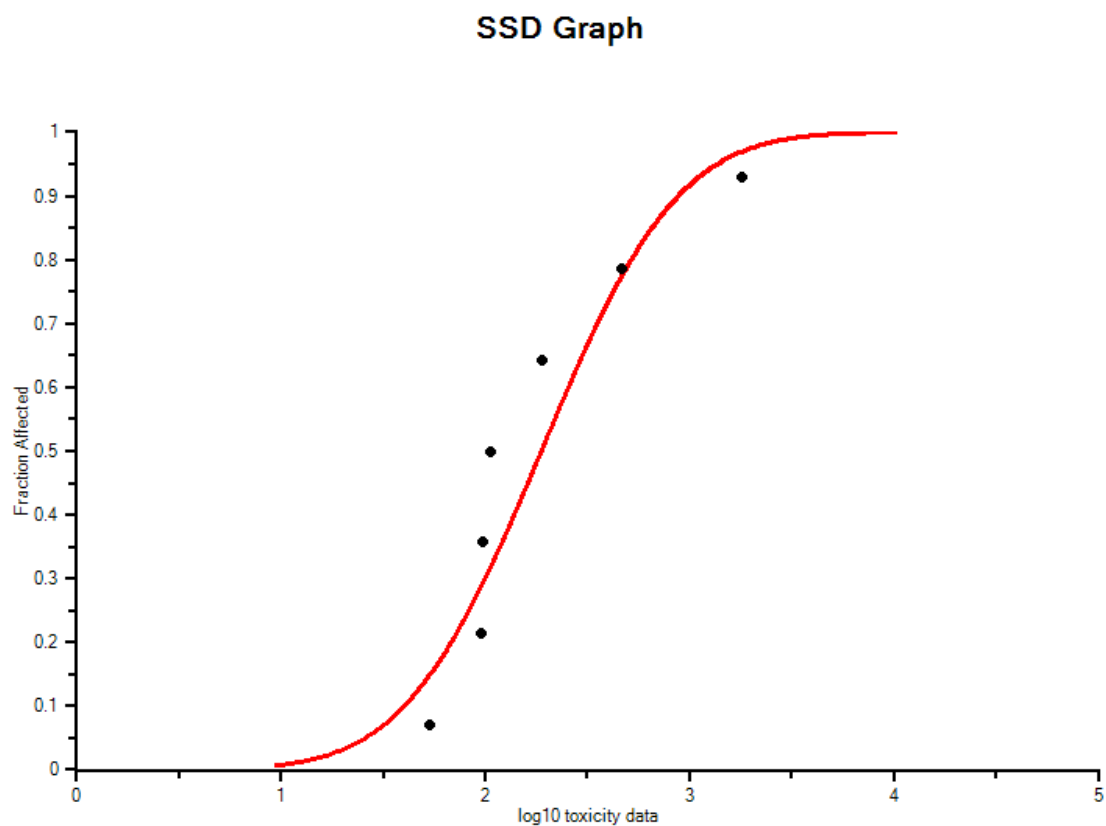


Figure 9.10-1: Species sensitivity distribution of vegetative vigour dry shoot weight EC_{50} values

The goodness-of-fit results are summarized in the table below.

Table 9.10-6: Goodness-of-fit of vegetative vigour EC₅₀ values

Test type	Significance level	Critical value for test statistic	Accepted or rejected
Anderson-Darling	0.05	0.752	Accepted
Kolmogorov-Smirnov	0.05	0.895	Accepted
Cramer von Mises	0.05	0.126	Accepted

The HC₅ calculated results are summarized in the table below.

Table 9.10-7: HC₅ calculation results

Description	Name	Value	log ₁₀ (Value)
lower estimate of the HC ₅	LL HC ₅	3.09723	0.490973
median estimate of the HC₅	HC₅	23.22428	1.365942
upper estimate of the HC ₅	UL HC ₅	61.89614	1.791664
spread of the HC ₅ estimate	sprHC ₅	19.98435	1.30069

The non-target terrestrial plant risk assessment was calculated using the median HC₅ value and a trigger of 1. This is presented in the table below.

Table 9.10-8: Assessment of the risk for non-target plants due to the use of formulation ADM.01851.H.1.A in crop sugar beet

Intended use	Sugar beet			
Active substance/product	Clopyralid and Ethofumesate/ADM.01851.H.1.A			
Application rate (g/ha)	3 x 0.66 L product/ha			
MAF	2.7			
Test species	HC₅ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 1
Vegetative vigour	23.22 (L prod./ha)	2.77	0.049 (L prod./ha)	470.4

MAF: Multiple application factor; HC₅: Hazardous concentration to 5% of species; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The probabilistic risk assessment considering species sensitivity distribution indicates acceptable risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A following application to sugar beet according to the proposed GAP.

zRMS comments:

Probabilistic risk assessment:

The above probabilistic risk assessment has been checked by zRMS using ETX program 2.3.1. it has been amendment taking into account the correct value of HR₅=23.22 mL/ha instead of 23.22 L/ha and corrected PER_{off-field} value.

The relevant calculations are presented below:

Table 9.10-9-1: Probabilistic risk assessment of the risk for non-target plants due to the use of formulation ADM.01851.H.1.A in crop sugar beet

Intended use	Sugar beet			
Active substance/product	Clopyralid and Ethofumesate/ADM.01851.H.1.A			
Application rate (g/ha)	3 x 0.66 L product/ha			
MAF	2.7			
Test species	HC₅ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 1
Vegetative vigour	0.02322	2.01	0.0133	1.75

MAF: Multiple application factor; HC₅: Hazardous concentration to 5% of species; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Based on the probabilistic risk assessment it is concluded that the use of the product ADM.01851.H.1.A will not produce unacceptable effects on terrestrial non-target plants growing near treated fields without risk mitigation measures when trigger 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.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risks to non-target terrestrial plants from exposure to ADM.01851.H.1.A when applied according to the GAP are acceptable without consideration of mitigation measures when probabilistic risk assessment is considered with AF of 1. 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.

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

No further data on effects of ethofumesate, clopyralid or formulation ADM.01851.H.1.A to other terrestrial organisms are available.

9.12 Monitoring data (KCP 10.8)

No further monitoring data on ethofumesate, clopyralid or formulation ADM.01851.H.1.A are available.

9.13 Classification and Labelling

The classification and labelling of the product ADM.01851.H.1.A has been determined in accordance with ECHA Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 6.0 Jan 2024.

Information on ingredients classification and concentration:

Ethofumesate	Acute 1	M=1	Chronic 1	M=1	42.74 % w/w
Clopyralid	-	-	Chronic 1	M=10	6.75 % w/w

Test data are available for the mixture as follows:

Acute	
Fish (<i>Oncorhynchus mykiss</i>)	96h LC ₅₀ = 31.8 mg/L _{nom}
Crustacea (<i>Daphnia magna</i>)	48h EC ₅₀ = 61.4 mg/L _{mm}
Algae (<i>Raphidocelis subcapitata</i>)	72h E _r C ₅₀ = 39.2 mg/L _{nom}
Aquatic plants (<i>Myriophyllum spicatum</i>)	14d E _r C ₅₀ = 1.20 mg/L _{nom} (main shoot length)
Chronic	No data available

The acute classification can be based on test data for the mixture as data are available for all trophic levels. Results are >1 mg/L for all trophic levels, therefore there is no need to classify for short-term

(acute) hazard.

The chronic classification must be based on the summation method as chronic test data are not available for the mixture. Ethofumesate is classified as Chronic 1 with an M factor of 1, at a concentration in the mixture of 42.74%. As the concentration of ethofumesate is >25%, the mixture must be classified as Chronic 1.

Labelling elements based on the classification are as follows:

GHS Pictogram	GHS09
Signal Word	Warning
Hazard Statement	Very toxic to aquatic life with long lasting effects
Precautionary Statements	P501
Additional Phrases	EUH401, SP1

zRMS comments:

zRMS agrees with the classification: **H410, Aquatic Chronic 1**, proposed for formulation ADM.01851.H.1.A:

We agree that the acute classification can be based on test data for the mixture as data are available for all trophic levels with toxicity endpoints > 1 mg product/L and there is no need to classify for acute hazard.

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 10.2/01	██████	2024a	Acute toxicity of ADM.01851.H.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour static test 23 48 AFA 0006 ██████ GLP Unpublished	Y	Adama
KCP 10.2/02	Renner P.	2024b	Acute toxicity of ADM.01851.H.1.A to <i>Daphnia magna</i> in a 48-hour static test 23 48 ADL 0020 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.2/03	Renner P.	2024c	Effects of ADM.01851.H.1.A on <i>Raphidocelis subcapitata</i> in an algal growth inhibition test 23 48 AAL 0014 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.2/04	Warning D.	2022	Effects of ADM.01851.H.1.A on <i>Myriophyllum spicatum</i> in a static water-sediment system 22 48 AMS 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.1.1/01	Poráčzki K.	2022	Acute toxicity of ADM.01851.H.1.A to the honeybee <i>Apis mellifera</i> L. under laboratory conditions 22 48 BAA 0079 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.1.2/01	Ruhland S.	2023	Chronic toxicity of ADM.01851.H.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions 22 48 BAC 0024 BioChem agrar, Machern OT Gerichshain, 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	Kleebaum K.	2022	ADM.01851.H.1.A – Repeated Exposure of Honey Bee (<i>Apis mellifera</i> L.) Larvae under Laboratory Conditions 22 48 BLC 0025 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.2.1/01	Röhlig U.	2024a	Effects of ADM.01851.H.1.A on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test 23 48 NTL 0010 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.2.1/02	Röhlig U.	2024b	Effects of ADM.01851.H.1.A on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEPHANI-PEREZ) in a laboratory test 23 48 NAL 0010 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.4.1.1/01	Friedrich S.	2018	Effects of ADM.01851.H.1.A on the reproduction of the earthworm <i>Eisenia fetida</i> in artificial soil 22 48 TEC 0019 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.4.2.1/01	Schulz L.	2024a	Effects of ADM.01851.H.1.A on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> , 23 48 THC 0023 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.4.2.1/02	Ruhland S.	2024g	Effects of ADM.01851.H.1.A on the mortality and reproduction of the collembolan <i>Folsomia candida</i> 23 48 TCC 0020 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	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 10.5/01	Schulz L.	2024b	Effects of ADM.01851.H.1.A on the activity of soil microflora (Nitrogen Transformation Test) 23 48 SMN 0023 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.6.2/01	Friedemann A.	2024a	Effects of ADM.01851.H.1.A on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions 23 46 PSE 0012 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.6.2/02	Friedemann A.	2024b	Effects of ADM.01851.H.1.A on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions 23 46 PVV 0011 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama

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

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

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the 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 Study 1: Acute toxicity to fish

Comments of zRMS:	The study was conducted in line with OECD 203 with no minor deviations. Recoveries of clopyralid and ethofumesate were sufficiently stable during the test ($\pm 20\%$ of nominal). Therefore, the results were based on nominal test substances concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 96 h LC_{50} = 31.8 mg product /L (based on nominal concentration)
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Reference: KCP 10.2.1/01

Report Acute toxicity of ADM.01851H.1.A to *Oncorhynchus mykiss* in a 96-hour static test, Renner P., 2024a,
23 48 AFA 0006, 000116374 (sponsor report number)

Guideline(s): Yes, OECD 203 (2019)

Deviations: No major deviations

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1

Active ingredient content	75.6 g/L clopyralid, 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/cm ³
Expiry date	09 Jan 2025
Reference substance	Potassium dichromate was tested in a separate study

Test organism:

Test species	<i>Oncorhynchus mykiss</i>
Origin	Forellenzucht Trostadt GbR, Reiroeth OT Trostadt, Germany
Acclimation	25 days
Mean fish weight at test start (g)	0.862 ± 0.163
Mean fish length at test start (cm)	4.67 ± 0.142 cm
Fish leading	0.585 g fish/L
No. of daphnia per test substance concentration	7
No. of test vessels (replicates) per test substance concentration	1

Test conditions:

Test substance concentrations	0 (control), 5.63, 11.3, 22.5, 45.0, 90.0 mg product/L
Control	Untreated test medium
Test duration	96 h
Test medium	Reconstituted water (OECD 203)
Test type	Static
Test medium pH	7.76-8.20
Water temperature	12.5-13.2°C
Dissolved oxygen	7.95-9.01 mg/L
Hardness	230 mg CaCO ₃ /L
Test solution appearance	Clear and transparent
Test vessel	Stainless steel container, approx. 12L volume
Test volume	10 L
Light intensity	20 µEm ⁻² s ⁻¹
Photoperiod	16 h light : 8 h dark
Feeding	None

Observations:

Fish observations	Mortality (including sub-lethal effects): 0, 2.5/5.5, 24, 30, 48, 54, 72, 78 and 96 h Fish wet weight and length: 96 h
Test substance concentration	0 and 96 h
Test conditions	pH and O ₂ content: 0, 24, 48, 72 and 96 h Temperature: continuously

Analytical method:

Method type	LC-MS/MS
Equipment	Shimadzu 8050 system
Column	ACE Excel3 C18 Amide 3 µm, 100 x 2.1mm
Column temperature	35°C
Detector	Detection: ESI positive MRM mass transitions: Ethofumesate: m/z 287.0 → 259.1 (quantifier); 287.0 → 161.0 (qualifier) Clopyralid: m/z 191.8 → 145.9 (quantifier); 191.8 → 110.0 (qualifier)
Flow rate	0.20 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid and 5 mM ammonium formate B: Methanol containing 0.1% formic acid and 5 mM ammonium formate 0.0 min 60% A, 40% B 1.5 min 50% A, 50% B 2.0 min 20% A, 80% B 3.0 min 0% A, 100% B 4.0 min 0% A, 100% B 4.1 min 60% A, 40% B

[illegible]

22.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45.0	0.0	0.0	85.7	100	100	100	100	100	100
90.0	100	100	100	100	100	100	100	100	100

Sub-lethal effects were found exclusively in the 45.0 mg/L treatment. After 2.5 and 5.5 hours after test start, fish were winding around their body axis showing abnormal, involuntary and uncontrolled contraction of muscles. After 24 hours when only one individual was alive in this treatment, it was paralysed and had irregular ventilation.

Based on these results, the following endpoints were obtained.

Table A2.2.1.1-3: Endpoints of the acute toxicity test with ADM.01851.H.1.A in *Oncorhynchus mykiss*

Endpoint	Nominal concentration (mg product/L)
96 h NOEC	22.5
96 h LOEC	45.0
96 h EC ₅₀ (95% confidence interval)	31.8 (22.5 – 45.0)

Conclusion

In this test on acute toxicity of ADM.01851.H.1.A in *Oncorhynchus mykiss*, the 96-hour LC₅₀ was determined to be 31.8 mg product/L (22.5 - 45.0 mg product/L, 95% confidence interval).

A 2.2.1.2 Study 2: Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study was conducted in line with OECD 202 with no minor deviations. Recoveries of clopyralid and ethofumesate were sufficiently stable during the test ($\pm 20\%$ of nominal). Therefore, the results were based on nominal test substances concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48 h EC₅₀ = 63.7 mg product /L (based on nominal concentration)
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Reference:	KCP 10.2.1/02
Report	Acute toxicity of ADM.01851.H.1.A to <i>Daphnia magna</i> in a 48-hour static test 23 48 ADL 0020, Renner P., 2024b, 23 48 ADL 0020, 000116373 (sponsor report number)
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No major deviations
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	75.6 g/L clopyralid, 517.4 g/L ethofumesate

Appearance	Off-white liquid
Density	1.154 g/cm ³
Expiry date	09 Jan 2025
Reference substance	Not required

Test organism:

Test species	<i>Daphnia magna</i> (Straus)
Origin	In-house laboratory culture
Age at test start	<24 hours
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: 2 mL test solution per <i>Daphnia</i>)
No. of daphnia per test substance concentration	20
No. of test vessels (replicates) per test substance concentration	4

Test conditions:

Test substance concentrations	0, 19.8, 29.6, 44.4, 66.7 and 100 mg product/L
Control	Untreated test medium
Test duration	48 h
Test medium	Reconstituted water (Elendt "M4")
Test type	Static
Test medium pH	7.98 - 8.08
Water temperature	19.9 – 20.1 °C
Dissolved oxygen	8.56 – 8.90 mg O ₂ /L
Hardness	230 mg CaCO ₃ /L
Alkalinity	0.8 mmol/L
Test vessel	Glass beakers (50 mL)
Test volume	10.5 mL
Light intensity	20 µEm ⁻² s ⁻¹
Photoperiod	16 h light : 8 h dark
Feeding	None

Observations:

Daphnia observations	Immobility (including sub-lethal effects): 3, 24 and 48 h
Test substance concentration	0 and 48 h
Test conditions	pH, O ₂ content: 0 and 48 h Temperature: continuously

Analytical method:

Method type	LC-MS/MS
Equipment	Shimadzu 8050 system
Column	ACE Excel3 C18 Amide 3 µm, 100 x 2.1mm
Column temperature	35°C
Detector	Detection: ESI positive MRM mass transitions: Ethofumesate: m/z 287.0 → 259.1 (quantifier); 287.0 → 161.0 (qualifier) Clopyralid: m/z 191.8 → 145.9 (quantifier); 191.8 → 110.0 (qualifier)
Flow rate	0.20 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid and 5 mM ammonium formate B: Methanol containing 0.1% formic acid and 5 mM ammonium formate 0.0 min 60% A, 40% B 1.5 min 50% A, 50% B 2.0 min 20% A, 80% B 3.0 min 0% A, 100% B 4.0 min 0% A, 100% B

	4.1 min 60% A, 40% B 8.0 min 60% A, 40% B
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Experimental dates: 11 to 13 July 2023

Calculations:

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

Statistics:

A sequence of pre-testing was performed before final statistical testing. These pre-tests for quantal data with binominal distribution will included testing of (1) monotonicity (trend analysis by contrasts, $p \leq 0.05$) and where appropriate (2) extra-binomial variance (Tarone's test, $p \leq 0.01$). As a result, a monotonous trend was found. Extra-binomial variances were not found. The usage of the Step-down Cochran-Armitage Test Procedure was justified.

LOECs were determined employing the Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$, one-sided smaller). The NOECs were derived from the LOECs.

Effect concentrations EC_x were determined by Weibull analysis using the maximum likelihood regression. The goodness of fit was justified by the significance of p(Chi²) and the p(F) statistics for the slope. As a result, the fit was good with p(Chi²) > 0.1 and the slope was significantly different from zero/chance (p(F) < 0.05).

Statistics and modelling were done using ToxRat professional version 3.3.0 (RATTE, 2018).

Results and discussions

Validity criteria:

- Immobility or other signs of disease or stress in control daphnids $\leq 10\%$
- Dissolved oxygen concentration at the end of the test in control and test vessels ≥ 3 mg O₂/L

Immobility in control daphnids was 0% and furthermore, no daphnid showed signs of disease or stress. Dissolved oxygen concentration was ≥ 5.56 mg O₂/L in in all treatment groups. Therefore, all validity criteria were met.

Test substance concentrations were determined by analysis of the analytes ethofumesate and clopyralid. Percentage recovery of the analytes in test solutions is presented in the table below.

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

Nominal concentrations (mg prod./L)	Clopyralid (% of nominal)		Ethofumesate (% of nominal)	
	0 hours	48 hours	0 hours	48 hours
Control	< 30% LOQ	< 30% LOQ	< 30% LOQ	< 30% LOQ
19.8	94.8	100.7	91.9	122.7
29.6	99.0	97.7	95.1	92.4
44.4	98.3	102.8	95.4	93.1
66.7	96.7	101.8	94.5	96.6
100	96.9	95.9	91.6	84.7

LOQ clopyralid = 0.2894 mg/L; LOQ ethofumesate = 1.981 mg/L

Recoveries of clopyralid and ethofumesate were not sufficiently stable during the test ($\pm 20\%$ of nominal). Therefore, the biological results were based on nominal and mean measured 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.01851.H.1.A

Product concentration nominal (mg product/L)	Immobility of <i>Daphnia magna</i> (%)		
	3 hours	24 hours	48 hours
Control	0	0	0
19.8	0	0	0
29.6	0	0	0
44.4	0	0	0
66.7	0	35	65
100	0	70	100

No visible signs of abnormalities of surviving daphnids were observed at any assessment.

Based on these results, the following endpoints were obtained.

Table A2.2.1.1-3: Endpoints of the acute toxicity test with ADM.01851.H.1.A in *Daphnia magna*

Endpoint	Nominal concentration (mg product/L)	Mean measured concentration (mg product/L)
48 h NOEC	44.4	42.2
48 h LOEC	66.7	64.1
48 h EC ₅₀ (95% confidence interval)	63.7 (59.3 – 68.7)	61.4 (57.0 – 65.9)

Potassium dichromate was tested as a reference substance in a separate study. The EC₅₀ value was 1.52 mg/L (1.38 -1.67 mg/L, 95% confidence interval).

