

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: CHR/H/DIK 480 SL

Product name(s): Macamba 480 SL, Dikambin 480 SL

Chemical active substance(s):

Dicamba, 480 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: May 2022

MS Finalisation date: 16/06/2023

Version history

When	What
January 2023	Dossier sent for evaluation
March 2023	Applicant update of dRR .Addition of new studies, additional calculations for aquatic species and proposition of the classification and labelling
April 2023	zRMS evaluation of dRR
June 2023	Final version prepared by zRMS after Commenting period

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9 Ecotoxicology (KCP 10)

zRMS comments:

This application was submitted by Innvigo Sp. z o.o. for approval of the formulation CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL containing 480 g/L of dicamba for use as a herbicide in maize.

This dRR report Part B reviews only ecotoxicological data (Annex III) and additional information that has not previously been considered within the EU review process.

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 is struck through and shaded for transparency.

In the following document, data for active substances - dicamba - was described during its inclusion on Annex 1 process in respectively 2009. Were reference to active substance data in the current risk assessment has been made, it was based on the data which protection for expired 10 years from date of inclusion of active substances on Annex I

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: devel- opmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. num- ber a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro- pods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Maize	F	Mono- and dicots weeds	Spray	BBCH 12- 16	a)1 b)1	n/a	a) 0.6 L/ha b) 0.6 L/ha	a) 288 g as/ha b) 288 g as/ha	200-300	n/a								
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
Minor uses according to Article 51 (field uses)																				
Minor uses according to Article 51 (interzonal uses)																				

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

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required

C	To be confirmed by cMS
N	No safe use

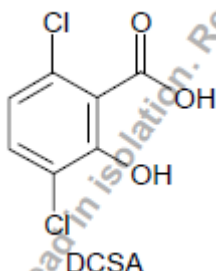
**Remarks
table:**

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

zRMS comments:

GAP presented in the Table 9.1-1 of this document is revised with consideration of the outcome of the evaluation performed in area of ecotoxicology.

9.1.1 Overall conclusions

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to
DCSA	207	 <p>DCSA</p>	Total Water and Sediment: - Soil: 24.2% molar basis with respect to the parent	Aquatic species, soil organisms

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)

CHR/H/DIK 480 SL no pose any unacceptable risk to birds and mammals.

The risk assessment performed for birds and mammals indicate acceptable acute and long-term risk to birds and mammals exposed to dicamba following application of CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL acc. to intended GAP.

As dicamba and its metabolite DCSA have a log Pow value of < 3 it was not necessary to consider the risk to birds and mammals from secondary poisoning.

No risk to birds or mammals via drinking water was identified, as the ratio of the effective application rate to relevant endpoints was < 50.

Regarding effects on other terrestrial vertebrate wildlife (reptiles and amphibians), no data/information available

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

CHR/H/DIK 480 SL no pose any unacceptable risk to aquatic species.

Based on PEC/RAC calculations, no unacceptable risk is indicated for aquatic organisms considering GAP use in maize. Risk mitigation measures are not required since risk assessment is acceptable already at the Step 2.

9.1.1.3 Effects on bees (KCP 10.3.1)

CHR/H/DIK 480 SL no pose any unacceptable risk to bees.

The evaluation of the risk for bees has been performed in line with SANCO/10329/2002 rev 2 final.

CHR/H/DIK 480 SL pose no unacceptable risk to bees according to the label.

Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on national level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

CHR/H/DIK 480 SL no pose any unacceptable risk to arthropods other than bees..

The risk assessment was conducted according to the ESCORT 2 Guidance Document (2000) and the

Guidance Document on Terrestrial Ecotoxicology (2002).

CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL poses an acceptable risk to non-target arthropods in both in-field and off-field areas without the need for risk mitigation measures.

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

CHR/H/DIK 480 SL no pose any unacceptable risk to soil organisms.

Chronic risk to earthworms arising from the application of CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 according to the intended GAP uses can be excluded as the trigger values of 5 for long-term risk were exceeded by far.

Additionally, performed long-term risk assessment for collembola and predatory mites indicates that TER_{lt} is above the trigger value of 5, indicating acceptable risk to soil organisms (other than earthworms) from the proposed uses of CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL

The risk to soil microorganisms is acceptable since effects on the nitrogen transformations are acceptable at concentration which is higher than the maximum relevant PEC soil for the maximum application rate of CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480.

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

Based on the predicted rates of CHR/H/DIK 480 SL in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/DIK 480 SL according to the GAP of the formulation CHR/H/DIK 480 SL achieve the acceptability criteria TER ≥, with applying:

- 20 m buffer zone
- 10 m and use of 50 % drift reducing nozzles
- 5 m and use of 75 % drift reducing nozzles

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of formulation grouped according to criterion

Group	Intended uses	relevant use parameters for grouping	relevant parameter or value
Terrestrial vertebrates (Birds and Mammals; 9.2 and 9.3)	According to GAP	Scenarios according to EFSA Birds and Mammals Guidance (2009)	Crop, application rate, number of applications, timing criterion
Aquatic organisms (9.5)	According to GAP	Crops according to FOCUS surface water guidance (2015) ¹	FOCUS modelling, for details see Part B 8
Bees (9.6)	Generic risk envelope covering all product	Risk assessments are based on the maximum single application	Maximum single application rate

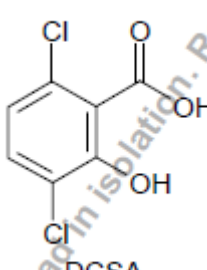
¹ FOCUS (2015): Generic guidance for FOCUS surface water Scenarios. Version 1.4.

Group	Intended uses	relevant use parameters for grouping	relevant parameter or value
	uses	rate	
Terrestrial non-target arthropods other than bees (9.7)	According to GAP In-field	In-field and off-field risk assessments are based on the maximum application rate for each type of crops	Application rate and number of uses
	According to GAP Off-field		Crop type (field), application rate and number of uses
Soil meso- and macrofauna / soil microorganisms (9.8 and 9.9)	Generic risk envelope covering all product uses	Risk assessments are based on the application rate of 1 x 0.288 kg s.a./ha in maize BBCH >10	Worst case PECsoil value taken from Section 8 (Environmental Fate)
Non-target terrestrial plants (9.10)	According to GAP	Risk assessments are based on the maximum single application rate for each type of crops	Application rate and drift rate

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 CHR/H/DIK 480 SL is indicated in the table.

Table 9.1-3 Metabolites of dicamba

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to
DCSA	207	 <p>DCSA</p>	Total Water and Sediment: - Soil: 24.2% molar basis with respect to the parent Water: > 10 % of a.s. Sediment: < 5 % of a.s.	Aquatic species, soil organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with dicamba and its relevant metabolites. Full details of these studies are provided in the respective EU DAR

However, the provision of further data on the formulation CHR/H/DIK 480 SL is not considered essential, because all relevant data is provided in EU reports.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Dicamba	Acute	LD ₅₀ = 216 mg as/kg bw/d	EFSA Journal 2011;9(1):1965
<i>Anas platyrhynchos</i>	Dicamba	Oral	LD ₅₀ = 1373	EFSA Scientific

Species	Substance	Exposure System	Results	Reference
(Mallard duck)		1 d Acute	mg/kg bw	Report 2011;9(1):1965
<i>Anas platyrhynchos</i> (Mallard duck), <i>Colinus virginianus</i> (Bobwhite quail)	Dicamba	Acute	Geometric mean of endpoints = 545 mg a.s./kg bw	
<i>Anas platyrhynchos</i>	Dicamba	Long term	NOEL = 89 mg as/kg bw/d	EFSA Journal 2011;9(1):1965
<i>Anas platyrhynchos</i> (Mallard duck)	Dicamba	Dietary 8 d Short-term	LD ₅₀ >1567 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
<i>Colinus virginianus</i> (Bobwhite quail)	Dicamba	Dietary 8 d Short-term	LD ₅₀ >995 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
<i>Colinus virginianus</i> (Bobwhite quail)	Dicamba	Dietary Reproductive toxicity	NOEL = 170 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
<i>Anas platyrhynchos</i> (Mallard duck), <i>Colinus virginianus</i> (Bobwhite quail)	Dicamba	Reproductive toxicity	LD/10 ie 54.5 mg/kg bw *	EFSA Scientific Report 2011;9(1):1965

*For the long-term assessment, the geometric mean LD₅₀/10 of 54.5 mg a.s./kg bw is used as an endpoint in the reproductive assessment, since this endpoint is lower than the lowest NOEL from the avian reproduction studies (89 mg a.s./kg bw/d)
 Endpoints in **bold** were used for the risk assessment

zRMS comments:

Consideration of acute endpoint use in RA for dicamba:

According to EFSA/2009/1438 the geometric mean should be used for the acute assessment, except when the endpoint for the most sensitive species is more than a factor of 10 below the geometric mean of all the tested species. Since this is not the case, the geometric mean was used for the risk assessment derived according the EFSA B&M guidance (Points: 2.4.1 and 2.4.2).

Before a geometric mean can be calculated it need to ensure that the studies are equivalent in terms of endpoint and in particular the vehicle/solvent used in dosing.

The studies were conducted in accordance with the same guidance documents by the same laboratory therefore the studies are equivalent and it is appropriate to calculate a geometric mean. The geometric mean of 1373 mg a.s./kg bw and 216 mg a.s./kg bw/d is 545 mg/kg bw.

Consideration of long-term endpoint use in RA for dicamba:

According to the EFSA B&M guidance an estimated reproductive endpoint should be derived by using the acute oral LD₅₀ (from a single species or geometric mean) and divided by 10 to obtain an LD₅₀/10. For dicamba the geometric mean LD₅₀/10 of 54.5 mg a.s./kg bw is used as an endpoint in the reproductive assessment, since this endpoint is lower than the lowest NOEL from the avian reproduction studies.

9.2.1.1 Justification for new endpoints

Not revelant.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: ~~Screening and~~ First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/DIK 480 SL in maize

Intended use		Maize				
Active substance/product		Dicamba				
Application rate (g/ha)		1 × 288				
Acute toxicity (mg/kg bw)		545 216				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Screening step	Small omnivorous bird	158.8	1	45.73	11.9 4.7	
Maize BBCH 10—19	Small insectivorous bird “wagtail” ground invertebrates without interception 50% ground arthropods, 50% foliar arthropods	26.8	–	–	28.0	
Maize BBCH 10—29	Medium granivorous bird "gamebird" Small seeds 100% seed	6.6	–	–	113.6	
Maize BBCH 10—29	medium herbivorous/granivorous bird "pigeon" Non grass herbs 100% leaves	55.6	–	–	13.5	
Maize BBCH 10—29	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	24.0	–	–	31.3	
Maize Leaf development BBCH 10 to 19	Small insectivorous/worm feeding species “thrush” ground invertebrates without interception 100% soil dwelling invertebrates	10.5	–	–	71.4	
Reprod. toxicity (mg/kg bw/d)		54.5 89				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Screening step	Small omnivorous bird	64.8	0.53	9.89	5.51 2.2	
Maize BBCH 10—19	Small insectivorous bird “wagtail” ground invertebrates without interception 50% ground arthropods, 50% foliar arthropods	11.3	–	–	12.5	

Maize BBCH 10–29	Medium granivorous bird "gamebird" Small seeds 100% seed	3.0	-	-	47.2
Maize BBCH 10–29	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves	22.7	-	-	6.2
Maize BBCH 10–29	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	-	-	13.0
Maize Leaf development BBCH 10 to 19	Small insectivorous/worm feeding species "thrush" ground invertebrates without interception 100% soil dwelling invertebrates	5.7	-	-	24.8

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

9.2.2.2 Higher-tier risk assessment

Not relevant.

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 CHR/H/DIK 480 SL 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 12.36, dicamba belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha) =	288			
Acute toxicity (mg/kg bw) =	545 216	quotient =		0.52 1.3
Reprod. toxicity (mg/kg bw/d) =	54.5 89	quotient =		5.28 3.24

the relevant trigger.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of dicamba amounts to 0.55 – 1.9 (at pH 5.0 – 8.9) 1.8 and for its metabolite DCSA is -0.84 (pH 6.8), 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

CHR/H/DIK 480 SL no pose any unacceptable risk for birds.

zRMS comments:

The acute and long-term risk assessment for birds performed by the Applicant was updated by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.

Based on screening \ step the acceptable acute and reproductive risk to birds was concluded for application of the formulation in maize s according to the intended uses.

CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL presents no unacceptable risk to birds resulting from exposure via drinking water. Since the log Pow value of dicamba and its relevant aquatic metabolite DCSA are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

However, the provision of further data on the formulation CHR/H/DIK 480 SL is not considered essential, because all relevant data is provided in EU reports.

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

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

Species	Substance	Exposure System	Results	Reference
Rat, female	Dicamba	Acute	LD50= 1581 mg/kg bw/d	EFSA Journal 2011;9(1):196
Rat	Dicamba	Long-term	NOAEL= 150 mg/kg bw/d	EFSA Journal 2011;9(1):196

Endpoints in **bold** were used for the risk assessment purposes

9.3.1.1 Justification for new endpoints

Not relevant

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment

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

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

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

Table 9.3-2: Screening ~~First-tier~~ assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/DIK 480 SL in maize

Intended use		Maize				
Active substance/product		Dicamba				
Application rate (g/ha)		1 × 288				
Acute toxicity (mg/kg bw)		1581				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Screening Step	Small herbivorous mammal	136.4	1	39.28	40.2	
Reprod. toxicity (mg/kg bw/d)		150				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Screening Step	Small herbivorous mammal	72.3	0.53	11.04	13.59	

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.

9.3.2.2 Higher-tier risk assessment

Not required.

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 12.36, dicamba belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha) = 288		
Acute toxicity (mg/kg bw) = 1581	quotient	= 0.18
Reprod. toxicity (mg/kg bw/d) = 150	quotient	= 1.92

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of dicamba amounts to 0.55 – 1.9 (at pH 5.0 – 8.9) ~~1.8~~ and for its metabolite DCSA is -0.84 (pH 6.8), 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

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

CHR/H/DIK 480 SL no pose any unacceptable risk to mammal.

zRMS comments:

The acute and long-term risk assessment for mammals performed by the Applicant was agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.

All TER_A and TER_{LT} values are above a trigger value of 5 or 10 respectively (Screening step).

CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL presents no unacceptable risk to mammals resulting from exposure via drinking water. Since the log Pow value of dicamba and its relevant aquatic metabolite DCSA are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

Not required.

zRMS comments:

This issue is not assessed at the product level.

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 dicamba and its relevant aquatic metabolites DCSA. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of CHR/H/DIK 480 SL were not evaluated as part of the EU assessment of dicamba. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
<i>Cyprinus carpio</i>	Dicamba	96 h, s	EC50 > 100 mg a.s./L nom	EFSA Journal 2011;9(1):1965
<i>Oncorhynchus mykiss</i>	DCSA	96 d, s	EC50 > 100 mg as/L	EFSA Journal 2011;9(1):1965

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Dicamba	21 d	NOEC = 180 mg as/L	EFSA Journal 2011;9(1):1965
<i>Daphnia magna</i>	Dicamba (tested as Banvel 480 SL)	48 h, s	EC₅₀ > 41 mg a.s./L_{nom}	EFSA Journal 2011;9(1):1965
<i>Daphnia magna</i>	Dicamba	21 d, ss	NOEC = 97 mg as/L	EFSA Journal 2011;9(1):1965
<i>Daphnia magna</i>	DCSA	48h, s	EC50 = 89 mg as/L	EFSA Journal 2011;9(1):1965
<i>Skeletonema costatum</i>	Dicamba	72 h, s	Biomass: EbC50 = 1.8 mg as/L Growth rate: ErC50 > 4.1 mg as/L	EFSA Journal 2011;9(1):1965
<i>Navicula pelliculosa</i>	Dicamba	72 h, s	Biomass: EbC50 > 3.8 mg as/L Growth rate: ErC50 > 3.8 mg as/L	EFSA Journal 2011;9(1):1965
<i>Anabaena flos-aque</i>	Dicamba	72 h, s	Biomass: EbC50 > 32 mg as/L Growth rate: ErC50 > 32 mg as/L	EFSA Journal 2011;9(1):1965
<i>Selenastrum capricornutum</i>	DCSA	72 h, s	Biomass: EbC50 = 118 mg as/L Growth rate: ErC50 = 138 mg as/L	EFSA Journal 2011;9(1):1965
<i>Myriophyllum spicatum</i>	Dicamba	26d, s	EbC50 > 0.45 mg as/L ErC50 > 0.45 mg as/L	EFSA Journal 2011;9(1):1965
<i>Lemna gibba</i>	Dicamba	7d, s	EbC50 > 3.25 mg as/L ErC50 = - mg as/L	EFSA Journal 2011;9(1):1965
<i>Lemna gibba</i>	DCSA	7d, s	EbC50 = 11.9 mg as/L ErC ₅₀ > 73 mg as/L	EFSA Journal 2011;9(1):1965
Higher-tier studies (micro- or mesocosm studies)				

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 – CHR/H/DIK 480 SL

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	CHR/H/DIK 480 SL	48h, s	EC ₅₀ > 100 mg <u>formulation</u> /L	T. Turek-Lipka, Study code: W-23-21
<i>Pseudokirchneriella subcapitata</i>	CHR/H/DIK 480 SL	72 h, s	ErC ₅₀ > 1000 mg <u>formulation</u> /L _{nom}	M. Czarnecka, Study code: W-24-21

Species	Substance	Exposure System	Results	Reference
			EyC ₅₀ = 333 375.2 mg formulation/L _{nom}	
<i>Anabaena flos-aquae</i>	CHR/H/DIK 480 SL	72 h, s	ErC ₅₀ > 1000 mg formulation/L _{nom} EyC ₅₀ = 716.5 mg formulation/L _{nom} ErC ₅₀ > 1000 mg formulation/L	M. Czarnecka, Study code: W-26-21
<i>Lemna Gibba</i>	CHR/H/DIK 480 SL	7d, s	ErC ₅₀ > 250 4000 mg/L _{nom}	M. Czarnecka, Study code: W-25-21
Higher-tier studies (micro- or mesocosm studies)				

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

zRMS comments:

Since the EyC₅₀ is lower than ErC₅₀ in W-26-21 study with the *Anabaena flos-aquae* and in the study W-24-21 with *Pseudokirchneriella subcapitata* zRMS agree to use this value as a worst case scenario for RA purposes.

