

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **102000037599**

Product name(s) Active substance(s): **Prohexadione-calcium OD 75 (75 g/L)**

Central Zone

Zonal Rapporteur Member State: **Poland**

CORE ASSESSMENT

Authorisation

Applicant: **Bayer CropScience Division**

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M-766712-01-1

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July 2021	Submission to Merit Mark for evaluation
January 2022	zRMS-PL finalised evaluation
April 2022	Final version prepared by zRMS after Commenting period

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The product Prohexadione-calcium OD 75 (75 g/L) (PRL OD 75 / Product Code 102000037599) has not been previously evaluated at zonal level. All data and information assessed during the EU re-evaluation of prohexadione-calcium is considered EU peer-reviewed data.

9 Ecotoxicology (KCP 10)

Review Comments:

This application was submitted by Bayer CropScience Division for approval of the formulation Prohexadione-calcium OD 75/PRL OD 75/ (product code: 102000037599) which contains 75 g/L of prohexadione calcium for use as plant growth regulator on winter oil seed rape.

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.

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, G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method Kind	Timing Growth stage of crop season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	AUT	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth stage							
4	CZE	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth stage							
7	DEU	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth stage							
10	HUN	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth stage							
13	POL	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth stage							
16	ROU	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													stage							
19	SVK	Rape, winter (BRSNW)	F	winter solidness, growth regulation of crop	spraying (broadcast, overall)	12-18	a) 1 b) 1	-	a) 1.2 b) 1.2	a) PRL 90 b) PRL 90	100-400	As per growth stage	PHI according to growth stage							

PRL: Prohexadione-Calcium

* 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:

- Numeration necessary to allow references
- Use official codes/nomenclatures of EU
- 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)
- 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
- 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
- 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
- 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
- The maximum number of application possible under practical conditions of use must be provided
- Minimum interval (in days) between applications of the same product.
- 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
- The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- If water volume range depends on application equipments (*e.g.* ULVA or LVA) it should be mentioned under “application: method/kind”.
- PHI - minimum pre-harvest interval
- Remarks may include: Extent of use/economic importance/restrictions

Review Comments:

GAP table 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

A summary of the conclusions and risk mitigation measures for the risk assessments conducted in this document is provided below.

All the general data and EU agreed endpoints for prohexadione-calcium used in this Section 9 can be found in the respective EU assessment report and EFSA Conclusion published for the EU review of the active substance (references provided in Appendix 1 of this document).

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

The risk for birds and mammals from dietary exposure after the use supported for the product Prohexadione-calcium OD 75 is acceptable. All TER values exceed the required regulatory trigger at Tier 1.

No unacceptable acute and long-term risk is expected for birds and mammals drinking water contaminated by residues from the use of Prohexadione-calcium OD 75.

The risk of secondary poisoning for birds and mammals via contaminated food (fish or earthworms) is considered to be low.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

For the intended use, calculated PEC/RAC ratios indicate an acceptable risk in all FOCUS Step 1 and 2 scenarios. PEC/RAC ratios for the formulated product also demonstrated acceptable risk for aquatic organisms. Based on the risk assessment presented in this document, it can be concluded that no mitigation is needed for the intended use in oil seed rape (winter).

9.1.1.3 Effects on bees (KCP 10.3.1)

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

The hazard quotients for both contact and oral exposure are below the trigger of concern ($QH \leq 50$) for the formulated product Prohexadione-calcium OD 75. Therefore, it can be concluded that no unacceptable risk to bees is expected using the product according to the proposed use pattern at a maximal application rate of 1 x 1.2 L product/ha in winter oil seed rape. Bumble bees did not exhibit greater sensitivity to Prohexadione-calcium OD 75 compared to the honeybee, rendering the honey bee risk assessment protective of bumble bees. Studies assessing the toxicity to adults following chronic exposure and larvae following repeated exposure to the formulated product did not indicate delayed or cumulative toxicity effects to adult bees or risk to honeybee larvae. Overall, the risk to bees due to applications of Prohexadione-calcium OD 75 in oil seed rape (winter) is considered to be low/acceptable.

Chronic and larvae data was provided with the formulation. Nevertheless, such studies were deemed not necessary to finalize the risk assessment.

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)

No unacceptable risk to non-target arthropods in the in-field and the off-field is to be expected based on the risk assessment. No mitigation measures are needed.

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

The long-term risk of the product was assessed for long-term exposure for earthworms and other soil macro-organisms (*Collembola*, *Hypoaspis*) and all TER values are greater than the relevant trigger values indicating a safe use of Prohexadione-calcium OD 75 in oil seed rape (winter) according to the proposed use pattern. Furthermore, no adverse effects on soil micro-organisms are to be expected.

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

All TER calculations are above the relevant trigger value of 5. Accordingly, a safe use of the product according to the use pattern can be concluded.

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

No further information is available or considered to be necessary.

9.1.2 Grouping of intended uses for risk assessment

Grouping is not applicable as only one use of 1 x 1.2 product/ha of Prohexadione-calcium OD 75 in oil seed rape (winter) is intended.

Table 9.1-2: Critical use pattern of Prohexadione-calcium OD 75 grouped according to crop

Grouping according to crop			
Group	Intended uses	Relevant use parameters for grouping	Relevant parameter or value for sorting
A	Oil seed rape (winter) 1 × 1.2 L product/ha BBCH 12-18	Crop type, application rate, number of applications, growth stage	Not applicable as only one use.

9.1.3 Consideration of metabolites

A metabolite, despropionyl prohexadione, was detected at 15.2% in a soil photolysis study under dry soil conditions. PEC values in soil were estimated for this metabolite in the EU review (EFSA Journal 2010; 8(3):1555), however it was noted in the EU review that the rapid rate of biodegradation under more realistic moist soil conditions would mean that it would not be expected to be formed under field conditions. No risk assessment was performed on this metabolite in the EU review; therefore, this metabolite is not considered in this assessment.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prohexadione-calcium. Full details of these studies are available in the respective EU assessment report.

Effects on birds of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. The submission of further data on the formulation Prohexadione-calcium OD 75 is not considered necessary, because studies done with mammals indicate that the formulation is not more toxic than expected based on its active substances (see 9.3.1). For this reason and also considering animal welfare, no acute oral toxicity study with the preparation was deemed necessary.

The studies and endpoints used for the risk assessment are in line with the endpoints listed for the EU review of the concerned active substance.

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>	Prohexadione-calcium	Oral 1 d Acute	LD ₅₀ > 2000 mg a.s./kg bw	EFSA Journal 2010; 8(3):1555
<i>Anas platyrhynchos</i>	Prohexadione-calcium	Oral 1 d Acute	LD ₅₀ > 2000 mg a.s./kg bw	EFSA Journal 2010; 8(3):1555
<i>C. virginianus</i>	Prohexadione-calcium	Dietary 5 d Short-term	LC ₅₀ > 1214 mg a.s./kg bw/d	EFSA Journal 2010; 8(3):1555
<i>A. platyrhynchos</i>	Prohexadione-calcium	Dietary 5 d Short-term	> 1352 mg a.s./kg bw/d	EFSA Journal 2010; 8(3):1555
<i>C. coturnix japonica</i>	Prohexadione-calcium	Dietary 6 w Reproductive toxicity	NOEC = 126 mg a.s./kg bw/d (parent and reproduction parameters)	EFSA Journal 2010; 8(3):1555
<i>C. virginianus</i>	Prohexadione-calcium	Dietary 22 w Reproductive toxicity	48.8 mg a.s./kg bw/d (effects on early embryonic mortality)	EFSA Journal 2010; 8(3):1555
<i>A. platyrhynchos</i>	Prohexadione-calcium	Dietary 23 w Reproductive toxicity	116.4 mg a.s./kg bw/d	EFSA Journal 2010; 8(3):1555

9.2.1.1 Justification for new endpoints

No deviation to EU agreed endpoints.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Prohexadione-calcium OD 75 in oil seed rape (winter)

Intended use		Oil seed rape (winter), 1 x 1.2 L/ha				
Active substance/product		Prohexadione-calcium				
Application rate (kg/ha)		1 × 0.09				
Acute toxicity (mg/kg bw)		>2000				
TER criterion						
Crop scenario	Indicator species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Oil seed rape	Small omnivorous bird	158.8	1.0	14.3	≥140	
Reprod. toxicity (mg/kg bw/d)		48.8				
TER criterion						
Crop scenario	Indicator species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Oil seed rape	Small omnivorous bird	64.8	1.0 × 0.53	3.09	15.8	

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.

The acute and long-term risk assessment for the use of the product in oil seed rape is acceptable.

9.2.2.2 Higher-tier risk assessment

Not required.

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 Prohexadione-calcium OD 75 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a $K(f)_{oc}$ of 182, prohexadione-calcium belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	90 ^A		
Acute toxicity (mg/kg bw)	=	> 2000	quotient =	< 0.045
Reprod. toxicity (mg/kg bw/d)	=	48.8	quotient =	1.84

^A Effective application rate for the use in oil seed rape; the MAF for 1 application is 1.0

9.2.2.4 Effects of secondary poisoning

Risk assessments for effects due to secondary poisoning are provided below for the substance with a log P_{ow} which exceeds the trigger value of 3. For the metabolites, only those with a log $P_{ow} > 3$ and relevant to the media have been considered, in accordance with EFSA guidance 2009.

The log P_{ow} of prohexadione-calcium amounts to -2.90 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The risk for birds from dietary exposure after the use supported for the product Prohexadione-calcium OD 75 is acceptable.

For prohexadione-calcium, TER values for all risk scenarios exceed the required regulatory trigger at Tier 1. No risk is discernible for birds drinking contaminated water and via secondary poisoning.

Review comments:

The acute and long-term risk assessment for birds performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009).

Endpoints used for acute and reproductive risk assessment provided by the Applicant represent worst-case scenario for birds thus they are accepted by zRMS. No formulation study was required.

Overall, acceptable acute and reproductive risk to birds may be concluded for application of PRL OD 75 on oilseed rape.

PRL OD 75 presents no unacceptable risk to birds resulting from exposure via drinking water. Since the log P_{ow} value of prohexadione calcium is 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 prohexadione-calcium. Full details of these studies are available in the respective EU assessment report as well as in Section 6 (Mammalian Toxicology).

Effects on mammals of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. New data submitted with this application in the core dossier are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology).

The studies and endpoints used for the risk assessment are in line with the endpoints listed for the EU review of the concerned active substance.

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

Species	Substance	Exposure System	Results	Reference
Rat	Prohexadione-calcium	Oral Acute	LD ₅₀ > 5000 mg a.s./kg bw	EFSA Journal 2010; 8(3):1555
Rat	Prohexadione-calcium	Dietary Reproductive toxicity Two-generation study	NOAEL = 35 mg a.s./kg bw/d	EFSA Journal 2010; 8(3):1555

Table 9.3-2: Mammalian toxicity data of the formulated product Prohexadione-calcium OD 75

Species	Substance	Exposure System	Results	Reference
Rat	PRL OD 75	Oral 14 d Acute	LD ₅₀ > 2000 mg prod./kg bw	Appendix 2 XXXXXX, 2020 M-690665-01-1

9.3.1.1 Justification for new endpoints

An acute rat study with the product Prohexadione-calcium OD 75 was performed and is submitted with this application (see Section 6). The endpoint from this study was used for the risk assessment.