Conclusion

In this test on acute toxicity of ADM.01851.H.1.A in *Daphnia magna*, the 48-hour EC₅₀ based on nominal test substance concentrations was determined to be 63.7 mg product/L (59.3 – 68.7 mg product/L 95% confidence interval) and based on mean measured test substance concentrations was 61.4 mg product/L (57.0 – 65.9 mg product/L 95% confidence interval).

A 2.2.1.3 Study 3: Effects on aquatic algae

Comments of zRMS:	The study was conducted in line with OECD 201 with no major deviation. Recoveries of clopyralid and ethofumesate were sufficiently stable during the test ($\pm$ 20% of nominal). Therefore, the results were based on nominal test substances concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: Growth rate: 72 h EC₁₀ (95% confidence interval) = 11.7 mg product/L (7.84 – 15.3) 72 h EC₂₀ (95% confidence interval) = 17.8 mg product/L (13.3 – 21.7) 72 h EC₅₀ (95% confidence interval) = 39.2 mg product/L (33.7 – 45.8) Yield: 72 h EC₁₀ (95% confidence interval) = 14.6 mg product/L (13.3 – 17.0) 72 h EC₂₀ (95% confidence interval) = 16.2 mg product/L (15.1 – 18.4) 72 h EC₅₀ (95% confidence interval) = 19.9 mg product/L (19.1 – 21.2)
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Reference: KCP 10.2.1/03

Report Effects of ADM.01851.H.1.A on *Raphidocelis subcapitata* in an algal

	growth inhibition test, Renner P., 2024c, 23 48 AAL 0014, 000116375 (sponsor report number)
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No major deviations
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	75.6 g/L clopyralid, 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/cm ³
Expiry date	09 Jan 2025
Reference substance	Not required

Test organism:

Test species	<i>Raphidocelis subcapitata</i> (KORSHIKOV), Strain No. 61.81 SAG (recently renamed from <i>Pseudokirchneriella subcapitata</i> and formerly known as <i>Selenastrum capricornutum</i>)
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 Additional vessels were set up for analysis and retained specimens. All additional test vessels were incubated under the same conditions as the regular test vessels.

Test conditions:

Test substance concentrations	0 (control), 6.25, 12.5, 25.0, 50.0, 100 mg product/L
Control	Untreated test medium
Test duration	72 h
Test medium	OECD 201 medium
Test type	Static
Test medium pH	8.12 – 8.19 at 0 h 8.09-9.25 at 72 h
Water temperature	22.9-23.0°C
Shaking	Continuously by magnetic stirrers
Test solution appearance	Test solutions were clear without precipitates
Test vessel	250-mL Erlenmeyer flasks covered with air-permeable stoppers
Test volume	100 mL
Sterility	The test was conducted under sterile conditions
Light intensity	107 µEm ⁻² s ⁻¹
Photoperiod	Continuous illumination

Observations:

Algal cell density and qualitative observations	0, 24, 48 and 72 h Algal cell density was determined using a Neubauer counting chamber
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Test substance concentration	0 and 72 h
Test conditions	Temperature: continuous pH: in each test group at the start and end of the test Light intensity: at test start

Analytical method:

Method type	LC-MS/MS
Equipment	Shimadzu 8050 system
Column	ACE Excel3 C18 Amide 3 µm, 100 x 2.1mm
Column temperature	35°C
Detector	Detection: ESI positive MRM mass transitions: Ethofumesate: m/z 287.0 → 259.1 (quantifier); 287.0 → 161.0 (qualifier) Clopyralid: m/z 191.8 → 145.9 (quantifier); 191.8 → 110.0 (qualifier)
Flow rate	0.20 mL/min
Mobile phase	A: HPLC water containing 0.1% formic acid and 5 mM ammonium formate B: Methanol containing 0.1% formic acid and 5 mM ammonium formate 0.0 min 60% A, 40% B 1.5 min 50% A, 50% B 2.0 min 20% A, 80% B 3.0 min 0% A, 100% B 4.0 min 0% A, 100% B 4.1 min 60% A, 40% B 8.0 min 60% A, 40% B

Experimental dates: 10 to 13 July 2023

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:

The data set (growth rate and yield inhibition) was found normally distributed (Shapiro-Wilk's test, $p \leq 0.01$); variances of the endpoints based on yield were homogen (Levene's test, $p \leq 0.01$). Endpoints based on growth rate, were found non-homogenous. The monotonicity criteria (endpoint yield) was fulfilled (trend analysis by contrasts, $p > 0.05$). Based on these pretesting results, the usage of Williams t-test ($p \leq 0.05$, one-sided smaller) and the Welch's t-test ($p \leq 0.05$, one-sided smaller) was justified.

Effect concentrations EC_x were determined by concentration-response modelling. EyC_x were based on Weibull regression while ErC₅₀ were done using the probit analysis (maximum-likelihood regression). The goodness of fit was justified by the significance of p(Chi²), the r²-value and the p(F) statistics for the slope. As a result, the fit was considered good with p(Chi²) > 0.1, r² > 0.85 and p(F) < 0.05. Confidence intervals were determined by Fieller's theorem.

Statistics were evaluated using ToxRat professional version 3.3.0 (RATTE, 2018).

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 78.1-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 20.3% and the coefficient of variation of average specific growth rates in replicate control cultures was 0.5%. Therefore, all validity criteria were met.

Recoveries of clopyralid and ethofumesate were not sufficiently stable during the test ($\pm 20\%$ of nominal). Therefore, the biological results were based on nominal and mean measured test substance concentrations.

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

Nominal concentrations (mg prod./L)	Clopyralid (% of nominal)		Ethofumesate (% of nominal)	
	0 hours	72 hours	0 hours	72 hours
Control	-	-	-	-
6.25	111.8	111.2	98.3	99.8
12.5	111.2	105.3	99.2	95.2
25.0	106.5	104.3	93.8	92.6
50.0	102.7	106.1	96.3	96.7
100.0	106.3	105.6	97.6	102.1

LOQ clopyralid = 0.08918 mg/L; LOQ ethofumesate = 0.6104 mg/L

Recoveries of clopyralid and ethofumesate were sufficiently stable during the test ($\pm 20\%$ of nominal). Therefore, the biological results were based on nominal test substance concentrations.

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

Table A2.2.1.2-2: Algal cell densities during exposure to ADM.01851.H.1.A.

Nominal concentrations (mg prod./L)	0 hours	Density of algal cells ($\times 10^4$ cells/mL)		
		24 hours	48 hours	72 hours
Control	0.5	2.46	12.4	39.5
6.25		2.42	12.3	39.0
12.5		2.58	12.4	38.7
25.0		1.67	3.83	7.25
50.0		0.833	1.00	2.42
100.0		0.833	1.00	1.42

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

Table A2.2.1.2-3: Growth rates of algae and their percentage inhibition

Nominal concentrations (mg prod./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	1.583	n.a.	1.606	n.a.	1.456	n.a.
6.25	1.574	0.6	1.599	0.4	1.452	0.3
12.5	1.638	-3.4	1.605	0.0	1.449	0.5
25.0	1.201	24.1*	1.01	36.7*	0.891	38.8*
50.0	0.501	68.3*	0.347	78.4*	0.522	64.1*
100.0	0.501	68.3*	0.336	79.1*	0.346	76.2*

Note: Negative “% Inhibition” values indicate an increase in growth relative to the control.

n.a. Not applicable

* Mean value significantly different from the control

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 algae and their percentage inhibition

Nominal concentrations (mg prod./L)	0 - 24 h		0 - 48 h		0 - 72 h	
	Yield y	% Inhibition	Yield y	% Inhibition	Yield y	% Inhibition
Control	1.958	n.a.	11.917	n.a.	38.958	n.a.
6.25	1.917	2.1	11.750	1.4	38.500	1.2
12.5	2.083	-6.4	11.917	0.0	38.167	2.0
25.0	1.167	40.4*	3.333	72.0*	6.750	82.7*
50.0	0.333	83.0*	0.500	95.8*	1.917	95.1*
100.0	0.333	83.0*	0.500	95.8*	0.917	97.6*

Note: Negative “% Inhibition” values indicate an increase in yield relative to the control.

n.a. Not applicable

* Mean value significantly different from the control

Test solutions appeared clear and non-turbid. The cell shape was not altered. Discolouration, clumping of cells or sedimentation were not found.

Based on these results, the following endpoints were obtained.

Table A2.2.1.2-5: Study endpoints of algae growth inhibition test with ADM.01851.H.1.A.

Parameter	Growth rate	Yield
Endpoints based on nominal concentrations		
72 h NOEC	12.5	12.5
72 h LOEC	25.0	25.0
72 h EC ₁₀ (95% confidence interval)	11.7 (7.84 – 15.3)	14.6 (13.3 – 17.0)
72 h EC ₂₀ (95% confidence interval)	17.8 (13.3 – 21.7)	16.2 (15.1 – 18.4)
72 h EC ₅₀ (95% confidence interval)	39.2 (33.7 – 45.8)	19.9 (19.1 – 21.2)

Conclusion

In this test to determine the growth inhibition of ADM.01851.H.1.A in the green algae *Raphidocelis subcapitata*, the 72-hour NOEC, E_rC₁₀, E_rC₂₀ and E_rC₅₀ based on growth rate were calculated to be 12.5, 11.7 (7.84 – 15.3, 95% confidence interval), 17.8 (13.3 – 21.7, 95% confidence interval) and 39.2 (33.7 – 45.8, 95% confidence interval) mg product/L, respectively. The 72-hour NOEC, E_yC₁₀, E_yC₂₀ and E_yC₅₀ based on yield were determined as 12.5, 14.6 (13.3 – 17.0, 95% confidence interval), 16.2 (15.1 – 18.4, 95% confidence interval) and 19.9 (19.1 – 21.2, 95% confidence interval) mg product/L, respectively.

A 2.2.1.4 Study 4: Effects on aquatic macrophytes

Comments of zRMS:	The study was conducted in line with OECD 239 with no major deviations.				
	Recoveries of clopyralid and ethofumesate were sufficiently stable during the test ($\pm$ 20% of nominal). Therefore, the results were based on nominal test substances concentrations.				
	All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:				
	Endpoint (mg product/L)	Inhibition fresh weight		Inhibition dry weight	
		Yield	Growth rate	Yield	Growth rate
	NOEC	0.045	0.045	0.143	0.143
	LOEC	0.143	0.143	0.458	0.458
	EC ₁₀ (95% CI)	0.066 (0.034-0.106)	0.108 (0.062-0.167)	0.141 (0.021-0.364)	0.267 (0.051-0.620)
	EC ₂₀ (95% CI)	0.172 (0.107-0.247)	0.318 (0.214-0.435)	0.710 (0.244-1.34)	1.50 (0.656-2.62)
	EC ₅₀ (95% CI)	0.108 (0.838-1.41)	2.49 (2.00-3.14)	15.6 (7.92-52.0)	40.6 (17.9-199.4)
		Inhibition main shoot length		Inhibition total shoot length	

		Yield	Growth rate	Yield	Growth rate
	NOEC	0.045	0.045	0.045	0.143
	LOEC	0.143	0.143	0.143	0.458
	EC ₁₀ (95% CI)	0.060 (0.034-0.091)	0.064 (0.030-0.110)	0.103 (0.070-0.140)	0.117 (0.072-0.170)
	EC ₂₀ (95% CI)	0.134 (0.088-0.183)	0.175 (0.101-0.262)	0.197 (0.147-0.248)	0.272 (0.190-0.360)
	EC ₅₀ (95% CI)	0.617 (0.491-0.773)	1.20 (0.898-1.61)	0.677 (0.573-0.799)	1.36 (1.12-1.67)
CI – 95 % confidence intervals (lower – upper)					

Reference: KCP 10.2.1/04

Report: Effects of ADM.01851.H.1.A on *Myriophyllum spicatum* in a static water-sediment system,
Warning, D., 2022,
22 48 AMS 0001, 000110709 (sponsor report number)

Guideline(s): Yes, OECD 239 (2014)

Deviations: Deviations to 239 (2014):
No major deviations

GLP: Yes

Acceptability: Yes

Duplication: -
(if vertebrate study)

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A (suspension concentrate formulation of the active substances clopyralid and ethofumesate)
Batch No.	U3111P1
Active ingredient content	Nominal / analysed: 75 / 74.8 g/L clopyralid Nominal / analysed: 500 / 525.1 g/L ethofumesate
Appearance	Off-white liquid
Density	1.1622 g/mL
Expiry date	30 Nov 2023
Reference substance	For evaluation of the sensitivity of the test system, the reference substance 3,5-dichlorophenol was tested twice a year in a separate study. The latest positive control test performed from 01.08. to 18.08.2021 resulted in E _x C ₅₀ values in the range of 4.77 to 5.96 mg/L which complies with the range as given by OECD 239 (2014) of 4.7 to 6.1 mg/L.

Test organism:

Test species	<i>Myriophyllum spicatum</i> L.
Origin	Purchased from Federal Environment Agency; Division IV: Chemical and Biological Safety, Germany, in January 2012
Acclimation	Pre-culture plants were prepared 1 – 2 weeks before test initiation, kept in artificial sediment and fresh medium, cultivated under test conditions.

Test conditions:

Test substance concentrations	0.045, 0.143, 0.458, 1.47, 4.69 and 15.0 mg product/L (nominal) A stock solution (270 g product/L) was prepared by dissolving ADM.01851.H.1.A in test medium. A further stock solution (8.2377 mg/L) was prepared from this stock solution and both stock solutions
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	were used to spike 1000 mL test medium to obtain the test substance concentrations.
Control	Untreated test medium and sediment
Test duration	14 d
Test medium	Smart and Barko medium: Macroelements: CaCl ₂ x 2 H ₂ O 91.7 mg/L MgSO ₄ x 7 H ₂ O 69.0 mg/L NaHCO ₃ 58.4 mg/L KHCO ₃ 15.4 mg/L
Test sediment	4% (dry weight) peat as close to pH 5.5 – 6 as possible 20% (dry weight) kaolin clay (kaolinite content >30%) 76% (dry weight) fine quartz sand (>50% particles between 50 – 200 µm) 2.00% (dry weight) total organic carbon Aqueous nutrient medium to obtain 200 mg/kg (dry sediment) ammonium chloride and 200 mg/kg (dry sediment) sodium phosphate and a moisture content of 30-50% CaCO ₃ to adjust the pH to 7.0 ± 0.5, pH (CaCl ₂) measured: 6.98
Test type	Static
Test medium pH at test start	7.96
Water temperature	18.8 – 19.9°C
Test vessel	2 L glass beakers with lids
Plant pots	160 mL glass beakers
Test volume	Approximately 1800 mL
No. replicates	1 shoot per plant pot 3 plant pots per replicate 6 control replicates 4 treated group replicates Additional vessels for analysis and retained specimens
Light intensity	Mean 131 µE/m ² /s ⁻¹ Differences from the selected light intensity over the test area did not exceed the range ± 15%.
Photoperiod	16/8 h light/dark

Observations:

Light intensity	Test start
Water temperature	Continuous
Macrophyte observations: Shoot length and number and length of shoot lateral branches Shoot fresh and dry weight	0, 14 d 0, 14 d
Qualitative observations: Change in plant development Test medium appearance Root appearance	0, 7, 14 d 0, 7, 14 d 0, 14 d
Test substance concentration	0, 14 d
pH and dissolved oxygen concentration	0, 7, 14 d

Analytical method:

Method type	HPLC-MS/MS
Equipment	Shimadzu HPLC system with triple quadrupole mass spectrometric detector
Column	Zorbax Eclipse XDB-C8, 150 x 4.6 mm, 5 µm
Detector	Shimadzu LCMS-8050 Detection: Clopyralid: ESI positive, [M+H] ⁺ MRM: m/z 191.8 → 110.0; 191.8 → 110.0 Ethofumesate: ESI positive, [M+H] ⁺ MRM: m/z 287.0 → 161.0; 287.0 → 259.1
Flow rate	0.0 min 0.200 mL/min 8.50 min 0.200 mL/min 8.75 min 0.400 mL/min 11.50 min 0.400 mL/min 11.51 min 0.200 mL/min 13.00 min 0.200 mL/min

Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 50% B 1.50 min 60% B 5.50 min 95% B 10.00 min 95% B 10.01 min 50% B 13.00 min 50% B Run time: 13.00 min
Retention time	Clopyralid: 8.9-9.0 min Ethofumesate: 11.0-11.1 min

Calculations:

To quantify test substance-related effects, growth in the test solutions was compared with the growth of the control plants, and the concentration causing a specified x% inhibition of growth was determined and expressed as the EC_x. Both average specific growth rate (estimated from assessments of main shoot length, total shoot length and shoot fresh weight and shoot dry weight) and yield (estimated from assessments of main shoot length, total shoot length, shoot fresh weight and shoot dry weight) of untreated and treated plants were determined. Specific growth rate (r) and yield (y) were subsequently used to determine the E_rC_x and E_yC_x values, respectively.

Statistics:

For estimation of the LOEC and hence the NOEC values, the treatment means were compared using analysis of variance (ANOVA) techniques. A sequence of pretesting was performed before final statistical testing. These pre-tests included testing of normal distribution (Shapiro-Wilks test, $\alpha = 0.01$), variance homogeneity (Levene's test, $\alpha = 0.01$) and monotonicity (trend analysis by contrasts, $\alpha = 0.05$). The mean for each concentration was then compared with the control mean using an appropriate multiple comparison (William's t-test for homogeneous variances, $\alpha = 0.05$).

Statistical analysis was performed using the statistical software ToxRat Professional Version 3.3 (20.10.2018).

Results and discussions

Validity criteria:

- Total shoot length and shoot fresh weight in control plants must at least double during the exposure phase (achieved: factor 4.3 for total shoot length and factor 3.2 for fresh weight)
- Mean coefficient of variation for yield fresh weight $\leq 35\%$ (achieved: 13.1%)
- Control plants must be free from chlorosis and contamination by other organisms (achieved)
- Test solutions, plants and sediment shall be free from bacterial, algal, fungal films (achieved)

Test substance concentrations are presented in the table below.

Table A2.2.1.1.1-1. Concentrations and % recovery rates of clopyralid and ethofumesate in test media

Product nominal (mg prod./L)	Clopyralid nominal (µg a.s./L)	0 h (fresh media)		14 d (old media)		Ethofumesate nominal (µg a.s./L)	0 h (fresh media)		14 d (old media)	
		Mean (mg a.s./L)	% recovery	Mean (mg a.s./L)	% recovery		Mean (mg a.s./L)	% recovery	Mean (mg a.s./L)	% recovery
Control	Control	< LOD	-	< LOD	-	Control	< LOD	-	< LOD	-
0.045	2.86	2.503	87.1	2.427	84.4	20.21	19.62	97.1	18.77	92.9
0.143	9.16	10.42	113.2	8.482	92.2	64.66	65.55	101.4	61.87	95.7
0.458	29.3	27.16	92.3	25.62	87.0	206.9	198.8	96.1	192.5	93.0
1.47	93.8	100.8	106.9	99.79	105.9	662.4	654.6	98.8	618.1	93.3
4.69	300.0	329.4	109.3	324.0	107.5	2119	2133	100.7	1995	94.2
15.0	960.0	1052	109.1	1039	107.7	6780	6737	99.4	6407	94.5

< LOD = < Limit of detection (0.1981 µg/L clopyralid; 1.391 µg/L ethofumesate)

Since recoveries of clopyralid and ethofumesate were within 80 - 120% of nominal for both fresh and old media, the biological results were based on nominal test substance concentrations.

Analysis of pore water and sediment were not performed because the dissipation of the active substances in the overlaying water measured in fresh and spent samples was less than 20% over time.

The percentage inhibition of yield and growth rates for fresh weight, dry weight, main shoot length and total shoot length in comparison to the control are presented in the table below.

Table A2.2.1.1.1-2. Percentage inhibition of yield and growth rates of *Myriophyllum spicatum*

Concentration product (mg product/L)	Fresh weight		Dry weight	
	Yield % inhibition	Growth rate % inhibition	Yield % inhibition	Growth rate % inhibition
Control	-	-	-	-
0.045	-9.9	-5.8	-14.3	-9.8
0.143	12.5*	7.6*	-1.0	-0.6
0.458	44.9*	31.6*	23.7*	18.2*
1.47	54.9*	40.6*	29.3*	22.1*
4.69	73.5*	60.5*	32.7*	25.1*
15.0	84.6*	74.8*	49.9*	40.7*
	Main shoot length		Total shoot length	
	Yield % inhibition	Growth rate % inhibition	Yield % inhibition	Growth rate % inhibition
Control	-	-	-	-
0.045	-0.2	0.6	-4.7	-1.6
0.143	15.9*	10.8*	8.0*	5.4
0.458	48.5*	36.2*	45.4*	30.8*
1.47	74.9*	64.0*	71.5*	56.5*
4.69	80.2*	68.8*	86.3*	74.4*
15.0	87.1*	79.2*	90.7*	82.1*

Note: Negative values indicate an increase in growth relative to the control

* Significantly different from the control (Williams t-test, $\alpha < 0.05$, one-sided smaller)

No changes in plant development (e.g., deformation of plants, appearance, necrosis and chlorosis) were observed in the control group and at the test concentrations ≤ 0.458 mg product/L at day 7 and at day 14 after test start. At the test concentration of 1.47 mg product/L, the whorls of the lateral branches were smaller at day 7 and 14 after test start. At the test concentrations ≥ 4.69 mg product/L, the whorls of the lateral branches were smaller and the main shoots were deformed at day 7 and 14 after test start.