In the study W-24-21 at the nominal concentration of 333 mg formulation/L already 50.6 % inhibition of yield was observed. At this concentration deformed cells (50% of rod-shaped cells and 50% of swollen cells) were reported as compared to the algae cells in the control. Therefore value of EyC₅₀= 333 mg formulation/L would be used for RA purposes as a worst case EyC₅₀ for yield parameter

In the study W-25-21 at the nominal concentration of 250 mg formulation/L already 56. % inhibition (based on the frond number) was observed. Therefore value of ErC₅₀= 250 mg formulation/L should be used for RA purposes as a worst case ErC₅₀ for growth yield parameter based on the frond number.

9.5.2 Risk assessment

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

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

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 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/H/DIK 480 SL in maize

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plant
Test species		<i>Cyprinus carpio</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Seletonema costatum</i>	<i>Myriophyllum spictatum</i>
Endpoint (µg/L)		LC ₅₀ 100 000	NOEC 180 000	EC ₅₀ -	NOEC 97 000	E _r C ₅₀ /E _y C ₅₀ 1 800	NOEC 450
AF		100	10	100	10	10	10
RAC (µg/L)		1000	1800	-	9700	180	45
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	97.09 97.41	0.00953 0.09741	0.00529 0.05412	-	0.00098 0.01004	0.05294 0.54117	0.21178 2.1647
Step 2							
N-Europe	9.53 9.56	<0.0001 0.00956	<0.0001 0.00531	-	<0.0001 0.00099	<0.0001 0.05311	0.00001 0.21244

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DCSA for each organism group based on FOCUS Steps 1 calculations for the use of CHR/H/DIK 480 SL in maize

Group		Fish acute	Inverteb. prolonged	Algae	Aquatic plant
Test species		<i>Cyprinus carpio</i>	<i>Daphnia magna</i>	<i>Seletonema costatum</i>	<i>Myriophyllum spictatum</i>
Endpoint (µg/L)		LC ₅₀ 100 000	NOEC 89 000	E _r C ₅₀ /E _y C ₅₀ 118000	NOEC 11900

Group		Fish acute	Inverteb. prolonged	Algae	Aquatic plant
AF		100	10	10	10
RAC (µg/L)		1000	8900	11800	1190
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	31.83 38.17	0.03183 0.03817	0.00358 0.00429	0.00270 0.00323	0.02675 0.03208

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CHR/H/DIK 480 SL for each organism group based on FOCUS Drift calculator calculations for the use of CHR/H/DIK 480 SL in maize

Group		Inverteb. acute	Algae	Algae	Aquatic plant
Test species		<i>Daphnia magna</i>	<i>Pseudokirchnella subcapitata</i>	<i>Anabaena flos-aque</i>	<i>Lemna gibba</i>
Endpoint		EC ₅₀	ErC ₅₀ /EyC ₅₀ *	EyC ₅₀ *	ErC ₅₀
(µg/L)		100000	333000 375200	716500	250000 1000
AF		100	10		10
RAC (µg/L)		1000	33300 37520	71650	25000 100
FOCUS Scenario	PEC _{gl-max} (µg/L)				
1 meters buffe zone					
	3.6882	0.00369	0.00010	0.00005	0.000147-0.03688
* Since the EyC50 is lower than ErC50 in study zRMS agree to use this value as a worst case scenario for RA purposes.					

9.5.3 Overall conclusions

CHR/H/DIK 480 SL no pose any unacceptable risk to aquatic organisms. No mitigation measures required.

zRMS comments:

The evaluation of the risk for aquatic was performed in accordance with Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009(EFSA Journal 2013;11(7):3290).

For the active substance dicamba and its metabolite DCSA calculated PEC/RAC ratios for maize did indicate an acceptable risk

For the formulated product, no potential risks are identified for aquatic organisms following application of CHR/H/DIK 480 SL to maize. No mitigation measures are required.

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 dicamba and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of CHR/H/DIK 480 SL were not evaluated as part of the EU assessment of dicamba. New data submitted with this application are listed in **Appendix 1** ~~Błąd! Nie można odnaleźć źródła odwołania.~~ and summarised in Appendix 2.

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>	Dicamba	Oral	LD ₅₀ > 100 µg/bee	EFSA Journal 2011;9(1):1965
<i>Apis mellifera</i>	Dicamba	Contact	LD ₅₀ > 100 µg/bee	EFSA Journal 2011;9(1):1965
<i>Apis mellifera</i>	CHR/H/DIK 480 SL	Oral	LD ₅₀ > 200 µg/bee	A. Fulczyk, Study code: B-57-21
<i>Apis mellifera</i>	CHR/H/DIK 480 SL	Contact	LD ₅₀ > 200 µg/bee	A. Fulczyk, Study code: B-58-21
Apis mellifera	CHR/H/DIK 480 SL	Chronic Oral	LC₅₀ > 667 mg/kg LDD₅₀ > 17.2 µg/bee/day	A. Fulczyk, Study code: B-63-21
Apis mellifera	CHR/H/DIK 480 SL	Larval Repeated Exposure	EC₅₀ > 649.4 mg/kg ED₅₀ > 100 µg/larva NOEC = 324.7 mg/kg NOED =50 µg/larva	A. Fulczyk, Study code: B-64-21
Higher-tier studies (tunnel test, field studies)				

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 (SAN-

CO/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 CHR/H/DIK 480 SL in maize

Intended use			
Active substance		Dicamba	
Application rate (g/ha)		1 × 288	
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	100	288	2.88
Contact toxicity	100		2.88
Product		CHR/H/DIK 480 SL	
Application rate (g/ha)		1 × 694.32	
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	200	694.32	3.47
Contact toxicity	200		3.47

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

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

Not relevant.

zRMS comments:

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

The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible risk associated with the exposure of bees to CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL.

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): the Applicant provided chronic test on bees and evaluation of effects on honey bee development with formulated product.

Concerned Member States must decide on the consideration of data requirements on national level.

9.6.3 Effects on bumble bees

Not relevant

zRMS comments:

According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.4 Effects on solitary bees

Not relevant.

zRMS comments:

According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.5 Overall conclusions

CHR/H/DIK 480 SL no pose any unacceptable risk to bees.

zRMS comments:

The evaluation has been performed in line with SANCO/10329/2002 rev 2 final. The risk assessment performed for active substance prohexadione calcium and the formulated product CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL is agreed by the zRMS.

The acute hazard quotients for the active substance and for the formulation are below the trigger value of 50 with large margins of safety, indicating an acceptable acute risk to bees from exposure to CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL in maize.

Concerned Member States must decide on the consideration of data requirements on national level.

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 dicamba and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of CHR/H/DIK 480 SL were not evaluated as part of the EU assessment of dicamba. 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)	CHR/H/DIK 480 SL	An extended laboratory test glass plates (2D)	LR ₅₀ > 0.6 L/ha, which is equivalent to 694.31 g/ha ER ₅₀ > 0.6 L/ha which is equivalent to 694.31 g/ha	A. Grande, Study code: B-59-21 B-69-21
<i>Aphidius rhopalosiphi</i> (adults)	CHR/H/DIK 480 SL	An extended laboratory test glass plates (2D)	LR ₅₀ > 0.6 L/ha, which is equivalent to 694.31 g/ha ER ₅₀ > 0.6 L/ha which is equivalent to 694.31 g/ha	A. Grande, Study code: B-60-21
<i>Coccinella septempunctata</i>	CHR/H/DIK 480 SL	An extended laboratory test glass plates (2D)	LR ₅₀ > 0.6 L/ha, which is equivalent to 694.31 g/ha ER ₅₀ > 0.6 L/ha which is equivalent to 694.31 g/ha	A. Fulczyk, Study code: B-61-21
<i>Chrysoperla carnea</i>	CHR/H/DIK 480 SL	An extended Laboratory test glass plates (2D)	LR ₅₀ > 0.6 L/ha, which is equivalent to 694.31 g/ha ER ₅₀ > 0.6 L/ha which is equivalent to 694.31 g/ha	M. Knapik, Study code: B-62-21

Species	Substance	Exposure System	Results	Reference
Field or semi-field tests				

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: ~~The Tier II based on extended laboratory studies~~ ~~First- and higher-tier assessment~~ of the in-field risk for non-target arthropods due to the use of CHR/H/DIK 480 SL in maize

Intended use		maize		
Active substance/product		CHR/H/DIK 480 SL		
Application rate (g/ha)		1 × 694.32		
MAF		1		
Test species Tier II	LR ₅₀ (lab.) (g/ha)	PER _{in-field} (g/ha)	HQ _{in-field} criterion: HQ ≤ 1	
<i>Typhlodromus pyri</i>	694.32	694.32	1	
<i>Aphidius rhopalosiphi</i>	694.32		1	
<i>Coccinella Septempunctata</i>	694.32		1	
<i>Chrysoperla Carnea</i>	694.32		1	

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.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

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 CHR/H/DIK 480 SL in maize

Intended use		Maize			
Active substance/product		CHR/H/DIK 480 SL			
Application rate (g/ha)		1 × 694.32			
MAF		1			
vdf		1			
Test species Tier II	LR ₅₀ (lab.) (g/ha)	Drift rate	PER _{off-field} (g/ha)	CF	HQ _{off-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i>	694.32	0.0277	19.23	5	0.1391
<i>Aphidius rhopalosiphi</i>	694.32				
<i>Coccinella Septempunctata</i>	694.32				
<i>Chrysoperla Carnea</i>	694.32				

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.

* If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

zRMS comments:

Since the first-tier risk assessment indicates that CHR/H/DIK 480 SL does not pose an unacceptable risk to non-target arthropods, further assessment is not necessary.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

MAF: Multiple application factor; PER: Predicted environmental rates; HQ: Hazard quotient; Criteria values shown in bold breach the relevant trigger.

9.7.3 Overall conclusions

CHR/H/DIK 480 SL no pose any unacceptable risk to non-target arthropods other than bees.

zRMS comments:

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the guidance document ESCORT 2.

At TIER 1 not acceptable in-field risk for *Typhlodromus pyri* and *Aphidius* was indicated. Additional study for *Chrysoperla carnea* and *Coccidella septapunctata* was performed indicated low risk for arthropods.

The HQ for recommended species: *Typhlodromus pyri*, *Aphidius rhopalosiphii*, *Chrysoperla carnea* and *Coccidella septapunctata* is below the ESCORT 2 trigger value of 1 indicating acceptable off-field risk to non-target arthropods at tier II level.

On this basis acceptable risk for in-field and off-field habitats may be concluded with no need for risk mitigation measures.

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 dicamba and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CHR/H/DIK 480 SL were not evaluated as part of the EU assessment of dicamba. 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>	Dicamba	Acute, 14d	LC ₅₀ > 1000 mg as/kg/dw soil	EFSA Journal 2011;9(1):1965
<i>Eisenia fetida</i>	Dicamba	Chronic, 8 w	Not required	EFSA Journal 2011;9(1):1965

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	DCSA	Acute	LC50 > 1000 mg DCSA/kg dw soil	EFSA Journal 2011;9(1):1965
<i>Eisenia fetida</i>	DCSA	Chronic	Not required	EFSA Journal 2011;9(1):1965
<i>Eisenia fetida</i>	CHR/H/DIK 480 SL	Chronic	NOEC > 1000 mg formulation/kg dw soil EC ₁₀ > 1000 mg formulation /kg dw soil	A. Gierbuszewska, Study code: G-09-21
<i>Folsomia candida</i>	CHR/H/DIK 480 SL	Chronic	NOEC _{reproduction} > 1000 mg formulation/kg dw	P. Pieczka, Study code: G-10-21
<i>Hypoaspis aculeifer</i>	CHR/H/DIK 480 SL	Chronic	NOEC _{reproduction} = 560 mg formulation/kg dw	M. Czarnynoga, Study code: G-11-21
Field studies				
Litter bag test				

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

9.8.1.1 Justification for new endpoints

Not relevant

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 does not need to be considered for dicamba.

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 CHR/H/DIK 480 SL in maize

Intended use			
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Not required			
Chronic effects on earthworms			

Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Dicamba	-	0.288	-
DCSA	-	0.203	-
CHR/H/DIK 480 SL	1000	0.6943	1440
Chronic effects on other soil macro- and mesofauna – Folsomia Candida			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Dicamba	-	0.288	-
DCSA	-	0.203	-
CHR/H/DIK 480 SL	1000	0.6943	1440
Chronic effects on other soil macro- and mesofauna – Hypoaspis aculeifer			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Dicamba	-	0.288	-
DCSA	-	0.203	-
CHR/H/DIK 480 SL	560	0.6943	806

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

zRMS comments:

A higher tier assessment is not required based on the low risk indicated in the chronic assessment on earthworms, collembolan, and soil mite.

9.8.3 Overall conclusions

CHR/H/DIK 480 SL no pose any unacceptable risk to soil organisms.

zRMS comments:

The risk assessment for earthworms exposed to formulation following application of CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL according to critical GAP was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology” (SANCO/10329/2002) and was accepted by the zRMS. Applicant presented also assessment for *Folsomia* and *Hypoaspis*.

The relevant PEC_{soil} for risk assessments is taken from Section 8 (Environmental Fate), for details please, refer to Section 8.

TER_{lt} values calculated for CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL were above the triggers indicating acceptable long-term risk to earthworms and other non-target soil organisms No further evaluation is deemed necessary.

Overall, acceptable risk could be concluded for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL in maize according to GAP.

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 dicamba and its relevant metabolites.

Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of CHR/H/DIK 480 SL were not evaluated as part of the EU assessment of dicamba. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Dicamba	28 d, aerobic soil type	0 % effect at day 28 at 6.4 and 2.4 mg a.s./kg dw soil, respectively	EFSA Journal 2011;9(1):1965
C-mineralisation	dicamba	28 d, aerobic soil type	0 % effect at day 28 at 6.4 and 2.4 mg a.s./kg dw soil, respectively	EFSA Journal 2011;9(1):1965
N-mineralisation	CHR/H/DIK 480 SL	42 d, aerobic soil type	On the basis of the results, it was concluded that CHR/H/DIK 480 SL at the concentrations corresponding to the PEC: 1.39 mg test item/kg dry weight of soil (i.e. 0.57 mg of dicamba/kg dry weight of soil) and 5 x PEC: 6.94 mg test item/kg dry weight of soil (i.e. 2.85 mg of dicamba/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	M. Czarnynoga, Study code: G-12-21

9.9.1.1 Justification for new endpoints

No relevant

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 CHR/H/DIK 480 SL in maize

Intended use	
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N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC_{soil} (mg/kg dw)	Risk acceptable?
Dicamba	0 % effect at day 28 at 6.4 and 2.4 mg a.s./kg dw soil, respectively	0.288	yes
CHR/H/DIK 480 SL	On the basis of the results, it was concluded that CHR/H/DIK 480 SL at the concentrations corresponding to the PEC: 1.39 mg test item/kg dry weight of soil (i.e. 0.57 mg of dicamba/kg dry weight of soil) and 5 x PEC: 6.94 mg test item/kg dry weight of soil (i.e. 2.85 mg of dicamba/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	0.6943	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC_{soil} (mg/kg dw)	Risk acceptable?
Not required			

9.9.3 Overall conclusions

CHR/H/DIK 480 SL no pose any unacceptable risk to soil microbial activity.

zRMS comments:

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) and was generally accepted by the zRMS. The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), for details please, refer to Section 8.

Based on the obtained results, soil nitrate formation rates were below the 25% trigger value. Thus, it is concluded that CHR/H/DIK 480 SL had no significant impact on soil microorganisms when applied at test item concentrations up to 6.94 mg formulation/kg dry weight of soil, did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

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 dicamba and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target terrestrial plants of CHR/H/DIK 480 SL were not evaluated as part of the EU assessment of dicamba. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

Species	Substance	Exposure System	Results (ml/product/ha)	Reference
<i>Flax</i>	CHR/H/DIK 480 SL	21 d Seedling emergence	ER50 = 54.473 ml/ha which is equivalent to 63.04 g /ha	A. Gierbuszewska, Study code: G-14-21
<i>Tomato</i>			ER50 = 5.063 ml/ha which is equivalent to 5.86 g /ha	
<i>Pea</i>			ER50 = 33.571ml/ha which is equivalent to 38.85 g /ha	
<i>Carrot</i>			ER50 = 90.976 ml/ha which is equivalent to 105.28 g /ha	
<i>Onion</i>			ER50 = 57.235 ml/ha which is equivalent to 66.23 g /ha	
<i>Wheat</i>			ER50 = 494.991 ml/ha which is equivalent to 572.8 g /ha	
<i>Flax</i>		21 d Vegetative Vigour	ER50 = 22.04 ml/ha which is equivalent to 25.5 g /ha	P. Pieczka, Study code: G-13-21
<i>Tomato</i>			ER50 = 15.28 ml/ha which is equivalent to 17.68 g /ha	
<i>Pea</i>			ER50 = 37.47 ml/ha which is equivalent to 43.36 g /ha	
<i>Carrot</i>			ER50 = 392.49 ml/ha which is equivalent to 454.19 g /ha	
<i>Onion</i>			ER50 = 260.48 ml/ha which is equivalent to 301.43 g /ha	
<i>Wheat</i>			ER50 >600 ml/ha which is equivalent to 63.04 g /ha	

m: monocotyledonous; d: dicotyledonous

in **bold** the lowest value from the study
formulation density- 1.1572 g/ml

9.10.1.1 Justification for new endpoints

Not relevant.