9.3.2 Risk assessment for spray applications

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

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

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

Table 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of Prohexadione-calcium OD 75 in oil seed rape (winter)

Intended use		Oil seed rape (winter), 1 x 1.2 L/ha				
Active substance/product		Prohexadione-calcium				
Application rate (kg/ha)		1 × 0.09				
Acute toxicity (mg/kg bw)		5000				
TER criterion		10				
Crop scenario Growth stage	Indicator species		SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Oil seed rape	Small herbivorous mammal		118.4	1.0	10.7	≥469
Reprod. toxicity (mg/kg bw/d)		35				
TER criterion		5				
Crop scenario Growth stage	Indicator species		SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Oil seed rape	Small herbivorous mammal		48.3	1.0 × 0.53	2.30	15.2

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

Acute risk assessment of the product

Table 9.3-4: Screening assessment of the acute risk for mammals due to the use of Prohexadione-Calcium OD 75 in oil seed rape (winter)

Intended use		Oil seed rape (winter), 1 x 1.2 L/ha			
Active substance/product		Prohexadione-calcium OD 75			
Application rate (kg/ha)		1 × 1.25 ^{a)}			
Acute toxicity (mg/kg bw)		>2000			
TER criterion		10			
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
Oil seed rape	Small herbivorous mammal	118.4	1.0	148	>13.5

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

^{a)} Product density: 1.038 g/mL

The acute and long-term risk assessment for the use of the product in oil seed rape is acceptable.

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 182, prohexadione-calcium belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	90 ^A		
Acute toxicity (mg/kg bw)	=	> 5000	quotient =	< 0.018
Reprod. toxicity (mg/kg bw/d)	=	35	quotient =	2.57

^A Effective application rate for the use in oil seed rape; the MAF for 1 application is 1.0

9.3.2.4 Effects of secondary poisoning

Risk assessments for effects due to secondary poisoning are provided below for the substance with a log P_{ow} which exceeds the trigger value of 3 (refer to 9.2.2.4).

The log P_{ow} of prohexadione-calcium amounts to -2.90 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The risk for mammals from dietary exposure after the use supported for the product Prohexadione-calcium OD 75 is acceptable.

For prohexadione-calcium and the product Prohexadione-calcium OD 75, TER values for all risk scenarios exceed the required regulatory trigger at Tier 1. No risk is discernible for mammals drinking contaminated water and via secondary poisoning.

Review comments:

The acute and long-term risk assessment for mammals performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009).

Endpoints used for acute and reproductive risk assessment provided by the Applicant represent worst-case

scenario for mammals thus they are accepted by zRMS.

Applicant performed also screening assessment of the acute risk for mammals due to the use of Prohexadione-Calcium OD 75 in oil seed rape (winter) on the basis of the results from the formulation study by xxxxxx, 2020 M-690665-01-1 (evaluated and accepted in Toxicological section).

Overall, acceptable acute and reproductive risk to birds may be concluded for application of PRL OD 75 on oilseed rape.

PRL OD 75 presents no unacceptable risk to mammals resulting from exposure via drinking water. Since the log Pow value of prohexadione calcium is 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)

Regarding the assessment of potential effects on reptiles and amphibians neither guidance documents nor testing guidelines are available at present. Therefore, no additional data on terrestrial vertebrate wildlife is presented here.

Review 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 prohexadione-calcium. Full details of these studies are available in the respective EU assessment report.

Effects on aquatic organisms of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. New data submitted with this application in this core dossier are listed in Appendix 1 and summarised in Appendix 2.

Where the selection of studies and endpoints for the risk assessment deviates from the results of the EU review process, justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Prohexadione-calcium	96 h, s	LC ₅₀ > 100 mg a.s./L _{nom}	EFSA Journal 2010; 8(3):1555
<i>Lepomis macrochyrus</i>	Prohexadione-calcium	96 h, ss	LC ₅₀ > 100 mg a.s./L _{nom}	EFSA Journal 2010; 8(3):1555
<i>Oncorhynchus mykiss</i>	Prohexadione-	28 d, f	NOEC = 100 mg	EFSA Journal 2010;

Species	Substance	Exposure System	Results	Reference
	calcium		a.s./L _{nom}	8(3):1555
<i>Daphnia magna</i>	Prohexadione-calcium	48 h, s	EC ₅₀ > 100 mg a.s./L _{nom}	EFSA Journal 2010; 8(3):1555
<i>Daphnia magna</i>	Prohexadione-calcium	21 d, ss	NOEC = 100 mg a.s./L _{nom}	EFSA Journal 2010; 8(3):1555
<i>Pseudokirchneriella subcapitata</i>	Prohexadione-calcium	120 h, s	72 h ErC ₅₀ > 100 mg a.s./L _{nom}	EFSA Journal 2010; 8(3):1555
<i>Anabaena flosaquae</i>	Prohexadione-calcium	120 h, s	EbC ₅₀ > 1.2 mg a.s./L _{nom}	EFSA Journal 2010; 8(3):1555
<i>Lemna gibba</i>	Prohexadione-calcium	14 d, ss	EC ₅₀ > 1.2 mg a.s./L _{mm}	EFSA Journal 2010; 8(3):1555

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 – Prohexadione-calcium OD 75

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	PRL OD 75	48 h, ss	EC ₅₀ = 7.88 mg prod./L _{mm}	Appendix 2 Riebschläger, 2021 M-764734-01-1
<i>Pseudokirchneriella subcapitata</i>	PRL OD 75	96 h, s	72 h ErC ₅₀ = > 20.4 mg prod./L _{mm} 72 h EyC ₅₀ = > 11.6 mg prod./L	Appendix 2 Kuhl, 2021 M-763760-01-1
<i>Lemna gibba</i>	PRL OD 75	7 d, s	EC ₅₀ = 45.8 mg prod./L _{mm}	Appendix 2 Schrader, 2021 M-763594-01-1
<i>Myriophyllum spicatum</i>	PRL OD 75	14 d, s Water/sediment	EC ₅₀ = 26.4 mg prod./L _{mm}	Appendix 2 Armbruster, 2021 M-764321-01-1

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

No acute toxicity study with fish was conducted with the formulated product Prohexadione-calcium OD 75 due to animal welfare reasons. The data on the active substance prohexadione-calcium demonstrates low toxicity to fish with both, acute and chronic tests, resulting in endpoints greater or equal to 100 mg a.s./L. Thus, the available data on the active substance toxicity did not indicate any risk for fish.

Review comments:

All the studies provided for the formulated product were evaluated and accepted by zRMS. For details please, refer to Appendix 2.

The endpoint ErC₅₀ is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guid-

ance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone." Please note that this issue will be addressed in the German national risk assessment.

9.5.1.1 Justification for new endpoints

Studies with the product Prohexadione-calcium OD 75 were performed and are submitted with this application. The endpoints from these studies are considered in the risk assessment in addition to the EU agreed endpoints for the active substance.

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 Regulation (EC) No 284/2013 entitled "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 PEC_{SW} values used for the risk assessment covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables 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 prohexadione-calcium for each organism group based on FOCUS Steps 1,2 calculations for the use of Prohexadione-calcium OD 75 in oil seed rape (winter) (1 x 90 g a.s./ha; modelling use oil seed rape (winter) - 1×90 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic plants
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Anabaena flos-aqua</i>	<i>Lemna gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀
(µg/L)		> 100000	100000	> 100000	100000	> 1200	> 1200
AF		100	10	100	10	10	10
RAC (µg/L)		> 1000	10000	> 1000	10000	> 120	> 120
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
- -	25.0	< 0.025	0.002	< 0.025	0.002	< 0.208	< 0.208
Step 2							
Northern Europe	0.828*	<0.001	<0.001	<0.001	<0.001	<0.007	<0.007
Southern Europe	0.828*	<0.001	<0.001	<0.001	<0.001	<0.007	<0.007

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

* Worst-case PEC value of Summer (Jun.-Sep.) and Autumn (Oct.-Feb.) scenario considered; FOCUS Step 1/2 PEC_{sw} values for the active substance prohexadione-calcium following single application to oilseed rape are summarized in Part B8 - Chapter 8.9.2.1

For the intended use, calculated PEC/RAC ratios for prohexadione-calcium indicate an acceptable risk for aquatic organisms **already** at FOCUS Step 1.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prohexadione-calcium for each organism group based on PEC calculations for the use of Prohexadione-calcium OD 75 in oil seed rape (winter) (1 x 1.2 L/ha; modelling use oil seed rape (winter) -- 1x1.2 L prod./ha)

Group		Inverteb. acute	Algae	Aquatic plants	Aquatic plants
Test species		<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Lemna gibba</i>	<i>Myriophyllum spicatum</i>
Endpoint (µg prod./L)		EC ₅₀ 7880	EC ₅₀ 20400	EC ₅₀ 45800	EC ₅₀ 26400
AF		100	10	10	10
RAC (µg prod./L)		78.80	2040	4580	2640
Scenario	PEC _{ini} (µg prod./L)				
Spray drift (1 m distance)	8.003 11.501	0.101 0.146	0.00392 0.00564	0.00174 0.00251	0.00303 0.00436

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

Initial PEC_{SW} values for the product Prohexadione-calcium OD 75 following single application to oil seed rape are summarized in Part B8 - Chapter 8.9.2.2

For the intended use, calculated PEC/RAC ratios for the product Prohexadione-calcium OD 75 indicate an acceptable risk for aquatic organisms without the need for any drift mitigation measures.

9.5.3 Overall conclusions

For the intended use, calculated PEC/RAC ratios indicate an acceptable risk in all FOCUS Step 1 ~~and 2~~ scenarios. PEC/RAC ratios for the formulated product also demonstrated acceptable risk for aquatic organisms. Therefore, no further assessment was considered necessary.

Based on the risk assessment provided above, it can be concluded that no mitigation is needed for the intended use in oil seed rape (winter).

Review 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).

Risk assessment was provided for the active substance and formulation and is considered acceptable. For prohexadione calcium and the product calculated PEC/RAC ratios for the use in winter oilseed rape indicated an acceptable risk already at FOCUS Step 1.

The PEC_{sw} for the formulation PRL OD 75 used in winter oil seed rape was updated according to Section 8.

Concluding the risk to aquatic organisms caused by the application of PRL OD 75 for use foreseen in critical GAP is acceptable without risk mitigation measures.

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 prohexadione-calcium. Full details of these studies are available in the respective EU assessment report.

Effects on bees of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. New data submitted with this application in the core dossier are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line the results of the EU review process. Where the selection of studies and endpoints for the risk assessment deviates from the results of the EU review process, justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Prohexadione-calcium	Oral 48 h	LD ₅₀ > 100 µg a.s./bee	EFSA Journal 2010; 8(3):1555
<i>Apis mellifera</i>	Prohexadione-calcium	Contact 48 h	LD ₅₀ > 100 µg a.s./bee	EFSA Journal 2010; 8(3):1555
<i>Apis mellifera</i>	PRL OD 75	Oral 48 h	LD ₅₀ > 76.5 µg a.s./bee	Appendix 2 Sekine, 2020 M-758769-01-1

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	PRL OD 75	Contact 72 h	LD ₅₀ = 14.4 µg a.s./bee	Appendix 2 Sekine, 2020 M-758769-01-1
<i>Apis mellifera</i>	PRL OD 75	Oral (10 d, chronic)	LC ₅₀ = 505 mg a.s./kg diet LDD ₅₀ = 4.33 µg a.s./bee/day NOEC = 280 mg a.s./kg diet NOEDD = 3.34 µg a.s./bee/day	Appendix 2 Kling, 2021 M-762860-01-1
<i>Apis mellifera</i> (larvae)	PRL OD 75	Oral (22 d, repeated feeding)	NOEC (emergence) = 19.2 mg a.s./kg diet NOED (emergence) = 2.96 µg a.s./larva	Appendix 2 Kling, 2021 M-762844-01-1
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

9.6.1.1 Justification for new endpoints

In order to complete the data set and the knowledge on adult bees after acute and chronic exposure and to investigate potential effects on developmental stages of honeybees, further studies with the product have been performed.