The control roots and the roots at the test concentrations ≤ 0.458 mg product/L showed a very good root growth. At the test concentration of 1.47 mg product/L, the root growth was moderate. At the test concentrations ≥ 4.69 mg product/L only few roots were observed.

The calculated study endpoints are given in the following table.

Table A2.2.1.1.1-3. Study endpoints of *Myriophyllum spicatum* test with ADM.01851.H.1.A.

Endpoint (mg product/L)	Inhibition fresh weight		Inhibition dry weight	
	Yield	Growth rate	Yield	Growth rate
NOEC	0.045	0.045	0.143	0.143
LOEC	0.143	0.143	0.458	0.458
EC ₁₀ (95% CI)	0.066 (0.034-0.106)	0.108 (0.062-0.167)	0.141 (0.021-0.364)	0.267 (0.051-0.620)
EC ₂₀ (95% CI)	0.172 (0.107-0.247)	0.318 (0.214-0.435)	0.710 (0.244-1.34)	1.50 (0.656-2.62)
EC ₅₀ (95% CI)	0.108 (0.838-1.41)	2.49 (2.00-3.14)	15.6 (7.92-52.0)	40.6 (17.9-199.4)
	Inhibition main shoot length		Inhibition total shoot length	
	Yield	Growth rate	Yield	Growth rate
NOEC	0.045	0.045	0.045	0.143

LOEC	0.143	0.143	0.143	0.458
EC ₁₀ (95% CI)	0.060 (0.034-0.091)	0.064 (0.030-0.110)	0.103 (0.070-0.140)	0.117 (0.072-0.170)
EC ₂₀ (95% CI)	0.134 (0.088-0.183)	0.175 (0.101-0.262)	0.197 (0.147-0.248)	0.272 (0.190-0.360)
EC ₅₀ (95% CI)	0.617 (0.491-0.773)	1.20 (0.898-1.61)	0.677 (0.573-0.799)	1.36 (1.12-1.67)

CI – 95 % confidence intervals (lower – upper)

At the end of the 14-day exposure, yield and growth rates based on fresh weight, yield and growth rate based on main shoot length and yield based on total shoot length were statistically significantly inhibited at 0.143 mg product/L and all higher concentrations. Therefore, the NOEC and LOEC for these parameters were determined as 0.045 and 0.143 mg product/L, respectively. Yield and growth rates based on dry weight and growth rate based on total shoot length were statistically significantly inhibited at 0.458 mg product/L and all higher concentrations. The NOEC and LOEC for these parameters were 0.143 and 0.458 mg product/L, respectively.

E_rC₁₀, E_rC₂₀, E_rC₅₀, E_yC₁₀, E_yC₂₀ and E_yC₅₀ values based on fresh weight, dry weight, shoot length and total shoot length are presented in the table above with 95% confidence intervals.

Conclusion

In this test to determine the effects of ADM.01851.H.1.A in the aquatic plant *Myriophyllum spicatum*, the 14-d NOEC, E_rC₁₀, E_rC₂₀ and E_rC₅₀ based on fresh weight were calculated to be 0.045, 0.108, 0.318 and 2.49 mg product/L, respectively. The 14-d NOEC, E_yC₁₀, E_yC₂₀ and E_yC₅₀ based on fresh weight were determined as 0.045, 0.066, 0.172 and 0.108 mg product/L, respectively. The 14-d NOEC, E_rC₁₀, E_rC₂₀ and E_rC₅₀ based on dry weight were calculated to be 0.143, 0.267, 1.50 and 40.6 mg product/L, respectively. The 14-d NOEC, E_yC₁₀, E_yC₂₀ and E_yC₅₀ based on dry weight were determined as 0.143, 0.141, 0.710 and 15.6 mg product/L, respectively. The 14-d NOEC, E_rC₁₀, E_rC₂₀ and E_rC₅₀ based on main shoot length were calculated to be 0.045, 0.064, 0.175 and 1.20 mg product/L, respectively. The 14-d NOEC, E_yC₁₀, E_yC₂₀ and E_yC₅₀ based on main shoot length were determined as 0.045, 0.060, 0.134 and 0.617 mg product/L, respectively. The 14-d NOEC, E_rC₁₀, E_rC₂₀ and E_rC₅₀ based on total shoot length were calculated to be 0.143, 0.117, 0.272 and 1.36 mg product/L, respectively. The 14-d NOEC, E_yC₁₀, E_yC₂₀ and E_yC₅₀ based on total shoot length were determined as 0.045, 0.103, 0.197 and 0.677 mg product/L, respectively.

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

Comments of zRMS:	The study was conducted in line with OECD 213 and 214 with minor deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The contact LD₅₀ (48 h) > 199 µg product/bee The oral LD₅₀ (48 h) > 200 µg product/bee
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Reference: KCP 10.3.1.1/01

Report: Acute toxicity of ADM.01851.H.1.A to the honeybee *Apis mellifera* L. under

laboratory conditions, Poráčki K., 2022, 22 48 BAA 0079, 000110705 (sponsor report number)

Guideline(s): OECD 213 (1998), OECD 214 (1998)

Deviations: Deviations to OECD 213 (1998), OECD 214 (1998)
No major deviations

GLP: Yes

Acceptability: Yes

Duplication: -
(if vertebrate study)

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3111P1
Active ingredient content	Nominal / analysed: 75 / 74.8 g/L clopyralid Nominal / analysed: 500 / 525.1 g/L ethofumesate
Appearance	Off-white liquid
Density	1.1622 g/mL
Expiry date	30 Nov 2023
Reference substance	Dimethoate EC 400 (Danadim® Progress) Nominal / analysed: 400 / 401.7 g/L dimethoate Expiry date: 15 Jul 2023

Test organism:

Test species	Honey bee, <i>Apis mellifera</i> L. subspecies Buckfast (Hymenoptera, Apoidea)
Origin	On-site apiary of the test facility (worker bees of a colony in good health condition and queen-right)
Collection	Bees were collected in the morning prior of use from the top of the bee hive. Ten bees were transferred into each test cage without anesthesia using a carousel with glass tubes as trapping device. The bees were acclimatised to the test conditions for about 1 hour (corresponding to the starvation period in the oral toxicity test).
Age at test start	Female, adult worker bees, 3-5 weeks old
Feeding	After exposure in the oral toxicity test, bees were fed with 50% w/v sucrose solution <i>ad libitum</i> . In the contact toxicity test, bees were fed with 50% w/v sucrose solution <i>ad libitum</i> for the whole test duration.
No. bees per replicate	10
No. replicates per test substance, reference substance or control	3

Test conditions:

Test substance dosage	Oral toxicity test Offered dose rates: 12.5, 25.0, 50.0, 100 and 200 µg product/bee Actually consumed dose rates: 12.5, 25.0, 50.0, 99.2 and 199 µg product/bee Contact toxicity test 12.5, 25.0, 50.0, 100 and 200 µg product/bee
Reference substance dosage	Oral toxicity test Offered dose rates: 0.183, 0.282, 0.434 and 0.668 µg product/bee, corresponding with 0.069, 0.106, 0.162 and 0.250 µg a.s./bee Actually consumed dose rates: 0.182, 0.282, 0.433 and 0.666 µg product/bee, corresponding with 0.068, 0.105, 0.162 and 0.249 µg a.s./bee Contact toxicity test

	0.282, 0.376, 0.501 and 0.668 µg product/bee, corresponding with 0.105, 0.141, 0.187 and 0.250 µg a.s./bee
Control	Oral toxicity test 50% w/v sucrose solution Contact toxicity test Water control: deionised water 1% v/v tween control: deionised water – 1% Tween®80
Administration	Oral toxicity test The bees were fed with a defined quantity of a 50% w/v sucrose solution including the test or reference substance. The control treatments were fed with 50% w/v sucrose solution. Groups of 10 bees were provided with 200 µL (corresponding to 0.2374 g) of application solution, which was offered to the bees in a glass ampoule. Due to their social feeding behaviour (trophallaxis), honeybees in each test unit are assumed to receive approximately the same amount of food (about 20 µL/bee) and consequently the same dose of the test/reference substance. After 3 hours of exposure, the application solutions were obviously completely consumed, the feeding tubes were removed, and the exact quantity of consumed application solution was determined. Thereafter, the bees were fed with untreated 50% w/v sucrose solution <i>ad libitum</i>. Contact toxicity test Before application, bees in each test cage were anaesthetised with CO₂ for approximately ½ minute. Anaesthetised bees were transferred from the cages into a large petri dish and turned around with forceps for thoracic application of a single 2 µL/bee droplet of the control (water control or 1% v/v tween control) or the test or reference substance solutions (the volume of 2 µL droplet was chosen to be more appropriate than the guideline recommendation of 1 µL). The doses were placed on the dorsal thorax using an Eppendorf Micropipette. After application bees were carefully transferred one by one into the test cages by means of forceps.
Test duration	48 hours
Test units	Disposable cardboard cages (95 mm x 50 mm x 65 mm, length x width x height) with holes in the bottom side for ventilation and a glass plate in front
Temperature	Nominal: 25±2°C, actual: 24.2-24.4°C
Relative humidity	Nominal: 50-70%, actual: 60.2-62.1%
Illumination	Constant darkness throughout the test (diffuse artificial light only during handling and assessments)
Ventilation	By the air-conditioning equipment of the climatic chamber

Observations:

Mortality	4, 24 and 48 hours after application
Behavioural abnormalities	4, 24 and 48 hours after application
Test conditions (temperature, relative humidity)	Continuously

Calculations:

Mean mortality (number of dead bees/replicate) in % for each treatment group was calculated. For each dose rate, the corrected mortality was calculated according to Abbott (1925) modified by Schneider-Orelli (1947).

Calculations for the oral toxicity test were based on actually consumed doses. The oral and contact LD₅₀ values for the test substance were determined directly from the raw data.

Statistics:

The oral and contact LD₅₀ and their 95% confidence limits for the reference substance were calculated by Probit analysis (linear maximum likelihood regression).

Statistical significance of mortality values was determined by Fisher's Exact Binomial Test with Bonferroni-Holm Correction ($\alpha = 0.05$, one-sided greater) for both the test substance and the reference substance.

Statistical calculations were performed with the computer program ToxRat Professional 3.3.0 (2018)

Results and discussions

Validity criteria of the oral toxicity test:

- The average mortality for the total number of controls must not exceed 10 per cent at the end of the test (achieved: 0%)
- The 24-h LD₅₀ of the toxic standard is in the range of 0.10-0.35 µg a.s./bee (achieved: 0.122 µg a.s./bee)

Validity criteria of the contact toxicity test:

- The average mortality for the total number of controls must not exceed 10 per cent at the end of the test (achieved: 0%)
- The 24-h LD₅₀ of the toxic standard is in the range of 0.10-0.30 µg a.s./bee (achieved: 0.143 µg a.s./bee)

Table A2.3.1.1-1: Acute oral and acute contact toxicity of ADM.01851.H.1.A to honey bees (*Apis mellifera* L.)

Oral test		Contact test	Oral test			Contact test		
target dose	actually consumed dose		Mortality [%]		Corr. ^a mortality [%]	Mortality [%]		Corr. ^a mortality [%]
[µg prod./bee]	[µg prod./bee]	[µg prod./bee]	24 h	48 h	48 h	24 h	48 h	48 h
Control (50% w/v sucrose solution)	0.0	-	0.0	0.0	-	-	-	-
Water control	-	0.0	-	-	-	0.0	0.0	-
1% v/v tween control	-	0.0	-	-	-	0.0	0.0	-
Test substance: ADM.01851.H.1.A								
12.5	12.5	12.5	0.0	0.0	-	0.0	0.0	-
25.0	25.0	25.0	0.0	0.0	-	0.0	0.0	-
50.0	50.0	50.0	0.0	0.0	-	0.0	0.0	-
100	99.2	100	0.0	0.0	-	0.0	0.0	-
200	199	200	0.0	0.0	-	0.0	0.0	-
Reference substance: Dimethoate EC 400								
[µg a.s./bee]	[µg a.s./bee]	[µg a.s./bee]	24 h	48 h	48 h	24 h	48 h	48 h
0.105	0.068	0.105	6.7	10.0	-	10.0	23.3*	-
0.141	0.105	0.141	33.3*	50.0*	-	60.0*	60.0*	-
0.187	0.162	0.187	73.3*	90.0*	-	80.0*	86.7*	-
0.250	0.249	0.250	100.0*	100.0*	-	93.3*	100.0*	-
Endpoints								
48-h LD ₅₀ oral (actually consumed)			> 199 µg product/bee					
48-h LD ₅₀ contact (nominal)			> 200 µg product/bee					

^a Corrected for mortality in the control according to Abbott (1925) modified by Schneider-Orelli (1947)

* Statistically significantly different from control (Fisher's Exact Binomial Test with Bonferroni-Holm Correction, $\alpha = 0.05$, one-sided greater)

Oral toxicity test

No mortality was observed in the control group and all test substance treatment groups during the 48-hour study period. Furthermore, no behavioural abnormalities occurred in the control group and all test groups treated with the test substance.

In the reference substance treatments, mortality ranged between 10.0% and 100.0% after 48 hours of exposure. The oral 48-hour LD₅₀ of the reference substance was calculated to be 0.105 µg dimethoate/bee (95% confidence interval: 0.094-0.116 µg dimethoate/bee).

Contact toxicity test

No mortality was observed in the water control group, the 1% v/v tween control group and all test substance treatment groups during the 48-hour study period. Furthermore, no behavioural abnormalities occurred in the control group and all test groups treated with the test substance.

In the reference substance treatments, mortality ranged between 23.3% and 100.0% after 48 hours of exposure. The contact 48-hour LD₅₀ of the reference substance was calculated to be 0.131 µg dimethoate/bee (95% confidence interval: 0.118-0.142 µg dimethoate/bee).

Conclusion

In this test on toxicity of ADM.01851.H.1.A to the honey bee (*Apis mellifera* L.), the oral LD₅₀ (48 h) was determined to be > 199 µg product/bee and the contact LD₅₀ (48 h) was found to be > 200 µg product/bee.

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Please refer to chapter A 2.3.1.1 above.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to chapter A 2.3.1.1 above.

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 major deviation. The concentrations of the active ingredients in the applied test item feeding solutions were within the required range of ± 20 % of the nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD₅₀ >476 µg product/bee/day NOEDD ≥ 476 µg product/bee/day
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Reference:	KCP 10.3.1.2/01
Report:	Chronic toxicity of ADM.01851.H.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions, Ruhland S., 2023, 22 48 BAC 0024, 000110706 (sponsor report number)
Guideline(s):	OECD 245 (2017)
Deviations:	Deviations to OECD 245 (2017) No major deviations
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	-

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3111P1
Active ingredient content	Nominal / analysed: 75 / 74.8 g/L clopyralid Nominal / analysed: 500 / 525.1 g/L ethofumesate
Appearance	Off-white liquid
Density	1.1622 g/mL
Expiry date	30 Nov 2023
Reference substance	Dimethoate EC 400 (Danadim Progress) Nominal / analysed: 400 / 401.7 g/L dimethoate Expiry date: 15 Jul 2023

Test organism:

Test species	Honey bee, <i>Apis mellifera</i> L. subspecies Buckfast (Hymenoptera, Apoidea)
Origin	On-site apiary of the test facility (worker bees from healthy, disease free, well fed and queen-right bee colonies)
Collection	On Day-2, brood combs with capped cells were taken from outside hives of three different colonies. A sufficient food supply was ensured either by honey and pollen, which were on the same brood comb, or by an additional comb containing food. These frames were placed without adult worker bees in a “five comb hive body” and incubated under controlled environmental conditions in a climatic chamber at $33 \pm 2^\circ\text{C}$ in darkness (until Day-1). Afterwards, the newly hatched worker bees were transferred into the test cages in groups of 10 bees per cage. Since combs from three different colonies were used, the bees were equitably distributed across the treatments. For the following 24 ± 2 hours (until Day 0), the bees were held in the test cages at $33 \pm 2^\circ\text{C}$ and 50 – 70% relative humidity. They were provided with 50% (w/v) sucrose solution for acclimatisation to test conditions. Before starting the experimental phase on Day 0, moribund and dead bees were rejected and replaced by healthy bees that were held in spare cages under the above-mentioned controlled environmental conditions.
Age at test start	Maximum 2 days old
Feeding	Young worker bees were provided continuously with treated or untreated feeding solution via plastic syringes (tips removed) <i>ad libitum</i> through a hole in the lateral wall of the test cages.
No. bees per replicate	10
No. replicates per test substance, reference substance or control	3

Test conditions:

Test substance dosage	Nominal doses: 20.5, 51.2, 128, 320 and 800 µg product/bee/day corresponding to 521, 1304, 3259, 8148 and 20370 mg product/kg food Actually consumed doses (average over 10 days): 18.8, 43.9, 113, 199 and 476 µg product/bee/day The test substance feeding solutions were prepared in 50% (w/v) sucrose solution + 0.1% (w/v) xanthan freshly prepared every day just before administration of food.
Reference substance dosage	Nominal dose: 72.9 ng product/bee/day, corresponding to 1.855 mg product/kg food Actually consumed dose (average over 10 days): 34.4 ng product/bee/day The reference substance stock solution was prepared in 50% (w/v) sucrose solution once at the start of the exposure period (Day 0) and stored in the refrigerator at about 6°C. The reference substance feeding solution was prepared in 50% (w/v) sucrose solution at least every 4 days and stored in the refrigerator at about 6°C.
Control	Control AC: untreated food (50% (w/v) aqueous sucrose solution) Control BC:

	untreated food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan) The 50% (w/v) sucrose solution and 50% (w/v) sucrose solution + 0.1% (w/v) xanthan for the preparation of dilutions and feeding of the control groups were freshly prepared every day.
Administration	The treated/untreated food was provided <i>ad libitum</i> in a plastic syringe, which had been weighed before application. The feeders with treated/untreated food remained in the test cages for about 24 hours ($\pm$ 2 hours). The actual consumption of administered feeding solution was determined by re-weighing the feeders containing the remaining feeding solution each day after removal from the test units. The possible evaporation of feeding solutions from the feeders was investigated in additional test cages without bees but with pre-weighed feeders containing diet of untreated control feeding solutions. At the daily feeder exchange, the feeders were re-weighed and replaced by new feeders with fresh food.
Test duration	10 days
Test units	Bees were kept in aluminium cages (95 mm x 70 mm x 60 mm, width x height x depth) with holes in the lateral walls for sufficient air supply and ventilation and two glass plates (one in front and one in the back) for observation of the bees.
Temperature	Nominal: 33 $\pm$ 2°C, actual: 32.6-33.2°C
Relative humidity	Nominal: 50-70%, actual: 57.7-62.8%
Illumination	Constant darkness throughout the test (diffuse artificial light only during assessments and exchange of feeders)
Ventilation	By the air-conditioning equipment of the climatic chamber

Observations:

Mortality	Daily at about the same time of the day (every 24 h $\pm$ 2 h)
Behavioural abnormalities	Daily at about the same time of the day (every 24 h $\pm$ 2 h)
Test conditions (temperature, relative humidity)	Continuously

Analytical method:

Samples	For verification of the exposure concentrations, the test substance feeding solutions of all test doses as well as the control feeding solution BC were sampled in duplicate as specimens for analysis and retain directly after preparation on each day of application (Day 0 to Day 9). Additionally, storage stability samples were prepared on the first day of application (Day 0) for verification of stability during storage for longer than 30 days.
Method type	Reversed-phase HPLC with MS/MS detection
Equipment	Agilent 1200 HPLC system
Column	ACE C18 Amide 3 μ m 100x2.1 mm
Column temperature	45°C
Detection	ESI positive, MRM Clopyralid : m/z 192 $\rightarrow$ 146 (Quantifier) m/z 192 $\rightarrow$ 110 (Qualifier) Ethofumesate : m/z 287 $\rightarrow$ 121 (Quantifier) m/z 287 $\rightarrow$ 259 (Qualifier)
Flow rate	0.40 mL/min
Mobile phase	A: Water containing 0.1% acetic acid B: Acetonitrile 0.00 min 90% A/10% B 0.50 min 90% A/10% B 3.00 min 40% A/60% B 5.00 min 0% A/100% B 6.00 min Stop
Retention time	approximately 2.04 min for clopyralid approximately 5.02 min for ethofumesate
Sample preparation	The samples of the feeding solutions and storage stability were extracted directly after sampling and the extracts were shipped deep frozen. The extracts were thawed, homogenised, diluted and injected in the HPLC system.