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”, (SAN-CO/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 CHR/H/DIK 480 SL in maize

Intended use		Maize		
Active substance/product		CHR/H/DIK 480 SL		
Application rate (g formulation /ha)		1 × 694.32		
MAF		1		
Test species	ER₅₀ (g formulation /ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
<i>Flax</i>	63.04	0.0277	19.23	3.27
<i>Tomato</i>	5.86	0.0277	19.23	0.30
<i>Pea</i>	38.85	0.0277	19.23	2.02
<i>Carrot</i>	105.28	0.0277	19.23	5.47
<i>Onion</i>	66.23	0.0277	19.23	3.44
<i>Wheat</i>	572.8	0.0277	19.23	29.79
<i>Flax</i>	25.5	0.0277	19.23	1.32
<i>Tomato</i>	17.68	0.0277	19.23	0.91
<i>Pea</i>	43.36	0.0277	19.23	2.24
<i>Carrot</i>	454.19	0.0277	19.23	23.61
<i>Onion</i>	301.43	0.0277	19.23	15.67

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.10-3: Risk assessment for non-target terrestrial plants due to the use of CHR/H/DIK 480 SL in maize considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Maize			
Active substance/product		CHR/H/DIK 480 SL			
Application rate (g formulation/ha)		1 × 694.32			
MAF		1			
Buffer strip (m)	Drift rate (%)	PER_{off-field} (g/ha)	PER_{off-field} 50 % drift red. (g/ha)	PER_{off-field} 75 % drift red. (g/ha)	PER_{off-field} 90 % drift red. (g/ha)
1	2.77	19.23	9.62	4.81	1.92
5	0.57	3.96	1.98	0.99	0.40
10	0.29	2.01	1.01	0.50	0.20
15	0.20	1.38	0.69	0.35	0.14
20	0.15	1.04	-	-	-
Toxicity value		TER			
ER ₅₀ = 5.86 g formulation/ha (lowest endpoint)		criterion: TER ≥ 5			
1		0.3	0.61	1.22	3.05
5		1.50	2.96	5.92	14.65
10		2.91	5.80	11.72	29.3
15		4.25	8.49	16.74	41.86
20		5.63	-	-	-

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

9.10.3 Overall conclusions

Based on the predicted rates of CHR/H/DIK 480 SL in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/DIK 480 SL according to the GAP of the formulation CHR/H/DIK 480 SL achieve the acceptability criteria TER ≥, with applying:

- 20 m buffer zone
- 10 m and use of 50 % drift reducing nozzles
- 5 m and use of 75 % drift reducing nozzles

zRMS comments:

Risk assessment performed by the Applicant for non-target terrestrial plants was accepted.

Acceptable risk for non-target terrestrial plants could be concluded for CHR/H/DIK 480 SL when following risk mitigation measures are applied:

For use in maize

- 20 m unsprayed buffer zone or,
- 10 m unsprayed buffer zone with use of 50 % drift reducing nozzles or,
- 5 m unsprayed buffer zone with use of 75 % drift reducing nozzles to non-agricultural land.

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

CHR/H/DIK 480 SL was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Classification according to CLP Regulation:

CLASSIFICATION	
Hazard classes, categories:	Aquatic Chronic 3, H412
LABELLING	
Hazard pictograms:	none
Signal word:	none
Hazard statements:	H412: Toxic to aquatic life with long lasting effects
Precautionary statements:	P391 – Collect spillage. P501 - Dispose of contents/container to an approved waste disposal plant.
EUH401	To avoid risks to man and the environment, comply with the instructions for use.

Standard phrases under Regulation (EU) No 547/2011

SPe 1	Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
SPe3	To protect non target terrestrial plants respect a: - 20 m buffer zone - 10 m and use of 50 % drift reducing nozzles - 5 m and use of 75 % drift reducing nozzles

zRMS comments:

Proposition of the classification has been accepted.

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	T. Turek-Lipka	2021	CHR/H/DIK 480 SL Daphnia magna, Acute Immobilisation Test W-23-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.2/02	M. Czarnecka	2021	CHR/H/DIK 480 SL Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test W-24-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.2/03	M. Czarnecka	2021	CHR/H/DIK 480 SL Anabaena flos-aquae UTEX B 1444, Growth inhibition test W-26-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.2/04	M. Czarnecka	2021	CHR/H/DIK 480 SL Lemna gibba CPCC 310, Growth inhibition test W-25-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP	A. Fulczyk	2021	CHR/H/DIK 480 SL Honeybees (Apis mellifera L.), Acute Oral Toxicity Test	N	Chemrol

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
10.3/01			B-57-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		
KCP 10.3/02	A. Fulczyk	2021	CHR/H/DIK 480 SL Honeybees (<i>Apis mellifera</i> L.), Acute Contact Toxicity Test B-58-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.3/03	A. Grande	2021	An extended laboratory test for evaluating the effects of CHR/H/DIK 480 SL on the predatory mite, <i>Typhlodromus pyri</i> (Sch.). B-59-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.3/04	A. Grande	2021	An extended laboratory test for evaluating the effects of CHR/H/DIK 480 SL on the parasitic wasp, <i>Aphidius rhopalosiphii</i> (De Stefani-Perez). B-60-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.3/05	A. Fulczyk	2021	An extended laboratory test for evaluating effects of CHR/H/DIK 480 SL on the ladybird beetle, <i>Coccinella septempunctata</i> L. B-61-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland	N	Chemrol

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
			GLP Unpublished		
KCP 10.3/06	M. Knapik	2021	An extended laboratory test for evaluating effects of CHR/H/DIK 480 SL on the green lacewing, <i>Chrysoperla carnea</i> (Steph.) B-62-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemiroł
KCP 10.3/07	A. Fulczyk	2022	CHR/H/DIK 480 SL Honeybees (<i>Apis mellifera</i> L.), Chronic Oral Toxicity Test B-63-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemiroł
KCP 10.3/08	A. Fulczyk	2022	CHR/H/DIK 480 SL Honeybees (<i>Apis mellifera</i> L.), Larval Toxicity Test, Repeated Exposure B-64-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemiroł
KCP 10.4/01	A. Gierbuszewska	2021	CHR/H/DIK 480 SL Earthworm reproduction test (<i>Eisenia andrei</i>) G-09-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemiroł
KCP 10.4/02	P. Pieczka	2021	CHR/H/DIK 480 SL Collembolan (<i>Folsomia candida</i>) Reproduction Test G-10-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna	N	Chemiroł

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
			Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished		
KCP 10.4/03	M. Czarnynoga	2021	CHR/H/DIK 480 SL Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil G-11-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.5/01	M. Czarnynoga	2021	CHR/H/DIK 480 SL Soil Microorganisms: Nitrogen Transformation Test G-12-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.6/01	A. Gierbuszewska	2021	CHR/H/DIK 480 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test G-14-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol
KCP 10.6/02	P. Pieczka	2021	CHR/H/DIK 480 SL Terrestrial Plant Test: Vegetative Vigour Test G-13-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	Chemrol

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
KCP 10.1/01	xxxxxxxxxx	1993	Technical Dicamba: an acute oral toxicity study with northern bobwhite Report No 131-179A xxxxxxxxxx GLP Unpublished	Y	Syn
KCP 10.1/02	xxxxxxxxxx	1977	Eight-day dietary LC50 – Bobwhite quail Report No 107-149 xxxxxxxxxx Not GLP Unpublished	Y	Syn
KCP 10.1/03	xxxxxxxxxx	1977	Eight-day dietary LC50 – mallard duck Report No 107-150 xxxxxxxxxx Not GLP Unpublished	Y	Syn
KCP 10.1/04	xxxxxxxxxx	1994	Technical Dicamba: a reproduction study with the Northern Bobwhite Report 131-182 xxxxxxxxxx GLP Unpublished	Y	Syn
KCP 10.1/05	xxxxxxxxxx	1994	Technical Dicamba: a reproduction study with the mallard Report No 131-183 xxxxxxxxxx GLP Unpublished	Y	Syn
KCP 10.2/01	xxxxxxxxxx	1993	3,6-dichlorosalicylic acid – 96 hour toxicity to rainbow trout (oncorhynchus mykiss) under semi-static conditions Repor SAZ 435©/930344	Y	Syn

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
			xxxxxxxxxx GLP Unpublished		
KCP 10.2/02	xxxxxxxxxx	2004	Acute toxicity of dicamba tech. (SAN 837) to common carp (Cyprinus carpio) in a 96-hour static test Report no. 848757 GLP Unpublished	Y	Syn
KCP 10.2/03	xxxxxxxxxx	1990	Dicamba – Study of Prolonged Toxicity (21d) to fish (Rainbow Trout) Report No 1544 xxxxxxxxxx GLP Unpublished	Y	SYN
KCP 10.2/04	Douglas, M.T	1993	3,6-dichlorosalicylic acid – 48 hour toxicity to Daphnia magna under semi-static conditions Report No SAZ 435(B)/930346 Novartis Crop Protection AG, Switzerland Huntingdon Research Centre, LTD GLP Unpublished	N	SYN
KCP 10.2/05	Douglas, M.T.	1993	Prolonged toxicity (21 day exposure) to Daphnia magna under semi-static conditions Novartis Crop Protection AG, Basel, Switzerland Huntingdon Research Centre Ltd., Huntingdon, United Kingdom, Report No SAZ 439/931396 GLP Not Published Syngenta File N° SAN837/5332	N	SYN
KCP 10.2/06	Douglas, M.T. <i>et al.</i>	1993	3,6-dichlorosalicylic acid - 72 hour algal growth inhibition under static conditions Novartis Crop Protection AG, Basel, Switzerland Huntingdon Research Centre Ltd., Huntingdon, United Kingdom, Report No SAZ 435(A)/930347 GLP Not Published Syngenta File N° NOA414746/0004	N	Syn

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 10.2/07	Hoberg, J.R.	1993	Dicamba technical - toxicity to the marine diatom, <i>Skeletonema costatum</i> Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No 93-3-4699 GLP Not Published Syngenta File N° SAN837/5224	N	SYN
KCP 10.2/08	Hoberg, J.R.	1993	Dicamba technical - toxicity to the freshwater diatom, <i>Navicula pelliculosa</i> Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No 92-11-4512 GLP Not Published Syngenta File N° SAN837/5229	N	Syn
KCP 10.2/09	Hoberg, J.R.	1993	Dicamba technical - toxicity to the freshwater green alga, <i>Selenastrum capricornutum</i> Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No 92-11-4498 GLP Not Published Syngenta File N° SAN837/5230	N	Syn
KCP 10.2/10	Grade, R.	2002	Growth Inhibition Test of NOA 414746 (Metabolite of SAN 837) to the Duckweed <i>Lemna gibba</i> G3 under Static Condition Syngenta Crop Protection AG, BASEL, Switzerland, Report No. 2011570 GLP Unpublished	N	SYN
KCP 10.2/11	Hoberg, J.R.	1993	Dicamba technical - toxicity to the duckweed, <i>Lemna gibba</i> Novartis Crop Protection AG, Basel, Switzerland Springborn Laboratories Inc., Wareham, United States, Report No 93-3-4665 GLP Not Published Syngenta File N° SAN837/5223	N	SYN
KCP	Volz, E.	2003	SAN 837 Tech: Toxicity test of Dicamba to an aquatic macrophyte, the eurasian watermilfoil <i>Myriophyllum</i>	N	SYN

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
10.2/12			lum spicatum in a static test system Syngenta Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 848730 / 2021781 GLP Not Published Syngenta File N° SAN837/6133		
KCP 10.3/01	Hillesheim, E.	1993	Laboratory studies on the oral toxicity of Dicamba (acid) and Banvel 4S (DMA-salt) to worker honeybees Novartis Crop Protection AG, Basel, Switzerland Sandoz Ltd., Agro Research, Witterswil, Switzerland, Report No 2/93 GLP Not Published Syngenta File N° SAN837/5339	N	SYN
KCP 10.4/01	Barth, M.	2001	Acute toxicity of Dicamba tech. to the earthworm Eisenia fetida Syngenta Crop Protection AG, Basel, Switzerland BioChem agrar, Gerichshain, Germany, Report No 011048003 GLP Not Published Syngenta File N° SAN837/5970	N	SYN
KCP 10.4/02	Barth, M.	2001	Acute toxicity of NOA 414746 (metabolite of dicamba tech.) to the earthworm Eisenia fetida Syngenta Crop Protection AG, Basel, Switzerland BioChem agrar, Gerichshain, Germany, Report No 011048004 GLP Not Published Syngenta File N° NOA414746/0007	N	SYN
KCP 10.5/01	Seyfried, B.	2002	The Effects of SAN 837 (DICAMBA TECH.) on Soil Respiration and Nitrification Syngenta CROP Protection AG, BASEL< Swietzerland RCC LTD., Itingen, Switzerland, Report NO 808086	N	SYN

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
			GLP Unpublished		
KCP 10.5/02	Scholtz, R.	1994	Dicamba – Test for Inhibition of Oxygen Consumption by Activated Sludge (OECD 209) – Respiration Inhibition Test Novartis Crop Protection AG, Basel, Switzerland MBT Umwelttechnik AG, Zurich, Switzerland, Report No MBT 236G,94 GLP Unpublished	N	SYN

Appendix 2 Detailed evaluation of the new studies

zRMS comments:

In order to provide sufficient details, where appropriate, the study summaries have been adapted by the zRMS from the full study reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

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 2.2.1.1.1	Study 1

zRMS comments:	<p>The study was conducted to OECD guideline 202 and according to the principles of GLP.</p> <p>In the definitive test the validity criteria were met according to OECD Guideline No. 202. No deviations from the guideline were noted during the study.</p> <p>The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations. Following endpoints based on nominal test item concentrations would be used for risk assessment purposes:</p> <p>EC50/48 h is > 100 mg formulation/L.</p>
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Reference:	KCP 10.2/01
Report	CHR/H/DIK 480 SL Daphnia magna, Acute Immobilisation Test, T. Turek-Lipka, 2021, Study code: W-23-21
Guideline(s):	according to the OECD Guideline No. 202 (2004)/EU method C.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

Immobilisation of *Daphnia magna* exposed to the test item, CHR/H/DIK 480 SL was investigated during a 48-hour static test. The *Daphnia magna* were observed for immobilization after 24 and 48 h of exposure. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel. At exposure termination in the test item concentration of 100 mg/L and the control, no immobilization of *Daphnia magna* was observed. No abnormal behavior of *Daphnia magna* was observed during exposure.

Materials and methods

Material and methods:

Test item: CHR/H/DIK 480 SL; batch no.: 012021; the content of dicamba: 475.25 g/L; density: 1.1568 g/cm³; manufacturing date: January 31, 2021, expiry date: January 31, 2023.

Test system: *Daphnia magna* Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna

Test design: Static test (48 h of exposure); 4 replicates per each test item concentration and the control; 5 *Daphnia magna* in each replicate.

Nominal test item concentration:
100 mg/L plus the control.

Test conditions: Temperature: 19.0 – 20.3°C; pH of the control: 7.79 – 7.91;
dissolved oxygen concentration in the control: 8.6 – 8.8 mg/L;
daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.

Chemical determinations:

The concentration of dicamba in the test item concentration was determined using a validated high performance liquid chromatographic method with DAD

Endpoint value: EC₅₀/48 h, LOEC, NOEC

Results and discussions

Immobilisation of *Daphnia magna* exposed to the test item, CHR/H/DIK 480 SL was investigated during a 48-hour static test. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentration or the control per replicate. The definitive test was performed with a single test item concentration of 100 mg/L as a limit test plus the control.

The *Daphnia magna* were observed for immobilisation after 24 and 48 h of exposure. The *Daphnia magna* were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.

At exposure termination in the test item concentration of 100 mg/L and the control, no immobilisation of *Daphnia magna* was observed. No abnormal behaviour of *Daphnia magna* was observed during exposure. The concentrations of dicamba in the test item concentration, were determined using a validated high performance liquid chromatographic method with DAD detection.

Table 1. Immobilization of *Daphnia magna*, definitive test

Nominal test item concentration [mg/L]	Number of <i>Daphnia magna</i>	Number of immobilised <i>Daphnia magna</i>								Total of immobilised <i>Daphnia magna</i> [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
100	20	0	0	0	0	0	0	0	0	0	0

Analytical measurements

Samples for chemical determination were collected from the test item concentration and the control at exposure initiation and at exposure termination.

In the sample collected at exposure initiation, the determined dicamba concentration was 107.1% of nominal concentration. Therefore, the test item concentration was prepared correctly.

In the sample collected at exposure termination, the determined dicamba concentration was 106.8% of nominal concentration. Therefore, the dicamba concentration was stable under test conditions.

Table 2. Concentration and stability of dicamba, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of dicamba [mg/L]	Average determined concentration of dicamba (n=3) in samples collected			
		at exposure initiation [mg/L]	[%] of nominal concentration	at exposure termination [mg/L]	[%] of nominal concentration
Control	---	< LoD	---	< LoD	---
100	41.1	44.0	107.1	43.9	106.8

LOQ = 10 mg/L
 LOD = 1 mg/L

The endpoint values were determined based on the nominal test item concentration.

Conclusion

The endpoint values based on nominal test item concentrations are given below:

the EC50/48 h is higher than 100 mg/L;

the LOEC/48 h is higher than 100 mg/L;

the NOEC/48 h is higher than or equal to 100 mg/L.

Table 3. Endpoints values based on the nominal test item concentration

Endpoint value [mg formulation/L]	Time of exposure	
	24 h	48 h
EC50	>100	>100
LOEC	>100	>100
NOEC	>100	>100

TEST VALIDITY CRITERIA

In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004) and EU Method C.2.:

- the percentage of immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 8.3 – 8.8 mg/L (criteri-

on: not less than 3 mg/L).