Table 9.6-2: Justification for new endpoints for honey bees

Species	Substance	Exposure system	Justification
<i>Apis mellifera</i>	PRL OD 75	Oral 48 h	New study endpoint: LD ₅₀ > 76.5 µg a.s./bee To provide information on the acute oral toxicity of the formulated product for the purposes of a risk assessment.
<i>Apis mellifera</i>	PRL OD 75	Contact 72 h	New study endpoint: LD ₅₀ = 14.4 µg a.s./bee To provide information on the acute contact toxicity of the formulated product for the purposes of a risk assessment.
<i>Apis mellifera</i>	PRL OD 75	Oral (10 d, chronic)	New study endpoints: LC ₅₀ = 505 mg a.s./kg diet LDD ₅₀ = 4.33 µg a.s./bee/day NOEC = 280 mg a.s./kg diet NOEDD = 3.34 µg a.s./bee/day To provide information on chronic toxicity to adult bees according to new data requirements.
<i>Apis mellifera</i> (larvae)	PRL OD 75	Oral (22 d, repeated feeding)	New study endpoints: NOEC (emergence) = 19.2 mg a.s./kg diet NOED (emergence) = 2.96 µg a.s./larva To provide information on toxicity to larvae according to new data requirements.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-3: First-tier assessment of the risk for bees due to the use of Prohexadione-calcium OD 75 in oil seed rape (winter)

Intended use	Oil seed rapes (winter), 1 × 1.2 L/ha		
Product	Prohexadione-calcium OD 75		
Application rate (g/ha)	1 × 90		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	>76.5	90	<1.18
Contact toxicity	14.4		6.25

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

Summary and further considerations regarding the risk to bees

Acute toxicity/effects of formulation

The active substance prohexadione-calcium is of low toxicity to bees, with an acute LD₅₀ contact value for adult bees of >100 µg a.s./bee and an acute LD₅₀ oral value for adult bees of > 100 µg a.s./bee. The formulated product Prohexadione-calcium OD 75 results in an acute oral LD₅₀ value for adult bees > 76.5 µg a.s./bee (after 48 h) and an acute contact LD₅₀ value for adult bees at 14.4 µg a.s./bee (after 72 h). HQ values based on the use in oil seed rape (winter) for the formulated product are lower than the levels regarded to indicate a risk to bees. Consequently, based on the available acute data, Prohexadione-calcium OD 75 does not pose an unacceptable risk to honeybee adults when applied to winter oilseed rape at a rate of 1.2 L product/ha (corresponding to 90 g a.s./ha).

As per the proposed GAP, a single foliar spray application of the formulated product is intended for pre-flowering winter oil seed rape (BBCH 12-18). As the product is only applied once into a non-blooming crop during autumn when foraging activity is greatly decreased, the probability of chronic exposure to the formulated product for either honeybee adults or larvae is considered to be low.

Nevertheless, the applicant has performed a chronic oral toxicity test (10-day feeding) as per OECD Guideline No. 245 as well as a chronic larvae laboratory study (repeated exposure) as per OECD Guidance Document No. 239 to address potential chronic toxicity to honey bees and effects on honey bee development and other honey bee life stages, respectively, in accordance with the data requirements as set out in Commission Regulation (EU) No. 284/2013. The findings of these studies are described below.

Chronic adult toxicity/effects of formulation

A 10-day laboratory feeding study investigating the effects of Prohexadione-calcium OD 75 was conducted to assess chronic toxicity to honeybees in accordance with OECD Guideline No. 245. The test comprised 5 test item treatment groups with nominal concentrations of 17.9, 44.8, 112, 280 and 700 mg a.s./kg feeding solution, corresponding to actual doses of 0.55, 1.22, 2.37, 3.34 and 4.99 µg a.s./bee/day. The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application. The study concluded that *ad libitum* feeding at concentrations up to 280 mg a.s./kg diet (corresponding to 3.34 µg a.s./bee/day) over a period of 10 days did not lead to statistically

significant mortality (actual mortalities $\leq 5\%$). Thus, the NOEC was determined as 280 mg a.s./kg diet and the corresponding NOEDD as 3.34 μg a.s./bee/day. The LDD_{50/20/10} were determined as 4.33, 3.87, and 3.60 μg a.s./bee/day, respectively. Daily dosing with 3.87 μg a.s./bee/day over 10 days (total dose of 38.7 μg a.s./bee) thus did not induce significantly higher mortality (20%) compared to a single acute oral exposure at 76.5 μg a.s./bee (actual mortality of 16.7%). Study results therefore do not indicate delayed or cumulative toxicity effects following chronic exposure to Prohexadione-calcium OD 75 compared with acute toxicity testing.

Chronic larval toxicity/effects on brood

A honeybee larval toxicity test assessing the effect of Prohexadione-calcium OD 75 on adult emergence following repeated feeding exposure was conducted to address effects on immature honeybee life stages and their development. The 22-day laboratory dose-response test assessed larval and pupal survival as well as adult emergence, following exposure to nominal concentrations of 7.68, 19.2, 48.0, 120 and 300 mg a.s./kg diet (corresponding to cumulative doses of 1.18, 2.96, 7.39, 18.5 and 46.2 μg a.s./larva per developmental period). The 22-day NOED (emergence) was determined to be 2.96 μg a.s./larva per developmental period, indicating no risk to honeybee development.

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

Not required.

Review Comments:

Since acceptable acute risk have been concluded for bees exposed to PRL OD 75 at the Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of PRL OD 75.

9.6.3 Effects on bumble bees

Review Comments:

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

A study on the toxicity to bumble bees has been carried out with Prohexadione-calcium OD 75. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.6-4: Endpoints and effect values relevant for the risk assessment for bumble bees

Species	Substance	Exposure System	Results	Reference
<i>Bombus terrestris</i>	PRL OD 75	Oral	LD ₅₀ > 33.2 μg a.s./bumble bee	Appendix 2 Chwiesko, 2021, M-761833-01-1
<i>Bombus terrestris</i>	PRL OD 75	Contact	LD ₅₀ > 63 μg a.s./bumble bee	Appendix 2 Chwiesko, 2021, M-761833-01-1

Table 9.6-5: Justification for new endpoints for bumble bees

Species	Substance	Exposure system	Justification
<i>Bombus terrestris</i> (adult)	PRL OD 75	Oral (48 h, acute)	New study endpoints: LC ₅₀ > 33.2 µg a.s./bumble bee To provide information on acute oral toxicity to adult bumble bees according to new data requirements
<i>Bombus terrestris</i> (adult)	PRL OD 75	Contact (48 h, acute)	New study endpoints: LC ₅₀ > 63 µg a.s./bumble bee To provide information on acute contact toxicity to adult bumble bees according to new data requirements

Acute contact and oral endpoints for bumble bees are similar and comparable to honeybee endpoints for Prohexadione-calcium OD 75. Hence, the findings indicate that bumble bees do not exhibit greater sensitivity to Prohexadione-calcium OD 75 compared to the honeybee. The risk assessment for honeybees was therefore considered to be protective of other bees and to cover the exposure of non-*Apis* bees such as *Bombus terrestris*.

9.6.4 Effects on solitary bees

Not relevant.

Review Comments:

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

9.6.5 Overall conclusions

The hazard quotients for both contact and oral exposure are below the trigger of concern ($QH \leq 50$) for the formulated product Prohexadione-calcium OD 75. Therefore, it can be concluded that no unacceptable risk to bees is expected using the product according to the proposed use pattern at a maximal application rate of 1 x 1.2 L product/ha in winter oil seed rape. Bumble bees did not exhibit greater sensitivity to Prohexadione-calcium OD 75 compared to the honeybee, rendering the honeybee risk assessment protective of bumble bees. Studies assessing the toxicity to adults following chronic exposure and larvae following repeated exposure to the formulated product did not indicate delayed or cumulative toxicity effects to adult bees or risk to honeybee larvae. Overall, the risk to bees due to applications of Prohexadione-calcium OD 75 in oil seed rape (winter) is considered to be low/acceptable.

Review 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 PRL OD 75 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 PRL OD 75 in oilseed rape.

The Applicant presented new chronic studies on bees and evaluation of effects on honey bee development and acute studies on bumblebees.

Chronic and larvae data was provided with the formulation and also new studies with bumble bees were provided. Nevertheless, such studies were deemed not necessary to finalize the risk assessment.

Since the risk assessment was performed according to SANCO/10329/2002 rev 2.
Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) 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 representative formulations of prohexadione-calcium. Full details of these studies are provided in the respective EU assessment report. Effects on non-target arthropods of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. New data submitted with this application in the core dossier 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)	PRL OD 75	Laboratory test glass plates (2D)	LR ₅₀ = 80.7 g a.s./ha ER ₅₀ > 57 g a.s./ha	Appendix 2 Waibel, 2020 M-761029-02-1
<i>Aphidius rhopalosiphi</i> (adults)	PRL OD 75	Laboratory test glass plates (2D)	LR ₅₀ = 26.1 g a.s./ha ER ₅₀ > 18 g a.s./ha	Appendix 2 Waibel, 2020 M-761030-02-1
<i>Typhlodromus pyri</i> (protonymphs)	PRL OD 75	Extended laboratory test bean leaves (2D)	LR ₅₀ > 180 g a.s./ha ER ₅₀ > 180 g a.s./ha	Appendix 2 Waibel, 2021 M-762531-02-1
<i>Aphidius rhopalosiphi</i> (adults)	PRL OD 75	Extended laboratory test barley plants (3D)	LR ₅₀ > 180 g a.s./ha ER ₅₀ > 180 g a.s./ha	Appendix 2 Waibel, 2021 M-762532-02-1
<i>Coccinella septempunctata</i>	PRL OD 75	Extended laboratory test bean leaves (2D)	LR ₅₀ > 180 g a.s./ha No effect on reproduction at 180 g a.s./ha	Appendix 2 Waibel, 2021 M-763934-02-1
<i>Chrysoperla carnea</i>	PRL OD 75	Extended laboratory test bean leaves (2D)	LR ₅₀ > 180 g a.s./ha No effect on reproduction at 180 g a.s./ha	Appendix 2 Waibel, 2021 M-762533-01-1

9.7.1.1 Justification for new endpoints

Studies with the product Prohexadione-calcium OD 75 were performed and are submitted with this application. The endpoints from these studies were used for the risk assessment.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the

Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of Prohexadione-calcium in oil seed rape (winter)

Intended use		Oil seed rape (winter), 1 × 90 g a.s./ha		
Active substance/product		Prohexadione-calcium		
Application rate		1 × 90 g a.s./ha		
MAF		1.0		
Test Tier 1	species	LR₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 2
	<i>Typhlodromus pyri</i>	80.7	90	1.1
	<i>Aphidius rhopalosiphi</i>	26.1		3.4
Test Higher-tier	species	Rate with ≤ 50 % effect* (g/ha)	PER_{in-field} (g/ha)	PER_{in-field} below rate with ≤ 50 % effect?
	<i>Typhlodromus pyri</i>	>180	90	yes
	<i>Aphidius rhopalosiphi</i>	>180	90	yes
	<i>Coccinella septempunctata</i>	>180	90	yes
	<i>Chrysoperla carnea</i>	>180	90	yes
Test Higher-tier	species	Rate with ≤ 50 % effect (g/ha) at xxx DALT	PER_{in-field} (g/ha)	PER_{in-field} below rate with ≤ 50 % effect?
-		-	-	-

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 is 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 Prohexadione-calcium in oil seed rape (winter)

Intended use		Oil seed rape (winter), 1 × 90 g a.s./ha				
Active substance/product		Prohexadione-calcium				
Application rate		1 × 90 g a.s./ha				
MAF		1.0				
VDF		10 (TIER 1) 5 (2D) / 1 (3D)				
Test Tier 1	species	LR₅₀ (lab.) (g a.s/ha)	Drift rate (%)	PER_{off-field} (g/ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
	<i>Typhlodromus pyri</i>	80.7	2.77	0.249	10	0.031 ≤ 0.1

<i>Aphidius rhopalosiphi</i>	26.1		2.49	5	0.1
Test species	Rate with $\leq 50\%$ effect* (g/ha)	Drift rate (%)	PER_{off-field} (g/ha)	CF	corr. PER_{off-field} with $\leq 50\%$ effect?
-	-	-	-	-	-

MAF: multiple application factor; VDF: vegetation distribution factor; (corr.) PER: (corrected) predicted environmental rate; CF: conversion 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 is considered in place of the rate with $\leq 50\%$ effect.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

No unacceptable risk to non-target arthropods in the in-field and the off-field is to be expected based on the risk assessment as provided above. No mitigation measures are needed.