Calculations:

The difference of the feeder weight at the start and end of each feeding period represents the food consumed by the bees in one cage during 24 hours. The respective mean evaporation figure was then subtracted from the calculated food uptake to give the real food uptake accounting for the loss by evaporation. Finally, this amount of food was divided by the number of living bees at the start of the corresponding exposure interval. In case the subtraction of the mean evaporation figure from the calculated food uptake led to a negative value, the food consumption of the respective day was considered to be “0” (no food consumption).

The percentage of cumulative mortality was calculated for each treatment group and assessment time from the number of dead individuals in relation to the number of introduced test organisms. The cumulative mortality of the test and reference substance treatments was corrected for the corresponding control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Statistics:

For statistical determination of NOEDD/NOEC, the Fisher’s Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$; one sided greater) was used. Due to the lack of effects in all dose groups, the LDD_{50/20/10} and LC_{50/20/10} values could not be calculated. They were estimated from the generated raw data.

Statistical calculations were made with the statistical programme ToxRatPro Version 3.2.1 (2015).

Results and discussions

Validity criteria:

- Average mortality across replicates for the untreated control and solvent control groups $\leq 15\%$ at the end of the test (achieved: Control AC 0.0% mortality, Control BC 0.0% mortality)
- Average mortality in the reference substance treated group $\geq 50\%$ at the end of the test (achieved: 66.7%)

Test substance concentrations in honey bee feeding solutions are presented in the table below.

Table A2.3.1.2-1: Concentrations and % recovery rates of clopyralid and ethofumesate in honey bee feeding solutions

Treatment	Day	Clopyralid			Ethofumesate		
		Nominal conc. (mg a.s./kg food)	Analysed conc. (mg a.s./kg food)	Mean recovery (% of nominal)	Nominal conc. (mg a.s./kg food)	Analysed conc. (mg a.s./kg food)	Mean recovery (% of nominal)
Control BC	Day 0 - 9	0.000	< 30% LOQ	-	0.000	< 30% LOQ	-
ET	Day 0 - 9	33.5	29.7-32.7	92.4	236	199-218	88.3
DT	Day 0 - 9	83.8	70.8-82.2	93.4	589	504-576	91.4
CT	Day 0 - 9	210	186-204	94.1	1473	1276-1388	90.5
BT	Day 0 - 9	524	481-509	94.9	3683	3407-3558	93.9
AT	Day 0 - 9	1310	1248-1286	96.6	9207	8501-8838	94.5
AT-St1-St3	-	1310	1175-1249	93.4	9207	8462-8719	92.9

St: stock solution

LOQ: limit of quantification: 15.7 mg/kg for clopyralid (corresponding to 21.6 µg/L), 110 mg/kg for ethofumesate (corresponding to 183 µg/L)

The mean recoveries of clopyralid ranged between 92.4% and 96.6% and the mean recoveries of ethofumesate ranged between 88.3% and 94.5%. No clopyralid and ethofumesate were detected in the control samples. Thus, the concentrations of active substances in the specimens of the biological part of the study were verified and endpoints were based on nominal test concentrations. Storage stability for longer than 30 days was verified as well.

Mean cumulative uptake of test and reference substance and mean mortality and behaviour of bees in the

chronic toxicity feeding test after 10 days are presented in the tables below.

Table A2.3.1.2-2 Mean cumulative uptake of test and reference substance amount in the course of the study

Treatment group		Control	Test substance					Reference substance		
Nominal daily dose (µg prod- uct/bee/day)		AC/BC	ET 20.5	DT 51.2	CT 128	BT 320	AT 800	Nominal daily dose (ng a.s./bee/day)		
Mean cumulative uptake (µg product/bee)	Day 1	-	5.92	17.0	60.0	94.8	339.5	Mean cumulative uptake (ng a.s./bee)	Day 1	17.8
	Day 2	-	24.1	47.3	163.4	367.5	874.5		Day 2	38.4
	Day 3	-	41.6	88.8	261.0	563.8	1366.1		Day 3	59.1
	Day 4	-	61.4	131.0	384.3	811.8	1938.5		Day 4	70.4
	Day 5	-	82.2	183.1	507.6	1036.7	2489.2		Day 5	76.8
	Day 6	-	104.0	231.3	626.4	1221.4	2904.7		Day 6	84.7
	Day 7	-	124.5	287.0	752.1	1446.3	3431.8		Day 7	101.4
	Day 8	-	144.2	335.9	864.2	1572.0	3895.3		Day 8	111.5
	Day 9	-	168.7	385.0	1003.5	1743.9	4315.5		Day 9	121.6
	Day 10	-	188.5	438.7	1130.8	1987.0	4755.2		Day 10	128.8
Consumed daily dose (µg prod- uct/bee/day)		-	18.8	43.9	113	199	476	Consumed daily dose (ng a.s./bee/day)		12.9

Note: Calculations were performed with non-rounded values and corrected for evaporation

Table A2.3.1.2-3 Mean mortality and behaviour of bees in the chronic toxicity feeding test after 10 days

Treatment group	Daily dose		Concentration		After 10 days		
	nominal	Consumed ^c			Mean mortality		Number of bees with behavioural abnormalities ^d
	(µg product/bee/day)		(g product/kg food)		absolute (%)	Corrected ^e (%)	
Control AC ^a	-	-	-		0.0	-	0 out of 30
Control BC ^b	-	-	-		0.0	-	0 out of 30
Test sub- stance	20.5	18.8	521		0.0	-	0 out of 30
	51.2	43.9	1304		0.0	-	0 out of 30
	128	113	3259		0.0	-	0 out of 30
	320	199	8148		0.0	-	0 out of 30
	800	476	20370		6.7	-	0 out of 28
	(ng prod- uct/bee/ day)	(ng a.s./bee/ day)	(mg prod- uct/kg food)	(mg a.s./kg food)			
Reference substance	72.9 34.4	27.3 12.9	1.855	0.694	66.7	-	1 out of 10

^a Untreated food (50% (w/v) aqueous sucrose solution)

^b Untreated food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan)

^c Taking into account the actual food uptake and evaporation

^d Number of bees with behavioural abnormalities referring to the number of remaining bees

^e Corrected according to the formula of Abbott (1925), modified by Schneider-Orelli (1947)

Based on the results of the study, the following endpoints were obtained.

Table A2.3.1.2-4: Endpoints of the honey bee chronic test on day 10

Endpoint	(µg consumed product/bee/day)
10 d LDD ₁₀ ^a	> 476
10 d LDD ₂₀ ^a	> 476
10 d LDD ₅₀ ^a	> 476
10 d NOEDD ^b	≥ 476
Endpoint	(mg product/kg food)
10 d LC ₁₀ ^a	> 20370
10 d LC ₂₀ ^a	> 20370
10 d LC ₅₀ ^a	> 20370
10 d NOEC ^b	≥ 20370

^a Due to mortalities less than 10% in the test substance treatment groups, the LDD_{50/20/10}/LC_{50/20/10} is considered to be higher than the highest dose/concentration

^b No observed effect dietary doses/concentrations were determined using Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$; one sided greater)

After 10 days, no mortality was observed in either control group. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees in the test substance treatment groups effectively consumed doses of 18.8, 43.9, 113, 199 and 476 µg product/bee/day which resulted in mortalities of 0.0, 0.0, 0.0, 0.0 and 6.7% after 10 days, respectively. None of the obtained mortalities was statistically significantly increased compared to the control group BC (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

In the course of the test as well as in the final assessment on the last day of the test, no treatment related abnormal behaviour was observed in any of the test substance treatment groups.

The effective reference substance dosage in the study was 12.9 ng a.s./bee/day, which caused a mean mortality of 66.7% after 10 days of exposure.

In the test substance treatment groups, the overall mean daily food consumption ranged between 23.3 and 36.1 mg feeding solution/bee/day which corresponds to 59.4% and 92.0% of the expected daily amount with a tendency of higher food uptake in the lower test substance doses. In control group AC, the bees consumed on average 38.6 mg feeding solution/bee/day (corresponding to 98.3% of the expected daily amount) and in the viscosifier control group BC, 33.4 mg feeding solution/bee/day (corresponding to 85.0% of the expected daily amount).

The daily mean evaporation of 50% (w/v) sucrose solution ranged between 53.0 and 59.0 mg per cage and between 49.0 mg and 60.3 mg for 50% (w/v) sucrose solution + 0.1% (w/v) xanthan. The food consumption per cage was corrected by subtracting the mean evaporation figure of the respective day of application.

Conclusion

In this chronic oral test with ADM.01851.H.1.A in the honey bee *Apis mellifera* L., the 10-day LDD₁₀, LDD₂₀ and LDD₅₀ values were all found to be > 476 µg consumed product/bee/day. This correspond to 10-day LC₁₀, LC₂₀ and LC₅₀ values of all being > 20370 mg product/kg food. The 10-day NOEDD was determined to be ≥ 476 µg consumed product/bee/day corresponding to a 10-day NOEC of ≥ 20370 mg product/kg food.

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 no major deviations.	
	All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:	
	Endpoint	(µg product/larva)
	22 d ED ₁₀ (95% confidence interval)	132.5 (97.7 – 179.7)
	22 d ED ₂₀ (95% confidence interval)	166.4 (131.1 – 211.2)
	22 d ED ₅₀ (95% confidence interval)	257.0 (211.9 – 311.8)
	22 d NOED	100.3

Reference: KCP 10.3.1.3/01

Report: ADM.01851.H.1.A – Repeated Exposure of Honey Bee (*Apis mellifera* L.) Larvae under Laboratory Conditions, Kleebaum K., 2022, 22 48 BLC 0025, 000110708 (sponsor report number)

Guideline(s): OECD 239 (2016)

Deviations: Deviations to OECD 239 (2016)
No major deviations

GLP: Yes

Acceptability: Yes

Duplication: -
(if vertebrate study)

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3111P1
Active ingredient content	Nominal / analysed: 75 / 74.8 g/L clopyralid Nominal / analysed: 500 / 525.1 g/L ethofumesate
Appearance	Off-white liquid
Density	1.1622 g/mL
Expiry date	30 Nov 2023
Reference substance	Dimethoate tech. Analysed: 99.32% w/w Expiry date: 15 Jul 2023

Test organism:

Test species	Honey bee, <i>Apis mellifera</i> ssp. Buckfast (Hymenoptera, Apoidae)
Origin	The larvae originated from colonies maintained at the test facility. For each test, larvae were collected from three different colonies, each representing a replicate in order to avoid genetic influences on the test outcome. The colonies were healthy and queen-right and had not received treatments with chemical substances for at least 4 weeks prior to use in the study. The bee colonies producing the larvae were held under field conditions following good beekeeping practices. In the hives, a healthy queen and brood in egg, larval and pupal stages as well as filled food

	combs (containing nectar and pollen) were present. A sufficient amount of food was present in the bee hives.
Collection	On Day -3, the respective queen of the colony was confined on an empty brood comb, which was fitted in an excluder cage and thereafter placed in the hive. The queen laid her eggs solely on this comb. The caging time was about 25 hours. In the afternoon of Day -2, the queen was released from the excluder cage. The comb was checked for the presence of freshly laid eggs, was confined in the excluder again in order to avoid any further egg laying, and was placed in the hive near to frames containing open brood. The eggs were incubated within the hive between Day -2 and Day 1 to ensure the definition and uniformity of the age of respective larvae on the day of grafting (Day 1).
Age at test start	First instar larvae (L1 during grafting)
No. bees per replicate	12
No. replicates per test substance, reference substance or control	3 (placed on one culture plate)

Test conditions:

Test substance dosage	Cumulative doses: 3.7, 11.1, 33.4, 100.3 and 301.3 µg product/larva corresponding to 23.4, 70.5, 211.4, 634.1 and 1904.3 mg product/kg food Test substance stock solutions were prepared daily. The test substance was mixed into aqueous sugar solution to prepare the test substance base stock solution. In a parallel dilution, several dilutions were prepared by adding further sugar solution. Then the royal jelly was added to each stock solution at a ratio of 1:1 (w/w) to manufacture the final diets.
Reference substance dosage	Cumulative dose: 7.4 µg dimethoate/larva, corresponding to 47 mg dimethoate/kg food The reference substance was mixed into aqueous sugar solution, to prepare the reference substance base stock solution, which was diluted with further aqueous sugar solution to produce the reference substance stock solution. The final diet was prepared by mixing the previously compounded stock solution with royal jelly at a ratio of 1: 1 (w/w).
Control	Untreated diet
Administration	Each larva was fed separately using a sterile pipette. The food drop was placed next to the larvae to avoid drowning. Before feeding, the final diets were warmed up to 34.5°C. During the process, the culture plate in operation was placed on a warming plate set to 35°C.
Test duration	22 days Pre-grafting (<i>in vivo</i>): Day -3 to Day 0 Grafting: Day 1 Pre-exposure (<i>in vitro</i>): Day 1 to Day 2 Exposure (<i>in vitro</i>): Day 3 to Day 6 Post exposure (<i>in vitro</i>): Day 7 to Day 22
Test units	Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm, depth 8 mm) were placed in 48-well plates. Grafting cells were disinfected followed by drying of the cells under a laboratory fume hood.
Grafting of larvae	On Day 1, the combs containing larvae were transported from the hive to an acclimatised laboratory room using a polystyrene box. Larvae were transferred from the combs to the cells using a suitable grafting tool. During grafting, the larvae were placed on the surface of the artificial diet within the grafting cells. Each replicate represented larvae originating from a different colony to exclude colony effects. The grafting was performed on a warming plate set to 35°C.
Temperature	Nominal: 34-35°C, actual: 34.2-34.9°C
Relative humidity	Nominal: 95% ± 5% at Day 1 - Day 8, 80% ± 5% at Day 8 – Day 15, 50-80% at Day 15 – Day 22 Actual: Day 1 – Day 8: 93.1-98.8% Day 8 – Day 15: 81.1-83.7% Day 15 – Day 22: 60.0-61.5%
Illumination	Constant darkness throughout the test (diffuse artificial light only during

	handling and assessments)
Feeding	The diet was prepared using the following ingredients: - Diet A (Day 1, 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 (Day 3, 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 Day 4 to Day 6, 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 Aqueous sugar solution A for use on Day 1 was prepared prior to usage on Day 1. The aqueous sugar solutions as one component of the artificial diets B and C were prepared freshly and stored in a fridge for use. These sugar solutions were mixed with royal jelly every day before each feeding occasion.

Observations:

Mortality	Number of dead larvae/pupae Daily on Day 4 to Day 8 (larvae) and on Day 15 (pupae)
Developmental effects	During mortality assessments, comprising, e.g.: F (food left, assessed only on Day 8) S (small body size) A (abnormal moving behaviour in terms of increased activity) B (black spots or other discolorations indicating sickness - the test substance's colour was not to be noted as abnormal discoloration of any larvae)
Adult emergence	Number of adult bees, alive or dead on Day 22
Test conditions (temperature, relative humidity)	Continuously

Analytical method:

Samples	The final diets, the control and samples for proving storage stability were sampled in duplicate as specimens for analysis and retention directly after preparation on Day 3, Day 4, Day 5 and Day 6. Additionally, each stock solution and pure aqueous sugar solution as dilution medium were sampled to provide retain samples directly after preparation on Day 3, Day 4, Day 5 and Day 6.
Method type	Reversed-phase HPLC with MS/MS detection
Equipment	Agilent 1200 HPLC system
Column	ACE C18 Amide 3 µm 100x2.1 mm
Column temperature	45°C
Detection	ESI positive, MRM Clopyralid : m/z 192 → 146 (Quantifier) m/z 192 → 110 (Qualifier) Ethofumesate : m/z 287 → 121 (Quantifier) m/z 287 → 259 (Qualifier)
Flow rate	0.40 mL/min
Mobile phase	A: Water containing 0.1% acetic acid B: Acetonitrile 0.00 min 90% A/10% B 0.50 min 90% A/10% B 3.00 min 40% A/60% B 5.00 min 0% A/100% B 6.00 min Stop
Retention time	approximately 2.45 min for clopyralid approximately 5.02 min for ethofumesate
Sample preparation	The samples of the biological part were extracted directly after sampling and the extracts were shipped deep frozen. The extracts were thawed, homogenised, diluted and injected in the HPLC system.

Calculations:

Mean mortality for each test substance group and the reference substance group was corrected for control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Statistics:

For statistical calculation of the mortality results and for determination of the NOEC/NOED for Day 22, the Step-down Rao-Scott Cochran-Armitage test was used. The accepted significance level was $\alpha = 0.05$ (one-sided greater). With Probit analysis using linear maximum likelihood regression, values of the $ED_{50/20/10}/EC_{50/20/10}$ (for Day 22) of the test substance along with the 95% confidence limits were calculated.

In order to correct for control mortality, any calculations were conducted with mortality data. The statistical calculations were performed with the computer programme ToxRat Professional 3.2.1 (Ratte, 2015).

Results and discussions

Validity criteria:

- Cumulative larval mortality in the control plates from Day 3 to Day 8 $\leq 15\%$ across all replicates (achieved: 8.3%)
- Adult emergence rate in the control plates on Day 22 $\geq 70\%$ across all replicates (achieved: 80.6%)
- Reference cumulative larval mortality $\geq 50\%$ across all replicates on Day 8 (achieved: 94.4%)

Test substance concentrations in the larval diet are presented in the table below.

Table A2.3.1.3-1: Concentrations and % recovery rates of clopyralid and ethofumesate in larval diet

Treatment	Day	Clopyralid			Ethofumesate		
		Nominal conc. (mg a.s./kg food)	Analysed conc. (mg a.s./kg food)	Mean recovery (% of nominal)	Nominal conc. (mg a.s./kg food)	Analysed conc. (mg a.s./kg food)	Mean recovery (% of nominal)
Control AC	Day 3 - 6	0.000	< 30% LOQ	-	0.000	< 30% LOQ	-
ET	Day 0 - 9	1.51	1.46-1.61	101	10.6	9.59-10.2	94.1
DT	Day 0 - 9	4.53	4.34-4.52	97.8	31.8	31.7-32.0	100
CT	Day 0 - 9	13.6	12.6-13.5	96.9	95.5	83.1-84.4	87.5
BT	Day 0 - 9	40.8	39.0-40.8	97.8	287	256-258	89.7
AT	Day 0 - 9	122	106-123	96.0	861	710-802	89.8

LOQ: limit of quantification: 0.702 mg/kg for clopyralid (corresponding to 8.43 $\mu\text{g/L}$), 4.94 mg/kg for ethofumesate (corresponding to 59.3 $\mu\text{g/L}$)

The mean recoveries of chlopyralid in the samples were between 96% and 101%. The mean recoveries of ethofumesate in the samples were between 87.5% and 100%. No clopyralid and ethofumesate were detected in the control samples. Thus, the concentrations of active ingredients in the specimens of the biological part of the study were verified and endpoints were based on nominal test concentrations.

Cumulative mortality and emergence rate in the control, the test substance and reference substance treatment groups as well as the study endpoints are presented in the tables below.

Table A2.3.1.3-2: Cumulative mortality and emergence rate of honey bee larvae following exposure to ADM.01851.H.1.A

Treatment	Test rates		On Day 8		On Day 15	On Day 22	
	Cumulative dose	Concentration	Mean mortality of larvae Day 3 – Day 8	Mean OO	Mean mortality of pupae Day 8 – Day 15 (%)	Mean mortality of larvae + pupae Day 3 – Day 22	Adult emergence rate

			(%)	(%)			(%)	(%)	
	(µg prod/ larva)	(mg prod/ kg food)	abso- lute	cor- rec- ted		abso- lute	cor- rec- ted	abso- lute	correc- ted
Control	-	-	8.3	0.0	6.1	3.3	0.0	19.4	0.0
Test sub- stance	3.7	23.4	5.6	0.0	2.8	8.3	5.2	13.9	0.0
	11.1	70.5	2.8	0.0	0.0	3.0	0.0	13.9	0.0
	33.4	211.4	2.8	0.0	0.0	8.6	5.4	16.7	0.0
	100.3	634.1	2.8	0.0	3.0	17.2	14.3	22.2	3.4
	301.3	1904.3	58.3	54.5	16.7	36.1	33.9	69.4	62.1
Ref. sub- stance	(µg a.s./larva)	(mg a.s./kg food)							
	7.4	47	94.4	93.9	0.0	0.0	0.0	94.4	93.1
									5.6

OO: Other observations

Absolute: Mortality as counted from the results

Corrected: Mean mortality for each test substance group and the reference substance group was corrected according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Negative values were set to 0.0

* Statistically significant in comparison to untreated control (Step-down Roa-Scott Cochran-Armitage test; $\alpha = 0.05$; one-sided greater)

Table A2.3.1.3-3: Endpoints of the honey bee chronic larvae test on Day 22

Endpoint	(µg product/larva)
22 d ED ₁₀ ^a (95% confidence interval)	132.5 (97.7 – 179.7)
22 d ED ₂₀ ^a (95% confidence interval)	166.4 (131.1 – 211.2)
22 d ED ₅₀ ^a (95% confidence interval)	257.0 (211.9 – 311.8)
22 d NOED ^b	100.3
Endpoint	(mg product/kg food)
22 d EC ₁₀ ^a (95% confidence interval)	837.7 (617.8 – 1135.9)
22 d EC ₂₀ ^a (95% confidence interval)	1051.6 (828.5 – 1334.7)
22 d EC ₅₀ ^a (95% confidence interval)	1624.5 (1339.1 – 1970.6)
22 d NOEC ^b	634.1

^a Probit analysis using linear max. likelihood regression

^b (Step-down Roa-Scott Cochran-Armitage test; $\alpha = 0.05$; one-sided greater)

On Day 8 of the test, mortality was 8.3% in the control group. In the test substance treatment groups, larval mortalities on Day 8 ranged between 2.8% and 58.3% (corrected for control mortality: 0.0% to 54.5%). Larval mortality in the reference substance group was above 50% across all replicates on Day 8, being 94.4% (corrected for control mortality: 93.9%).