A 2.2.1.1.2 Study 2

zRMS comments:	<p>The study was conducted to OECD guideline 201 and according to the principles of GLP.</p> <p>In the definitive test the validity criteria were met according to OECD Guideline No. 201. In the experimental part of the study, no deviations occurred from the guideline.</p> <p>The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment.</p> <p>The ErC50/72 h value is higher than the highest test item concentration used for the exposure, i.e. 1000 mg/L.</p> <p>The LOEC/72 h value for growth rate is 111 mg/L.</p> <p>The NOEC/72 h value for growth rate is 37 mg/L.</p> <p>The EyC50/72 h value is 716.5 mg/L (95% confidence interval: 360.9 – >1000).</p> <p>The LOEC/72 h value for yield is 111 mg/L.</p> <p>The NOEC/72 h value for yield is 37 mg/L.</p>
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Reference:	KCP 10.2/02
Report	CHR/H/DIK 480 SL <i>Anabaena flos-aquae</i> UTEX B 1444, Growth inhibition test, M. Czarnecka, 2021, Study code: W-26-21
Guideline(s):	according to the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The growth of the cyanobacteria *Anabaena flos-aquae* exposed to the test item, CHR/H/DIK 480 SL was investigated during a 72-hour test. The number of cyanobacterial cells was determined with a direct method, which involves counting the number of cells in the Bürker chamber under a microscope. In case of each replicate, the number of cells was determined after 24, 48, and 72 h of exposure. Morphology observations of the cyanobacteria cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and color of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control.

Materials and methods

Test item: CHR/H/DIK 480 SL; batch no. 012021; the content of dicamba: specification 480.0 ± 24.0 g/L, result of the test 475.25 g/L; density at 20°C: 1.1568 g/cm³; manufacturing date: January 31, 2021, expiry date: January 31, 2023.

Test system: The freshwater cyanobacteria, *Anabaena flos-aquae* (Lyng.) Bréb UTEX B 1444 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The culture was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA.

Test design: 72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial cyanobacterial cell density: 1 x 10⁴ cells/mL.

Nominal test item concentrations:

1000, 333, 111, 37 and 12.3 mg/L plus the control.

Test conditions: Temperature: 22.4 – 22.8°C; pH of the control: 7.24 – 8.41;

mean light intensity: 3415 - 3580 lux; constant illumination and

shaking; medium: AAP.

Statistics: Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure.

Endpoint values: ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

Results and discussions

The growth of the cyanobacteria *Anabaena flos-aquae* exposed to the test item, CHR/H/DIK 480 SL was investigated during a 72-hour test. The test was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the cyanobacteria was 1 x 10⁴ cells/mL. The definitive test was performed using the following test item concentrations: 1000, 333, 111, 37 and 12.3 mg/L (with a spacing factor of 3.0) plus the control.

The number of cyanobacterial cells was determined with a direct method, which involves counting the number of cells in the Bürker chamber under a microscope. In case of each replicate, the number of cells was determined after 24, 48, and 72 h of exposure.

Table 8. Growth rate and yield inhibition – definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure (growth rate)	[%] inhibition after 72 h of exposure (yield)
Control	0.0	0.0
12.3	5.9	16.0
37	0.3	1.5
111	10.1	29.2
333	11.2	32.3
1000	25.5	58.9

Morphology observations of the cyanobacteria cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control. The concentrations of dicamba were chemically determined using the validated high performance liquid chromatographic method (HPLC) with Diode Array Detection (DAD).

Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined. At exposure initiation, the determined concentrations of dicamba was in the range of 105.0 – 106.3% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of dicamba was in the range of 106.9 – 108.9% of nominal concentration. Therefore, the concentrations of dicamba was stable under test conditions.

Table 4. Results from analysis of active substance in test samples

Nominal test item concentration [mg/L]	Nominal concentration of dicamba [mg/L]	Mean concentration of dicamba determined (n=3) in samples collected			
		T0 (11.10.2021) [mg/L]	% ± RSD of nominal concentration	T72 (14.10.2021) [mg/L]	% ± RSD of nominal concentration
Control	0.00	< LoD	---	< LoD	---
12.3	5.05	5.33	105.5±0.2	5.4	106.9±0.2
37	15.2	16.15	106.3±0.1	16.32	107.4±0.1
111	45.6	48.40	106.1±0.1	49.08	107.6±0.0
333	136.8	145.23	106.2±0.0	147.20	107.6±0.0
1000	410.8	431.16	105.0±0.0	447.17	108.9±0.1

LoQ = 0.2 mg/L

LoD = 0.1 mg/L

--- not calculated

The endpoint values were determined based on nominal test item concentrations.

Conclusion

The endpoint values based on the nominal test item concentrations are given below:

The ErC50/72 h value is higher than the highest test item concentration used for the exposure, i.e. 1000 mg/L.

The LOEC/72 h value for growth rate is 111 mg/L.

The NOEC/72 h value for growth rate is 37 mg/L.

The EyC50/72 h value is 716.5 mg/L (95% confidence interval: 360.9 – >1000).

The LOEC/72 h value for yield is 111 mg/L.

The NOEC/72 h value for yield is 37 mg/L.

Table 9. Growth rate endpoint values based on the nominal test item concentrations – definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC₅₀	282.1 (181.2-332.5)	580.0 (443.4-761.2)	>1000
E_rC₂₀	182.9 (40.3-238.7)	314.8 (179.5-416.8)	657.4 (393.9->1000)
E_rC₁₀	145.8 (17.8-207.5)	228.7 (105.1-324.0)	169.8 (46.7-296.6)
LOEC	>1000	>1000	111
NOEC	≥1000	≥1000	37

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]

Table 10. Yield endpoint values based on the nominal test item concentrations – definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC₅₀	240.9 (169.6-283.4)	287.1 (172.5-508.3)	716.5 (360.9->1000)
E_yC₂₀	165.2 (79.0-212.2)	79.5 (22.2-138.9)	77.0 (11.9-160.5)
E_yC₁₀	135.6 (52.2-185.0)	40.6 (6.4-83.5)	24.0 (1.0-67.2)
LOEC	333	1000	111
NOEC	111	333	37

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]

TEST VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU method C.3. were met:

- the biomass in the control increased by a factor of 27.0 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 3.9% (criterion: it must not exceed 10%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.5% (criterion: it must not exceed 35%).

A 2.2.1.1.3 Study 3

zRMS comments:	<p>The study was conducted to OECD guideline 201 and according to the principles of GLP.</p> <p>In the definitive test the validity criteria were met according to OECD Guideline No. 201. No deviations from the guideline were noted during the study.</p> <p>The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason end-points are expressed as nominal concentrations. The study is reliable and suit-able for the risk assessment. All results refer to nominal concentrations. Following endpoints based on nominal test item concentrations would be used for risk assessment purposes:</p> <p>At the nominal concentration of 333 mg formulation/L already 50.6 % inhibition of yield was observed. at this concentration deformed cells (50% of rod-shaped cells and 50% of swollen cells) were reported as compared to the algae cells in the control. Therefore value of E_yC₅₀= 333 mg formulation/L should be used for RA</p>
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	purposes as a worst case EyC ₅₀ for yield parameter. ErC ₅₀ = > 100 mg formulation/L EyC ₅₀ = 333 mg formulation/L
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Reference:	KCP 10.2/03
Report	CHR/H/DIK 480 SL Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test, M. Czarnecka, 2021, Study code: W-24-21
Guideline(s):	according to the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The growth of the green algae Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) exposed to the test item, CHR/H/DIK 480 SL was investigated during a 72-hour test. The initial density of the algae was 1 x 10⁴ cells/mL. The definitive test was performed with the following test item concentrations: 1000, 333, 111, 37, 12.3 mg/L plus the control.

Materials and methods

Test item: CHR/H/DIK 480 SL; batch no. 012021; the content of dicamba: specification 480.0 ± 24.0 g/L, result of the test 475.25 g/L; density at 20°C: 1.1568 g/cm³; manufacturing date: January 31, 2021, expiry date: January 31, 2023.

Test system: The unicellular freshwater green algae, Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata (Korshikov) Hindák, Selenastrum capricornutum Prinz) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.

Test design: 72 hours of exposure; three replicates per each test item concentration; six replicates per control; a background for each treatment; initial algal cell density: 1 x 10⁴ cells/mL.

Nominal test item concentrations: 1000, 333, 111, 37, 12.3 mg/L plus the control.

Test conditions: Temperature: 21.9 – 22.8°C; pH of the control: 7.52 – 8.06; mean light intensity: 6500 – 6593 lux; constant illumination and shaking; medium: AAP.

Statistics: Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure.

Endpoint values: ErC₅₀/72 h, EyC₅₀/72 h, NOEC/72 h, LOEC/72 h.

Results and discussions

The growth of the green algae Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) exposed to the test item, CHR/H/DIK 480 SL was investigated during a 72-hour test. The test

was performed in glass flasks with a capacity of 250 mL containing 100 mL of either the test item concentration, or the control, per replicate. The initial density of the algae was 1×10^4 cells/mL. The definitive test was performed with the following test item concentrations: 1000, 333, 111, 37, 12.3 mg/L plus the control.

The number of algal cells was determined with indirect method, which involves a spectrophotometric measurement of the absorbance of algal suspension at 670 nm and converting its value into the number of cells using a standard curve. The absorbance for each replicate of each test item concentration and the control were measured after 24, 48, and 72 h of exposure. Morphology observations of the algae cells were performed at exposure termination.

Calculated inhibition of growth rate for the test item concentrations ranging from 12.3 to 1000 mg/L after 72 h of exposure was in the range of 0.2 – 28.7% when compared to the control. Inhibition of yield for the test item concentrations ranging 12.3 to 1000 mg/L after 72 h of exposure was in the range of 1.3 – 76.3% when compared to the control.

In the test item concentrations of 12.3 and 37 mg/L no differences in shape, size and colour of algal cells were reported as compared to the algae cells in the control. In the test item concentration of 111 mg/L deformed cells (50% of swollen cells) were reported as compared to the algae cells in the control. In the test item concentration of 333 mg/L deformed cells (50% of rod-shaped cells and 50% of swollen cells) were reported as compared to the algae cells in the control. Moreover, in the test item concentration of 1000 mg/L deformed cells (75-80% of comma-shaped cells and 25-20% of rod-shaped cells) were also reported as compared to the algae cells in the control.

Table 9. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure	
	growth rate	yield
Control	0.0	0.0
12.3	1.0	4.9
37	0.2	1.3
111	2.4	11.5
333	14.2	50.6
1000	28.7	76.3

The concentrations of dicamba were chemically determined using the validated high performance liquid chromatographic method (HPLC) with Diode Array Detection (DAD).

Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined.

At exposure initiation, the determined concentrations of dicamba were in the range of 105.5 – 117.4% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of dicamba were in the range of 105.9 – 109.7% of the nominal concentration. Therefore, the concentrations of dicamba were stable under test conditions.

The endpoint values were determined based on the nominal test item concentrations.

Conclusion

The endpoint values based on nominal test item concentrations are given below:

The ErC50/72 h value is higher than the highest test item concentration used for the exposure, i.e. 1000 mg/L.

The EyC50/72 h value is 333 mg formulation/L 375.2 mg/L (95% confidence interval: 328.1 – 430.5).

The LOEC/72 h values for growth rate and yield are 111 mg/L.

The NOEC/72 h values for growth rate and yield are 37 mg/L.

TEST VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) and EU Method C.3 were met:

- the biomass in the control increased by a factor of 138.9 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.7% (criterion: it must not exceed 7%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 15.3% (criterion: it must not exceed 35%).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.2.1.1 Study 1

zRMS comments:	<p>CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL <i>Lemna gibba</i> Growth inhibition test was conducted to OECD guideline 221 and according to the principles of GLP. No deviations from the guideline were noted during the study.</p> <p>In the definitive test all the validity criteria were met. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason end-points are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment.</p> <p>At the nominal concentration of 250 mg formulation/L already 56. % inhibition (based on the frond number) was observed. Therefore value of ErC_{50} = 250 mg formulation/L should be used for RA purposes as a worst case ErC_{50} for growth yield parameter based on the frond number.</p>
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From Reference:	KCP 10.2/04
Report	CHR/H/DIK 480 SL <i>Lemna gibba</i> CPCC 310, Growth inhibition test, M. Czarnecka, 2021, Study code: W-25-21
Guideline(s):	according to the OECD Guideline No. 221 (2006)/ EU Method C.26.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

The growth of *Lemna gibba* exposed to the test item, CHR/H/DIK 480 SL, was investigated in a 7 day static test. The total number of fronds in each test vessel was counted twice during exposure (day 2 and 5) and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time.

Materials and methods

Test item: CHR/H/DIK 480 SL; batch no. 012021; the content of dicamba: specification 480.0 ± 24.0 g/L, result of the test 475.25 g/L; density at 20°C: 1.1568 g/cm³; manufacturing date: January 31, 2021, expiry date: January 31, 2023.

Test system: Freshwater aquatic plant *Lemna gibba* L. specification CPCC 310, cultured in the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Aquatic Organisms Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.

Test design: Static system (7 days of exposure); three replicates for each test item concentration and six replicates for the control.

Nominal test item concentrations:

1000, 250, 62.5, 15.6, and 3.9 mg/L plus control.

Test conditions: Temperature: 23.0 – 23.6°C; pH of the control: 7.63 – 8.86;

light intensity: 7983 – 8180 lux; constant illumination; test vessels: glass beakers with a capacity of 600 mL containing 400 mL of each treatment; initial frond number: 9, i.e. 3 plants per 3 fronds; medium: 20X AAP.

Endpoint value: ErC50, ErC20, ErC10, EyC50, EyC20, EyC10, LOEC and NOEC (for growth rate based on the dry weight).

Results and discussions

The growth of *Lemna gibba* exposed to the test item, CHR/H/DIK 480 SL, was investigated in a 7 day static test. The test was performed in glass beakers with a capacity of 600 mL containing 400 mL of either test item concentration or the control. The initial frond number in each test item concentration and the control was nine. The following test item concentrations were used: 1000, 250, 62.5, 15.6, and 3.9 mg/L plus the control.

The total number of fronds in each test vessel was counted twice during exposure (day 2 and 5) and at exposure termination. The observations of plant development, i.e. size of fronds, necrosis, chlorosis, colony break-up, gibbosity, changes in the appearance of roots were performed at the same time.

At exposure termination, in the test item concentrations of 3.9 mg/L, no distinctive changes from the normal development of plants in the control were observed. In the test item concentrations of 15.6 and 62.5 mg/L, bending down of colonies were observed. In the test item concentrations of 250 and 1000 mg/L, overlapping of colonies was observed.

The concentrations of dicamba were chemically determined using the validated high performance liquid chromatographic method (HPLC) with Diode Array Detection (DAD). Samples of all test item concentrations and the control collected at exposure initiation and at exposure termination were chemically determined.

At exposure initiation, the determined concentrations of dicamba were in the range of 106.1 – 115.0% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.

At exposure termination, the determined concentrations of dicamba were in the range of 106.6 – 118.1% of the nominal concentration. Therefore, the concentrations of dicamba were stable under test conditions.

The endpoint values were determined based on the nominal test item concentrations.

Conclusion

The endpoint values based on the nominal test item concentrations:

Endpoints based on the frond number:

The ErC50/7 d value is higher than the highest test item concentration used for the exposure, i.e. 1000 mg/L.

The ErC20/7 d value is 110.0 mg/L (95% confidence interval 58.4 – 182.4).

The ErC10/7 d value is 11.2 mg/L (95% confidence interval 2.7 – 25.3).

~~The EyC50/7 d value is 250 mg/ formulation/L 294.8 mg/L (95% confidence interval 168.9 – 632.0).~~

The EyC20/7 d value is 8.0 mg/L (95% confidence interval 2.2 – 17.4).

The EyC10/7 d value is 1.2 mg/L (95% confidence interval 0.2 – 3.9).

For growth rate and yield, the NOEC/7 d value is lower than 3.9 mg/L, whereas LOEC/7 d value is lower or equal to 3.9 mg/L.

Endpoints based on the dry weight:

The ErC50/7 d value is higher than the highest test item concentration used for the exposure, i.e. 1000 mg/L.

The ErC20/7 d value is 654.7 mg/L (95% confidence interval 322.5 – >1000).

The ErC10/7 d value is 71.2 mg/L (95% confidence interval 14.2 – 153.6).

The EyC50/7 d value is 613.3 mg/L (95% confidence interval 278.6 – >1000).

The EyC20/7 d value is 20.4 mg/L (95% confidence interval 3.3 – 49.4).

The EyC10/7 d value is 3.4 mg/L (95% confidence interval 0.2 – 12.7).

For growth rate and yield, the NOEC/7 d value is lower than 3.9 mg/L, whereas LOEC/7 d value is lower or equal to 3.9 mg/L.

VALIDITY CRITERIA

In the definitive test, the following validity criteria specified in the OECD Guideline No.221/ EU method C.26. were met:

- the doubling time of frond number in the control was 2.1 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 10.5),
- the average specific growth rate in the control between day 0 and day 7 was 0.336 d⁻¹ (minimum requirement: higher than 0.275 d⁻¹).

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.2 Study 1

zRMS comments:	The study was conducted to OECD guideline 213 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met according to OECD Guideline No. 213. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: LD ₅₀ /48 h oral > 200.0 µg formulation/honeybee
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Reference: KCP 10.3/01

Report CHR/H/DIK 480 SL Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test, A. Fulczyk, 2021, Study code: B-57-21

Guideline(s): according to the OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: CHR/H/DIK 480 SL

content: 475.25 g/L of dicamba (CAS No. 1918-00-9)

batch no.: 012021

production date: 31.01.2021

expiry date: 31.01.2023

Biological test system: the honeybee, *Apis mellifera* L., strain: carnica

– age: approximately 3 weeks

– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,

Test design: – the test item:

☐ exposure duration: 48 hours

☐ number of doses: 5 doses and a control

☐ number of replicates: 3 replicates

☐ number of bees: 10 bees/replicate

– the reference item:

☐ exposure duration: 24 hours

☐ number of doses: 3 doses

☐ number of replicates: 3 replicates

☐ number of bees: 10 bees/replicate

Test item doses: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Reference item doses: 0.1, 0.2 and 0.4 µg dimethoate/bee

Test conditions:

– temperature: 23.5 ☐ 25.0°C

– relative air humidity: 59.5 ☐ 63%

Place: Dark room

Statistical analysis: regression analysis using the log-probit method

Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,

– the oral LD₅₀/24 h of the reference item (dimethoate).