Review Comments:

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

The HQ for recommended species: *Typhlodromus pyri* and *Aphidius rhopalosiphi* is below the ESCORT 2 trigger value of 2, indicating acceptable in-field and off-field risk to non-target arthropods already at tier I level.

Overall 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 prohexadione-calcium. Full details of these studies are available in the respective EU assessment report.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. 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>	Prohexadione-calcium	14 d, acute	LC ₅₀ > 1000 mg a.s./kg dw	EFSA Journal 2010; 8(3):1555
<i>Eisenia fetida</i>	PRL OD 75	Mixed into substrate 56 d, chronic 10 % peat content	NOEC ≥ 1000 mg prod./kg dw	Appendix 2 Büttner, 2021 M-763276-01-1
<i>Folsomia candida</i>	PRL OD 75	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 562 mg prod./kg dw EC ₁₀ = 649 mg prod./kg dw	Appendix 2 Richter, 2021 M-762156-01-1
<i>Hypoaspis aculeifer</i>	PRL OD 75	Mixed into substrate 14 d, chronic 5 % peat content	NOEC ≥ 1000 mg prod./kg dw	Appendix 2 Richter, 2020 M-687925-01-1

9.8.1.1 Justification for new endpoints

Studies with the product Prohexadione-calcium OD 75 were performed and are submitted with this application. The endpoints from these studies were used for the risk assessment in addition to the EU agreed endpoint for the active substance.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for prohexadione-calcium.

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 PRL OD 75 - C-EU, PGR in winter OSR, single application in oil seed rape (winter) (use group oil seed rape)

Intended use	foliar spray oil seed rape (winter)		
Acute effects on earthworms			
Product/active substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw) ^A	TER _a (criterion TER ≥ 10)
Prohexadione-calcium	> 1000	0.072	> 13889
Chronic effects on earthworms			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} [*] (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
PRL OD 75 - C-EU, PGR in winter OSR, single application	≥ 1000	0.996	≥ 1004
Chronic effects on other soil macro- and mesofauna			
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} [*] (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
PRL OD 75 - C-EU, PGR in winter OSR, single application <i>Folsomia candida</i>	562	0.996	564
PRL OD 75 - C-EU, PGR in winter OSR, single application <i>Hypoaspis aculeifer</i>	≥ 1000	0.996	≥ 1004

^A PEC_{accumulation} = PEC_{actual} + PEC_{soil plateau}

* Formulation density 1038g/L and 40 % interception were taken into consideration in the calculations.

9.8.2.2 Higher-tier risk assessment

Not relevant.

Review 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

The long-term risk of the product was assessed for earthworms and other soil macro-organisms (Collembola, *Hypoaspis*) and all TER values are by far greater than the relevant trigger values, indicating a safe use of Prohexadione-calcium OD 75 in oil seed rape (winter) according to the proposed use pattern.

Review comments:

The risk assessment for earthworms and other soil macro-organisms exposed to PRL OD 75 was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology” (SANCO/10329/2002) and accepted by the zRMS.

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

refer to Section 8.
 TERIt values calculated for PRL OD 75 were above the respective trigger indicating acceptable long-term risk to earthworms and other soil macro-organisms. No further evaluation is deemed necessary.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with prohexadione-calcium. Full details of these studies are provided in the respective EU assessment report.

Effects on soil microorganisms of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. New data submitted with this application in the core dossier 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	Prohexadione-calcium	28 d, aerobic	No unacceptable effects on N-transformation at 0.696 mg a.s./kg soil dw	EFSA Journal 2010; 8(3):1555
N-mineralisation	PRL OD 75	28 d, aerobic loamy sand	No unacceptable effects on N-transformation at 12 L prod./ha (≥ 16.61 mg prod./kg soil dw)	Appendix 2 Schulz, 2020 M-758535-01-1

9.9.1.1 Justification for new endpoints

Studies with the product Prohexadione-calcium OD 75 were performed and are submitted with this application. The endpoints from these studies were used for the risk assessment in addition to the EU agreed endpoint for the active substance.

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).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of PRL OD 75 - C-EU, PGR in winter OSR, single application in oil seed rape (winter) (use group oil seed rape)

Intended use	foliar spray oil seed rape (winter)		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25% (mg/kg dw)	PEC _{soil} (mg/kg dw) ^A	Risk acceptable ?

Prohexadione-calcium	≥ 0.696 (at 28 d)	0.072	yes
PRL OD 75 - C-EU, PGR in winter OSR, single application	≥ 16.61 (at 28 d)	0.996 *	yes
C-mineralisation			
Not required according to Regulation (EC) 1107/2009.			

^A $PEC_{accumulation} = PEC_{actual} + PEC_{soil\ plateau}$

* Formulation density 1038g/L and 40 % interception were taken into consideration in the calculations.

9.9.3 Overall conclusions

No adverse effects on soil micro-organisms are to be expected if the product is used in oil seed rape as recommended.

Review 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 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 PRL OD 75 had no significant impact on soil microorganisms when applied at test item concentrations up to 16.61 mg formulation/kg dw 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 representative formulations of prohexadione-calcium. Full details of these studies are provided in the respective EU assessment report.

Effects on non-target terrestrial plants of Prohexadione-calcium OD 75 were not evaluated as part of the EU assessment of prohexadione-calcium. New data submitted with this application in the core dossier 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	Reference
<i>Beta vulgaris</i> [1] d <i>Brassica rapa</i> [1] d <i>Cucumis sativus</i> [1] d	PRL OD 75	21 d Seedling emergence Tier 1	[1] $ER_{50} > 90$ g a.s./ha	Appendix 2 Köhler, 2020, M-691185-01-1

Species	Substance	Exposure System	Results	Reference
<i>Glycine max</i> [1] d <i>Lactuca sativa</i> [1] d <i>Solanum lycopersicum</i> [1] d <i>Allium cepa</i> [1] m <i>Avena sativa</i> [1] m <i>Lolium perenne</i> [1] m <i>Zea mays</i> [1] m				
<i>Beta vulgaris</i> [1] d <i>Brassica rapa</i> [1] d <i>Cucumis sativus</i> [2] d <i>Glycine max</i> [1] d <i>Lactuca sativa</i> [1] d <i>Solanum lycopersicum</i> [1] d <i>Allium cepa</i> [1] m <i>Avena sativa</i> [1] m <i>Lolium perenne</i> [1] m <i>Zea mays</i> [1] m	PRL OD 75	21 d Vegetative vigour Tier 1	[1] ER ₅₀ > 90 g a.s./ha [2] ER ₅₀ plant height < 90 g a.s./ha	Appendix 2 Köhler, 2020, M-691184-02-1
<i>Cucumis sativus</i> d	PRL OD 75	21 d Vegetative vigour Tier 2	ER ₅₀ plant height = 48.8 g a.s./ha	Appendix 2 Köhler, 2020, M-758398-01-1

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Studies with the product Prohexadione-calcium OD 75 were performed and are submitted with this application. The endpoints from these studies were used for the risk assessment.

9.10.2 Risk assessment

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

Seedling emergence and vegetative vigour studies at rates of 1 × 90 g a.s./ha were conducted as limit tests with Prohexadione-calcium OD 75. In the seedling emergence study, the effects were below the critical threshold of 50% adverse effects. In the vegetative vigour study, effects were below the critical threshold of 50% adverse effects as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002) for all species except for *Cucumis sativus*. Therefore, a vegetative vigour study in dose-response design was conducted with *Cucumis sativus* which led to a lowest ER₅₀ of 48.8 g a.s./ha.

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Deterministic assessment of the risk for non-target plants due to the use of Prohexadione-calcium OD 75 in oil seed rape (winter)

Intended use	Oil seed rape (winter) 1 × 90 g a.s./ha				
Product	Prohexadione-calcium OD 75				
Application rate (g a.s./ha)	1 × 90				
MAF	1.0 (single application)				
Test species	ER ₅₀ (g a.s./ha)	Drift (%)	rate	PER _{off-field} (g a.s./ha)	TER criterion: TER ≥ 5*
All species -seedling emergence	> 90	2.77		2.5	>36.1
<i>Cucumis sativus</i> -vegetative vigour	48.8	2.77		2.5	19.6

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

* TER ≥ 5 for deterministic risk assessment based on ER₅₀

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

All TER calculations are above the relevant trigger value of 5. Accordingly, a safe use of the product according to the use pattern can be concluded.

Review comments:

Risk assessment performed by the Applicant for non-target terrestrial plants was accepted. Based on the predicted rates of Prohexadione-calcium OD 75 in off-field areas, the TER values describing the risk for non-target plants following exposure to formulation according to the GAP achieve the acceptability criteria TER ≥ 5. No mitigation measures are required.

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

No further information is available or considered to be necessary.


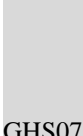
9.12 Monitoring data (KCP 10.8)

No monitoring data is available on organisms in the environment.

9.13 Classification and Labelling

Classification in accordance with Regulation (EC) N° 1272/2008 on classification, labelling and packaging of substances and mixtures, as amended

Hazard class(es), categories:	Chronic aquatic toxicity: Category 3
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Hazard pictograms:	  GHS07
Signal word:	Warning
Hazard statement(s):	H412 – Harmful to aquatic life with long lasting effects.
Precautionary statement(s):	P391 - Collect spillage. P501 Dispose of contents/container in accordance with local regulation.
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]

Standard phrases under Regulation (EU) No 547/2011

SP 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).
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Appendix 1 Lists of data considered in support of the evaluation

All the general data and EU agreed endpoints for prohexadione-calcium assessed in this Section 9 can be found in the following EU assessment report and EFSA Conclusions published for the EU review of the active substance:

Assessment report – public version - June 2009– Initial risk assessment provided by the rapporteur Member State France for the existing active substance prohexadione-calcium, Vol. 3 – B.9. Available online: <https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-prohexadione-calcium>
EFSA Journal 2010;8(3):1555 - Conclusion on the peer review of the pesticide risk assessment of the active substance prohexadione (considered variant prohexadione-calcium)

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 10.1.2.1 / 01 ... also filed: KCP 7.1.1 / 01	██████████	2020	Prohexadione-calcium OD 75 (75 g/L): Acute oral toxicity study in rats (up and down procedure) Report No.: 20/102-001P, Edition Number: M-690665-01-1 xxxxxxxxxx GLP/GEP: Yes unpublished	Yes	Bayer
KCP 10.2.2 / 01	Riebschläger, T.	2021	Acute toxicity of prohexadione-calcium OD 75 (75 g/L) to the waterflea Daphnia magna in a static renewal laboratory test system Report No.: EBPW0004, Edition Number: M-764734-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.2.2 / 02	Kuhl, K.	2021	Pseudokirchneriella subcapitata growth inhibition test with prohexadione-calcium OD 75 (75 g/L) Report No.: E 201 05583-4, Edition Number: M-763760-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