On Day 8, 6.1%, 2.8%, 3.0% and 16.7% (one or two individual each) of the remaining larvae treated with the control and 3.7, 100.3 and 301.3 µg product/larva, respectively, were observed to have food left and/or have a smaller body size.

Between Day 8 and Day 15, pupal mortality was 3.3% in the control group, and ranged between 3.0% and 36.1% (corrected for control mortality: 0.0% and 33.9%) in the test substance treatment groups.

After 22 days, adult emergence rate in the untreated control was 80.6% (mortality 19.4%). In the test substance treatment groups, adult emergence rate was 86.1%, 86.1%, 83.3%, 77.8% and 30.6% (from the lowest to the highest dose/concentration). The respective total mortality was 13.9%, 13.9%, 16.7%, 22.2% and 69.4% (corrected for control mortality: 0.0%, 0.0%, 0.0%, 3.4% and 62.1%). Statistically significant differences in adult emergence/total mortality on Day 22 compared to the control group occurred in the 301.3

µg product/larva treatment group only.

Conclusion

In this chronic larva test with ADM.01851.H.1.A in the honey bee *Apis mellifera* L., the 22-day NOED, was determined to be 100.3 µg product/larva with corresponding NOEC of 634.1 mg product/kg food. The 22-day ED₁₀, ED₂₀ and ED₅₀ were calculated as 132.5, 166.4 and 257.0 µg product/larva with corresponding EC₁₀, EC₂₀ and EC₅₀ of 837.7, 1051.6 and 1624.5 mg product/kg food, respectively.

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 honeybees
A 2.3.2	KCP 10.3.2	Effects on non-target arthropods other than bees
A 2.3.2.1	KCP 10.3.2.1	Standard laboratory testing for non-target arthropods
A 2.3.2.1.1	Study 1: Effects on <i>Typhlodromus pyri</i>	

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 endpoints relevant for the risk assessment: LR₅₀ > 3 L prod./ha ER₅₀ reproduction > 3 L prod./ha
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Reference:	KCP 10.3.2.1/01
Report	Effects of ADM.01851.H.1.A on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test, Röhlig U., 2024a, 23 48 NTL 0010, 000116382 (sponsor report number)
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 <i>Typhlodromus pyri</i> 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:	No major deviations.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	30 Nov 2023
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) Batch No. 10214034 400 g dimethoate/L (nominal), 357.4 g dimethoate/L (actual) Expiry date: 15 Mar 2025

Test organism:

Test species	<i>Typhlodromus pyri</i> SCHEUTEN (Acari: Phytoseiidae)
Origin	Culture reared at the test facility, originally purchased from “Katz Bio-tech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany on 07 July 2023
Age at test start	Protonymphs (< 24 hours)
No. of protonymphs per replicate	20
No. of replicates per test substance, reference substance or control	5
Sex ratio	The numbers of male and female mites in each replicate were recorded at days 7, 9, 11 and 14.

Test conditions:

Test substance concentration	285.8, 514.4, 926, 1667 and 3000 mL product/ha The test concentrations were chosen based on the results of a range-finding test. Application in 200 L water/ha The test substance was diluted in deionised water. The highest application solution served as stock solution to prepare the lower application solutions by dilution in deionised water.
Reference substance concentration	16.8 mL product/ha Application in 200 L water/ha The reference substance was diluted with deionised water to obtain the application solution.
Control	Deionised water at 200 L/ha
Application method	Spray by a laboratory track-sprayer (Schachtner, 71640 Ludwigsburg, Germany), 1 nozzle (Lechler ES 90-015), 3.4 bar spray pressure, 2.25 km/h spraying speed, 37 cm distance between the target area and the nozzle, calibrated with deionised water to achieve an application rate of 200 L/ha $\pm$ 10% for three consecutive applications without adjustment
Test duration	Day 0 to 7: mortality test Day 7 to 14: reproduction test
Test arena	Two cover glass plates (50 mm x 22 mm) per replicate were sprayed with deionised water, the test or reference substance at the respective rate. After air-drying of the spray deposit, the cover glass plates were laid along their longitudinal side parallel to one another and touching. The treated surface was placed upwards on moistened filter paper on a sponge placed in a plastic tray. A band of sticky insect glue (Temmen-Insektenleim) was applied around the edges of the arena to form a barrier to prevent the mites from escaping.
Procedure	After setting up the test arenas, the protonymphs were put in the test arenas by means of a moistened brush.
Temperature	25 $\pm$ 2°C (nominal), 23-25°C (actual)
Relative humidity	60-90% (nominal), 71-80% (actual)
Light intensity	2030 lux
Photoperiod	16 hours light : 8 hours dark
Food and feeding regime	Pollen (pine (<i>Pinus nigra</i>) and birch (<i>Betula pendula</i>), 1:1) was supplied as a food source at test start and at each assessment

	day (<i>ad libitum</i>). The mites obtained the necessary moisture by the upward capillary movement of water in the space between the two adjacent cover glass plates.
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Observations:

Mortality and no. of trapped (in the insect glue) or missing mites	Days 3, 7, 9, 11 and 14
No. of eggs laid and hatched juveniles	Days 9, 11 and 14
Test conditions	Temperature and relative humidity: continuously Light intensity: once at the start of the test

Calculations:

The mean percentage mortality (dead and escaped mites) after 7 days was calculated for each treatment group and then corrected for any losses in the control using Abbott's formula (Abbott, 1925).

Reproduction assessment was performed for those test substance rates, where the corrected mortality was $\leq 50\%$, i.e., for all test substance rates. The number of eggs per female was determined by counting the number of females, eggs and juveniles (larvae) at the three assessment days following day 7. Eggs laid up to and including day 7 were removed from the test arena and were not counted. The number of eggs and larvae per replicate during the reproduction period until day 14 (inclusive) were summed and the cumulative mean reproduction per treatment was calculated taking into account the reproduction of all replicates per treatment. Reproduction performance in the respective test substance treatment groups was also expressed in percent reduction relative to the control group.

Statistics:

For statistical calculation of the results, the computer program ToxRat Professional 3.3.0 (RATTE, 2018) was used.

Mortality was analysed for statistical significance using the Step-down COCHRAN-ARMITAGE Test for the test substance and the Chi²-2x2 Table test for reference substance as distribution-free tests ($\alpha = 0.05$, one-sided greater) which do not require testing for normality or homoscedasticity prior to analysis.

Reproduction was analysed for statistical significance using WILLIAMS Multiple Sequential t-Test ($\alpha = 0.05$, one-sided smaller) following SHAPIRO-WILK's test for normal distribution, LEVENE's test procedure for variance homogeneity and monotonicity of rate-response using Trend analysis by contrasts.

Due to effects $< 50\%$, a calculation of the LR₅₀ (median lethal rate) and ER₅₀ (medial effect rate) was not possible.

Results and discussions

Validity criteria:

- Mean mortality in the control $\leq 20\%$ (dead and escaped mites) on day 7 (achieved: 2.0%)
- Cumulative mean mortality (control corrected) in the reference substance treatment 50-100% on day 7 (achieved: 77.6%)
- Mean cumulative number of eggs in the control ≥ 4 per female between 7 and 14 days (achieved: 6.11 eggs per female)

Observations of mortality and reproduction are presented in the table below.

Table A2.3.2-1: Observations of mortality and reproduction of *T. pyri* following exposure to ADM.01851.H.1.A

Treatment (mL product/ha)	Mortality		Reproduction	
	Mean (%)	Corrected ^a (%)	Mean no. of eggs per female	Inhibition of reproduction (%)
Test substance				
Control	2.0	-	6.11	-
285.8	1.0	-1.0	6.39	-4.6
514.4	2.0	0	5.92	3.1
926	2.0	0	6.09	0.3
1667	4.0	2.0	5.89	3.6
3000	46.0*	44.9	5.61	8.2
Reference substance				
16.8	78.0*	77.6	-	-

Note: Reproduction in the test substance treatment groups was not statistically significantly reduced compared to the control group (WILLIAMS Multiple Sequential t-Test, $\alpha = 0.05$, one-sided smaller, following SHAPIRO-WILK's test for normal distribution, LEVENE's test procedure for variance homogeneity and monotonicity of rate-response using Trend analysis by contrasts)

* Mortality statistically significantly different from control (test substance: Step-down COCHRAN-ARMITAGE Test, $\alpha = 0.05$, one-sided greater; reference substance: Chi²-2x2 Table test, $\alpha = 0.05$, one-sided greater)

^a Corrected according to Abbott's formula.

Based on the study results, the following endpoints were obtained.

Table A2.3.2-2: Endpoints for ADM.01851.H.1.A in the standard laboratory test with *T. pyri*

Endpoint	(mL product/ha)
LR ₅₀	> 3000
NOER (mortality)	1667
ER ₅₀ (reproduction)	> 3000
NOER (reproduction)	≥ 3000

At day 7, mortality in the highest test substance treatment group was statistically significantly reduced compared to the control. No unusual observations regarding behaviour were noted in the control and the test substance treatment groups at any observation point during the test. At the end of the test after 14 days, reproduction was not statistically significantly reduced compared to the control in any test substance treatment group.

Conclusion

In this standard laboratory test (use of glass plates as substrate) to determine the effects of exposure to ADM.01851.H.1.A on mortality and reproduction of *Typhlodromus pyri*, the LR₅₀ and ER₅₀ (reproduction) were both found to be higher than the highest test dose of 3000 mL product/ha. The NOER with respect to mortality was determined as 1667 mL product/ha and the NOER with respect to reproduction was determined as ≥ 3000 mL product/ha.

A 2.3.2.1.2 Study 2: Effects on *Aphidius rhopalosiphi*

Comments of zRMS:	The study was conducted in line with the respective guideline with no major deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ > 3 L prod./ha ER₅₀ reproduction > 3 L prod./ha
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Reference:	KCP 10.3.2.1/02
Report	Effects of ADM.01851.H.1.A on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEPHANI-PEREZ) in a laboratory test, Röhlig U., 2024b, 23 48 NAL 0010, 000116376 (sponsor report number)
Guideline(s):	Mead-Briggs, M. et al.: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (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.
Deviations:	No major deviations
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	09 Jan 2025
Reference substance	DANADIM PROGRESS (EC formulation containing the active substance dimethoate) Batch No. 10214034 400 g dimethoate/L (nominal), 357.4 g dimethoate/L (actual) Expiry date: 15 Mar 2025

Test organism:

Test species	<i>Aphidius rhopalosiphi</i> (DESTEPHANI-PEREZ) (Hymenoptera, Braconidae)
Origin	Culture reared at the test facility, originally purchased from “Katz Biotech AG”, An der Birkenpfehlheide 10, 15837 Baruth, Germany on 28 July 2023
Age at test start	Adults, within 48 hours of their emergence
No. of adults per replicate	10 (7 females + 3 males)
No. of replicates per test substance, reference substance or control	4 For the subsequent reproduction assessments, the performance of 15 individually-confined female wasps was evaluated per treatment.
Sex ratio	7 females : 3 males

Test conditions:

Test substance concentration	285.8, 514.4, 926, 1667 and 3000 mL product/ha The test concentrations were chosen based on the results of a range-finding test. Application in 200 L water/ha The test substance was diluted in deionised water. The highest application solution served as stock solution to prepare the lower application solutions by dilution in deionised water.
Reference substance concentration	0.34 mL product/ha Application in 200 L water/ha The reference substance was diluted with deionised water to obtain the

	application solution.
Control	Deionised water at 200 L/ha
Application method	Spray by a laboratory track-sprayer (Schachtner, 71640 Ludwigsburg, Germany), 1 nozzle (Lechler ES 90-015), 3.4 bar spray pressure, 2.25 km/h spraying speed, 37 cm distance between the target area and the nozzle, calibrated with deionised water to achieve an application rate of 200 L/ha $\pm$ 10% for three consecutive applications without adjustment
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: reproduction phase (11-day reproduction test after removal of the wasps)
Test arena	<u>Mortality phase:</u> Glass plates (13 cm x 13 cm) held apart by an aluminium frame (13 cm x 13 cm x 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/ min). <u>Reproduction phase (including parasitisation):</u> Acrylic cylinder (about 11 cm Ø, 20 cm high) with approx. 20 wheat seedlings (<i>Triticum</i>), e.g., variety “Tambor” (8 days old) planted in a pot containing potting soil, infested with > 100 adult and nymphal aphids of <i>Rhopalosiphum padi</i> (reared in the laboratory of the test facility) and covered with gauze at the top of the cylinder
Procedure	The test solution was sprayed onto one side of the glass plates. Following drying of the spray deposits (at room temperature within 1 hour after application), the two glass plates and an aluminium frame (containing gauze covered holes) were assembled to a test arena, with the treated surfaces directed to the inside. Thereafter, 7 females and 3 males of impartially selected <i>Aphidius</i> were confined in each test unit and the holes of the test units were closed. Reproduction assessments were initiated at 48 hours using surviving females (15 per treatment) from the control and from all test substance treatment groups (corrected mortality $\leq$ 50%). The wasps were transferred to the reproduction arenas, where they were confined individually. The adult wasps were removed after 24 hours and the aphid-infested plants were then maintained in the climatic room for a further 11 days, before the number of parasitised aphids (aphid mummies) was recorded.
Temperature	20 $\pm$ 3°C (nominal), 20-22°C
Relative humidity	50-90% (nominal), 69-76% (actual)
Light intensity	1040 lux (exposure phase) 2620 lux (parasitisation phase) 6710 lux (reproduction phase)
Photoperiod	16 hours light : 8 hours dark
Food and feeding regime	Exposure phase: 1:3 v/v solution of honey and water

Observations:

Mortality and condition	2, 24 and 48 hours after start of exposure
No. of parasitized aphids (mummies)	Day 14 (end of reproduction phase)
Test conditions	Temperature and relative humidity: continuously Light intensity: at the start of the test

Calculations:

From the mortality data at 48 hours after start of exposure, adult mortality (in %) was calculated and corrected for any losses in the control using Abbott’s formula (Abbott, 1925).

For reproduction assessment, the mean number of mummies produced per surviving female was calculated for each treatment (including standard deviation). Furthermore, the percentage change in numbers of mummies in the test substance treatments in comparison to the control was calculated.

Statistics:

For statistical analysis of the results, the computer program ToxRat Professional 3.3.0 (RATTE, 2018) was used.

Mortality was analysed for statistical significance using the Multiple Sequentially-rejective FISHER Test after BONFERRONI-HOLM (test substance) and Chi² 2x2 Table Test (reference substance) as distribution-free tests ($\alpha = 0.05$, one-sided greater) which do not require testing for normality or homoscedasticity prior to analysis.

Reproductive capacity was analysed for statistical significance using DUNNETT'S t-Test ($\alpha = 0.05$, one-sided smaller) following SHAPIRO-WILK's test on normal distribution, LEVENE's test on variance homogeneity as well as Tend analysis for monotonicity.

Since there were only low effects for mortality and reproduction assessments in the test substance treatment groups which were tested, a calculation of the LR₅₀ (median lethal rate) and ER₅₀ (median effect rate) was not possible.

Results and discussions

Validity criteria:

- Control mortality $\leq 13\%$ (48 hours) (achieved: 0%)
- Corrected reference mortality $> 50\%$ and preferably $< 100\%$ (48 hours; based on study protocol) (achieved: 100%)
- Control reproduction ≥ 5 mummies per female (achieved: 18.1 mummies per female) and no more than two wasps producing zero values (achieved: 2)

Observations of mortality and reproduction are presented in the table below.

Table A2.3.2-3: Observations of mortality and reproduction of *A. rhopalosiphi* following exposure to ADM.01851.H.1.A

Treatment	Mortality		Reproduction		
	Mean (%)	Corrected ^a (%)	No. of females used for reproduction assessment	Mean number of mummies per female	Effects on reproduction (%)
Test substance					
Control	0	-	15	18.1	-
285.8	0	0	15	18.0	0.6
514.4	2.5	2.5	15	18.7	-3.3
926	2.5	2.5	15	17.5	3.3
1667	2.5	2.5	15	16.9	6.6
3000	20.0*	20.0	15	16.9	6.6
Reference substance					
0.34	100*	100	n.p.	n.p.	n.p.

Note: For reproduction, there were no statistically significant differences in the assessed test substance treatments in comparison to the control (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller)

n.p. not performed (reproduction assessment performed for treatment groups with $\leq 50\%$ corrected mortality)

* Mortality statistically significantly different from control (Multiple Sequentially-rejective FISHER Test after BONFERRONI-HOLM (test substance) and Chi² 2x2 Table Test (reference substance), $\alpha = 0.05$, one-sided greater)

^a Calculated using Abbott's formula.

Based on the study results, the following endpoints were obtained.

Table A2.3.2-4: Endpoints for ADM.01851.H.1.A in the standard laboratory test with *A. rhopalosiphi*

Endpoint	(mL product/ha)
LR ₅₀	> 3000
NOER (mortality)	1667
ER ₅₀ (reproduction)	> 3000
NOER (reproduction)	≥ 3000

At 48 hours, mortality in the highest test substance treatment group was statistically significantly reduced compared to the control. At the end of the test after 14 days, reproduction was not statistically significantly reduced compared to the control in any test substance treatment group.

Conclusion

In this standard laboratory test (use of glass plates as substrate) to determine the effects of exposure to ADM.01851.H.1.A on mortality and reproduction of *Aphidius rhopalosiphi*, the LR₅₀ and ER₅₀ were both determined to be > 3000 mL product/ha. The NOER with respect to mortality was found to be 1667 mL product/ha and the NOER with respect to reproduction was determined as ≥ 3000 mL product/ha.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.2.5 KCP 10.3.2.4 Other routes of exposure for non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted in line with OECD 222 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: EC₁₀ (reproduction) (95% confidence limits) =29.82 mg product/kg dws (16.02 – 55.49) EC₂₀ (reproduction) (95% confidence limits) =59.05 mg product/kg dws (41.05 – 84.95) EC₅₀ (reproduction) (95% confidence limits) => 120 mg product/kg dws NOEC (reproduction)=37.04 mg product/kg dws
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Reference: KCP 10.4.1.1/01

Report: Effects of ADM.01851.H.1.A on the reproduction of the earthworm *Eisenia fetida* in artificial soil,
Friedrich, S., 2018,
22 48 TEC 0019, 000110707 (sponsor report number)

Guideline(s): OECD 222 (2016)

Deviations: None

GLP: Yes

Acceptability: **Yes**
Duplication: **No**
(if vertebrate study)

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3111P1
Active ingredient content	Nominal / analysed: 75 / 74.8 g/L clopyralid Nominal / analysed: 500 / 525.1 g/L ethofumesate
Appearance	Off-white liquid
Density	1.1622 g/mL
Expiry date	30 Nov 2023
Reference substance	The reference substance Maypon Flow (Carbendazim, SC 500) was evaluated in a separate study. In the most recent study reported 28 January 2022, the number of juveniles was reduced by 51.6% and 99.9% at concentrations of 5 and 10 mg product/kg soil dry weight, respectively, after 8 weeks of test duration when compared to the control. As results are in line with the test guideline, sensitivity of the test system was sufficiently demonstrated.

Test organism:

Test species	Earthworm <i>Eisenia fetida</i> (Savigny, 1826)
Origin	Purchased from Bias Labs Ltd, Unit 19, Enterprise Centre, Myregormie Place, Fife, UK, KY1 3PF
Age at test start	Adult worms (approximately 6 months old with clitellum)
Weight at test start	300 – 469 mg/worm
Acclimatisation	Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start.
Diet	Air-dried and finely ground horse manure One day after application, 5 g manure was scattered on the soil surface of each test vessel, which was moistened with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (approximately 5 g) depended on the feeding activity. After four weeks and removal of the adult worms from the test vessels, the soil of each vessel was mixed carefully with 5 g manure, which was the last feeding occasion of the test.