Results and discussions

The acute oral toxicity study of CHR/H/DIK 480 SL was conducted to determine the LD₅₀. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results.

Each group of 10 bees (3 replicates containing 10 bees each) was fed with 100 µL of 50% sucrose solution, containing the test item at the doses mentioned above, using a micropipette. During the entire experiment, the insects were caged in groups of 10.

The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure. After the administration, the insects were observed for mortality and other signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute oral toxicity test finished after the 48-hour observation.

Results:

The acute oral toxicity study of the test item, CHR/H/DIK 480 SL on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h after the beginning of the treatment		LD ₅₀ [µg/bee]
		Total		
		[no.]	[%]	
0.0 (Control)	30	0	0.0	> 200.0
12.5	30	2	6.7	
25.0	30	2	6.7	
50.0	30	2	6.7	
100.0	30	4	13.3	
200.0	30	5	16.7	

Validity criteria

The following validity criteria were met during the test:

- the mortality for the control was 0.0% at the end of the experiment (criterion: it must not exceed 10%).
- the LD₅₀/24 h of the reference item (dimethoate) was 0.16 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

Conclusion

The median lethal doses LD₅₀/24 h and LD₅₀/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee.

A 2.3.1.1.3 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.4 Study 1

zRMS comments:	<p>The study was conducted to OECD guideline 214 and according to the principles of GLP.</p> <p>According to the OECD 214 and EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anaesthesia was replaced with mechanical immobilisation. This deviation had no impact on the results.</p> <p>In the definitive test all the validity criteria were met according to OECD Guideline No. 214.</p> <p>The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: LD₅₀/48 h contact > 200.0 µg formulation/honeybee.</p>
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Reference: KCP 10.3/02

Report CHR/H/DIK 480 SL Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test, A. Fulczyk, 2021, Study code: B-58-21

Guideline(s): according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)

Deviations: No

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

Materials and methods

Test item: CHR/H/DIK 480 SL
content: 475.25 g/L of dicamba (CAS No. 1918-00-9)
batch no.: 012021
production date: 31.01.2021
expiry date: 31.01.2023
Biological test system: the honeybee, *Apis mellifera* L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,
Test design: – the test item:
☐ exposure duration: 48 hours
☐ number of doses: 5 doses and one control
☐ number of replicates: 3 replicates
☐ number of bees: 10 bees/replicate
– the reference item:
☐ exposure duration: 24 hours
☐ number of doses: 3 doses
☐ number of replicates: 3 replicates
☐ number of bees: 10 bees/replicate
Test item doses:
12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses: 0.1, 0.2 and 0.4 µg dimethoate/bee
Test conditions:
– temperature: 23.5 - 25°C
– relative air humidity: 59.5 - 63%
16 hours light : 8 hours dark
Place: dark room
Statistical analysis: regression analysis using the log-probit method
Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,
– the contact LD50/24 h of the reference item (dimethoate).

Results and discussions

Mortality of honeybees, *Apis mellifera*, exposed to CHR/H/DIK 480 SL was investigated during 48-hour test. Five doses of the test item were used. These included: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee. The range of doses was selected on the basis of the preliminary non-GLP range-finding test results. A microapplicator was used to apply the test item. The volume was 1 µL/bee. During the experiment, the insects were caged in groups of 10. The recommended reference item, i.e. dimethoate was used to verify the sensitivity of the honeybees and the precision of the test procedure. After the application, the insects were observed for mortality and signs of toxicity. These observations were made 4, 24 and 48 hours after the beginning of the treatment. The acute contact toxicity test finished after the 48-hour observation.

The acute contact toxicity study of the test item, CHR/H/DIK 480 SL on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Dose [µg/bee]	Number of tested bees [no.]	Mortality after 48 h of exposure		LD ₅₀ [µg/bee]
		Total		
		[no.]	[%]	
0.0 (control)	30	0	0.0	> 200.0
12.5	30	0	0.0	
25.0	30	0	0.0	
50.0	30	0	0.0	
100.0	30	1	3.3	
200.0	30	1	3.3	

Validity criteria

The following validity criteria were met during the test:

- the mortality for the control was 0.0% after 48 h (criterion: it must not exceed 10.0%),
- the LD₅₀/24 h of the reference item (dimethoate) was 0.26 µg a.i./bee (criterion: 0.10 – 0.30 µg dimethoate/bee).

Conclusion

The median lethal doses LD₅₀/24 h and LD₅₀/48 h are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

zRMS comments:	<p>The study was conducted to OECD guideline 245 and according to the principles of GLP.</p> <p>No deviation from the guideline were noted. All the validity criteria were met.</p> <p>The study is reliable with following endpoints:</p> <p>LC₅₀ = 667 mg formulation/kg diet</p> <p>LDD₅₀ = 17.2 µg formulation/bee/day</p>
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Reference:

KCP 10.3/07

Report

CHR/H/DIK 480 SL Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, A. Fulczyk, 2022, Study code: B-63-21

Guideline(s):

according to the OECD Guideline No. 245 (2017)

according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)

Deviations:

No

GLP:

Yes

Acceptability:

No

Duplication

No

(if vertebrate study)

Test item: CHR/H/DIK 480 SL
content: 475.25 g/L of dicamba
batch no.: 012021
production date: 31.01.2021
expiry date: 31.01.2023

Biological test system: species: the honeybee, *Apis mellifera* L.; strain: carnica, source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; age: freshly emerged worker honeybees (max. 2 days old) from the same queen-right colony

Experimental design: - the test item:
number of concentrations: 1 and the control
number of replicates: 5
number of insects: 10 bees/replicate

- the reference item:
number of concentrations: 1
number of replicates: 3
number of insects: 10 bees/replicate
exposure duration: 10 days
Nominal concentration of the test item: 666.7 mg/kg
Nominal dose of the test item: 20.0 µg/bee/day
Test item dietary dose: 17.2 µg/bee/day
Nominal concentration of the reference item (dimethoate): 0.8 mg/kg
Nominal dose of the reference item (dimethoate): 0.024 µg/bee/day
Reference item dietary dose: 0.014 µg/bee/day
Test conditions: temperature: 32.3 – 34.5°C;
relative humidity: 51.3 – 67.5%;
Statistical method: Chi2-Contingency test
Endpoints: honeybee mortality after 10 days of exposure

The mortality of honeybees exposed to CHR/H/DIK 480 SL was investigated during 10-days chronic oral toxicity test. The design of the definitive test was selected on the basis of the preliminary range-finding non-GLP test results. One dose of the test item was used (limit test). The nominal concentration was 666.7 mg/kg of diet (corresponding to the nominal dose of 20.0 µg/30 mg/day).

Daily dose, consumed by the bees in the group treated with the test item at the nominal concentration of 666.7 mg/kg (20 µg/30 mg/day) was 17.2 µg/bee/day (dietary dose). Daily dose was calculated on the basis of average consumption of a treated 50% sucrose solution and the nominal dose used for the treatment. Each group of bees (5 replicates/group; 10 bees/replicate) was fed with 2 mL of a 50% sucrose solution containing the test item at the concentration of 666.7 mg/kg or 50% sucrose solution alone (control group) for 10 days.

Dimethoate, which is a recommended reference item, was used to verify the sensitivity of the bees and the precision of the test procedure. The group treated with the reference item (3 replicates per 10 bees) was fed with 2 mL of a 50% sucrose solution containing reference item at the nominal concentration of 0.8 mg/kg (corresponding to the nominal dose of 0.024 µg/30 mg). Daily weighed feeders were used. During the experiment, the insects were caged in groups of 10. Daily dose, consumed by the bees in the group treated with the reference item at the nominal concentration of 0.8 mg/kg (0.024 µg/30 mg/day) was 0.014 µg/bee/day (dietary dose).

The insects were observed for mortality and behavioral abnormalities (signs of intoxication) at daily intervals up to 10 days of exposure. Average consumption of a 50% sucrose solution in the control group was 24.73 mg/bee/day. Average consumption in the group treated with the test item at the concentration of 666.7 mg/kg was 25.82 mg/bee/day. Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.8 mg/kg was 17.26 mg/bee/day.

The concentrations of dicamba were chemically determined using the validated high performance liquid

chromatographic method with DAD detection. Fresh samples of the test item concentration and the control were chemically analyzed at test initiation and at the end of the maximum storage period (i.e. after 4 days). At exposure initiation, in the fresh sample of the test item of 666.7 mg/kg, the determined concentration of dicamba was 89.4% of nominal concentration. The results confirm that the test item concentration was prepared correctly.

After 4 days of the storage period, in the sample of the test item of 666.7 mg/kg, the determined concentration of dicamba was 89.9% of nominal concentration. Based on the results of chemical analyses, the concentration of dicamba was stable under storage conditions

The validity criterion concerning mortality was met, because mortality in the control was 4.0% after 10 days of exposure. The percentage of mortality of the honeybees exposed to the test item, at the concentration of 666.7 mg/kg (dietary dose 17.2 µg/bee/day) at exposure termination (after 10 days), was -2.1%, after Abbott's correction.

On the basis of the obtained mortality results the LC₅₀ is higher than 666.7 mg/kg, and the LDD₅₀ value is higher than 17.2 µg/bee/day, there is no statistically significant differences in mortality between group treated with the test item at the dose of 666.7 mg/kg (dietary dose 17.2 µg/bee/day) and the control group (Chi²-Contingency test, p(Chi)²>α). The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, because mortality was equal to 100% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

The effects of CHR/H/DIK 480 SL on mortality of honeybees are summarized below:

Nominal test item concentration/ dose		Ingested ^a dose [µg/bee/day]	Number of tested bees [no]	Total mortality		LC ₅₀ [mg/kg]	LDD ₅₀ [µg/bee/day]
[µg/30 mg/day] [µg/bee/day]	[mg/kg]			No.	[%]		
CHR/H/DIK 480 SL							
0.0 (Control)			50	2	4.0	> 666.7	> 17.2
20.0	666.7	17.2	50	1	-2.1*		
Dimethoate (reference item)							
0.024	0.8	0.014	30	30	100	not determined	

^a: ingested doses (dietary doses) were calculated on the basis of the concentrations of the test item / reference item and average sucrose solution consumption

*: corrected according Abbott's formula [7], the negative value means that in the tested dose there was lower mortality than in the control group

TEST VALIDITY CRITERIA

The following validity criteria were met during the test:

- At the end of the experiment average mortality of the control groups was 4.0% (criterion: it must not exceed 15%)
- After 10 days of exposure corrected mortality of the honeybees exposed to the reference item at the concentration of 0.8 mg/kg (0.014 µg/bee/day) was 100% (criterion: it must be ≥ 50% on day 10 of exposure).

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

zRMS comments:	The study was conducted according to OECD 239 guideline and according to the principles of GLP. In the definitive test all the validity criteria were met. The study is reliable with following endpoints:
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	EC ₅₀ > 649.4 mg/kg ED ₅₀ > 100 µg/larva NOEC = 324.7 mg/kg NOED = 50 µg/larva
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Reference:	KCP 10.3/08
Report	CHR/H/DIK 480 SL Honeybees (<i>Apis mellifera</i> L.), Larval Toxicity Test, Repeated Exposure, A. Fulczyk, 2021, Study code: B-58-21
Guideline(s):	according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	No
Duplication (if vertebrate study)	No

Test item: CHR/H/DIK 480 SL

content: 475.25 g/L of dicamba (CAS No. 1918-00-9)

batch no.: 012021

production date: 31.01.2021

expiry date: 31.01.2023

Biological test

system:

the honeybee, *Apis mellifera* L.; strain: carnica; source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; age: one-day-old larvae

Experimental

design:

– the test item: number of cumulative doses: 5 and a control;

number of replicates: 3; number of larvae: 12/replicate

– the reference item: number of cumulative doses: 1; number of

replicates: 3; number of larvae: 12/replicate

Test item doses: 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva + control

Reference item

dose: 7.39 µg dimethoate/larva

Test duration: 22 days

Test conditions: temperature: 34.0 – 35.0°C;

relative air humidity: D1 – D8: 90.5 – 97.5%

D8 – D15: 77.3 – 84.9%

D15 – D22: 50.3 – 79.7%

Endpoints:

mortality of larvae and pupae, adult emergence, EC₁₀/ED₁₀,

EC₂₀/ED₂₀ EC₅₀/ED₅₀, NOEC/NOED on day 22 (D22)

Statistical method: Probit analysis using max. likelihood regression, Levene's Test on

Variance Homogeneity, Multiple Sequentially-rejective t-test after

Bonferroni-Holm

Statistical

software: ToxRat Professional 3.3.0.

The larval toxicity test of CHR/H/DIK 480 SL was conducted to determine the median effective concentration/dose, i.e. EC₅₀/ED₅₀ or any other effective concentration/dose, EC_x/ED_x, as well as the no observed effect concentration/dose (NOEC/NOED) after 22 days of the experiment. Five cumulative doses

of the test item were used. These were 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva. They were selected on the basis of the preliminary non-GLP range-finding test.

From day 3 (D3) to day 6 (D6) of the experiment, each larva (3 replicates; 12 larvae/replicate) was fed with treated diet in the volume of 20, 30, 40 or 50 µL, respectively (total volume of treated diet was 140 µL). During the experiment, the larvae were kept in grafting cells placed into 48-well plates.

The plates were kept in a desiccator, in an incubator. A toxic standard, i.e. dimethoate, was used to verify the sensitivity of the larvae and the precision of the test procedure. Mortality of the larvae was recorded daily from day 4 (D4) – to day 8 (D8) and at day 10, 11, 12, 13 and 14 (D10, D11, D12, D13, D14). On day 15 (D15) mortality of pupae was recorded. The test was ended on day 22 when the emergence of adults was evaluated. Mortality of the control group on day 8 (D8) of the test was 0.0% (criterion: ≤ 15%). The percentages of mortality of the honeybee larvae, exposed to the test item, CHR/H/DIK 480 SL at the cumulative doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg test item/larva at D8 were: 0.0, 0.0, 2.8, 5.6 and 0.0%, respectively. The percentage of larval mortality on D8 in the reference item group was 94.4%.

Pupal mortality of the control group on day 15 (D15) of the test was 2.8%. The percentages of mortality of the honeybee pupae, exposed to the test item, CHR/H/DIK 480 SL at the cumulative doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg/larva at D15, after Abbott's correction were: 0.0, 0.0, 5.7, 17.1 and 25.7%, respectively. The percentage of pupal mortality on D15 in the reference item group was 100.0%. The emergence of adults (emergence rate) at the end of the test (on D22) in the control group was 88.9%. In the groups treated with the test item at the cumulative doses of 6.25, 12.5, 25.0, 50.0 and 100.0 µg test item/larva the adult emergence rates were: 86.1, 88.9, 75.0, 69.4 and 41.7%, respectively.

The endpoint values at the end of the assessment (D22):

- ED50 value is higher than 100.0 µg test item /larva,
- EC50 value is higher than 649.4 mg /kg,
- NOED value is equal to 50.0 µg test item/larva,
- NOEC value is equal to 324.7 mg test item/kg.

The effects of CHR/H/DIK 480 SL on mortality of honey bee larvae are summarized below:

Cumulative dose [µg test item/larva]	Concentration [mg test item/kg food]	Number of tested larvae [no.]	Total mortality (larval and pupal) on day 22 (D22)				
			Number [no.]	[%]	Corr ^a [%]	Number of emerged adults [No.]	Emergence rate [%]
Test item: CHR/H/DIK 480 SL							
Control (0.0)		36	4	11.1	–	32	88.9
6.25	40.6	36	5	13.9	3.1	31	86.1
12.5	81.2	36	4	11.1	0.0	32	88.9
25.0	162.3	36	9	25.0	15.6	27	75.0
50.0	324.7	36	11	30.6	21.9	25	69.4
100.0 ⁺	649.4	36	21	58.3	53.1	15	41.7
ED ₅₀ [µg test item/larva]		> 100					
EC ₅₀ [mg test item/kg]		> 649.4					
NOED [µg test item/larva]		50.0					
NOEC [mg test item/kg]		324.7					
Reference item: Technical dimethoate mortality on day 8 (D8)							
7.39	48.0	36	34	94.4	94.4	not determined	

⁺: statistical significant difference [8], [SOP/B/67]

^a: Mortality corrected according to the Abbott formula [7]

TEST VALIDITY CRITERIA

The following validity criteria were met:

- Cumulative larval mortality in the control group was 0.0% at day 8 (D8) (criterion: ≤ 15%).
- Mortality of the larvae treated with the reference item at day 8 (D8) (dimethoate) was 94.4% (criterion: ≥ 50%).
- Emergence rate in the control group on D22 was 88.9% (criterion: ≥ 70%).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.4.1 Study 1

zRMS comments:	<p>The study follows the guideline specified by Blümel et al. (2000) and according to the principles of GLP.</p> <p>The short term deviations (< 2 hours per day) from the recommended temperature values are unavoidable and should not affect the integrity or outcome of the test</p> <p>According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen was used. However, in the experiment additional food</p>
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	<p>in the form of the two-spotted spider mite (<i>T. urticae</i>) eggs, was used. Another food source prevents the mites from escaping from discs. Since the definitive test all the validity criteria were met it didn't impact the results of the study. Considering the current test guideline (Blümel et al., 2000) the study is considered valid. $LR_{50} > 0.6$ L formulation/ha $NOER_{mortality} < 0.3$ L formulation/ha $ER_{50} = > 0.6$ L formulation/ha $NOER_{reproduction} < 0.15$ L formulation/ha</p>
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Reference:	KCP 10.3/03
Report	An extended laboratory test for evaluating the effects of CHR/H/DIK 480 SL on the predatory mite, <i>Typhlodromus pyri</i> (Sch.), A. Grande, 2021, Study code: B-59-21
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
 CHR/H/DIK 480 SL
 Active substance:
 475.25 g/L of dicamba
 Batch number:
 012021
 Manufacture date:
 31.01.2021
 Expiry date:
 31.01.2023
 Biological test system:
 the predatory mite, *Typhlodromus pyri* (Sch.) (Acari: Phytoseiidae)
 – age:
 24-hour-old protonymphs
 – source:
 a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented from a commercial breeder
 Experimental design:
 5 study groups:
 – a control group (0.0 L/ha)
 – CHR/H/DIK 480 SL at the rate of 0.15 L/ha
 – CHR/H/DIK 480 SL at the rate of 0.3 L/ha
 – CHR/H/DIK 480 SL at the rate of 0.6 L/ha
 – reference item: dimethoate at the rate of 4.0 g/ha
 number of replicates: 3/group

number of mites in each replicate: 20

Test conditions:

– temperature:

22 – 25 °C

– relative air humidity:

65 – 79 %

– photoperiod:

16 h light : 8 h dark

– light intensity:

899 lx

Statistical analysis:

Probit analysis using linear max. likelihood regression

Step-down Cochran-Armitage test procedure

Shapiro Wilk's Test on Normal Distribution

Levene's Test on Variance Homogeneity (with Residuals) Williams Multiple Sequential t-test Procedure

Chi2 2x2 Table Test with Bonferroni Correction

Endpoints:

– mite mortality after 7 days of the treatment

– LR50 and NOERMortality

– reproduction reduction (Pr) after 14 days of the treatment

– ER50 and NOERreproduction

Results and discussions

The aim of the extended laboratory test was to evaluate the effects of the test item, CHR/H/DIK 480 SL on mortality and reproduction of the predatory mite, *T. pyri* (Sch.).