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 10.2.3 / 01	Schrader, D.	2021	Lemna gibba G3 - Growth inhibition test with prohexadione-calcium OD 75 (75 g/L) under static conditions Report No.: E 221 05564 - 5, Edition Number: M-763594-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.2.3 / 02	Armbruster, H.; Emnet, P.	2021	Prohexadione-calcium OD 75 (75 g/L): Toxicity to the aquatic plant Myriophyllum spicatum in a semi-static growth inhibition test with a prior rooting phase Report No.: 152171215, Edition Number: M-764321-01-1 IBACON GmbH, Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.1.1 / 01	Sekine, T.	2020	Prohexadione-calcium OD 75 (75 g/L): Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory - Final report - Report No.: 152171035, Edition Number: M-758769-01-1 IBACON GmbH, Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.1.1 / 02	Chwiesko, D.; Kowalczyk, F.	2021	Prohexadione-calcium OD 75 (75 g/L): Effects (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory - Final report Report No.: 152171105, Edition Number: M-761833-01-1 IBACON GmbH, Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.1.2 / 01	Kling, A.	2021	Prohexadione-calcium OD 75 (75 g/L): Honey bee (Apis mellifera L.) chronic oral toxicity test 10 day feeding test in the laboratory - Final report - Report No.: S20-00682, Edition Number: M-762860-01-1 Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.3 / 01	Kling, A.	2021	Prohexadione-calcium OD 75 (75 g/L): Honey bee (<i>Apis mellifera</i> L.) 22 day larval toxicity test (repeated exposure) - Final report - Report No.: S20-00850, Edition Number: M-762844-01-1 Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.2.1 / 01	Waibel, J.	2021	Amendment no. 01: Toxicity to the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) using a laboratory test; prohexadione-calcium OD 75 (75 g/L) Report No.: CW20/019, Edition Number: M-761029-02-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany ... amended: 2021-02-24 GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.2.1 / 02	Waibel, J.	2021	Amendment no. 01: Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) using a laboratory test; prohexadione-calcium OD 75 (75 g/L) Report No.: CW20/020, Edition Number: M-761030-02-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany ... amended: 2021-02-24 GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.2.2 / 01	Waibel, J.	2021	Amendment no. 01: Toxicity to the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) using an extended laboratory test on bean; prohexadione-calcium OD 75 (75 g/L) Report No.: CW20/034, Edition Number: M-762531-02-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany ... amended: 2021-02-24 GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.2.2 / 02	Waibel, J.	2021	Amendment no. 01: Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) using an extended laboratory test on barley; prohexadione-calcium OD 75 (75 g/L) Report No.: CW20/035, Edition Number: M-762532-02-1	No	Bayer

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
			Bayer AG, Crop Science Division, Frankfurt am Main, Germany ... amended: 2021-02-24 GLP/GEP: Yes unpublished		
KCP 10.3.2.2 / 03	Waibel, J.	2021	Amendment no. 01: Toxicity to the ladybird beetle <i>Coccinella septempunctata</i> (Coleoptera: Coccinellidae) using an extended laboratory test on bean; prohexadione-calcium OD 75 (75 g/L) Report No.: CW20/041, Edition Number: M-763934-02-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany ... amended: 2021-02-24 GLP/GEP: Yes unpublished	No	Bayer
KCP 10.3.2.2 / 04	Waibel, J.	2021	Toxicity to the green lacewing <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae) using an extended laboratory test on bean; prohexadione-calcium OD 75 (75 g/L) Report No.: CW20/040, Edition Number: M-762533-01-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.4.1.1 / 01	Büttner, G.	2021	Prohexadione-calcium OD 75 (75 g/L): Effects on survival, growth and reproduction of the earthworm <i>Eisenia fetida</i> tested in artificial soil Report No.: E 312 05562-4, Edition Number: M-763276-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.4.2.1 / 01	Richter, A.	2021	Prohexadione-calcium OD 75 (75 g/L): Influence on mortality and reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil Report No.: E 314 05536-7, Edition Number: M-762156-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.2.1 / 02	Richter, A.	2020	Prohexadione-calcium OD 75 G (75 g/L): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil Report No.: E 428 05529-5, Edition Number: M-687925-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.5 / 01	Schulz, L.	2020	Prohexadione-calcium OD 75 (75 g/L): Effects on the activity of soil microflora (nitrogen transformation test) Report No.: 20 48 SMN 0038, Edition Number: M-758535-01-1 BioChem agrar GmbH, Gerichshain, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.6.1 / 01	Köhler, P.	2020	Effects on the seedling emergence and growth of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L) Report No.: SE20/022, Edition Number: M-691185-01-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 10.6.1 / 02	Köhler, P.	2020	Amendment no. 01: Effects on the vegetative vigor of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L) Report No.: VV20/021, Edition Number: M-691184-02-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany ... amended: 2020-09-07 GLP/GEP: Yes unpublished	No	Bayer
KCP 10.6.2 / 01	Köhler, P.	2020	Effects on the vegetative vigor of the non-target terrestrial plant species Cucumis sativus (Tier 2); prohexadione-calcium OD 75 (75 g/L) Report No.: VV20/039, Edition Number: M-758398-01-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany GLP/GEP: Yes	No	Bayer

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

Appendix 2 Detailed evaluation of the new studies

Review Comment:

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

No additional study submitted.

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional study submitted.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional study submitted.

Summarised in Section 6 (Mammalian Toxicology)

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

No additional study submitted.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

Comments of zRMS:	The study has been assessed in toxicology part of this Core dossier. For details please, see section 6 (Mammalian Toxicology).
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Reference:	KCP 10.1.2.1/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Acute oral toxicity study in rats (up and down procedure)
Report:	xxxxxxx
Authority registration No:	
Guideline(s):	OECD 425 (2008); US-EPA 712-C-02-190, OPPTS 870.1100 (2002); Commission Regulation (EC) 440/2008, B.1 tris (2008)
Deviations:	None
GLP/GEP:	yes
Acceptability:	see section 6 (Mammalian Toxicology).
Duplication (if vertebrate study):	

For a summary of this study please see Section 6 (Mammalian Toxicology) of this core dossier.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional study submitted.

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

No additional study submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 Fish

No additional study submitted. No acute toxicity study with fish was conducted with the formulated product Prohexadione-calcium OD 75 due to animal welfare reasons. The data on the active substance prohexadione-calcium demonstrates low toxicity to fish with both, acute and chronic tests, resulting in endpoints greater or equal to 100 mg a.s./L. Thus, the available data on the active substance toxicity did not indicate any risk for fish.

A 2.2.1.2 Aquatic invertebrates

Comments of zRMS:	<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 outside the range 80-120% of nominal and for this reason endpoints are expressed as measured concentrations. The study is reliable and suitable for the risk assessment. Following endpoints based on measured test item concentrations would be used for risk assessment purposes:</p> <p>EC₅₀/48 h is > 7.88 mg prod./L</p>
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Reference:	KCP 10.2.2/01
Title:	Acute toxicity of prohexadione-calcium OD 75 (75 g/L) to the waterflea <i>Daphnia magna</i> in a static renewal laboratory test system
Report:	Riebschläger, T.; 2021; EBPW0004; M-764734-01-1
Authority registration No:	
Guideline(s):	OECD Guideline No. 202 (Guideline for Testing of Chemicals, "Daphnia sp., Acute Immobilisation Test (April 13, 2004") OCSPP Guideline 850.1010: Aquatic invertebrate acute toxicity test, freshwater daphnids (October 2016 (modified)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	No

Executive Summary

The study was performed to detect possible effects of the test item Prohexadione-calcium OD 75 (75 g/L) on the mobility of *Daphnia magna* after 48 hours of exposure in a static-renewal laboratory test system, expressed as EC₅₀ for immobilisation. For this purpose, nominal test concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0 mg prod./L, corresponding to 75.7, 151, 303, 606 and 1211 µg a.s./L were used. The water samples were analyzed with HPLC-MS/MS. Recovery rates ranged from 68 to 86% of nominal values in the fresh prepared solutions (0 and 24 h) and from 46 to 68% of nominal values in aged solutions (24 and 48 h), respectively. Thus, geometric mean measured concentrations were calculated to 0.71, 1.43, 2.66, 4.92 and 9.24 mg prod./L, corresponding to 53.4, 108, 201, 373, 699 µg a.s./L. The study fulfils all validity criteria of OECD 202 guideline.

At the highest test concentration of 16.0 mg prod./L, 73.3% affected daphnids were found. The 48h-EC₅₀ determined at 7.88 mg prod./L (geometric mean measured).

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

Guideline(s) adaptation	None
Test species	Waterflea <i>Daphnia magna</i>
Culturing conditions	Climate-controlled environment under study conditions. Feeding regime was three times per week with living cells of the green alga <i>Desmodesmus subspicatus</i> in aqueous suspension.
Organism age at study initiation	1 st instars < 24 hours old neonates
Test solutions	Test medium: fully defined, artificial water (type "Elendt M7") Nominal concentrations: 1.0, 2.0, 4.0, 8.0 and 16.0 mg product/L Corresponding mean measured concentrations: 0.71, 1.43, 2.66, 4.92 and 9.24 mg prod./L Controls: untreated dilution water Evidence of undissolved material: No precipitates during exposure were

	observed.
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Organisms per replicate	5
Exposure	Test type: static-renewal (one renewal after 24 hours) Total exposure duration: 48 h
Feeding during test	None
Test conditions	Temperature: 19.7 – 20.7 °C Photoperiod: 16:8 hours light-dark cycle Light intensity: max. 960 - 964 lux pH: 7.8 - 7.9 Water hardness: 213.4 mg/L CaCO ₃ Alkalinity: 53.4 mg/L CaCO ₃ Dissolved oxygen: 7.5 – 8.7 mg O ₂ /L Conductivity: 599 - 601 µS/cm
Parameters Measured / Observations	Observations of mobility and sublethal effects of the treatment levels and the controls after 24 and 48 hours. Measurements of pH and dissolved oxygen were determined for all freshly prepared solutions and again in the aged solutions at the end of exposure. Water temperatures within the test system were recorded at start and end of exposure from one vessel of the untreated control group and of the highest treatment group. Daily visual examination on features of the test-item in water like coloration, turbidity & signs of inhomogeneous distribution of the test item in the test media (e.g. precipitation of undissolved material).
Sampling for chemical analysis	Samples were analyzed before distribution to the test vessels (fresh solution: at 0 h and 24 h) and at the end of exposure period (aged solution: at 24 h and 48 h). They were a composite of all replicates within each treatment level and control group. The water samples were analysed with HPLC-MS/MS.
Data analysis	Probit analysis, fitted by an iterative weighed linear regression according to the Maximum Likelihood principle. Data adjustment: In cases where less than 3 test concentrations with immobility rates between 0% and 100% remained, a replacement of the 0% and 100% rates, the so-called “fudging” by slightly deviating values (e.g. 0.1% / 99.9%) were performed. Software: “Excel® for Office 365 MSO” and ToxRatPro® Version 3.2.1

Results and discussions

Validity criteria according to OECD 202 (2004)	Required	Obtained
Immobilisation and sub-lethal effects in control during test	≤ 10%	0%
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	≥ 8.6 mg/L

Analytical results:

Recovery rates ranged from 68 to 86% of nominal values for fresh solutions and from 46 to 68% of nominal values for aged solutions, respectively. Biological results are based on geometric mean measured concentrations.

No residues of prohexadione-calcium were found in the control and solvent control samples above the limit of detection (7.6 µg a.s./L).

Nominal test concentrations		Measured test concentrations					
mg prod./L	µg a.s./L	Start of exposure period (fresh solutions)		End of exposure period (aged solutions)		Geometric mean measured	
		µg a.s./L	% of nominal	µg a.s./L	% of nominal	µg a.s./L	mg prod./L
1.0	75.7	59.7	79%	47.8	63%	53.4	0.71
		55.0	73%	51.8	68%		
2.0	151	112	74%	102	68%	108	1.43
		119	79%	98.7	65%		
4.0	303	261	86%	158	52%	201	2.66
		217	72%	183	60%		
8.0	606	425	70%	353	58%	373	4.92
		434	72%	296	49%		
16.0	1211	820	68%	559	46%	699	9.24
		868	72%	600	50%		

Biological results:

Observations:

No immobility or other effects on behavior occurred within 48 hours of exposure.