Test conditions:

Test substance concentrations	1.96, 3.53, 6.35, 11.43, 20.58, 37.04, 66.67 and 120 mg product/kg soil dw An exactly weighed amount of the test substance was dispersed in deionised water to prepare a stock solution without addition of solubility mediators immediately before application. This stock solution was diluted with deionised water to prepare further test solutions (serial dilution).
Reference substance concentration	The reference substance was tested in a separate study at 5 and 10 mg product/kg soil dry weight.
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 10% Sphagnum peat (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content) - 20% kaolin clay (kaolinite content > 30%) - 0.5% calcium carbonate - 69.5% industrial quartz sand (> 50% of particles between 50 and 200 µm) One day before test start, the mixed dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. On the day of the test start, the test substance was introduced by dispersing the quantity of test substance required to obtain the desired test concentration in the volume of water required to

	hydrate the soil to 40-60% of maximum water-holding capacity (WHC). The control substrate contained the corresponding amount of deionised water only.
No. of test organisms per replicate (test vessel)	10
No. of replicates per treatment	8 for the control, 4 for the test substance treatments
Application method	The test substance application solutions (or deionised water for the control) were added to the pre-moistened artificial soil. Each test vessel was then filled with the treated or untreated soil.
Test duration	8 weeks After four weeks, adult worms were removed from the test vessels and the soil was returned to the original test vessels for reproduction assessment during the second four weeks of the test.
Test vessels	Plastic vessels (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light Each test vessel contained 810 g wet weight corresponding to 600 g dry weight of artificial soil with a water content corresponding to 40-60% of WHC
Temperature	20.0°C nominal, 19.7-21.2°C actual
Illumination	16 h light (artificial light at intensity 590 lux) : 8 h dark photoperiod
Water content of artificial soil	Test start: 34.9 – 35.0 g/100 g soil dw (equivalent to 55.5% – 55.6% of WHC) Test end: 34.2 – 34.6 g/100 g soil dw (equivalent to 54.4% – 55.0% of WHC)
pH of artificial soil	Test start: 5.95 – 5.99 Test end: 5.75 – 5.81

Observations:

Adult mortality	After 4 weeks
Behaviour of earthworms	At test start
Behavioural and pathological symptoms	Weekly during the first 4 weeks (including the feeding activity) After 4 weeks and after 8 weeks (including morphological alterations)
Biomass development of adults	The fresh weight of worms was determined individually at test start and per replicate after 4 weeks.
Reproduction	The numbers of juvenile earthworms and unhatched cocoons per replicate were determined after 8 weeks.
Temperature	Recorded continuously by a data logger
pH of the artificial soil	At test start and end
Moisture content of the artificial soil	At test start and end

Calculations:

The endpoints of the test were mortality, change in biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for biomass, mortality and reproduction were calculated. Furthermore, the percentage change in reproductive performance of the treated groups in comparison to the control treatment was calculated.

Statistics:

Statistical analyses were performed using the software ToxRat Professional 3.3.0 (Ratte 2018).

The EC₁₀, EC₂₀ and EC₅₀ values (number of juveniles) were calculated using Probit analysis using the maximum likelihood method. Confidence limits (95%) of the EC_x values were computed by normal approximation. For determination of NOEC values, the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (mortality) or the Williams-t-test (biomass, reproduction) were used to compare the control with the independent test substance treatment groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and discussions

Validity criteria:

- Each control replicate produces ≥ 30 juveniles by the end of the test (achieved: 119-203 juveniles)
- The coefficient of variation of reproduction in the control is $\leq 30\%$ (achieved: 19.9%)
- Mean mortality of adults in the control is $\leq 10\%$ over the initial 4 weeks of the test (achieved: 0%)

Observations of mortality, biomass change and reproduction are presented in the table below.

Table A2.4.1.1-1: Effects of ADM.01851.H.1.A on mortality, biomass change and reproduction of *Eisenia fetida*

Treatment (mg product/kg soil dw)	Mortality after 4 weeks of exposure (%)	Mean biomass change after 4 weeks of exposure (%)	Reproduction after 8 weeks	
			(mean number of juveniles/replicate)	Change compared to control (%)
0 (control)	0.0	27.2	161.9	-
1.96	0.0	28.3	161.8	0.1
3.53	0.0	24.7	166.0	-2.5
6.35	2.5	24.1	152.3	5.9
11.43	2.5	30.1	148.5	8.3
20.58	0.0	26.8	164.5	-1.6
37.04	0.0	23.4	147.0	9.2
66.67	0.0	29.2	116.5*	28.0
120	0.0	25.9	108.3*	33.1

Note 1: There were no statistically significant differences in mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater) and biomass (Williams-t-test, $\alpha = 0.05$, one-sided smaller) between test substance treatments and control

Note 2: Negative values for change in reproduction indicate an increase relative to the control.

* Statistically significantly different compared to control regarding reproduction (Williams-t-test, $\alpha = 0.05$, one-sided smaller)

Based on the study results, the following endpoints were obtained.

Table A2.4.1.1-2: Endpoints for effects of ADM.01851.H.1.A on mortality, biomass change and reproduction of *Eisenia fetida*

Endpoints (mg product/kg soil dw)	
EC ₁₀ (reproduction) ^a (95% confidence limits)	29.82 (16.02 – 55.49)
EC ₂₀ (reproduction) ^a (95% confidence limits)	59.05 (41.05 – 84.95)
EC ₅₀ (reproduction) ^b (95% confidence limits)	> 120
NOEC (reproduction)	37.04
LOEC (reproduction)	66.67
NOEC (mortality, biomass change)	120
LC ₅₀ (mortality) ^b	> 120

^a Based on Probit analysis

^b Based on estimation of data

After four weeks, there was no significant adult mortality in any of the treatment concentrations up to and including 120 mg product/kg soil dw, the maximum tested (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Furthermore, there was no statistically significant difference in biomass change of adult worms at all tested concentrations in comparison to the control during the first four weeks. No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test for the control and all test substance concentrations.

The numbers of juveniles were statistically significantly reduced relative to the control in the treatment concentrations of 66.67 and 120 mg product/kg soil dw (Williams-t-test, $\alpha = 0.05$, one-sided smaller). Thus, with respect to reproduction, the LOEC was 66.67 mg product/kg soil dw and the NOEC was 37.04 mg product/kg soil dw.

Conclusion

In this test on sub-lethal effects of ADM.01851.H.1.A on the earthworm *Eisenia fetida*, the EC₁₀, EC₂₀, and EC₅₀ for reproduction were determined to be 29.82, 59.05 and > 120 mg product/kg soil dw, respectively. The NOEC for reproduction was determined as 37.04 mg product/kg soil dw and the NOEC for mortality and biomass change was 120 mg product/kg soil dw.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 1: Effects on *Hypoaspis aculeifer*

Comments of zRMS:	The study was conducted in line with OECD 226-with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC_{reproduction} > 1000 mg prod./kg soil dw. EC₁₀ > 1000 mg prod./kg soil dw
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Reference:	KCP 10.4.2.1/01
Report	Effects of ADM.01851.H.1.A on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> , Schulz L., 2024a, 23 48 THC 0023, 000116379 (sponsor report number)
Guideline(s):	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	09 Jan 2022
Reference substance	The reference substance Dimethoate 400 EC was tested in a separate study

Test organism:

Test species	Predatory mite <i>Hypoaspis aculeifer</i>
Origin	Culture maintained at the test facility
Age at test start	Adults from a synchronised culture with an age difference of 2 days
Diet	Cheese mites <i>ad libitum</i> (2-3 times per week) throughout the test.

Test conditions:

Test substance concentration	1000, 556, 309, 171, 95.3, 52.9, 29.4 and 16.3 mg product/kg soil dw,
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 5% w/w <i>Sphagnum</i> peat (air-dried, finely ground and with no visible plants remaining) - 20% w/w kaolinite clay (kaolinite content > 30%) - 0.225% w/w calcium carbonate (for pH adjustment) - 74.775% w/w sand (air-dried, > 50% of particles between 50 and 200 µm)
No. of test organisms per replicate (test vessel)	10
No. of replicates per treatment	8 for the control, 4 for the test substance treatments
Application method	An exact weighed amount of the test item was mixed with deionised water, immediately prior to application. The test item solution was then thoroughly mixed with the prepared artificial soil by means of a hand stirrer.
Test duration	14 days
Test vessels	160 mL WECK-jar with glass lid
Temperature	20.0 - 20.9 °C
Illumination	16 h light: 8 h dark photoperiod 523 lux
Water content of artificial soil	Maintained at 40-60% of maximum water holding capacity (MWHC), which was 39.6 g/100g soil d.w.
pH of the soil samples (in 1 M KCl)	Test start: 6.2 – 6.3 Test end: 5.7 – 5.8

Measurements:

Adult mortality and reproduction	On day 14, the number of both surviving adults and of F1 progeny (i.e. juvenile test mites) in each test arena were assessed. The adult and juvenile mites were extracted from the soil using a MacFayden high-gradient extractor. The mites were collected and preserved in vials containing 70% v/v ethanol beneath the funnels.
Temperature	Continuous recording
pH of the soil samples	At test start and end
Weight of test vessels	Vessels were weighed every 2-3 days and water added when required.

Experimental dates: 23 August to 14 September 2023

Calculations:

Mortality (number of dead adults) in % for each treatment group was calculated. Missing mites were counted as dead. The reproductive output for the test item treatment group was calculated in % compared to control.

Statistics:

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018). Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (for mortality) and the Dunnett's Multiple t-test Procedure (for reproduction) were used to compare the control to the independent test item groups.

Results and discussions

Validity criteria:

- Mean adult mortality in the control $\leq 20\%$ 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 $\leq 30\%$ at the end of the test

Mean adult mortality in the control was 3.8% at the end of the test. The mean number of juveniles per replicate in the control was 327.3 at the end of the test. The coefficient of variation of reproduction per replicate in the control was 14.2% 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 A2.4.2.1-1: Effects of ADM.01851.H.1.A on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg product/kg soil dw)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
Mean adult mortality (%) (day 14)	3.9	7.5	2.5	5.0	0.0	0.0	7.5	5.0	2.5
Mean No. juveniles (day 14)	327.3	347.5	350.3	306.8	349.5	349.5	323.5	331.8	293.5
Reproduction (% of control)	100	106	107	94	107	107	99	101	90

Based on the study results, the following endpoints were obtained.

Table A2.4.2.1-2: Endpoints for effects of ADM.01851.H.1.A on mortality and reproduction of *Hypoaspis aculeifer*

Endpoints (mg product/kg soil dw) / (mg ethofumesate/kg soil dw) / (mg phenmedipham/kg soil dw)	
14 d EC ₁₀ / EC ₂₀ / EC ₅₀ (reproduction)	>1000
14 d NOEC ^a (reproduction)	>1000
14 d LC ₅₀	>1000
14 d NOEC ^b (mortality)	>1000

Conclusion

In this test on sub-lethal effects of ADM.01851.H.1.A on the predatory mite *Hypoaspis aculeifer*, the EC₅₀, EC₂₀ and EC₁₀ for reproduction as well as the LC₅₀ were estimated to be all > 1000 mg product/kg soil dw. The NOEC for reproduction and the NOEC for mortality were both determined as 1000 mg product/kg soil dw.

A 2.4.2.1.2 Study 2: Effects on *Folsomia candida*

Comments of zRMS:	The study was conducted in line with OECD 232 with no major deviations All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC_{reproduction} = 32.7 mg prod./kg soil_{dw}. 28 d EC₁₀ reproduction (95% confidence interval) = 56.9 (48.9-66.2) 28 d EC₂₀ reproduction (95% confidence interval) = 82.3 (75.2-90.1) 28 d EC₅₀ reproduction (95% confidence interval) = 167 (147-190)
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Reference:	KCP 10.4.2.1/02
Report	Effects of ADM.01851.H.1.A on the mortality and reproduction of the collembolan <i>Folsomia candida</i> , Ruhland, S., 2024g, 23 48 TCC 0020, 000116377 (sponsor report number)
Guideline(s):	OECD 232 (2016)
Deviations:	No major deviations
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study) No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	09 Jan 2025
Reference substance	The reference substance boric acid was routinely tested. In the most recent study, the EC ₅₀ (reproduction) was determined to be 106 mg/kg soil dry weight and the LC ₅₀ was determined to be 141 mg/kg soil dry weight. The NOEC for mortality and the NOEC for reproduction were both determined to be 44 mg/kg soil dry weight. The EC ₅₀ (reproduction) was close to the value of 100 mg/kg soil dry weight as stated by OECD 232 (2016).

Test organism:

Test species	Collembolan <i>Folsomia candida</i> (Willem)
Origin	Reared at the test facility; originally obtained from “Biologische Bundesanstalt (BBA)”, Berlin-Dahlem, Germany in May 2000
Age at test start	Juvenile collembolans, 9-12 days old
Acclimatisation	Test organisms of a uniform age were obtained by transferring egg clusters from the breeding containers to new containers with fresh breeding substrate 12 days before starting the test. After 72 hours, these egg clusters were removed from the containers and the juveniles that had hatched during the preceding 72 hours were fed with granulated dry yeast. After a further 9 days, the test organisms were collected and used for the test.
Diet	Springtails were fed with approximately 2 mg of granulated dry yeast per test vessel at the start of the test and after 14 days.

Test conditions:

Test substance concentration	5.0, 8.0, 12.8, 20.4, 32.7, 52.3, 83.8 and 134 mg product/kg soil dw The test substance was dispersed in water to prepare a stock solution. This stock solution was diluted with deionised water to prepare further test solutions (serial dilution).
Reference substance concentration	The reference substance was tested in a separate study at 44, 67, 100, 150 and 225 mg/kg soil dw.
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 5% sphagnum peat (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content) - 20% kaolin clay (kaolinite content > 30%) - 0.3% calcium carbonate - 74.7% industrial quartz sand (predominantly fine sand with more than 50% of the particles between 50 and 200 µm)
No. of test organisms per replicate (test vessel)	10
No. of replicates per treatment	8 for the control (+ 2 replicates without Collembolans for measurement purposes), 4 for the test substance treatments (+ 2 replicates without Collembolans for measurement purposes)
Application method	The test solutions were thoroughly mixed with the artificial soil separately for each treatment group by intensive stirring in a laboratory mixer. Thus, the water content of the artificial soil was adjusted to 40-60% of the maximum water holding capacity (WHC). Subsequently, 30 g (dry weight) of the treated artificial soil were placed into each test vessel.
Test duration	28 days
Test vessels	Glass containers (approximately 150 mL) covered with a lid; surface area of soil: 18.9 cm ²
Temperature	20 ± 2°C (nominal), 18.8-20.8°C (actual)

Illumination	16 h light (light intensity: 580 lux) : 8 h dark photoperiod
Water content of artificial soil	Nominal: 40-60% WHC Actual: Test start: 22.8-23.0 (equivalent to 53.9-54.4% of WHC) Test end: 22.2-22.6 (equivalent to 52.5-53.4% of WHC)
pH of artificial soil	Test start: 5.85-5.93 Test end: 5.72-5.77
Aeration	The test vessels were opened briefly twice a week to allow aeration.

Measurements:

Adult mortality and reproduction	At day 28 The test substrate of each replicate was poured into an individual container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring, the number of parental and juvenile collembolans floating on the surface was determined. Missing parental collembolans were assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system. The extraction efficiency of the method was determined to be 99% in a separate extraction run.
Temperature	Continuously
pH of the soil samples	At test start and end
Water content of the soil samples	At test start and end The water content was checked weekly by reweighing the additional test vessels, and water loss was compensated if exceeding 2% of the initial water content.

Calculations:

The endpoints of the test were mortality and reproduction (number of juveniles). The arithmetic means per treatment for both endpoints were calculated. For reproduction, the reproductive output for each test substance treatment group was calculated in % compared to the control.

Statistics:

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018). Step-down Cochran-Armitage test and Williams-t-test were used to compare the control with the independent test substance treatment groups. The LC₁₀, LC₂₀ and LC₅₀ values (mortality) were estimated by Logit analysis using the maximum likelihood method. The EC₁₀, EC₂₀ and EC₅₀ values (number of juveniles) were estimated by Probit analysis using the maximum likelihood method. Confidence limits (95%) of the LC_x/EC_x values were computed by normal approximation.

Results and discussions

Validity criteria:

- Mean adult mortality in the control $\leq 20\%$ at the end of the test (achieved: 2.5%)
- Mean number of juveniles per replicate in the control ≥ 100 at the end of the test (achieved: average of 939/vessel)
- Coefficient of variation of reproduction per replicate in the control $\leq 30\%$ at the end of the test (achieved: 8.0%)

Observations of adult mortality and reproduction are presented in the table below.

Table A2.4.2.1-3: Effects of ADM.01851.H.1.A on mortality and reproduction of *Folsomia candida*

Treatment (mg product/kg soil dw)	Mean parental mortality after 28 days of exposure (%)	Reproduction after 28 days	
		(mean number of juveniles/replicate)	Reduction of reproduction compared to control (%)
0 (control)	2.5	939	-
5.0	0.0	958	-2.0
8.0	2.5	946	-0.8
12.8	0.0	938	0.1
20.4	2.5	956	-1.8
32.7	2.5	926	1.3
52.3	15.0*	863*	8.1
83.8	40.0*	763*	18.7
134	55.0*	557*	40.6

* Statistically significantly different compared to control (Step-down Cochran-Armitage for mortality, $\alpha = 0.05$, one-sided greater; Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)

Based on the study results, the following endpoints were obtained.

Table A2.4.2.1-4: Endpoints for effects of ADM.01851.H.1.A on mortality and reproduction of *Folsomia candida*

Endpoints (mg product/kg soil dw)	
28 d NOEC ^b (reproduction)	32.7
28 d NOEC ^b (mortality)	32.7
28 d EC ₁₀ ^a (reproduction) (95% confidence interval)	56.9 (48.9-66.2)
28 d EC ₂₀ ^a (reproduction) (95% confidence interval)	82.3 (75.2-90.1)
28 d EC ₅₀ ^a (reproduction) (95% confidence interval)	167 (147-190)
28 d LC ₅₀ ^b (mortality) (95% confidence interval)	40.8 (31.6-52.7)
28 d LC ₅₀ ^b (mortality) (95% confidence interval)	60.0 (49.6-72.5)
28 d LC ₅₀ ^b (mortality) (95% confidence interval)	116 (93.2-144)

^a Based on Probit analysis

^b Based on Logit analysis

After 28 days of exposure, mean parental mortality was statistically significantly reduced in the three highest test substance treatment groups of 52.3, 83.8 and 134 mg product/kg soil dw (Step-down Cochran-Armitage, $\alpha = 0.05$, one-sided greater). No effects on behaviour of the Collembolans were observed during the test. Reproduction was statistically significantly reduced also at the three highest test substance treatment groups of 52.3, 83.8 and 134 mg product/kg soil dw (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

Conclusion

In this test on sub-lethal effects of ADM.01851.H.1.A on the collembolan *Folsomia candida*, the EC₁₀, EC₂₀, and EC₅₀ for reproduction were determined to be 56.9, 82.3 and 167 mg product/kg soil dw, respectively. The LC₁₀, LC₂₀, and LC₅₀ were calculated as 40.8, 60.0 and 116 mg product/kg soil dw, respectively. The NOEC for reproduction and the NOEC for mortality were both determined as 32.7 mg product/kg soil dw.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 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 with the following endpoints relevant for the risk assessment: The test substance ADM.01851.H.1.A 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 13.0 mg product/kg soil dw.
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Reference:	KCP 10.5/01
Report	Effects of ADM.01851.H.1.A on the Activity of Soil Microflora (Nitrogen Transformation test), Schulz L., 2024b, 23 48 SMN 0023, 000116378 (sponsor report number)
Guideline(s):	OECD 216 (2000)
Deviations:	No major deviations
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	09 Jan 2025
Reference substance	Dicyandiamide was tested in a separate study

Test soil:

Origin	Wassergut Canitz, Saxony, Germany
Cultivation	Fallow grassland
History	No pesticides or organic or mineral fertilizer had been used on the soil since 2003.
Batch	2/2023
Soil sampling	From the top 20 cm
Soil preparation	After arrival at the sampling laboratory, the soil was air dried and sieved (mesh 2 mm) at room temperature.
Soil storage	The soil was stored at 4°C in the dark
Soil pre-incubation	At 20±2°C for 25 days
Physico-chemical properties	pH: 6.2 Corg: 1.42% Total N: 0.14% Cation exchange capacity: 11.1 cmol/kg dw Max. water holding capacity: 38.87 g/100g soil dw Particle size distribution (ISO 11277): Clay: 11.3% Silt: 37.2%

	Sand: 51.6%
	Soil texture: Loamy sand

Test conditions:

Test substance concentration	1.30 (low dose) and 13.0 (high dose) mg product/kg dry soil
Control	Deionised 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 hand stirrer. For the control, ultrapure water and additionally 0.5% lucerne meal (related to soil dry weight) were mixed into the soil. The lucerne meal had a carbon to nitrogen ratio of 13.2:1.
Test duration	28 days
Test vessels	Wide-mouth glass flasks (500 mL) with screw caps that permit air exchange
Temperature	18.9 – 20.7°C
Illumination	Constant darkness
Water content of the soil	16.84 – 17.22 g/100g soil dw equivalent to 43.33 – 44.31 % of MWHC
pH of soil	5.9 – 6.0

Measurements:

NH ₄ ⁺ -N, NO ₂ ⁻ -N and NO ₃ ⁻ -N contents of the soil samples	Soil samples were taken within 3 hours (day 0) and on days 7, 14 and 28 after application. An amount of 10 g soil was extracted with 50mL 1 M KCl solution and agitated for one hour. After centrifugation, the extracts were stored deep frozen. Frozen extract samples were thawed and mineralised nitrogen was determined using a SEAL Analytical Autoanalyzer.
Test conditions	Temperature: Continuously Soil water content: 0, 7, 14, 21 and 28 d pH: days 0 and 28

Experimental dates: 26 June to 24 July 2023

Calculations:

The mean nitrogen-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date.