On the basis of the preliminary test results, it was decided to use three rates of the test item in the definitive test. These were 0.15, 0.3 and 0.6 L/ha.

The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to leaf discs. The mites were fed with pine pollen (*Pinus* sp.) and *T. urticae* eggs. Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and groups treated with the test item at rates 0.15, 0.3 and 0.6 L/ha were made after 8, 11, and 14 days of the treatment.

Mortality of *T. pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints.

To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 4.0 g/ha. The control group was treated with distilled water.

In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to CHR/H/DIK 480 SL at rates of 0.15, 0.3 and 0.6 L/ha, the percentages of *T. pyri*, mortality, were 1.7, 0.0 and 43.3%, respectively.

There were no statistically significant differences in mortality between the groups treated with the test item at the rates of 0.15 and 0.3 L/ha in comparison to the control group. There was a statistically significant difference in mortality between group treated with the test item at the rate of 0.6 L/ha in comparison to the control group (Step-down Cochran-Armitage test procedure, $p(\text{trend}) < \alpha 0.05$).

The LR50 value is higher than 0.6 L/ha and the NOERMortality is 0.3 L/ha.

After 7 days of exposure to dimethoate at the rate of 4.0 g/ha mortality was 93.3%. Therefore, the validity criterion specified in the method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

Reproduction of the surviving mites from the control group and the groups treated with test item at the rates of 0.15, 0.3 and 0.6 L/ha, was assessed since mortality of these groups was < 50.0%.

The mean reproduction rate (Rr) in the control group was 7.7 eggs/female. The mean Rr after 14 days of exposure to test item at the rates of 0.15, 0.3 and 0.6 L/ha were 7.6, 6.2 and 5.1 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by test item at the rates of 0.15, 0.3 and 0.6 L/ha were 1.1, 19.9 and 33.5%, respectively.

There were no statistically significant differences in reproduction between the group treated with the test item at the rate of 0.15 L/ha and the control group. There were statistically significant differences in reproduction between the group treated with the test item at the rates of 0.3 and 0.6 L/ha and the control group (Williams Multiple Sequential t-test Procedure, $|t| > |t^*|$).

On the basis of the obtained reproduction results, the ER₅₀ value is higher than 0.6 L/ha and the NOER_{reproduction} is 0.15 L/ha.

Test item rate [L/ha]	Parameter (endpoint)			
	Mortality after 7 days	Reproduction		
	Total [%]	Test item rate [L/ha]	Mean number of eggs/ female (Rr) [no.]	Repro- duction reduction Pr [%]
0.0 (control)	0.0	0.0 (control)	7.7	-
0.15	1.7	0.15	7.6	1.1
0.3	0.0	0.3 ⁺	6.2	19.9
0.6 ⁺	43.3	0.6 ⁺	5.1	33.5
LR ₅₀	> 0.6 L/ha	ER ₅₀	> 0.6 L/ha	
NOER _{mortality}	0.3 L/ha	NOER _{reproduction}	0.15 L/ha	
Reference item: dimethoate				
Rate [g/ha]	Total mortality [%]	Reproduction		
4.0	93.3	not assessed		

*: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [12], [SOP/B/67]

Conclusion

Based on the results it can be stated that CHR/H/DIK 480 SL at the rate of 0.6 L/ha has an adverse effect on mortality of the mites. The test item at the rates of 0.3 and 0.6 L/ha has an adverse effect on reproduction of the mites.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study [3]:

- mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%),
- mortality of the mites exposed to the reference item at the rate of 4.0 g/ha, was 93.3% on day 7 of exposure (criterion: from 50 to 100%),
- the mean number of eggs per female in the control group was 7.7 (required: ≥ 4 eggs per female).

A 2.3.1.4.2 Study 1

zRMS comments:	<p>The extended study follows the guideline specified by Mead Briggs M.A. et al. (2000) and according to the principles of GLP. No deviations to the guideline were noted. The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/DIK 480 SL on mortality and fecundity of the parasitic wasp, <i>Aphidius rhopalosiphi</i>. Considering the current test guideline (Mead Briggs M.A. et al, 2000) the study is considered valid.</p> <p>LR₅₀ > 0.6 L formulation/ha NOER_{mortality} \geq 0.6 L formulation/ha ER₅₀ > 0.6 L formulation/ha NOER_{reproduction} < 0.15 L formulation/ha</p>
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Report	An extended laboratory test for evaluating the effects of CHR/H/DIK 480 SL on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani-Perez); A. Grande, 2021, Study code: B-60-21
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: CHR/H/DIK 480 SL

Active substance: 475.25 g/L of dicamba

Batch number: 012021

Manufacture date: 31.01.2021

Expiry date: 31.01.2023

Biological test system:

the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: Braconidae, Aphidinae
– age:

imago (24 – 48 hours after emerging from mummies)

– source:

the culture was obtained from a commercial breeder (Katz Biotech AG)

Experimental design:

5 study groups:

– a control group (0.0 L/ha)

– CHR/H/DIK 480 SL at the rate of 0.15 L/ha

– CHR/H/DIK 480 SL at the rate of 0.3 L/ha

– CHR/H/DIK 480 SL at the rate of 0.6 L/ha

– Reference item: dimethoate at the rate of 4.0 g/ha

mortality assessment: 6 replicates/group, 5 females/replicate

fecundity assessment: 15 replicates/group, 1 female/replicate

Test conditions:

– temperature:

18 – 20.5°C

– relative air humidity:

65 – 87%

– photoperiod:

16 hours light : 8 hours dark

– light intensity:

mortality and oviposition assessment: 1674 lx

fecundity phase: 4307 lx

Statistical analyses:

– Logit analysis using max. likelihood regression

– Estimated parameters of the 3-param. normal CDF

– Shapiro-Wilk's Test on Normal Distribution

– Levene's Test on Variance Homogeneity

– Chi2 2x2 Table Test with Bonferroni Correction

– Williams Multiple Sequential t-test Procedure

– Multiple Sequentially-rejective U-test After Bonferroni-Holm

Endpoints:

- wasp mortality after 48 hours of exposure,
- LR50 and the NOERmortality,
- ER50 and the NOERfecundity.
- reduction in fecundity (Pr) of the surviving female wasps exposed to test item, 12 days after the oviposition period

Results and discussions

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/DIK 480 SL on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphii*. On the basis of the results of the preliminary range - finding test, it was decided to use three rates of the test item in the definitive test. These were 0.15, 0.3 and 0.6 L/ha.

Adult wasps were exposed to the test item applied to barley plants. Observations of settling behavior were made during the initial 3 hours of exposure. The aims were to determine repellent effects of CHR/H/DIK 480 SL and to check if the test insects had contact with barley plants sprayed with the test item. Settling behavior of wasps from each replicate was observed five times. Mortality was determined 2, 24 and 48 hours after the introduction of the wasps to the test arenas.

Females which survived the 48-hour exposure to test item and the ones from the control group were subjected to fecundity assessments. Fifteen female wasps from the four group treated with the test item and the control were individually introduced into the fecundity units containing barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded.

Mortality after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints.

To verify the sensitivity of the biological test system and the precision of the test procedure, dimethoate, which is an insecticide, was used as a reference item. The rate of the reference item was 4.0 g/ha. The control group was treated with distilled water.

In the definitive test, after 48 hours, mortality of the control wasps was 0.0%.

After 48 hours of the exposure to CHR/H/DIK 480 SL at the rates of 0.15, 0.3 and 0.6 L/ha, the percentages mortality of *A. rhopalosiphii*, were 0.0%, 0.0% and 3.3%, respectively.

At the significance level of 0.05, there were no statistically significant difference in mortality between the wasps exposed to the test item at the rates of 0.15, 0.3 and 0.6 L/ha and the control group (Chi2 2x2 Table Test with Bonferroni Correction, $p(z) > \alpha$).

Based on the obtained mortality results it could be assumed that the LR50 is higher than 0.6 L/ha and the NOERmortality is higher than or equal to 0.6 L/ha.

The mortality of the wasps exposed to dimethoate at the rate of 4.0 g/ha was 73.3% after 48 hours. Therefore, the validity criterion specified in the method description was met [6]. The results showed that the test organisms were sensitive to dimethoate.

The fecundity assessment showed that the mean number of mummies per female in the control group was 25.2 (after 12 days after oviposition). As for the wasps treated with test item at the rates of 0.15, 0.3 and 0.6 L/ha the mean number of mummies per female were 22.9, 19.5 and 18.9, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.15, 0.3 and 0.6 L/ha were 9.3, 22.5 and 25.1%, respectively.

At the significance level of 0.05, there were statistically significant differences in fecundity between the wasps exposed to the test item at the rates of 0.3 and 0.6 L/ha and the control group (Williams Multiple Sequential t-test Procedure, $|t| > |t^*|$).

The ER50 value is higher than 0.6 L/ha and the NOERfecundity is 0.15 L/ha of the test item.

Parametr (endpoint)						
Mortality after 48 h			Fecundity			
Test item rate [L/ha]	Total [%]	LR ₅₀ [L/ha]	Test item rate [L/ha]	Mean no. of mummies /female	Fecundity reduction Pr [%]	ER ₅₀ [L/ha]
0.0 (control)	0.0	–	0.0 (control)	25.2	–	–
0.15	0.0	> 0.6	0.15	22.9	9.3	> 0.6
0.3	0.0		0.3 ⁺	19.5	22.5	
0.6	3.3		0.6 ⁺	18.9	25.1	
NOER _{mortality}	≥ 0.6 [L/ha]		NOER _{fecundity}	0.15 [L/ha]		
Reference item: dimethoate						
Rate [g/ha]	Total mortality [%]		Fecundity			
4.0	73.3		not assessed			

*: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [3], [SOP/B/67].

Conclusion

On the basis of the obtained results it can be concluded that CHR/H/DIK 480 SL at the rates of 0.15, 0.3 and 0.6 L/ha has no adverse effect on the mortality of the wasps. The test item at the rate of 0.15 L/ha has no adverse effect on the fecundity of the wasps, however, at the rates of 0.3 and 0.6 L/ha such an effect is observed.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study [6]:

- after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
- after 48 hours, mortality of the group treated with the reference item at the rate of 4.0 g/ha was 73.3% (criterion: a minimum of 50%),
- all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- the mean number of mummies per female in the control group was 25.2 (criterion: a minimum of 5.0 mummies/female),
- all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

A 2.3.1.4.3 Study 1

zRMS comments:	<p>The study follows the guideline specified by. Schmuck et al. (2000) in Candolfi (2000) guidelines according to the principles of GLP.</p> <p>In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Schmuck V., et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs.</p> <p>Since the definitive test all the validity criteria were met. The study is considered</p>
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	valid. In the definitive test all the validity criteria were met. LR ₅₀ > 0.6 L product/ha
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Reference:	KCP 10.3/05
Report	An extended laboratory test for evaluating effects of CHR/H/DIK 480 SL on the ladybird beetle, <i>Coccinella septempunctata</i> L.; A. Fulczyk, 2021, Study code: B-61-21
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Schmuck et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: CHR/H/DIK 480 SL
 content: 475.25 g/L of dicamba (CAS No. 1918-00-9)
 batch no.: 012021
 production date: 31.01.2021
 expiry date: 31.01.2023
 Biological test system: the ladybird beetle, *C. septempunctata* L. (Arthropoda: Coccinellidae)
 – age: 4-day-old larvae
 – source: breeding of ladybird beetle at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; beetles were obtained from commercial breeder (Katz Biotech AG, Germany)
 Experimental design: 5 study groups:
☐ a control group (0.0 L/ha)
☐ CHR/H/DIK 480 SL at the rates of:
 - 0.15 L/ha
 - 0.3 L/ha
 - 0.6 L/ha
☐ dimethoate at the rate of 3.2 g/ha
 number of replicates: 40 replicates/group
 number of larvae: 1 larva of *Coccinella septempunctata* /replicate
 Test conditions:
 – temperature: 23.0 – 27.0°C
 – relative air humidity: 60.4 ☐ 84.0%
 – photoperiod: 16 hours light : 8 hours dark
 – light intensity 2853 lx
 Statistical analysis: Probit analysis using linear max. likelihood regression, Chi2 2x2 Table Test with Bonferroni Correction
 Endpoints: – preimaginal mortality of the ladybird beetles
 – LR50
 – NOERMortality

– reproductive performance of the moulted beetles over a period of 9 days (the mean number of fertile eggs/female/day)
 reproduction reduction (Pr)

Results and discussions

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/DIK 480 SL on mortality and reproductive capacity of the ladybird beetle, *Coccinella septempunctata*. In a definitive test, three test item application rates of 0.15, 0.3 and 0.6 L/ha were used.

To assess mortality of the ladybird beetles, *Coccinella septempunctata* L., 4-day-old larvae were exposed to the test item applied to leaf discs. There were 40 replicates of each treated group. Each replicate contained 1 larva of *C. septempunctata* L. The larvae were fed with the fresh aphids, *Acyrtosiphon pisum* until pupation. During the exposure phase, survival, condition and development of the ladybird beetles were regularly assessed until the end of pupation. After emergence of the adults, pre-imaginal mortality was calculated on the basis of the numbers of dead larvae, pupae, and adults which died during emergence.

After completion of mortality assessment, healthy hatched beetles from the control group and from group treated with the test item at the rates of application rates of 0.15, 0.3 and 0.6 L/ha were subjected to evaluate the reproductive performance. To allow egg-laying, adult ladybirds were transferred to separate reproduction units. The beetles had continuous access to food in the form of a honey-water solution (2:1), pine pollen (*Pinus* sp.) and the broad bean plants infested with the aphid, *A. pisum*. Reproductive performance observations, concerning the numbers of eggs laid and their fertility were made over a period of 9 days.

To check the relative susceptibility of the test system and the sensitivity of the test method, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 3.2 g/ha. Control beetles had contact with leaf discs sprayed with distilled water.

Study group	Parameters (endpoints)					
	Mortality			Reproduction		
Test item [L/ha]	[%]	[%] ^a	LR ₅₀ [L/ha]	Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]
Control (0.0)	2.5	–	> 0.6	8.9	7.4	–
0.15	2.5	0.0		7.4	5.7	23.0
0.3	7.5	5.1		12.9	10.5	-41.9*
0.6	10.0	7.7		6.5	5.0	32.4
NOER _{mortality}	≥ 0.6 [L/ha]					
dimethoate						
Reference item [g/ha]	100.0	100.0		–		
3.2						

^a: mortality was corrected according Abbott's equation [1]

*: the negative value means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group

Conclusion

The validity criterion concerning mortality was met, because mortality of the ladybird beetle, *Coccinella septempunctata* L. in the control group was equal to 2.5% ($\leq 30.0\%$). The mortality of the ladybird beetles exposed to the test item at the rates of 0.15, 0.3 and 0.6 L/ha, after Abbott's correction, were 0.0, 5.1 and 7.7%, respectively.

At the significance level of 0.05, there were no statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.15, 0.3 and 0.6 L/ha of CHR/H/DIK 480 SL and the control group (Chi2 2x2 Table Test with Bonferroni Correction, ($\alpha=0.05$, $p(z)>\alpha$)).

The LR50 value is above 0.6 L/ha of CHR/H/DIK 480 SL. The NOERMortality is higher or equal to 0.6 L/ha of CHR/H/DIK 480 SL.

The mortality of the ladybird beetles exposed to the reference item at the rate of 3.2 g of dimethoate/ha, after Abbott's correction, was equal to 100.0%. Therefore, the validity criterion was met. The results showed that the insects were sensitive to dimethoate.

The mean number of fertile eggs/female/day in the control group was 7.4 (criterion: ≥ 2 eggs/female/day). The mean numbers of fertile eggs/female/day in the group treated with the of CHR/H/DIK 480 SL at the rates of 0.15, 0.3 and 0.6 L/ha were equal to 5.7, 10.5 and 5.0 it refers to 23.0, -41.9 and 32.4% reproduction reduction. The negative value means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group.