Nominal test concentration (mg prod./L)	Geometric mean measured concentration (mg prod./L)	Exposed daphnids	Immobilised daphnids	
			24 h	48 h
Control		30	0	0
1.0	0.71	30	0	0
2.0	1.43	30	0	0
4.0	2.66	30	0	0
8.0	4.92	30	0	1
16.0	9.24	30	13	22

Conclusions

The study meets all validity criteria and the 48h-EC₅₀ was determined at 7.88 mg prod./L based on geometric mean measured concentrations.

Summary of endpoints

Results based on geometric mean measured concentrations:	
EC ₅₀ -48 hours [95 % C.I.]:	7.88 mg prod./L [6.99 – 8.84 mg prod./L]

A 2.2.1.3 Effects on aquatic algae

Comments of zRMS:	<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 outside 80-120% of nominal (On day 0, recovery rates ranged from 69.0 - 91.0% of nominal) and for this reason endpoints are expressed as measured concentrations. The study is reliable and suitable for the risk assessment. Following endpoints based on measured test item concentrations would be used for risk assessment purposes:</p> <p>ErC₅₀ = > 20.4 mg prod./L</p> <p>EyC₅₀ = > 11.6 mg prod./L</p>
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Reference:	KCP 10.2.2/02
Title:	Pseudokirchneriella subcapitata growth inhibition test with prohexadione-calcium OD 75 (75 g/L)
Report:	Kuhl, K.; 2021; E 201 05583-4; M-763760-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (July 28, 2011) OCSPP Guideline 850.4500: "Algal Toxicity" (January 2012)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive Summary

The aim of the study was to determine the influence of the test item Prohexadione-calcium OD 75 (75 g/L) on exponentially growing populations of *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate and further endpoints of algal biomass. *Pseudokirchneriella subcapitata* with an initial cell density of 1.0×10^4 cells/mL were exposed for 96 hours to nominal concentrations of 1.46, 4.10, 11.5, 32.1 and 90.0 mg prod./L. The cell density in each replicate was estimated photometrically after 24, 48, 72 and 96 hours. The water samples were analyzed for test item with HPLC-MS/MS. Recoveries ranged from 69.0 to 92.8%. Geometric mean measured concentrations were 1.34, 3.61, 10.2, 26.6 and 71.4 mg prod./L. No control contamination was detected. The study fulfils all validity criteria of the OECD 201 guideline. The endpoints were based on geometric mean measured concentrations. The 72-h ErC₅₀ value was calculated to be 20.4 mg prod./L. The 72-h NOEC was determined to be 10.2 mg prod./L. The 96-h ErC₅₀ value was calculated to be 27.9 mg prod./L. The 96-h NOEC was determined to be 10.2 mg prod./L.

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

Guideline(s) adaptation	None
Test species	Green algae (<i>Pseudokirchneriella subcapitata</i>) Strain 61.81
Culturing conditions	Cultivation: Every 7-10 days, old stock culture was transferred into a 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium. Pre-cultures were prepared from stock culture 3 days before test start using OECD medium Light intensity: 24 hours light (4.50 - 7.00 klux) Temperature: 22 ± 2 °C
Test solutions	Nominal concentrations: 1.46, 4.10, 11.5, 32.1 and 90.0 mg prod./L Corresponding to 72 h geometric mean measured concentrations: 1.34, 3.61, 10.2, 26.6 and 71.4 mg prod./L Controls: untreated test medium Evidence of undissolved material: yes, turbidity at 11.5 to 90 mg prod./L.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	Test type: static Total exposure duration: 96 hours
Initial cells density	1.0 × 10 ⁴ cells/mL
Test conditions	Vessels: 300 mL Erlenmeyer flasks Temperature: 22.4 – 23.3 °C Photoperiod: 24 hours light Light intensity: 4450– 4910 lux pH: 8.0 – 9.2 (control); 7.6 – 9.3 (test solutions) Water hardness measured values: not reported
Parameters Measured / Observations	The cell density in each replicate was counted after 24, 48, 72 and 96 hours. Morphological examinations of cells were made after 24, 48, 72 and 96 hours. The pH value was measured at exposure initiation, after 72 hours of exposure, and at termination. The temperature was continuously determined. Observations of test medium appearance were made every 24 hours.
Sampling for chemical analysis	Samples were analysed for the actual concentration of test item present in the test medium of all treatment levels and the control after 0, 72 and 96 hours. The water samples were analyzed with HPLC-MS/MS.
Data analysis	ToxRat Professional was used. ECx values were calculated using a linear regression analysis. NOEC/LOEC values were determined using suitable multiple comparison tests (α=0.05, one-sided smaller).

Results and discussions

Validity criteria (according to OECD 201, 2011)	Required	Obtained
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Biomass in the control within the 72-hour test period	≥16	63.9
Mean coefficient of variation for section-by-section specific growth rates in the control within the 72-hour test period	≤ 35%	18.0%
Coefficient of variation of average specific growth rates within 72-hour test period in control replicates	≤ 7%	1.5%

Analytical results:

On day 0, recovery rates ranged from 69.0 - 91.0% of nominal. After 72 hours, analytical findings were 86.6 – 91.9% of nominal and after 96 hours, analytical findings were 81.9 – 92.8 % of nominal. Therefore, all reported results are based on geometric mean measured concentration of the formulation.

No residues of test item were measured in the control samples above the limit of quantification (0.02 mg a.s./L).

Nominal conc. [mg prod./L]	Nominal conc. [mg a.s./L]	Fresh 0 h		Aged 72 h		Geometric mean measured concentration [mg prod./L]
		Mean measured concentration [mg a.s./L] ^A	% of nominal	Mean measured concentration [mg a.s./L] ^A	% of nominal	
1.46	0.111	0.101	91.0	0.102	91.9	1.34
4.10	0.310	0.265	85.5	0.278	89.7	3.61
11.5	0.871	0.752	86.3	0.782	89.8	10.2
32.1	2.43	1.87	77.0	2.13	87.7	26.6
90.0	6.81	4.70	69.0	5.90	86.6	71.4

^A Average of two determinations.

Nominal concentration [mg prod./L]	Nominal concentration [mg a.s./L]	Aged 96 h	
		Mean measured concentration [mg a.s./L] ^A	% of nominal
1.46	0.111	0.103	92.8
4.10	0.31	0.278	89.7
11.5	0.871	0.768	88.2
32.1	2.43	1.99	81.9
90.0	6.81	5.75	84.4

^A Average of two determinations.

Biological results:

Observations:

No visual abnormal observations of the test media were made in the lower test concentrations. Turbidity was observed at test start from 11.5 to 90 mg prod./L. No morphological change in algae was observed during the first 72 hours in all test concentrations. After 96 hours morphological changes (cell deformation) were only observed in the two highest test concentrations, where clear growth inhibition occurred already.

% Inhibition of biomass endpoints: Growth rate and yield

Nominal [mg prod./L]	conc.	Growth rate inhibition (%)		Yield inhibition (%)	
		72 h	96 h	72 h	96 h
Control		-	-	-	-
1.46		-0.2	0.1	-2.0	0.3
4.10		3.2	-1.5	12.5 [#]	-7.3
11.5		11.4	-1.4	38.2 [#]	-6.7
32.1		68.0 [#]	48.6 [#]	93.8 [#]	90.9 [#]
90.0		100.0 [#]	91.1 [#]	99.8 [#]	99.5 [#]

[#]significantly ($\alpha=0.05$, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

Negative % inhibition: increase in growth relative to the pooled control

Conclusions

The study meets the validity criteria and the endpoints based on geometric mean measured concentrations are:

Summary of results:

Results – 0 to 72 hours based on geometric mean measured concentration	
E_rC₅₀ - 72 hours (95 % CI):	20.4 mg prod./L (17.2 – 23.8 mg prod./L)
E _r C ₂₀ -72 hours (95 % C.I.):	12.4 mg prod./L (8.65 – 15.1 mg prod./L)
E _r C ₁₀ -72 hours (95 % C.I.):	9.55 mg prod./L (5.85 – 12.3 mg prod./L)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate)	26.6 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate)	10.2 mg prod./L
E_yC₅₀ - 72 hours (95 % CI):	11.6 mg prod./L (10.2 – 13.3 mg prod./L)
E _y C ₂₀ -72 hours (95 % C.I.):	6.65 mg prod./L (5.01 – 7.91 mg prod./L)
E _y C ₁₀ -72 hours (95 % C.I.):	4.97 mg prod./L (3.35 – 6.32 mg prod./L)
LOEC - 72 hours: lowest concentration with an effect (based on yield)	3.61 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on yield)	1.34 mg prod./L
Results – 0 to 96 hours based on geometric mean measured concentration	
E_rC₅₀ - 96 hours (95 % CI):	27.9 mg prod./L (26.0 – 30.1 mg prod./L)
E _r C ₂₀ -96 hours (95 % C.I.):	17.0 mg prod./L (14.6 – 18.9 mg prod./L)
E _r C ₁₀ -96 hours (95 % C.I.):	13.1 mg prod./L (10.6 – 15.1 mg prod./L)
LOEC – 96 hours: lowest concentration with an effect (based on growth rate)	26.6 mg prod./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate)	10.2 mg prod./L
E _y C ₅₀ - 96 hours (95 % CI):	17.4 mg prod./L (16.5 – 18.5 mg prod./L)
E _y C ₂₀ -96 hours (95 % C.I.):	12.3 mg prod./L (11.5 – 13.1 mg prod./L)

E _y C ₁₀ -96 hours (95 % C.I.):	10.3 mg prod./L (9.48 – 11.1 mg prod./L)
LOEC – 96 hours: lowest concentration with an effect (based on yield)	26.6 mg prod./L
NOEC - 96 hours: highest concentration without an effect (based on yield)	10.2 mg prod./L

CI: confidence interval

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

No additional study submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Comments of zRMS:	<p>Prohexadione-calcium OD 75 (75 g/L) <i>Lemna gibba</i> growth inhibition test was conducted to OECD guideline 221 and according to the principles of GLP. No deviations were noted during the study.</p> <p>In the definitive test all the validity criteria were met.</p> <p>The analytical measurements demonstrated that the test item concentrations throughout the test was outside the range 80-120% of nominal and for this reason endpoints are expressed as measured concentrations. The study is reliable and suitable for the risk assessment. Following endpoints based on measured test item concentrations would be used for risk assessment purposes:</p> <p>ErC₅₀ = > 45.8 mg prod./L</p> <p>The endpoint ErC₁₀ and NOEC were selected from the study based on the statistical analysis.</p> <p>Visual analysis of data showed effect >10%, which could be biologically significant, already at 5.75 mg product/L (and nearly 10% at 1.94 mg product/L). Thus it can be concluded that realistic EC₁₀ would be between 1.94 and 5.75 rather than 10.1 mg product/L. and Thus calculated ErC₁₀ should not be provided as 10.1 mg product/L.</p>
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Reference:	KCP 10.2.3/01
Title:	<i>Lemna gibba</i> G3 - Growth inhibition test with prohexadione-calcium OD 75 (75 g/L) under static conditions
Report:	Schrader, D.; 2021; E 221 05564 - 5; M-763594-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 221 (March 23, 2006) US EPA OCSPP 850.4400
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	No

Executive Summary

The aim of the study was to determine the influence of the test item on exponentially growing *Lemna gibba* G3 expressed as NOEC, LOEC and EC_x for growth rate of the measurement variables, frond

number and dry weight of plants. 4 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 0 (control), 0.596, 1.91, 6.10, 19.5, 62.5 and 200 mg prod./L. Determination of fronds and visual observations were conducted on days 2, 5 and 7. Dry weight was determined at start and end of the test. The water samples were analyzed with HPLC-MS/MS. The analytical findings of Prohexadione-calcium OD 75 (75 g/L) found in all freshly prepared test levels on day 0 ranged between 70.9 and 109% of nominal. In aged test levels on day 7, analytical findings ranged between 81.5 and 119% of nominal. The geometric mean measured concentrations were 0 (control), 0.675, 1.94, 5.75, 17.7, 49.9 and 152 mg prod./L. All reported results are based on geometric mean measured concentrations. The study fulfils all validity criteria of OECD 221 guideline. Sublethal effects on plants were observed in the three highest concentrations of 17.7, 49.9 and 152 mg prod./L (geometric mean measured concentration). The E_rC_{50} for effects on mean growth rate of frond number and dry weight of plant was 45.8 mg prod./L corresponding to 69.7 mg prod./L, respectively. The lowest statistically observed NOE_rC was 0.675 mg prod./L (geometric mean measured) and was based on statistical data analysis of the mean growth rate of frond number.