Furthermore, the nitrogen transformation rate per time interval and the nitrogen transformation rate/time interval/day (day 0 - 7, 7 - 14, 14 - 28) were calculated for each treatment group.

The % deviations in the quantities of nitrogen formed between the control and the test item treatment groups were determined for each time interval as follows:

% deviation to control = ((test item rate - control rate)/control rate) x 100 %

Statistics:

A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level). The statistical analysis was performed with the software ToxRat Professional 3.3.0 (Ratte 2018).

Results and discussions

Validity criterion:

- Variation between replicate control samples: $\leq \pm 15\%$

The coefficients of variation in the control group of the nitrogen test were maximum 2.7 %. Therefore, the

validity criterion was met.

NH₄⁺-N, NO₂⁻-N and NO₃⁻-N contents and nitrate formation rates are presented in the following two tables.

Table A2.6-1: NH₄⁺-N, NO₂⁻-N and NO₃⁻-N contents in the nitrogen transformation test

Test group	Control		1.30 mg product/kg dw	13.0 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	0.62	n/p	0.60	0.59
7	<LOQ	n/a	<LOQ	<LOQ
14	<LOQ	n/a	<LOQ	<LOQ
28	<LOQ	n/a	<LOQ	<LOQ
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	<LOQ	n/a	<LOQ	<LOQ
7	<LOQ	n/a	<LOQ	<LOQ
14	<LOQ	n/a	<LOQ	<LOQ
28	<LOQ	n/a	<LOQ	<LOQ
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	21.47	2.7	20.47	21.17
7	49.67	1.6	51.83	51.40
14	61.97	2.5	62.83	63.37
28	77.90	1.3	79.20	82.77

Note: The values are means of triplicate samples.
Measured values below LOQ were set at LOQ.
LOQ: 0.13 mg NH₄⁺-N/kg dw
LOQ: 0.126 mg NO₂⁻-N/kg dw
LOQ: 0.25 mg NO₃⁻-N/kg dw
n/p: not provided, n/a not applicable

Table A2.6-2: Nitrate formation rates in the nitrogen transformation test

Test group	Control	1.30 mg product/kg dw		13.0 mg product/kg dw	
Time interval (days)	NO ₃ ⁻ -N formation rate	NO ₃ ⁻ -N formation rate		NO ₃ ⁻ -N formation rate	
	Mean (mg/kg dw/day)	Mean (mg/kg dw/day)	Dev. from control (%)	Mean (mg/kg dw/day)	Dev. from control (%)
0-7	4.03	4.48*	-11.2	4.32	+7.2
7-14	1.76	1.57	-10.6	1.17	-2.7
14-28	1.14	1.17	+2.7	1.39	+21.8

* statistically significantly different to control

Conclusion

In this nitrogen transformation test, the test substance ADM.01851.H.1.A 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 13.0 mg product/kg soil dw.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 1: Seedling Emergence

Comments of zRMS:	The study was conducted in line with OECD 208 with no minor deviations. Analytical recoveries of the active substances, clopyralid and ethofumesate, were within $100\% \pm 20\%$ of nominal. Thus, study endpoints were calculated and reported in terms of nominal application rates of ADM.01851.H.1.A. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The lowest ER₅₀ (shoot dry weight) = 239 mL product/ha (oat) <u>Phytotoxicity:</u> It should be noted that for wheat, soybean and field bean phytotoxicity effects >50% is observed at 188 mL/ha. As no calculations of ER₅₀ values are available it can be assumed that it will be at range between 85.4 -188 ml/ha. For MSs that needs ER₅₀ values from phytotoxicity effects the relevant calculations are required.
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Reference:	KCP 10.6.2/01
Report	Effects of ADM.01851.H.1.A on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions Friedemann A., 2024a, 23 46 PSE 0012, 000116380 (sponsor report number)
Guideline(s):	OECD 208 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U33 01P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	09 Jan 2025

Test organism:

Test species	Monocotyledons: Oat, <i>Avena sativa</i> (Poaceae) Barley, <i>Hordeum vulgare</i> (Poaceae) Wheat, <i>Triticum aestivum</i> (Poaceae) Onion, <i>Allium cepa</i> (Liliaceae) Dicotyledons: Cress, <i>Lepidum sativum</i> (Brassicaceae) Soybean, <i>Glycine max</i> (Fabaceae) Tomato, <i>Solanum Lycopersicon</i> (Solanaceae) Field bean, <i>Vicia faba</i> (Fabaceae) Lettuce, <i>Lactuca sativa</i> (Asteraceae)
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	Buckwheat, <i>Fagopyrum esculentum</i> (Polygonaceae)
No. of seeds per pot	2 (soybean, tomato, field bean, lettuce, buckwheat) 4 (oat, barley, wheat, onion, cress)
No. of pots treatment	15 (soybean, tomato, field bean, lettuce, buckwheat) 8 (oat, barley, wheat, onion, cress)
Test vessels	Non-porous plastic pots (15 cm diameter)
Preparation	Prior to test substance application, the required number of seeds of each species was directly sown into pots
Fertilisation	0.1% nutrient solution (Hakaphos blau) as required
Watering	Plants were watered by addition of water in the saucers below the pots.

Test soil:

Soil batch	G 01/2023
Soil type	Silty loamy sand
Physico-chemical properties	
Soil Texture (% w/w):	
Clay (<0.002 mm)	11.8
Silt (0.002-0.063 mm)	47.0
Sand (0.063-2.0 mm)	41.3
Organic carbon (% w/w):	1.25
pH (CaCl ₂):	6.1
Salt content (mg KCl/100 g soil dw)	67.8
MWHC (g/100 g soil dw)	30.7

Test conditions:

Test substance concentration	17.6, 38.8, 85.4, 188, 413, 909, 2000 mL product/ha Application in 200 L water/ha
Control	Deionised water
Application time	Pre-emergence after sowing
Application method	Applications were made using an automatic spray cabin (Schachtner Gerätetechnik)
Test duration	21 days after emergence of 50% of seedlings in the control
Test facility type	Greenhouse
Temperature	16.4 – 30.1 °C
Relative humidity	47 – 84%
Light intensity	301 – 384 µmol/m ² /s
Photoperiod	minimum 16 hours light

Observations:

Observation period (BBCH growth stage, emergence, mortality and visual phytotoxicity)	7, 14 and 21 days following 50% emergence in the control plants
Plant height	Day 21
Shoot dry weight	Day 21

Analytical method:

Samples	Samples of the control and highest test substance concentration solution were taken for analytical dose verification
Method type	HPLC-PDA
Equipment	Shimadzu LC-20 system
Column	Phenomenex Synergi, Polar-RP (C ₁₂ -OPh), 150 x 2.0 mm (4µm)
Column temperature	40°C
Flow rate	0.00 min 0.28 mL/min 5.20 min 0.40 mL/min 6.50 min 0.40 mL/min 8.51 min 0.280 mL/min
Mobile phase	A: water/phosphoric acid (1000:1 v/v) B: acetonitrile/phosphoric acid (1000:1 v/v)
Gradient	0.00 min 70% A 30% B 3.10 min 70% A 30% B 7.00 min 40% A 60% B 8.50 min 40% A 60% B

	8.51 min 05% A 95% B 9.50 min 05% A 95% B 9.51 min 70% A 30% B 13.0 min 70% A 30% B
Wavelength	224 nm
Injection volume	7 µL
Retention time	Approximately 3.11 min (clopyralid) and 8.64 min (ethofumesate)
Sample preparation	Samples were stored frozen until analysis. After thawing, a portion of the sample was diluted with deionised water prior to analysis.

Experimental dates: 17 November to 15 December 2023

Calculations:

Mean and standard deviation of assessment data were calculated and rounded using Microsoft Excel. The measurements and observations were compared to those of untreated control plants.

If negative effects could be determined on 21 DAE further statistical analysis were performed by software ToxRat Professional (ToxRatPro Version 3.3.0).

For calculation of effective rates (ER₁₀, ER₂₀ and ER₅₀) of quantal data a linear regression (Probit analysis using linear max. likelihood regression) was performed. The 95%-confidence limits were calculated by normal approximation.

Statistics:

For statistical evaluation of seedling emergence and plant survival, data were tested for monotonicity of rate/response using a qualitative trend analysis by contrasts as well as for extra-binomial variance using Tarone's test. Depending on the outcome of the pre-testing sequences, the Multiple sequentially rejective Fisher test after Bonferroni-Holm (one-sided greater, $\alpha=0.05$), the Chi² 2x2 Table test with Bonferroni correction (one-sided greater, $\alpha=0.05$) or the Step-down Cochran-Armitage test (one-sided greater, $\alpha=0.05$) was used.

For statistical evaluation of plant height and shoot dry weight the data were tested for Normal Distribution (Shapiro-Wilk's Test or Kolmogorov-Smirnov-test, $\alpha=0.01$), Variance Homogeneity (with Residuals) (Levene's Test, $\alpha=0.01$) and Monotonicity of Rate/Response (Trend analysis by Contrasts, $\alpha=0.05$). Depending on the outcomes of the pre-testing sequences, the Williams Multiple Sequential t-test (onesided smaller, $\alpha=0.05$), the Dunnett's multiple t-test (one-sided smaller, $\alpha=0.05$), the multiple sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, $\alpha=0.05$) or the Multiple Sequentially-rejective Median (2x2-Table) Test After Bonferroni-Holm (one-sided smaller, $\alpha=0.05$) was used.

For calculation of effective rates (ER₁₀, ER₂₀ and ER₅₀) of metric data a linear regression (Logit or Weibull analysis using linear max. likelihood regression) was performed. The 95%-confidence limits were calculated by normal approximation or estimated according to Fieller's theorem. Or a non-linear regression without weighting was performed (3-param. logistic CDF or 3-param. normal CDF (optimization method: Levenberg-Marquardt). The 95%-confidence limits were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

The NOER for the observed visual injury was estimated. For this purpose, a threshold value of 10% was assumed (mean value per treatment, effects greater than or equal 10% are evaluated as significant).

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 97% to 100%. The control seedlings did not exhibit visible phytotoxic effects. Mean survival of emerged control seedlings was 100% for the duration of the study. Environmental conditions per species were identical and growing media were the same. Therefore, the validity criteria were met.

Analytical verification of test substance ADM.01851.H.1.A (measured as clopyralid and ethofumesate) concentrations in the highest application solution and the control application solution are summarised in the table below.

Table A2.6.2-1: Verification of test substance concentrations

Application solution	Nominal concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
Clopyralid			
23 46 PSE 0012-1	0.00	n.d.	-
23 46 PSE 0012-3	766.5	788.0	103
Ethofumesate			
23 46 PSE 0012-1	0.00	n.d.	-
23 46 PSE 0012-3	5246	5335	102

n.d. = not detected; LOQ for clopyralid = 5.065 mg/L; LOQ for ethofumesate = 34.67 mg/L

Analytical recoveries of the active substances, clopyralid and ethofumesate, were within 100% ± 20% of nominal. Thus, study endpoints were calculated and reported in terms of nominal application rates of ADM.01851.H.1.A.

Effects of ADM.01851.H.1.A on seedling emergence, seedling mortality/survival, plant height, plant fresh weight and visual phytotoxicity are summarised in the tables below.

Table A2.6.2-2: Seedling emergence of plants exposed to ADM.01851.H.1.A

Seedling emergence of plants exposed to A. fabae infestation								
Application rate (mL product/ha)		Oat		Barley		Cress		
		Mean No. of emerged plants per replicate	% emerged	Mean No. of emerged plants per replicate	% emerged	Mean No. of emerged plants per replicate	% emerged	
Control		4.0	100	4.0	100	4.0	100	
38.8		4.0	100	4.0	100	4.0	100	
85.4		3.8	93.8	3.6	90.6	4.0	100	
188		4.0	100	3.9	96.9	4.0	100	
413		4.0	100	4.0	100	4.0	100	
909		4.0	100	3.9	96.9	4.0	100	
Application rate (mL product/ha)		Onion		Tomato		Buckwheat		
		Mean No. of emerged plants per replicate	% emerged	Mean No. of emerged plants per replicate	% emerged	Mean No. of emerged plants per replicate	% emerged	
Control		4.0	100	2.0	100	2.0	100	
85.4		4.0	100	2.0	100	2.0	100	
188		3.9	96.9	2.0	100	2.0	100	
413		4.0	100	2.0	100	2.0	100	
909		4.0	100	2.0	100	1.9	96.7	
2000		3.8	93.8	2.0	100	1.9	96.7	
Application rate (mL product/ ha)	Wheat		Soybean		Field bean		Lettuce	
	Mean No. of emerged plants per repli- cate	% emerged	Mean No. of emerged plants per repli- cate	% emerged	Mean No. of emerged plants per repli- cate	% emerged	Mean No. of emerged plants per repli- cate	% emerged

Control	3.9	100	2.0	100	2.0	100	2.0	100
17.6	3.9	100	1.9	96.7	2.0	100	2.0	100
38.8	3.9	100	2.0	100	1.9	93.3	2.0	100
85.4	3.9	100	1.9	96.7	1.9	96.7	2.0	100
188	4.0	103.2	2.0	100	1.9	93.3	2.0	100
413	4.0	103.2	2.0	100	1.9	93.3	1.9	93.3

* Statistically significantly different from control ($p \leq 0.05$)

Table A2.6.2-3: Seedling mortality/survival of plants exposed to ADM.01851.H.1.A

Application rate (mL product/ha)		Oat	Barley	Cress	
		% survival of emerged plants			
Control		100	100	100	
38.8		100	100	100	
85.4		100	100	100	
188		100	100	100	
413		87.5	96.9	100	
909		87.5	77.7	100	
Application rate (mL product/ha)		Onion	Tomato	Buckwheat	
		% survival of emerged plants			
Control		100	100	100	
85.4		100	100	100	
188		100	100	100	
413		100	100	100	
909		100	100	100	
2000		100	100	100	
Application rate (mL product/ha)		Wheat	Soybean	Field bean	Lettuce
		% survival of emerged plants			
Control		100	100	100	100
17.6		100	100	100	100
38.8		100	100	100	100
85.4		100	100	100	100
188		96.9	100	100	100
413		75.0	96.7	96.4	96.4

Table A2.6.2-4: Effects on phytotoxicity from exposure to ADM.01851.H.1.A

Species	End-point	Application rate (mL product/ha)							
		0.0	17.6	38.8	85.4	188	413	909	2000
Oat	Symp-toms	None	-	None	None	CNS	CNS	CNS	-
	EPPO (%)	0.0	-	0.0	0.0	53.8	68.8	78.8	-
Barley	Symp-toms	None	-	None	S	S	CNDS	CNDS	-
	EPPO (%)	0.0	-	0.0	1.3	13.8	26.3	37.5	-
Wheat	Symp-toms	None	None	None	None	CDS	CNDS	-	-
	EPPO (%)	0.0	0.0	0.0	0.0	52.5	80.0	-	-
Onion	Symp-toms	None	-	-	None	None	None	None	None
	EPPO (%)	0.0	-	-	0.0	0.0	0.0	0.0	0.0
Cress	Symp-toms	None	-	None	None	S	CNDS	CNDS	-
	EPPO (%)	0.0	-	0.0	0.0	11.3	56.3	72.5	-
Soybean	Symp-toms	None	None	CDS	CDS	CDS	CDS	-	-
	EPPO (%)	0.0	0.0	12.0	32.0	69.3	79.3	-	-
Tomato	Symp-toms	None	-	-	None	None	CDS	CDS	CDS

	EPPO (%)	0.0	-	-	0.0	0.0	19.3	72.7	80.0
Field bean	Symp-toms	None	None	D	CDS	CDS	CNDS	-	-
	EPPO (%)	0.0	0.0	10.0	40.7	70.7	75.3	-	-
Lettuce	Symp-toms	None	None	None	DS	DS	CNDS	-	-
	EPPO (%)	0.0	0.0	0.0	7.3	7.3	60.7	-	-
Buck-wheat	Symp-toms	None	-	-	None	None	C	C	CNDS
	EPPO (%)	0.0	-	-	0.0	0.0	5.3	10.0	32.0

Symptoms: C=chlorosis; N=necrosis; D=deformation; S=stunting

Table A2.6.2-5: Effects on shoot dry weight from exposure to ADM.01851.H.1.A

Application rate (mL product/ha)	Oat		Barley		Cress			
	Dry weight (g)	% of control	Dry weight (g)	% of control	Dry weight (g)	% of control		
Control	2.309	n.a.	2.926	n.a.	2.427	n.a.		
38.8	2.291	0.8	2.734	6.6	2.518	-3.7		
85.4	2.236	3.1	2.721	7.0	2.434	-0.3		
188	1.407*	39.0	2.655	9.3	2.258	7.0		
413	0.510*	77.9	2.404*	17.9	1.587*	34.6		
909	0.308*	86.7	1.510*	48.4	0.784*	67.7		
Application rate (mL product/ha)	Onion		Tomato		Buckwheat			
	Dry weight (g)	% of control	Dry weight (g)	% of control	Dry weight (g)	% of control		
Control	0.143	n.a.	1.423	n.a.	4.269	n.a.		
85.4	0.139	3.3	1.464	-2.8	4.691	-9.9		
188	0.138	4.0	1.416	0.5	4.542	-6.4		
413	0.144	-0.3	1.266*	11.0	4.474	-4.8		
909	0.137	4.2	0.404*	71.6	4.517	-5.8		
2000	0.125	12.8	0.113*	92.0	3.580*	16.1		
Application rate (mL product/ha)	Wheat		Soybean		Field bean		Lettuce	
	Dry weight (g)	% of control	Dry weight (g)	% of control	Dry weight (g)	% of control	Dry weight (g)	% of control
Control	2.042	n.a.	2.660	n.a.	2.714	n.a.	0.679	n.a.
17.6	2.005	1.8	2.583	2.9	2.822	-4.0	0.729	-7.4
38.8	2.042	0.0	2.439	8.3	2.474	8.8	0.797	-17.4
85.4	1.981	3.0	2.314*	13.0	2.193*	19.2	0.711	-4.7
188	1.391*	31.9	1.603*	39.7	1.531*	43.6	0.895	-31.8
413	0.367*	82.0	0.727*	72.7	1.291*	52.4	0.460	32.2

n.a. not applicable

* Statistically significantly different from control ($\alpha \leq 0.05$)

Table A2.6.2-6: Effects on plant height from exposure to ADM.01851.H.1.A

Application rate (mL product/ha)	Oat		Barley		Cress	
	Plant height (cm)	% of control	Plant height (cm)	% of control	Plant height (cm)	% of control
Control	40.3	n.a.	39.4	n.a.	11.1	n.a.
38.8	41.2	-2.2	38.5	2.3	11.4	-2.8
85.4	42.3	-5.0	38.6	2.0	11.4	-2.2
188	32.7*	18.9	37.5	4.7	8.7*	21.6
413	24.0*	40.5	36.1*	8.4	6.3*	43.0
909	20.4*	49.5	26.1*	33.8	4.3*	61.2
Application rate	Onion		Tomato		Buckwheat	

(mL product/ha)	Plant height (cm)	% of control	Plant height (cm)	% of control	Plant height (cm)	% of control		
Control	18.5	n.a.	12.9	n.a.	57.8	n.a.		
85.4	18.0	2.4	13.0	-0.5	60.6	-4.8		
188	18.7	-1.5	13.1	-1.3	58.1	-0.5		
413	18.5	0.0	12.4	3.9	59.8	-3.5		
909	18.3	0.7	9.6*	25.6	60.7	-5.1		
2000	18.2	1.2	4.9*	61.8	54.9	5.0		
Application rate (mL product/ha)	Wheat		Soybean		Field bean		Lettuce	
	Plant height (cm)	% of control	Plant height (cm)	% of control	Plant height (cm)	% of control	Plant height (cm)	% of control
Control	37.2	n.a.	22.8	n.a.	29.8	n.a.	8.8	n.a.
17.6	38.5	-3.4	22.5	1.3	30.5	-2.1	8.7	1.5
38.8	38.8	-4.2	22.5	1.2	32.4	-8.5	8.9	-0.8
85.4	38.2	-2.5	21.8	4.4	33.1	-10.8	8.7	1.5
188	29.6*	20.4	20.1*	11.7	30.8	-3.2	9.1	-3.0
413	14.5*	61.1	14.9*	34.8	27.1	9.1	7.8	11.7

n.a. not applicable

* Statistically significantly different from control ($\alpha \leq 0.05$)

Calculated ER₅₀, ER₂₀, ER₁₀ and NOER values for the different parameters for each plant species are presented in the table below.