It can be concluded that CHR/H/DIK 480 SL at the rates of 0.15, 0.3 and 0.6 L/ha had no adverse effect on the reproduction capacity of the ladybird beetle.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study [6]:

- ☐ pre-imaginal mortality of the control group was 2.5% (criterion: a maximum of 30.0%),
- ☐ mean corrected mortality of the reference item group was 100.0% (criterion: a minimum of 40%),
- ☐ fertility (the mean number of fertile eggs/female/day) in the control group was 7.4 (criterion: ≥ 2 fertile eggs/female).

A 2.3.1.4.4 Study 1

zRMS comments:	<p>The study follows the guideline specified by Vogt et al. and according to the principles of GLP.</p> <p>In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Vogt H. et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. Since the definitive test all the validity criteria were met. The study is considered valid.</p> <p>LR= > 0.6 L formulation/ha</p>
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Reference:	KCP 10.3/06
Report	An extended laboratory test for evaluating effects of CHR/H/DIK 480 SL on the green lacewing, <i>Chrysoperla carnea</i> (Steph.); M. Knapik, 2021, Study code: B-62-21
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Vogt H. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication No
(if vertebrate study)

Materials and methods

Test item:

CHR/H/DIK 480 SL

content: 475.25 g/L of dicamba,

batch no.: 012021

production date: 31.01.2021

expiry date: 31.01.2023

Biological test system:

the green lacewing, *Chrysoperla carnea* (Steph.), Neuroptera: Chrysopidae

– age:

first instars' larvae (2 days old)

– source:

a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented by commercial breeder

Experimental design:

5 study groups:

– a control group (0.0 L/ha)

– CHR/H/DIK 480 SL at the rates of

- 0.07 L/ha

- 0.2 L/ha

- 0.6 L/ha

– dimethoate at the rate of 15.0 g/ha

number of replicates: 30 replicates/group

number of larvae: 1 larva of *Chrysoperla carnea* /replicate

Test conditions:

– temperature: 23.5 – 27.0°C

– relative air humidity: 61.0 – 84.0%

– photoperiod:

16 hours light : 8 hours dark

– light intensity

3065 lux

Statistical analysis:

Probit analysis using linear max. likelihood regression, Step-down Cochran-Armitage Test Procedure

Endpoints:

– cumulative mortality of larvae, pupae, and adults after emergence

– LR50 value

– reproduction of the lacewings:

– fecundity (mean number of eggs/female/day)

– fertility (mean hatching rate)

Results and discussions

The extended laboratory test involved the evaluation of the effects of the test item, CHR/H/DIK 480 SL on mortality and reproductive capacity of the green lacewing, *Chrysoperla carnea*. In a definitive test, three test item application rates of 0.07, 0.2 and 0.6 L/ha were used.

To assess mortality, 2-day-old larvae of *Chrysoperla carnea* were exposed to dry residues of the test item on leaf discs. Eggs of the mill moth *Ephestia kuehniella* were offered as food. After emergence of adults, total mortality was calculated on the basis of the numbers of dead larvae, pupae, and adults which died during emergence. There were 30 replicates of each treated group. Each of them contained 1 larva of *Chrysoperla carnea*.

To determine possible adverse effects of the test item on fecundity and fertility of the lacewings, repro-

ductive performance was conducted during 13 days.

Total mortality of the lacewings, the mean number of eggs laid per female lacewing per day, and the mean hatching rate were the endpoints.

To control the sensitivity of the biological test system, an insecticide, dimethoate was used as a reference item. The rate of the reference item was 15.0 g/ha. Control lacewings had contact with discs sprayed with distilled water.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study [5]:

- pre-imaginal mortality of the control group was 0.0% (criterion: a maximum of 20.0%),
- mean mortality of the reference item group was 83.3% (criterion: a minimum of 50%),
- the mean number of eggs per female per day in the control group (fecundity) was 19.4 (criterion: ≥ 15.0),
- the mean hatching rate in the control group (fertility) was 74.7 (criterion: $\geq 70\%$).

Study group [application rate]	Parameter (endpoints)				
	Mortality		Reproduction		
Test item [L/ha]	[%]	LR ₅₀ [L/ha]	Mean number of eggs/female /day [no.]	Mean hatching rate [%]	Fecundity reduction relative to the control (Pr) [%]
Control (0.0)	0.0	> 0.6	19.4	74.7	-
0.07	0.0		15.7	75.8	-1.5
0.2	6.7		13.2	74.1	0.8
0.6	0.0		12.8	76.4	-2.3
NOER _{mortality}	≥ 0.6 [L/ha]				
Reference item [g/ha]	Dimethoate				
15.0	83.3	-			-

Conclusion

The validity criterion concerning mortality was met, because mortality of the green lacewings, *Chrysoperla carnea* (Steph.) in the control group was 0.0%. The mortality of the green lacewings exposed to the test item at the rates of 0.07, 0.2 and 0.6 L/ha of CHR/H/DIK 480 SL was 0.0, 6.7 and 0.0%, respectively.

There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.07, 0.2 and 0.6 L/ha in comparison to the control group (Step-down Cochran-Armitage Test Procedure, $p > \alpha$, ($\alpha=0.05$)).

The LR₅₀ value is higher than 0.6 L/ha. The NOER_{mortality} value is equal or higher than 0.6 L/ha.

The percentage of mortality of *Ch. carnea* (Steph.) exposed to dimethoate at rate of 15.0 g/ha, was 83.3%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate.

Reproduction of the lacewings from the control group and the groups treated with the test item at the rates of 0.07, 0.2 and 0.6 L/ha was assessed, since the mortality were $< 50\%$.

The mean number of fertile eggs/female/day in the control group was equal to 19.4 (criterion: ≥ 15.0). The mean numbers of fertile eggs/female/day in the groups treated with CHR/H/DIK 480 SL at the rates of 0.07, 0.2 and 0.6 L/ha were equal to 15.7, 13.2 and 12.8, respectively. The mean hatching rate in the control group was 74.7% (criterion: $\geq 70\%$). The mean hatching rate in the groups treated with the test

item at the rates of 0.07, 0.2 and 0.6 L/ha were 75.8, 74.1 and 76.4%, respectively.

Based on the results, it can be presumed that CHR/H/DIK 480 SL at the rates of 0.07, 0.2 and 0.6 L/ha had no adverse effect on the hatching rate, but CHR/H/DIK 480 SL at the rates of 0.2 and 0.6 L/ha had adverse effect on the numbers of fertile eggs/female/day.

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 2.4	KCP 10.4	Effects on non-target soil meso- and macrofauna
A 2.4.1	KCP 10.4.1	Earthworms
A 2.4.1.1	KCP 10.4.1.1	Earthworms - sub-lethal effects
A 2.4.1.1.1	Study 1	

zRMS comments:	<p>The study was conducted to OECD guideline 222 and according to the principles of GLP.</p> <p>No deviation has been noted in the study.</p> <p>In the definitive test all the validity criteria were met. The study is reliable and suitable for the risk assessment.</p> <p>Overall, the study is considered acceptable with following endpoints</p> <p>NOEC_{reproduction} = ≥ 1000.0 mg formulation/kg dw soil</p> <p>NOEC_{survival} = ≥ 1000.0 mg formulation/kg dw soil</p> <p>EC₁₀ = > 1000.0 mg formulation/kg dw soil</p>
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Reference:	KCP 10.4/01
Report	CHR/H/DIK 480 SL Earthworm reproduction test (<i>Eisenia andrei</i>); A. Gierbuszewska, 2021, Study code: G-09-21
Guideline(s):	According to the OECD Guideline No. 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

CHR/H/DIK 480 SL

batch no.: 012021

Active substance:

dicamba – 475.25 g/L

Artificial soil:

10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand

Test organism:

the earthworm, *Eisenia andrei* obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology

Test design:

Concentrations of the test item:

test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate

control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artifi-

cial soil

Test conditions:

temperature: 20 – 22°C;

pH at the beginning of the experiment: 5.50 – 5.65;

pH at the end of the experiment: 5.58 – 5.72;

soil moisture content at the beginning of the experiment: 18.2 – 22.4% (45.5 – 55.9% of the maximum water holding capacity);

soil moisture content at the end of the experiment: 18.7 – 23.1% (46.7 – 57.7% of the maximum water holding capacity);

light-dark cycle: 16h : 8h;

light intensity at the beginning of the experiment: 670.4 – 720.7 lux

light intensity at the end of the experiment: 668.3 – 704.1 lux

Statistical analysis:

EC10, EC20, EC50, LC50 – probit analysis using linear max. likelihood regression

NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Williams Multiple Sequential t-test Procedure,

NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction

LOEC: a values suggested by the ToxRat Professional 2.10 statistical computer software

Endpoint:

EC10, EC20, EC50, NOEC, LOEC (reproduction)

LC50, NOEC, LOEC (survival)

Results and discussions

The aims of the study were to assess the impact of CHR/H/DIK 480 SL on reproduction of the earthworm, *Eisenia andrei* and to determine EC10, EC20, EC50 and NOEC. The test item in the form of an aqueous solution was mixed with a suitable amount of the artificial soil. The concentrations of the test item were: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil. Each of them was divided into four replicates. There was also one untreated control group with the deionised water only. Control group was divided into eight replicates. The experiment lasted 8 weeks. After 4 weeks, all of adult earthworms were removed from the test containers and observed. All changes in their behavior and morphology were recorded. The number of earthworms and their body weights were also determined. The impact of the test item on reproduction was evaluated after the additional 4 week period on the basis of the number of juveniles hatched from cocoons during the experiment.

At concentrations ranging from 5.6 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 and 12.5%. As for the control group, mortality of the adult earthworms was equal to 1.3%.

The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 1000.0 mg/kg dry weight of the artificial soil (above 410.83 mg of dicamba/kg dry weight of the artificial soil).

No changes in the appearance (morphology) and behaviour of the living adult earthworms were noticed.

After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of artificial soil, the body weight increase was between 13.5 and 38.6%. As for the control group, the body weight increase was equal to 18.2%.

After 8 weeks of the experiment, the obtained results led to the following conclusions:

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 123.5 and 141.5 per replicate. The mean number of juveniles in the control group was equal to 134.8 per replicate.

After 8 weeks of the experiment, it was concluded that CHR/H/DIK 480 SL had no a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil.

The endpoint values showing the impact of the test item on reproduction and survival of adult earthworms are presented in the table given below.

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of dicamba/kg dry weight of artificial soil]
EC ₁₀	> 1000.0	> 410.83
EC ₂₀	> 1000.0	> 410.83
EC ₅₀	> 1000.0	> 410.83
NOEC (reproduction)	≥ 1000.0	≥ 410.83
LOEC (reproduction)	> 1000.0	> 410.83
LC ₅₀	> 1000.0	> 410.83
NOEC (survival)	≥ 1000.0	≥ 410.83
LOEC (survival)	> 1000.0	> 410.83

VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced from 117 to 172 juveniles (134.8 mean) at the end of the exposure period (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 13.0% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 1.3% (criterion: ≤ 10%).

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 2.4.2.1.1 Study 1

zRMS comments:	<p>The study was conducted to OECD guideline 232 and according to the principles of GLP.</p> <p>Following deviations from the OECD Guideline No. 232 (2016) occurred:</p> <ul style="list-style-type: none"> - culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016) - at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) <p>However, noted deviations did not affect the results of the study since all the validity criteria were met.</p> <p>The study is reliable and suitable for the risk assessment.</p>
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Report	CHR/H/DIK 480 SL Collembolan (<i>Folsomia candida</i>) Reproduction Test; P. Pieczka, 2021, Study code: G-10-21
Guideline(s):	according to the OECD Guideline No. 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

CHR/H/DIK 480 SL

batch no.: 012021

Active substances:

dicamba 475.25 g/L (Appendix No. 1)

Artificial soil:

5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand,

Test organism:

the collembolan, *Folsomia candida* obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Organisms Toxicology. The collembolans used in the study were between 9 to 12 days old.

Test design:

test duration: 28 days

number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate

Concentrations of the test item:

a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg of the test item/kg of dry weight of the artificial soil

Test conditions:

temperature: 20.6 – 22.0°C;

pH at the beginning of the test: 5.69 – 5.78;

pH at the end of the test: 5.53 – 5.66;

soil moisture content at the beginning of the test: 17.7 – 18.5% (46.2 – 48.3% of the maximum water holding capacity); soil moisture content at the end of the test: 17.0 – 18.1% (44.4 – 47.2% of the maximum water holding capacity);

lighting: 16 h light and 8h dark;

light intensity at the beginning of the experiment: 590.7 – 726.0 lux;

light intensity at the end of the experiment: 660.7 – 756.3 lux;

Statistical analysis:

EC10, EC20, EC50, LC10, LC20 and LC50 – probit analysis using linear max. likelihood regression

NOEC (number of juveniles):

- Shapiro-Wilk's Test on Normal Distribution,
- Bartlett's Test Procedure on Variance Homogeneity,
- Williams Multiple Sequential t-test Procedure

NOEC (survival):

- Fisher's Exact Binomial Test with Bonferroni Correction.

Endpoints:

EC10, EC20, EC50, NOEC

LC10, LC20, LC50, NOEC

Results and discussions

The aims of the study were to assess the impact of CHR/H/DIK 480 SL on reproduction of the collembolans, *Folsomia candida* and to determine the EC10, EC20, EC50, and NOEC. Ten concentrations of the test item were used. These were 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0, and 1000.0 mg of the test item/kg of dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in form of aqueous solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The exposure period lasted 28 days. After that, the collembolans were extracted from the artificial soil. The numbers of adults and juveniles were determined separately.

Conclusion

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 0 to 10.0%. As for the control group, it was equal to 3.8%.

The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]
LC ₁₀	>1000.0	>410.83
LC ₂₀	>1000.0	>410.83
LC ₅₀	>1000.0	>410.83
NOEC	≥1000.0	>410.83

After the exposure of collembolans to the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 1021.5 – 1121.5 per replicate. As for the control group, the number of juveniles was equal 1059.9 per replicate.

The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the table given below.

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]
EC ₁₀	>1000.0	>410.83
EC ₂₀	>1000.0	>410.83
EC ₅₀	>1000.0	>410.83
NOEC	≥1000.0	>410.83

VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the controls:

- mean adult mortality: 3.8% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 1059.9 (criterion: ≥100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 13.5% (criterion: ≤ 30%).

A 2.4.2.1.2 Study 1

zRMS comments:	<p>The study was conducted to OECD guideline 226 and according to the principles of GLP.</p> <p>Following deviations from the OECD Guideline No. 226 occurred:</p> <ol style="list-style-type: none"> 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and reweighing at the beginning, after 7 days of the test and at the end of the test 2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution 3. Due to the use of the temperature extraction method, there was no impossible to record the symptoms with behavioural and morphology changes of the extracted predatory mites <p>However, noted deviations did not affect the results of the study since all the validity criteria were met.</p> <p>The study is reliable and suitable for the risk assessment.</p>
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Reference:	KCP 10.4/03
Report	CHR/H/DIK 480 SL Predatory mite (<i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i>) reproduction test in soil, M. Czarnynoga, 2021, Study code: G-11-21
Guideline(s):	according to the OECD Guideline No. 226 (2016)
Deviations:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
 CHR/H/DIK 480 SL
 batch number: 012021
 Active substance:
 dicamba: 475.25 g/L (Appendix No. 1)
 Artificial soil:
 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand
 Test organism:
 the predatory mites, *Hypoaspis* (*Geolaelaps*) *aculeifer* (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult.
 Test design:
 test duration: 14 days
 number of replicates:
 4 replicates / concentration + 8 replicates / control;
 number of mites: 10 mites / replicate

Concentrations of the test item:

a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg test item/kg dry weight of the artificial soil.

Test conditions:

temperature: 20.0 – 22.0°C

pH at the beginning of the test: 5.66 – 5.69

pH at the end of the test: 5.62 – 5.67

soil moisture content at the beginning of the test: 17.3 – 18.3% (45.2 – 47.8% of the maximum water holding capacity)

soil moisture content in the middle of the test: 16.5 – 18.6% (43.1 – 48.6% of the maximum water holding capacity)

soil moisture content at the end of the test: 17.1 – 18.0% (44.6 – 47.0% of the maximum water holding capacity)

light-dark cycle: 16 h light and 8 h dark

light intensity at the beginning of the test: 462.5 – 527.1 lux

light intensity at end of the test: 533.3 – 567.1 lux

Statistical analysis:

EC10, EC20, EC50 – nonlinear regression using the 4-parameter logistic

LC10, LC20, LC50 – probit analysis using linear max. likelihood regression

NOEC:

- offspring number – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Williams Multiple Sequential t-test Procedure

- survival – Fisher's Exact Binominal Test with Bonferroni Correction

Endpoints:

EC10, EC20, EC50, NOEC

LC10, LC20, LC50, NOEC

Results and discussions

The aims of the study were to assess the impact of CHR/H/DIK 480 SL on reproduction of the predatory mite, *Hypoaspis* (Geolaelaps) *aculeifer* and to determine the EC10, EC20, EC50, and NOEC.

Ten concentrations of the test item were used. These included: 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil. Each concentration was divided into four replicates. There was also an untreated control group divided into eight replicates. The test item in the form of water solution was mixed with the artificial soil. The control artificial soil was mixed with deionized water alone. The experiment lasted 14 days. After that, the mites were extracted from the artificial soil (48-hour extraction). The numbers of adults and juveniles were determined separately.

Conclusion

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil was between 0.0% and 5.0%. Mortality of the control group was equal to 1.3%.

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil the mean number of juveniles was between 103.8 – 160.3 per replicate. The mean number of juveniles in the control group was equal to 131.5 per replicate.