Materials and Methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

Guideline(s) adaptation	None
Test species	Duckweed (<i>Lemna gibba</i>) strain G3
Culturing conditions	20 x AAP medium Light intensity: 6500 – 7000 lux Temperature of 23 - 26°
Test solutions	Nominal concentrations: 0 (control), 0.596, 1.91, 6.10, 19.5, 62.5 and 200 mg prod./L Geometric mean measured concentrations: 0 (control), 0.675, 1.94, 5.75, 17.7, 49.9 and 152 mg prod./L Control: water Evidence of undissolved material: yes: turbidity at 17.7 to 152 mg prod./L
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Organisms per replicate	12 fronds per vessel (3 – 4 fronds per plant)
Exposure	Test type: static Total exposure duration: 7 days
Test vessel loading	12 fronds per 150 mL
Test conditions	Incubation chamber used: multitron, growth incubator Temperature: 23.7 °C to 24.4 °C Photoperiod: permanent light Light quality: bank light containing fluorescent lamps Light intensity: 6.65 klux pH in the controls: 7.5-8.8 Water hardness: not specified Dissolved oxygen: not specified

	Conductivity: not specified Growth medium: 20X AAP
Parameters Measured / Observations	The numbers of fronds in each replicate were counted on days 2, 5 and 7. Dry weight was determined on day 7 Visual observations of sub-lethal effects on days 2, 5 and 7. At test start the dry weight of a sample of fronds similar to those used to inoculate the test vessels was determined. At the end of the test the dry weight of all plants from each vessel was determined. Temperature was determined by a continuous measurement. The pH was measured in all freshly prepared and all aged test levels and the controls. The light was measured at least once during the test.
Sampling for chemical analysis	Day 0: duplicate samples (A+B) were taken from the prepared volume of each test treatment level Day 7: after removing the plant material from the test vessels, all replicates of a treatment level were combined and duplicate samples (A+B) were taken of the combined replicates. A-samples were analyzed, B-samples were stored as retain samples. On day 0, the B-samples of the two highest test concentrations were measured to verify the results of the A-samples. The water samples were analyzed with HPLC-MS/MS
Data analysis	ECx calculations were performed by probit analysis. Effect thresholds (e.g. NOECs) were determined by Williams Multiple Sequential t-test Procedure. All statistical evaluations were done with ToxRat Professional Version 3.2.1.

Results and Discussions

Validity criteria according to OECD 221 (2006)	Required	Obtained
Doubling time	< 2.5 days	2.1 days
Control CV for yield and growth rate at test termination	< 20%	≤ 8.5

Analytical results:

The analytical findings of Prohexadione-calcium OD 75 found in all freshly prepared test levels on day 0 ranged between 70.9 and 109% of nominal. In aged test levels on day 7, analytical findings ranged between 81.5 and 119% of nominal. As not all recoveries were within the range of 80 – 120% of nominal, the biological results are based on geometric mean measured concentrations of Prohexadione-calcium OD 75 (75 g/L). No residues of the test item were measured in the control and solvent control samples above 0.0005 mg a.s./L, which was used as the lowest standard concentration.

Nominal test concentrations		Measured test concentrations				
mg prod./L	mg a.s./L	Day 0 (New)		Day 7 (Old)		Geometric mean measured mg prod./L
		mg a.s./L	% of nominal	mg a.s./L	% of nominal	
0.596	0.0451	0.049	109	0.0533	119	0.675
1.91	0.144	0.142	89.6	0.153	106	1.94
6.10	0.462	0.412	88.3	0.460	99.6	5.75
19.5	1.48	1.32	88.5	1.37	92.6	17.7

62.5	4.73	3.60	76.1	3.96	83.7	49.9
200	15.1	10.7	70.9	12.3	81.5	152

Biological results:

Observations

Sublethal effects on plants were observed in the three highest concentrations of 17.7, 49.9 and 152 mg prod./L (geometric mean measured concentration).

Statistically significant inhibition could be determined on *Lemna* for the endpoint mean growth rate of frond number and mean dry weight. The EC_x values were directly determined from the raw data. The LOEC and NOEC values were calculated *via* statistical data analysis.

Nominal test concentration [mg prod./L]	Geometric mean measured concentration [mg prod./L]	Frond number (day 7) mean values from 4 replicates	Dry weight of plants (day 7) mean values from 4 replicates [mg]	% Inhibition	
				Mean growth rate for frond number	Mean growth rate for dry weight of plants
Control		126	17.9	--	--
0.596	0.675	128	16.8	-0.8	2.5
1.91	1.94	100	17.2	9.8*	1.9
6.10	5.75	89.8	16.6	14.4*	3.2
19.5	17.7	73.8	13.5	22.8*	11.4*
62.5	49.9	46.3	9.4	42.8*	25.7*
200	152	13.5	1.6	95.1*	94.7*

Negative inhibition values indicate an increase in growth relative to the control

* significantly ($\alpha=0.05$, one-sided smaller) reduced, based on Williams multiple sequential t-test

Conclusions

The study meets the validity criteria and endpoints were calculated based on geometric mean measured concentrations.

Summary of endpoints

Results based on geometric mean measured concentration:		
Endpoint (0-7 day)	Effect on mean growth rate of frond number [mg prod./L]	Effect on mean growth rate of dry weight of plants [mg prod./L]
E _r C ₅₀ (CL 95%)	45.8 mg prod./L (37.2 – 56.7 mg prod./L)	69.7 mg prod./L (63.0 – 78.8 mg prod./L)
E _r C ₂₀ (CL 95%)	16.9 mg prod./L (11.3 – 22.2 mg prod./L)	44.3 mg prod./L (38.3 – 49.4 mg prod./L)
E _r C ₁₀ (CL 95%)	10.1 mg prod./L (5.75 – 14.3 mg prod./L)	35.0 mg prod./L (28.5 – 40.2 mg prod./L)
LOE _r C lowest concentration with an effect	1.94 mg prod./L	17.7 mg prod./L
NOE _r C highest concentration without adverse effects	0.675 mg prod./L	5.75 mg prod./L

CL = Confidence limits

Comments of zRMS:	<p>The study was conducted to OECD guideline 239 and according to the principles of GLP.</p> <p>In the definitive test the validity criteria were met according to OECD Guideline No. 239. 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 outside 80-120% of nominal and for this reason endpoints are expressed as measured concentrations. The study is reliable and suitable for the risk assessment. Following endpoints based on measured test item concentrations would be used for risk assessment purposes:</p> <p>ErC₅₀ = > 26.4 mg prod./L</p>
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Reference:	KCP 10.2.3/02
Title:	Prohexadione-calcium OD 75 (75 g/L): Toxicity to the aquatic plant <i>Myriophyllum spicatum</i> in a semi-static growth inhibition test with a prior rooting phase
Report:	Armbruster, H.; Emnet, P.; 2021; 152171215; M-764321-01-1
Authority registration No:	
Guideline(s):	<p>OECD Guideline 239: Water-Sediment <i>Myriophyllum spicatum</i> Toxicity Test, September 26, 2014</p> <p>SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of Directive 91/414</p>
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item Prohexadione-calcium OD 75 (75 g/L) on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum*. Plants of *Myriophyllum spicatum* were exposed in a semi-static test to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 14 days. The purpose of the analytical part of this study was to verify the concentration of the test item in the test medium.

After a pre-rooting phase of 7 days, 1 plant per replicate was incubated for 14 days under semi-static conditions to the nominal concentrations of 0 (control), 0.317, 1.0, 3.17, 10.0, 31.6 and 100 mg prod./L. The shoot length was determined at test start, at day 7 and at test end. Sublethal parameters were assessed at test start, once during the test (day 7) and at test end. At test end fresh and dry weight of each replicate was determined. The water samples were analyzed with HPLC-MS/MS At the start of the test and at the renewal periods on day 4 and day 9 the recoveries of the nominal test concentrations ranged between 80 – 102% (all test concentrations considered). The test item was therefore dosed correctly. In the corresponding aged samples, the recoveries ranged between 73 – 91% (all test concentrations considered). The geometric mean measured concentrations were 0 (control), 0.275, 0.861, 2.71, 8.35, 27.2 and 83.7 mg prod./L. All reported results are based on geometric mean measured concentrations. The study fulfils all validity criteria of OECD 239 guideline. Sublethal effects on plants were observed from 2.71 mg prod./L upwards (geometric mean measured concentration) The lowest 14-day EC₅₀ values were obtained for the parameter total shoot length, E_rC₅₀ = 26.4 mg prod./L and E_yC₅₀ = 14.2 mg prod./L. The lowest NOE_rC of 2.71 mg prod./L was obtained for total shoot length. The lowest NOE_yC was 2.71 mg prod./L and was obtained for total shoot length.

Materials and Methods

Test item: Prohexadione-calcium OD 75 (75 g/L)

Type of formulation: OD (oil dispersion)
Sample description: TOX 21574-00
Active substance (analysed content): prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.: 102000037599
Supplier batch no.: 2020-001264
Density: 1.038 g/mL

Guideline(s) adaptation	None
Test species	Myriophyllum spicatum In-house laboratory culture
Culturing conditions	Modified Andrews' medium containing 3 % sucrose Photoperiod: 16 h : 8 h light : dark Light intensity: 4110 lux Temperature of 22.9°C
Test solutions	Nominal concentrations: 0 (control), 0.317, 1.00, 3.17, 10.0, 31.6 and 100 mg prod./L Geometric mean measured concentrations: 0 (control), 0.275, 0.861, 2.71, 8.35, 27.2 and 83.7 mg prod./L Control: water Evidence of undissolved material: yes: Turbidity in freshly prepared and aged test media of 27.2 and 83.7 mg prod./L. The intensity was concentration dependent. In the aged test media of 8.35, 27.2 and 83.7 mg prod./L test item was observed floating on the surface. The observations were only slightly pronounced at the lower concentration
Replication	No. of vessels per concentration (replicates): 5 No. of vessels per control (replicates): 10
Organisms per replicate	1 plant per vessel
Exposure	Test type: semi-static Total exposure duration: 14 days
Test vessel	Small plastic plant pots (approx. 9.0 cm diameter, 7 cm high and with a volume of approx. 400 mL) were used as containers for potting the plants into the sediment. Test beakers of 2000 mL volume (approx. 11.5 cm diameter, 24 cm high) with approximately 1800 mL test medium were used to provide a minimum overlaying water depth of 12 cm. The exposed sediment surface area covers approximately 61% of the vessel surface area.
Test conditions	Water temperature: 20.3 - 21.9 °C Light regime: 16 h light : 8 h dark, mean light intensity: 8786 lux (8190- 9860 lux) pH values at the start of the pre-rooting phase: 7.7 - 7.9 pH values of the control: 7.9 - 8.1 (freshly prepared) and 8.6 - 10.0 (aged test media) pH values of test item treatments: 7.7 - 8.0 (freshly prepared) and 7.3 - 9.9 (aged test media). oxygen concentrations: 8.1 - 9.1 mg/L (freshly prepared) and 0.1 - 14.4 mg/L (aged test media). 8786 lux (mean value) with a range of 8190 - 9860 lux during exposure
Artificial Sediment:	Sediment was prepared according to OECD test guideline 239, based on dry weight: 4.50% sphagnum moss peat: as close to pH 5.5 - 6.0 as possible, no visible