Table A2.6.2-7: ER₅₀, ER₂₀, ER₁₀ and NOER for various endpoints for seedlings exposed to ADM.01851.H.1.A

	Endpoint (mL product/ha)									
	Oat	Barley	Wheat	Onion	Cress	Soy-bean	Tomato	Field bean	Lettuce	Buck-wheat
Seedling emergence										
ER ₅₀	>909	>909	>413	>2000	>909	>413	>2000	>413	>413	>2000
ER ₂₀	>909	>909	>413	>2000	>909	>413	>2000	>413	>413	>2000
ER ₁₀	>909	>909	>413	>2000	>909	>413	>2000	>413	>413	>2000
NOER	≥909	≥909	≥413	≥2000	≥909	≥413	≥2000	≥413	≥413	≥2000
Survival										
ER ₅₀	>909	>909	>413	>2000	>909	>413	>2000	>413	>413	>2000
ER ₂₀	>909	860	370	>2000	>909	>413	>2000	>413	>413	>2000
ER ₁₀	>909	659	279	>2000	>909	>413	>2000	>413	>413	>2000
NOER	≥909	413	188	≥2000	≥909	≥413	≥2000	≥413	≥413	≥2000
Phytotoxicity										
NOER	85.4	85.4	85.4	≥2000	85.4	17.6	188	17.6	188	413
Shoot dry weight										
ER ₅₀	239	>909	245	>2000	582	246	702	294	>413	>2000
ER ₂₀	122	488	154	>2000	275	115	469	72.4	237	>2000
ER ₁₀	86.3	335	117	>2000	177	77.3	380	34.8	64.9	1378
NOER	85.4	188	85.4	≥2000	188	38.8	188	38.8	188	909
Plant height										
ER ₅₀	693	>909	329	>2000	575	>413	1545	>413	>413	>2000
ER ₂₀	191	680	181	>2000	188	269	778	>413	>413	>2000
ER ₁₀	89.6	475	128	>2000	105	173	521	>413	>413	>2000
NOER	85.4	188	85.4	≥2000	85.4	85.4	413	≥413	≥413	≥2000

Conclusion

In this seedling emergence test with ADM.01851.H.1.A at various application rates on ten plant species,

there were no statistically significant effects on seedling emergence. Effects on survival were only observed in barley and wheat, though all ER₅₀ values were higher than the highest tested concentrations. The lowest NOER for phytotoxicity was observed in soybean and field bean at 17.6 mL product/ha. The lowest ER₅₀ for shoot dry weight was observed in oat at 239 mL product/ha. The lowest observed ER₅₀ for plant height was observed in wheat at 329 mL product/ha.

A 2.6.2.2 Study 1: Vegetative Vigour

Comments of zRMS:	The study was conducted in line with OECD 227 with minor deviations. Analytical recoveries of the active substances, clopyralid and ethofumesate, were within 100% ± 20% of nominal. Thus, study endpoints were calculated and reported in terms of nominal application rates of ADM.01851.H.1.A. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The lowest ER₅₀ = 53.7 mL product/ha (field bean) <u>Phytotoxicity:</u> It should be noted that for wheat, soybean and field bean phytotoxicity effects >50% is observed at 17.6 ml/ha in field bean As no calculations of ER₅₀ values are available it can be assumed that it will be at range between 8.02-17.6 ml/ha. For MSs that needs ER₅₀ values from phytotoxicity effects the relevant ER₅₀ calculations is required.
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Reference:	KCP 10.6.2/02
Report	Effects of ADM.01851.H.1.A on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions, Friedemann A., 2024b, 23 46 PVV 0011, 000116381(sponsor report number)
Guideline(s):	Yes, OECD 227 (July 2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	ADM.01851.H.1.A
Batch No.	U3301P1
Active ingredient content	Nominal / analysed: 75 / 75.6 g/L clopyralid Nominal / analysed: 500 / 517.4 g/L ethofumesate
Appearance	Off-white liquid
Density	1.154 g/mL
Expiry date	09 Jan 2025

Test organism:

Test species	Monocotyledons: Oat, <i>Avena sativa</i> (Poaceae) Barley, <i>Hordeum vulgare</i> (Poaceae) Wheat, <i>Triticum aestivum</i> (Poaceae) Onion, <i>Allium cepa</i> (Liliaceae)
	Dicotyledons: Cress, <i>Lepidium sativum</i> (Brassicaceae) Soybean, <i>Glycine max</i> (Fabaceae) Tomato, <i>Solanum Lycopersicon</i> (Solanaceae) Field bean, <i>Vicia faba</i> (Fabaceae) Lettuce, <i>Lactuca sativa</i> (Asteraceae) Buckwheat, <i>Fagopyrum esculentum</i> (Polygonaceae)
No. of plants per pot	2 (soybean, tomato, field bean, lettuce, buckwheat) 4 (oat, barley, wheat, onion, cress)
No. of pots per treatment	15 (soybean, tomato, field bean, lettuce, buckwheat) 8 (oat, barley, wheat, onion, cress)
Test vessels	Non-porous plastic pots (15 cm diameter)
Preparation	The required number of seeds per species was sown per pot so that all species reached BBCH 12-14 at the same time.
Fertilisation	0.1% nutrient solution (Hakaphos blau) as required
Watering	Plants were watered by addition of water in the saucers below the pots.

Test soil:

Soil batch	G 01/2023
Soil type	Silty loamy sand
Physico-chemical properties	
Soil Texture (% w/w):	
Clay (<0.002 mm)	11.8
Silt (0.002-0.063 mm)	47.0
Sand (0.063-2.0 mm)	41.3
Organic carbon (% w/w):	1.25
pH (CaCl ₂):	6.1
Salt content (mg KCl/100 g soil dw)	67.8
MWHC (g/100 g soil dw)	30.7

Test conditions:

Test substance concentration	3.64, 8.02, 17.6, 38.8, 85.4, 188, 413, 909, 2000 mL product/ha Application in 200 L water/ha
Control	Deionised water
Application time	Post-emergence when the plants reached BBCH growth stage 12 – 14
Application method	Applications were made using an automatic spray cabin (Schachtner Gerätetechnik)
Test duration	21 days after application
Test facility type	Greenhouse
Temperature	14.4 - 26.8°C
Relative humidity	49 – 84%
Light intensity	310 – 703 µmol/m ² /s
Photoperiod	minimum 16 hours light

Observations:

Observation period (BBCH growth stage, mortality and visual phytotoxicity)	7, 14 and 21 days following 50% emergence in the control plants
Plant height	Day 21
Shoot dry weight	Day 21

Analytical method:

Samples	Samples of the control and highest test substance concentration solution were taken for analytical dose verification
Method type	HPLC-PDA

Equipment	Shimadzu LC-20 system
Column	Phenomenex Synergi, Polar-RP (C12-OPh), 150 x 2.0 mm (4µm)
Column temperature	40°C
Flow rate	0.00 min 0.28 mL/min 5.20 min 0.40 mL/min 6.50 min 0.40 mL/min 8.51 min 0.280 mL/min
Mobile phase	A: water/phosphoric acid (1000:1 v/v) B: acetonitrile/phosphoric acid (1000:1 v/v)
Gradient	0.00 min 70% A 30% B 3.10 min 70% A 30% B 7.00 min 40% A 60% B 8.50 min 40% A 60% B 8.51 min 05% A 95% B 9.50 min 05% A 95% B 9.51 min 70% A 30% B 13.0 min 70% A 30% B
Wavelength	224 nm
Injection volume	7 µL
Retention time	Approximately 3.11 min (clopyralid) and 8.64 min (ethofumesate)
Sample preparation	Samples were stored frozen until analysis. After thawing, a portion of the sample was diluted with deionised water prior to analysis.

Experimental dates: 21 December 2023 to 22 January 2024

Calculations:

Mean and standard deviation of assessment data were calculated and rounded using Microsoft Excel. The measurements and observations were compared to those of untreated control plants.

If negative effects could be determined on 21 DAT further statistical analysis were performed by software ToxRat Professional (ToxRatPro Version 3.3.0).

Statistics:

For statistical evaluation of plant survival, data were tested for monotonicity of rate/response using a qualitative trend analysis by contrasts. Depending on the outcome of the pre-testing sequences, the Multiple sequentially-rejective Fisher test after Bonferroni-Holm (one-sided greater, $\alpha=0.05$), was used.

For statistical evaluation of plant height and shoot dry weight the data were tested for Normal Distribution (Shapiro-Wilk's Test, $\alpha=0.01$), Variance Homogeneity (with Residuals) (Levene's Test, $\alpha=0.01$) and Monotonicity of Rate/Response (Trend analysis by Contrasts, $\alpha=0.05$). Depending on the outcomes of the pre-testing sequences, the Williams Multiple Sequential t-test (one-sided smaller, $\alpha=0.05$) or the multiple sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, $\alpha=0.05$) was used.

For calculation of effective rates (ER10, ER20 and ER50) of metric data a linear regression (Probit, Logit or Weibull analysis using linear max. likelihood regression) was performed. The 95%-confidence limits were calculated according to Fieller's theorem.

Or a non-linear regression without weighting was performed (3-param. normal CDF (optimization method: Levenberg-Marquardt). The confidence limits of the ER50 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ERx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).

The NOER for the observed visual injury was estimated. For this purpose, a threshold value of 10% was assumed (mean value per treatment, effects greater than or equal 10% are evaluated as significant).

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

The seedling emergence criterion is not relevant for this study, since the seeds were germinated in separate trays and, when the plants had reached the cotyledon or first leaf stage, they were transplanted into the bioassay pots in the requisite numbers. This ensured that the plants were all similar and the test was started with 100% of the required plant material. The control plants did not exhibit visible phytotoxic effects. Mean survival of control plants was 100% for the duration of the study. Environmental conditions per species were identical and growing media were the same. Therefore, the validity criteria were met.

Analytical verification of test substance ADM.01851.H.1.A (measured as clopyralid and ethofumesate) concentrations in the highest application solution and the control application solution are summarised in the table below.

Table A2.6.2-8: Verification of test substance concentrations

Application solution	Nominal concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
Clopyralid			
23 46 PSE 0012-1	0.00	n.d.	-
23 46 PSE 0012-3	766.5	788.0	103
Ethofumesate			
23 46 PSE 0012-1	0.00	n.d.	-
23 46 PSE 0012-3	5246	5335	102

n.d. = not detected; LOQ for clopyralid = 5.065 mg/L; LOQ for ethofumesate = 34.67 mg/L

Analytical recoveries of the active substances, clopyralid and ethofumesate, were within 100% ± 20% of nominal. Thus, study endpoints were calculated and reported in terms of nominal application rates of ADM.01851.H.1.A.

Effects of ADM.01851.H.1.A on plant mortality/survival, plant height, plant fresh weight and visual phytotoxicity are summarised in the tables below.

Table A2.6.2-9: Mortality/survival of plants exposed to ADM.01851.H.1.A

Application rate (mL product/ ha)	Oat	Barley	Onion
	% survival of emerged plants		
Control	100	100	100
85.4	100	100	100
188	100	100	100
413	100	100	100
909	100	100	100
2000	100	100	100
Application rate (mL product/ha)	Cress	Soybean	Tomato
	% survival of emerged plants		
Control	100	100	100
3.64	100	100	100
8.02	100	100	100
17.6	100	100	100
38.8	100	100	100
85.4	100	100	100
188	100	100	100
Application rate (mL product/ha)	Field bean	Lettuce	Buckwheat
	% survival of emerged plants		

Control	100	100	100
3.64	100	100	100
8.02	100	100	100
17.6	100	100	100
38.8	100	100	100
85.4	100	100	100
188	100	100	100
Application rate (mL product/ha)	Wheat		
	% survival of emerged plants		
Control	100		
38.8	100		
85.4	100		
188	100		
413	100		
909	100		

Table A2.6.2-10: Effects on phytotoxicity from exposure to ADM.01851.H.1.A

Species	End-point	Application rate (mL product/ha)									
		0.0	3.64	8.02	17.6	38.8	85.4	188	413	909	2000
Oat	Symptoms	None	-	-	-	-	None	None	None	DS	CDS
	EPPO (%)	0.0	-	-	-	-	0.0	0.0	0.0	15.0	36.3
Barley	Symptoms	None	-	-	-	-	None	None	None	CDS	CDS
	EPPO (%)	0.0	-	-	-	-	0.0	0.0	0.0	33.8	66.3
Wheat	Symptoms	None	-	-	-	DS	CDS	CDS	CDS	CNDS	-
	EPPO (%)	0.0	-	-	-	6.3	30.0	60.0	68.8	80.0	-
Onion	Symptoms	None	-	-	-	-	None	None	None	S	CS
	EPPO (%)	0.0	-	-	-	-	0.0	0.0	0.0	3.8	11.3
Cress	Symptoms	None	None	None	None	None	CNDS	CNDS	-	-	-
	EPPO (%)	0.0	0.0	0.0	0.0	0.0	35.0	70.0	-	-	-
Soy-bean	Symptoms	None	None	None	CDS	CNDS	CNDS	CNDS	-	-	-
	EPPO (%)	0.0	0.0	0.0	40.0	50.0	60.0	70.0	-	-	-
Tomato	Symptoms	None	None	None	D	D	CD	CDS	-	-	-
	EPPO (%)	0.0	0.0	0.0	1.3	20.0	60.0	70.0	-	-	-
Field bean	Symptoms	None	CD	CD	CDS	CDS	CNDS	CNDS	-	-	-
	EPPO (%)	0.0	7.3	30.7	55.3	62.0	70.0	83.3	-	-	-
Lettuce	Symptoms	None	None	None	None	D	CDS	CDS	-	-	-
	EPPO (%)	0.0	0.0	0.0	0.0	15.3	50.0	70.0	-	-	-
Buck-wheat	Symptoms	None	None	C	CND	CND	CNDS	CNDS	-	-	-
	EPPO (%)	0.0	0.0	4.0	8.7	16.7	24.7	46.7	-	-	-

Symptoms: C=chlorosis; N=necrosis; D=deformation; S=stunting

Table A2.6.2-11: Effects on plant height from exposure to ADM.01851.H.1.A

Application rate (mL product/ ha)	Oat		Barley		Onion	
	Plant height (cm)	% inhibition	Plant height (cm)	% inhibition	Plant height (cm)	% inhibition
Control	45.1	n.a.	52.5	n.a.	40.3	n.a.
85.4	44.1	2.1	53.3	-1.4	40.1	0.5
188	46.0	-2.1	51.3	2.2	40.7	-0.9
413	44.7	0.8	52.4	0.1	38.7	4.0
909	46.8	-4.0	43.7*	16.8	36.9*	8.4
2000	38.2*	15.3	31.4*	40.1	35.9*	10.9
Application rate (mL product/ha)	Cress		Soybean		Tomato	
	Plant height (cm)	% inhibition	Plant height (cm)	% inhibition	Plant height (cm)	% inhibition
Control	29.9	n.a.	69.7	n.a.	40.0	n.a.
3.64	29.3	2.1	67.7	3.0	39.9	0.2
8.02	27.9	6.6	63.5*	8.9	39.3	1.6
17.6	27.8	7.1	55.0*	21.1	38.4	3.8
38.8	28.0	6.4	37.9*	45.7	42.1	-5.3
85.4	22.4*	25.2	30.1*	56.8	42.4	-6.2
188	15.5*	48.2	26.2*	62.5	11.4*	71.4
Application rate (mL product/ha)	Field bean		Lettuce		Buckwheat	
	Plant height (cm)	% inhibition	Plant height (cm)	% inhibition	Plant height (cm)	% inhibition
Control	40.6	n.a.	15.5	n.a.	108.6	n.a.
3.64	43.3	-6.6	15.6	-0.2	106.3	2.1
8.02	42.8	-5.3	15.3	1.7	106.6	1.9
17.6	42.9	-5.6	14.9	3.9	105.7	2.7
38.8	38.4	5.4	15.0	3.4	102.6*	5.5
85.4	26.4*	35.0	17.0	-9.4	100.6*	7.4
188	14.1*	65.4	12.5*	19.7	87.5*	19.5
Application rate (mL product/ha)	Wheat					
	Plant height (cm)	% inhibition				
Control	42.3	n.a.				
38.8	40.1*	5.2				
85.4	39.1*	7.6				
188	31.7*	25.1				
413	22.6*	46.6				
909	19.3*	54.4				

n.a. not applicable

* Statistically significantly different from control ($\alpha \leq 0.05$)

Table A2.6.2-12: Effects on shoot dry weight from exposure to ADM.01851.H.1.A

Application rate (mL product/ ha)	Oat		Barley		Onion	
	Shoot weight (g)	% inhibition	Shoot weight (g)	% inhibition	Shoot weight (g)	% inhibition
Control	5.926	n.a.	6.527	n.a.	1.946	n.a.
85.4	5.580	5.8	6.301	3.5	2.066	-6.1
188	5.784	2.4	6.193	5.1	2.050	-5.4
413	5.563	6.1	6.352	2.7	1.795	7.8
909	5.312*	10.4	4.964*	23.9	1.649*	15.3
2000	3.777*	36.3	2.920*	55.3	1.597*	17.9
Application rate (mL product/ha)	Cress		Soybean		Tomato	
	Shoot weight (g)	% inhibition	Shoot weight (g)	% inhibition	Shoot weight (g)	% inhibition
Control	4.657	n.a.	10.595	n.a.	8.102	n.a.
3.64	4.726	-1.5	11.023	-4.0	8.049	0.7
8.02	4.535	2.6	9.888	6.7	7.898	2.5
17.6	4.598	1.3	8.912*	15.9	7.768*	4.1
38.8	4.609	1.0	7.295*	31.1	7.041*	13.1
85.4	3.629*	22.1	6.011*	43.3	4.259*	47.4
188	2.370*	49.1	3.824*	63.9	1.755*	78.3
Application rate (mL product/ha)	Field bean		Lettuce		Buckwheat	
	Shoot weight (g)	% inhibition	Shoot weight (g)	% inhibition	Shoot weight (g)	% inhibition

Control	5.013	n.a.	6.530	n.a.	8.282	n.a.
3.64	4.656*	7.1	6.445	1.3	8.350	-0.8
8.02	4.153*	17.2	6.347	2.8	8.016	3.2
17.6	3.803*	24.1	6.245	4.4	7.998	6.4
38.8	3.084*	38.5	5.413*	17.1	7.550*	8.8
85.4	2.225*	55.6	3.893*	40.4	7.114*	14.1
188	0.742*	85.2	1.707*	73.9	5.540*	33.1
Application rate (mL product/ha)	Wheat					
	Shoot weight (g)	% inhibition				
Control	4.388	n.a.				
38.8	4.522	-3.1				
85.4	4.288	2.3				
188	3.374*	23.1				
413	2.455*	44.0				
909	1.330*	69.7				

n.a. not applicable

* Statistically significantly different from control ($p \leq 0.05$)

Calculated ER_{50} , ER_{20} , ER_{10} and NOER values for the different parameters for each plant species are presented in the table below.

Table A2.6.2-7: ER_{50} , ER_{20} , ER_{10} and N,NOER for various endpoints for seedlings exposed to ADM.01851.H.1.A

	Endpoint (mL product/ha)									
	Oat	Barley	Wheat	Onion	Cress	Soy-bean	Tomato	Field bean	Lettuce	Buck-wheat
Survival										
ER_{50}	>2000	>2000	>909	>2000	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188
ER_{20}	>2000	>2000	>909	>2000	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188
ER_{10}	>2000	>2000	>909	>2000	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188
NOER	≥ 2000	≥ 2000	≥ 909	≥ 2000	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188	≥ 188
Phytotoxicity										
NOER	413	413	38.8	909	38.8	8.02	17.6	3.64	17.6	17.6
Shoot dry weight										
ER_{50}	239	>909	245	>2000	582	246	702	294	>413	>2000
ER_{20}	122	488	154	>2000	275	115	469	72.4	237	>2000
ER_{10}	86.3	335	117	>2000	177	77.3	380	34.8	64.9	1378
NOER	85.4	188	85.4	≥ 2000	188	38.8	188	38.8	188	909
Plant height										
ER_{50}	>2000	>2000	607	>2000	>188	71.2	162	120	>188	>188
ER_{20}	>2000	1079	138	>2000	61.5	13.3	129	55.0	>188	>188
ER_{10}	836	686	63.4	1402	31.8	5.51	115	36.6	117	77.7
NOER	909	413	<38.8	413	38.8	3.64	85.4	38.8	85.4	17.6

Conclusion

In this vegetative vigour test with ADM.01851.H.1.A at various application rates on ten plant species, there were no statistically significant effects on survival. The lowest NOER for phytotoxicity was observed in soybean at 8.02 mL product/ha. The lowest ER_{50} for plant height was observed in soybean at 71.2 mL product/ha. The lowest ER_{50} for shoot dry weight was observed in field bean at 53.7 mL product/ha.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data