Concentration [mg/kg dry weight of the artificial soil]	Adult mites			Number of juveniles (mean)
	Number of tested mites	Dead mites after 14 days		
		No.	%	
control	80	1	1.3	131.5
5.6	40	0	0.0	144.8
10.0	40	2	5.0	126.3
18.0	40	1	2.5	134.3
32.0	40	1	2.5	152.3
56.0	40	2	5.0	142.0
100.0	40	0	0.0	144.8
180.0	40	0	0.0	138.3
320.0	40	1	2.5	160.3
560.0	40	0	0.0	144.5
1000.0	40	0	0.0	103.8

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites (*Hypoaspis aculeifer*).

Endpoint	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg of dicamba/kg dry weight of the artificial soil]
EC ₁₀	950.2	390.4
EC ₂₀	984.7	404.5
EC ₅₀	> 1000.0	> 410.8
NOEC (reproduction)	560.0	230.1
LC ₁₀	> 1000.0	> 410.8
LC ₂₀	> 1000.0	> 410.8
LC ₅₀	> 1000.0	> 410.8
NOEC (survival)	≥ 1000.0	≥ 410.8

VALIDITY CRITERIA

The results are considered valid because the following criteria were satisfied in the control:

- mean adult mortality: 1.3% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 131.5 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation for the number of juveniles: 12.9% (criterion: ≤ 30%).

A 2.4.2.2 KCP 10.4.2.1 Species level testing

A 2.4.2.3 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1.1.1 Study 1

zRMS comments:	<p>The study was conducted to OECD guideline 216 and according to the principles of GLP.</p> <p>Following deviations in the study occurred.</p> <ol style="list-style-type: none"> 1. The predicted environmental concentration (PEC) was calculated assuming 2.5 cm of the soil depth. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000) and EU Method C.21, where the PEC is cal-
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	<p>culated by using 5 cm of the soil depth.</p> <p>2. According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm and time duration between 18 to 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer.</p> <p>3. According to OECD Guideline No. 216 (2000, the control and the treated soils should be incubated in a dark room at temperature of $20 \pm 2^{\circ}\text{C}$. In fact the temperature once exceed 22.0°C and was 22.3°C.</p> <p>The deviations mentioned above did not affect the final results of the study.</p> <p>On the basis of the results, it was concluded that CHR/H/DIK 480 SL / Macamba 480 SL, Dikambin 480 SL at the concentrations of 6.94 mg/kg of the soil did not have any long-term ad-verse effects on the process of nitrogen transformation in aerobic surface soils.</p>
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Reference:	KCP 10.5/01
Report	CHR/H/DIK 480 SL Soil Microorganisms: Nitrogen Transformation Test, M. Czarnynoga, 2021, Study code: G-12-21
Guideline(s):	according to the OECD Guideline No. 216 (2000)/EU Method C.21
Deviations:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material:

CHR/H/DIK 480 SL

batch no.: 012021

Active substance:

dicamba: 475.25 g/L (Appendix No. 1)

Soil:

Agricultural soil collected from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna.

Test design:

Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 42 days.

Concentrations of the test item:

control;

PEC: 1.39 mg test item/kg dry weight of soil (i.e. 0.57 mg of dicamba/kg dry weight of soil)

5 x PEC: 6.94 mg test item/kg dry weight of soil (i.e. 2.85 mg of dicamba/kg dry weight of soil)

Test conditions:

temperature: $20.0 - 22.3^{\circ}\text{C}$,

soil moisture: 41.1 – 49.4% of the maximum water holding capacity, incubation in darkness

Endpoints:

The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28 and 42 days of incubation.

The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days.

Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days.

Statistical analysis:

- Shapiro-Wilk's test on Normal Distribution
- Levene's Test on Variance Homogeneity (with Residuals)
- Williams Multiple Sequential t-test Procedure

Results and discussions

The aim of the study was to detect long-term adverse effects of CHR/H/DIK 480 SL on the processes of nitrogen transformation in aerobic surface soils.

The freshly collected agricultural soil was used in the experiment. It was manually cleared of large objects and sieved to a particle size of 2 mm.

Two concentrations of the test item were used, i.e.:

- PEC: 1.39 mg test item/kg dry weight of soil (i.e. 0.57 mg of dicamba/kg dry weight of soil)
- 5 x PEC: 6.94 mg test item/kg dry weight of soil (i.e. 2.85 mg of dicamba/kg dry weight of soil)

The treated and the control soils were divided into three replicates.

On days 0, 7, 14, 28 and 42 of incubation, soil samples were collected to determine the quantities of nitrate.

The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using deionised water. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used.

The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

On 28 day of analysis the percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at the concentration corresponding to the PEC and 5 x PEC exceeded 25%, therefore, according to the OECD No. 216, EU Method C.21 and the study plan, the experiment was continued.

The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 1.39 mg test item/kg dry weight of soil (i.e. 0.57 mg of dicamba/kg dry weight of soil) and 5 x PEC: 6.94 mg test item/kg dry weight of soil (i.e. 2.85 mg of dicamba/kg dry weight of soil) did not exceed 25% on 42 day of analysis.

Table 11. Deviations from the control based on nitrate formation rate for selected time intervals [%].

Time interval [d]	PEC	5 x PEC
0 – 7	12.41	11.16
0 – 14	23.23	-41.69
0 – 28	-27.60	-37.93
0 – 42	13.53	-16.76

Values obtained using ToxRat 2.10. computer software.

"-" - values of nitrate formation rate higher than the ones obtained for the control group

Conclusion

On the basis of the results, it was concluded that CHR/H/DIK 480 SL at the concentrations corresponding

to the PEC: 1.39 mg test item/kg dry weight of soil (i.e. 0.57 mg of dicamba/kg dry weight of soil) and 5 x PEC: 6.94 mg test item/kg dry weight of soil (i.e. 2.85 mg of dicamba/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

VALIDITY CRITERION

The coefficients of variation (CV) in the control group were 3.0, 4.6, 0.7, 3.4 and 2.7%, after 0, 7, 14, 28 and 42 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than 15%.

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 2.6.2.1.1 Study 1

zRMS comments:	<p>The Seedling Emergence study was conducted to OECD guideline 208 and according to the principles of GLP</p> <p>According to OECD Guideline No. 208 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between $77.5 - 169.5 \mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviation did not affect the results of the study</p> <p>All the validity criteria were met. The study is considered acceptable for the risk assessment purposes.</p>
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Reference:	KCP 10.6/01
Report	CHR/H/DIK 480 SL Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, A. Gierbuszewska, 2021, Study code: G-14-21
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:
 CHR/H/DIK 480 SL
 batch number: 012021
 active substances: dicamba 475.25 g/L (Appendix No. 1)
 Test species:
 flax (*Linum usitatissimum*), tomato (*Solanum lycopersicon*), pea (*Pisum sativum*), carrot (*Daucus carota*), onion (*Allium cepa*), wheat (*Triticum aestivum*)
 Soil:
 Sandy loam
 Study design:
 number of rates: 8 + control for all test species,

Number of replicates:

- 4 for flax, carrot, onion, wheat,
- 7 for pea,
- 10 for tomato.

The total number of seeds per application rate:

- 20 for flax, tomato, carrot, onion and wheat,
- 21 for pea;

test termination: 14 days after the emergence of 50% of the control seedlings.

Application rates:

- 0.00 (control), 0.27, 0.82, 2.47, 7.41, 22.22, 66.67, 200.00, and 600.00 mL/ha.

Volume of deionized water:

volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha

Test conditions:

temperature: 19.3 – 24.6°C, humidity: 48.8 – 69.8%, lighting: 16 h light : 8 h dark; light intensity: 77.5 – 169.5 $\mu\text{E}/\text{m}^2/\text{s}$; carbon dioxide concentration: 357 – 390 ppm

Statistical analysis:

ER25, ER50 (plant emergence, shoot length and dry weight) – probit analysis using linear max. likelihood regression

NOER:

In order to determine the NOER values for the plant number at the end of the experiment of flax, tomato, pea, carrot, onion, wheat the Fisher's Exact Binomial Test with Bonferroni Correction was used.

In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) the following statistical tests were used:

Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure

In order to determine the NOER values for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:

Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure or Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment

Endpoints:

ER25, ER50, NOERResults and discussions

The study, aimed at evaluating the effect CHR/H/DIK 480 SL on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. The test item was sprayed onto the soil surface. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots. There were 5 (flax, carrot, onion and wheat) or 3 (pea) or 2 (tomato) seeds/pot. The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every 1 to 2 days to the emergence of 50% of the control seedlings and after then every 2 – 3 days) and visual phytotoxicity (after 7 and 14 days after the emergence of 50% of the control seedlings). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60°C, and weighed.

The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER₂₅, ER₅₀, and NOER.

Results and conclusions

The ER₅₀ and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below.

	Flax <i>Linum usitatissimum</i>	Tomato <i>Solanum lycopersicon</i>	Pea <i>Pisum sativum</i>	Carrot <i>Daucus carota</i>	Onion <i>Allium cepa</i>	Wheat <i>Triticum aestivum</i>
Plant number at the end of the experiment						
ER₅₀	> 600.000*	165.516 (121.416 – 226.501)	1438.569	> 600.000*	882.402 (180.740 – 11647998.000)	> 600.000*
NOER	≥ 600.000	66.670	200.000	≥ 600.000	66.670	≥ 600.000
Shoot length (plants without roots)						
ER₅₀	113.705 (59.946 – 250.056)	24.167 (13.558 – 44.310)	74.192 (55.272 – 101.304)	481.950 (305.188 – 1103.06)	64.777 (44.404 – 96.998)	1180.091 (658.989 – 39265.754)
NOER	7.410	0.270	2.470	22.220	0.820	66.670
Plant dry weight (plants without roots)						
ER₅₀	54.473 (17.010 – 204.420)	5.063 (2.318 – 10.814)	33.571 (20.462 – 55.252)	90.976 (41.103 – 243.286)	57.235 (41.025 – 81.680)	494.991 (384.429 – 685.831)
NOER	2.470	0.270	2.470	2.470	2.470	22.220

The ER₅₀ and NOER values were calculated using the ToxRat Professional 2.10 computer software.

*the value could not be determined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 600.000 mL/ha

Conclusion

On the basis of the obtained results it was proved that the test item i.e. CHR/H/DIK 480 SL had varied impact on seedling emergence and seedling growth of the test plant species.

For the selected application rates, seedling emergence of tomato, pea, carrot and onion was delayed when compared with the control. The death of tomato, carrot and onion at the rates between 66.67 and 600.00

mL/ha was observed during the experiment. At the rate equal to 600.00 mL/ha mortality of all plants was noticed. The death of flax, pea and wheat was not observed.

The lowest ER50 value determined on the basis of the plant emergence at the end of the experiment, was observed for tomato and it was equal to 165.516 mL of the test item/ha.

The lowest ER50 value determined on the basis of the plant shoot length at the end of the experiment, was observed for tomato and it was equal to 24.167 mL of the test item/ha.

The lowest ER50 value determined on the basis of the plant shoot weight at the end of the experiment, was observed for tomato and it was equal to 5.063 mL of the test item/ha.

Significant and moderate inhibition of shoot length and weight was observed for flax, tomato, pea, carrot, onion and wheat.

Phytotoxic symptoms of plants, at selected application rates, were observed during the experiment. It was stunted growth, deformations, wilting, chlorosis and mortality of plants.

The following order of the test plant sensitivity was noticed:

tomato > onion > pea > flax > carrot > wheat.

VALIDITY CRITERIA

On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of CHR/H/DIK 480 SL on seedling emergence and seedling growth of terrestrial plants were met:

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:

- 90% – flax,

- 100% – tomato,

- 100% – pea,

- 100% – carrot,

- 100% – onion,

- 95% – wheat,

- the mean survival of the emerged control seedlings was 100% for flax, tomato, pea, carrot, onion and wheat (validity criterion: 90%);

- the control seedlings did not exhibit any visible phytotoxic effects;

- environmental conditions for all plants of the same species were identical.

A 2.6.2.1.2 Study 1

zRMS comments:	<p>The Vegetative vigour study was conducted to OECD guideline 227 and according to the principles of GLP</p> <p>According to OECD Guideline No. 227 (2006), the light intensity should be $350 \pm 50 \mu\text{E}/\text{m}^2/\text{s}$. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between $89.8 - 170.2 \mu\text{E}/\text{m}^2/\text{s}$. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. These deviations did not affect the results of the study.</p> <p>All the validity criteria were met. The study is considered acceptable for the risk assessment purposes.</p>
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Reference: KCP 10.6/02

Report CHR/H/DIK 480 SL Terrestrial Plant Test: Vegetative Vigour Test, P. Pieczka, 2021, Study code: G-13-21

Guideline(s): according to the OECD Guideline No. 227 (2006)

Deviations: No

GLP: Yes

Acceptability: Yes
Duplication No
(if vertebrate study)

Materials and methods

Test item:

CHR/H/DIK 480 SL

batch number: 012021

active substance: dicamba 475.25 g/L (Appendix No. 1)

Test species:

pea (*Pisum sativum*), tomato (*Solanum lycopersicon*), flax (*Linum usitatissimum*), carrot (*Daucus carota*), onion (*Allium cepa*), wheat (*Triticum aestivum*)

Soil:

Sandy loam

Study design:

number of rates: 8 + control; number of replicates/rate: 7 (pea), 4 (carrot, flax, onion and wheat) and 10 (tomato). The total number of plants per application rate – 21 (pea) or 20 (tomato, carrot, flax, onion, wheat)

exposure termination: 21 days after spraying

Application rates:

- a control,
- 0.27 mL of the test item /ha (0.13 g of dicamba/ha),
- 0.82 mL of the test item /ha (0.39 g of dicamba/ha),
- 2.47 mL of the test item /ha (1.17 g of dicamba/ha),
- 7.41 mL of the test item /ha (3.52 g of dicamba/ha),
- 22.22 mL of the test item /ha (10.56 g of dicamba/ha),
- 66.67. mL of the test item /ha (31.68 g of dicamba/ha),
- 200.00 mL of the test item /ha (95.05 g of dicamba/ha),
- 600.00 mL of the test item /ha (285.15 g of dicamba/ha).

volume of deionized water used to prepare the highest rate corresponded to 300 L spraying liquid/ha.

Test conditions:

temperature: 19.3 – 24.6°C, humidity: 48.8 – 69.8%, lighting: 16 h light : 8 h dark; light intensity: 89.12 – 170.2 µE/m²/s; carbon dioxide concentration: 329 – 348 ppm

Statistical analysis:

ER25, ER50 – probit analysis, 2-param. Normal CDF, 3-param. Normal CDF or 4-param. Normal CDF

NOER:

In order to determine the NOER values, the following tests were used:

- for the emergence of plants: Fisher's Exact Binomial Test with Bonferroni Correction, Qualitative Trend Analysis by Contrasts (Monotonicity of Rate/Response), Tarone's Test Procedure, Step-down Cochran-Armitage test Procedure, Step-down Rao-Scott-Cochran-Armitage Test Procedure
- for the shoot length: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm,
- for the plant weight: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Trend analysis by Contrasts (Monotonicity of Rate/Response), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsh t-test After Bonferroni-Holm.

Endpoints:

ER25, ER50, NOER

Results and discussions

The study, aimed at evaluating the effect of CHR/H/DIK 480 SL on vegetative vigour of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. Seeds of the test plant spe-

cies were sown in plastic pots (6 seeds/pot for pea and tomato; 10 seeds/pot for carrot, flax, onion and wheat). The plants were grown to the 2- to 4- true leaf stage. Then, some of them were removed. As a result, the number of plants per pot as well as the total number of plants per concentration were:

- pea: 3 plants/pot – 21 plants/application rate (7 pots/application rate);
- tomato: 2 plants/pot – 20 plants/application rate (10 pots/application rate);
- carrot: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- flax: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- onion: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate);
- wheat: 5 plants/pot – 20 plants/ application rate (4 pots/ application rate).

The pot is defined as a replicate. The test item was sprayed onto the plants. For each species, eight application rates were used. Untreated control group was conducted simultaneously. The treated and the control groups were divided into four replicates for carrot, flax, onion and wheat; 7 replicates for pea and 10 replicates for tomato. The experiment was conducted in a plant growth room where suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for visual phytotoxicity (7, 14 and 21 days after the test item application). The experiment finished 21 days after the spraying. At the end of the experiment, the number of surviving plants was counted. Next, the plants were cut down, and the lengths of their shoots were determined. Finally, they were dried at 60°C to a constant weight and weighed.

The results concerning the shoot length, the dry weight, and the number of plants at the end of the experiment were statistically analyzed to determine the ER₂₅, ER₅₀ and NOER.

The ER₅₀ and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below.

	Pea <i>Pisum sativum</i>	Tomato <i>Solanum lycopersicon</i>	Flax <i>Linum usitatissimum</i>	Carrot <i>Daucus carota</i>	Onion <i>Allium cepa</i>	Wheat <i>Triticum aestivum</i>
Plant number at the end of the experiment						
ER₅₀	67.46	61.22	109.37	582.71	>600.00	>600.00
NOER	22.22	22.22	22.22	200.00	>600.00	≥600.00
Shoot length						
ER₅₀	43.88	61.83	67.73	>600.00	>600.00	>600.00
NOER	7.41	22.22	22.22	200.00	200.00	66.67
Plant dry weight						
ER₅₀	22.04	15.28	37.47	392.49	260.48	>600.00
NOER	2.47	0.82	22.22	22.22	66.67	66.67

Conclusion

The test item, i.e. CHR/H/DIK 480 SL, applied at rates ranging from 0.27 to 600.00 mL/ha, had an impact on vegetative vigour of all tested plant species.

The mortality of plants was noticed in cultivation of pea, tomato, flax, carrot and wheat.

During the experiment the phytotoxic symptoms of the test item in cultivation of all testes plant species were observed. Among plant damages, these were: stunted growth (pea, tomato, flax, carrot, onion, wheat), chlorosis (tomato, flax, onion), wilting (pea, tomato, flax, onion), deformations (pea, tomato, flax, carrot, wheat), spots (tomato).

- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

A 2.8 KCP 10.8 Monitoring data