	<p>plant remains, finely ground and air dried</p> <p>74.75% quartz sand (grain size: > 50% of the particles were in the range of 50 - 200 µm)</p> <p>20.00% kaolin clay (kaolinite content ≥30%)</p> <p>CaCO₃ (0.75 %), of chemically pure quality was added to adjust the pH of the final mixture of the sediment to 6.8.</p> <p>Aqueous nutrient medium containing Na₃PO₄ and NH₄Cl was added to obtain a moisture content of the final mixture in a range of 30 - 50%.</p> <p>The organic carbon content of the final mixture was 2.2% by dry weight.</p>
Parameters Measured / Observations	<p>At test start (day 0) and end the shoot length above the sediment and the length of any lateral branch was measured for all the plants used in the test.</p> <p>At test end the fresh and dry weight of every test plant was measured in the same manner as at the test start.</p> <p>Any sublethal symptoms e.g. growth abnormalities, chlorosis or necrosis were recorded at test start, once during the test (day 7) and at the end of the test.</p> <p>At test end the existence of roots and their appearance was also recorded.</p>
Sampling for chemical analysis	<p>One sample from the freshly prepared stock solution and duplicate samples from the overlying water of all test concentrations and the control were taken at the start of the test and at the test medium renewal on day 4 and day 9.</p> <p>One additional sample of the control was taken at each sampling time without any sample treatment.</p> <p>Sediment samples from all test concentrations and the control were taken at the start and end of the test.</p> <p>The water samples were analyzed with HPLC-MS/MS</p>
Data analysis	<p>EC_x calculations were performed by probit analysis.</p> <p>Effect thresholds (e.g. NOECs) were determined by Williams t-test for growth rates based on total shoot length, fresh weight and dry weight at each test concentration. The test was chosen as data showed normal distribution (Shapiro-Wilk's Test) and variance homogeneity (Levene's Test) and the analysis of contrasts revealed a linear trend.</p> <p>The yield based on total shoot length, fresh weight and dry weight was compared to the control by Welch t-test after Bonferroni-Holm-adjustment as variance homogeneity check failed (Levene's Test) while normal distribution requirements were fulfilled (Shapiro-Wilk's Test).</p> <p>All statistical evaluations were done with ToxRat Professional Version 3.3.0.</p>

Results and Discussions

Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.

Validity criteria according to OECD 239 (2014)	Required	Obtained
Increase in total shoot length of control plants	Doubling	7.2
Increase in fresh weight of control plants	Doubling	6.0
Coefficient of variation for yield (based on fresh weight of control plants)	≤ 35%	29.0%
Sublethal effects in control plants	Control must not show any sign of e.g. chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial film.	Control plants did not show any sign of sublethal effects and were visibly free from contamination by other organisms such as algae and/or bacterial film.

Analytical results:

At the start of the test and at the renewal periods on day 4 and day 9 the recoveries of the nominal test concentrations ranged between 80 – 102% (all test concentrations considered). The test item was therefore dosed correctly. In the corresponding aged samples, the recoveries ranged between 73 – 91% (all test concentrations considered). No residues of the test item were measured in the control and solvent control samples above the limit of quantification (0.15 mg prod./L, corresponding to 11 µg a.s./L).

Nominal test concentrations	Measured test concentrations		
mg prod./L	Day 0 (New) % of nominal ¹	Day 14 (Old) % of nominal ¹	Geometric mean measured mg prod./L
0.317	89	84	0.275
1.00	91	82	0.861
3.17	89	82	2.71
10.0	89	78	8.35
31.6	91	80	27.2
100	89	79	83.7

¹ mean value of all measured samples per treatment group

Biological results:

Observations

Observed effects followed a concentration-response-relationship. Phytotoxic symptoms were observed from nominal 2.71 mg prod./L upwards. Symptoms observed were shortened shoot tips and shortened roots (2.71 and 8.35 mg prod./L), chlorosis and shortened roots (27.2 mg prod./L) and necrosis, less and shortened roots (83.7 mg prod./L). Statistically significant inhibition could be determined on *Myriophyllum spicatum* for the endpoints total shoot length, fresh weight and dry weight. The EC_x values were directly determined from the raw data. The LOEC and NOEC values were calculated *via* statistical data analysis.

Nominal test concentration [mg prod./L]	Geometric mean measured concentration [mg prod./L]	% Inhibition					
		Total Shoot Length yield	Total Shoot Length Growth rate	Fresh weight yield	Fresh weight Growth rate	Dry weight yield	Dry weight Growth rate
0.317	0.275	-43.6	-14.7	-17.2	-8.7	-18.5	-11.3
1.00	0.861	-42.5	-15.8	-16.5	-6.8	-31.0	-15.8
3.17	2.71	-25.3	-11.2	-4.1	-2.6	-24.7	-13.8
10.0	8.35	32.6 +	15.9 *	18.8	8.6	-18.4	-11.1
31.6	27.2	73.1 +	49.7 *	54.4 +	33.4 *	-5.7	-3.7
100	83.7	95.1 +	86.7 *	95.1 +	87.7 *	84.9 +	74.4 *

Negative inhibition values indicate an increase in growth relative to the control

* mean value significantly different from the control (tested with Williams t-test, $\alpha = 0.05$, one-sided)

+ mean value significantly different from the control (tested with Welch t-test after Bonferroni-Holm adjustment, $\alpha = 0.05$, one-sided)

Conclusions

The influence of Prohexadione-calcium OD 75 (75 g/L) on the growth of the dicotyledonous freshwater plant *Myriophyllum spicatum* was assessed in a semi-static concentration-response test. The lowest 14-day EC₅₀ values were obtained for the parameter total shoot length, E_rC₅₀ = 26.4 mg test item/L and E_yC₅₀ = 14.2 mg test item/L. The lowest NOE_rC of 2.71 mg test item/L was obtained for total shoot length. The lowest NOE_yC was 2.71 mg test item/L and was obtained for total shoot length.

The correct application of the test item and the maintenance of the exposure concentrations during the test were verified in the analytical part. All reported results refer to geometric mean concentrations, since not all test item concentrations were within ± 20% of the nominal concentrations during the test.

Summary of endpoints

Results based on geometric mean measured concentration:						
Endpoint (0-14 day)	Yield (total shoot length) [mg test item/L]	Growth rate (total shoot length) [mg test item/L]	Yield (fresh weight) [mg test item/L]	Growth rate (fresh weight) [mg test item/L]	Yield (dry weight) [mg test item/L]	Growth rate (dry weight) [mg test item/L]
EC ₅₀ (CL 95%)	14.2 (13.2 - 15.3)	26.4 (25.1 - 27.9)	22.0 (18.0 - 26.9)	36.3 (32.5 - 40.7)	52.5 (45.7 - 59.1)	62.5 (55.0 - 68.0)
EC ₂₀ (CL 95%)	6.07 (5.38 - 6.73)	10.6 (9.74 - 11.5)	9.21 (6.26 - 11.9)	18.9 (15.4 - 21.9)	35.9 (28.9 - 41.8)	42.8 (32.8 - 49.9)
EC ₁₀ (CL 95%)	3.89 (3.31 - 4.46)	6.62 (5.87 - 7.36)	5.84 (3.44 - 8.11)	13.4 (10.2 - 16.3)	29.5 (22.5 - 35.3)	34.3 (24.1 - 41.9)
14-day NOEC	2.71	2.71	8.35	8.35	27.2	27.2
14-day LOEC	8.35	8.35	27.2	27.2	83.7	83.7

CL = Confidence limits

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

Comments of zRMS:	<p>The studies were conducted to OECD guideline 213 and 214 and according to the principles of GLP.</p> <p>In the definitive test all the validity criteria were met according to OECD Guideline No. 213 and 214.</p> <p>The studies are reliable and suitable for the risk assessment with following endpoints:</p> <p>LD₅₀/48 h oral > 76.5 µg a.s./bee</p> <p>LD₅₀/72 h contact 14.4 µg a.s./bee</p>
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Reference:	KCP 10.3.1.1/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Effects (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory - Final report -
Report:	Sekine, T.; 2020; 152171035; M-758769-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 213 and 214 (1998)
Deviations:	Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. This deviation is not expected to have impacted the study results. All validity criteria were met.
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive summary

The purpose of this study was to determine the acute contact and oral toxicity of Prohexadione-calcium OD 75 (75 g/L) to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions, a total of 30 worker honey bees (*Apis mellifera* L.) per treatment were exposed for 72 hours to doses of 100.0, 45.5, 20.7, 9.4, 4.3 and 1.9 µg a.s. per bee by topical application (contact dose response test). A separate batch of 30 worker bees per treatment was exposed for 48 hours to doses of 76.5, 52.2, 26.7, 13.5 and 6.8 µg a.s. per bee by feeding (oral dose response test, value based on the actual intake of the test item). In both tests a toxic reference item (dimethoate) was included.

In the contact toxicity test the LD₅₀ value (72 h) of Prohexadione-calcium OD 75 (75 g/L) was 14.4 µg a.s./bee. The oral LD₅₀ value (48 h) of Prohexadione-calcium OD 75 (75 g/L) was > 76.5 µg a.s./bee.

The study fulfils all validity criteria of current Guidelines OECD 213 (1998) and OECD 214 (1998).

Material and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
Sample description: TOX 21574-00
Type of OD (oil dispersion) formulation:
Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
(analysed content):
Specification no.: 102000037599
Supplier batch no.: 2020-001264
Density: 1.038 g/mL

Test species: Honey bee (*Apis mellifera* L.); female worker bees from a healthy and queen-right colony.

Test design: Under laboratory conditions, a total of 30 worker honey bees (*Apis mellifera* L.) per treatment were exposed for 72 hours to doses of 100.0, 45.5, 20.7, 9.4, 4.3 and 1.9 µg a.s. per bee by topical application (contact dose response test). A separate batch of 30 worker bees per treatment was exposed for 48 hours to doses of 76.5, 52.2, 26.7, 13.5 and 6.8 µg a.s. per bee by feeding (oral dose response test, value based on the actual intake of the test item).

The controls used for the contact and oral tests were tap water containing 0.5% Adhäsit and 50% w/v sucrose solution (500 g/L tap water), respectively. As a toxic reference dimethoate (400.0 g/L nominal, 408 g/L analytical) was applied at nominal dose levels of 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee in the contact test and at nominal doses of 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee in the oral test.

In the contact and oral toxicity test each treatment group (test item, water control and reference item) comprised 3 replicates including 10 bees each.

Application in the contact test: In the contact toxicity test the test item was dissolved in tap water with 0.5% Adhäsit and applied as one 5 µL droplet onto the dorsal thorax of bees using a calibrated pipette. For the control, one 5 µL droplet of tap water containing 0.5% Adhäsit was used. The reference item was applied as one 5 µL droplet of dimethoate, dissolved in tap water with 0.5% Adhäsit. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. Bees were shortly anaesthetized with CO₂ until they were immobilized immediately before application.

Application in the oral test: Following a 15 minute starvation period, the test item and reference item were applied in 50% w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50% w/v sucrose solution was offered to the bees, following the starvation period. This diet was offered in syringes which were weighed before and after introduction into the cages. After a maximum of 6 hour, the test item treated food was removed and replaced by fresh, untreated food offered *ad libitum*.

Dose levels:

Nominal doses of the test item: 100.0, 45.5, 20.7, 9.4, 4.3 and 1.9 µg a.s./bee (contact test),
100.0, 50.0, 25.0 12.5 and 6.3 µg a.s./bee (oral test)

Actual dose of the test item (oral test): 76.5, 52.2, 26.7, 13.5 and 6.8 µg a.s./bee (based on the actual food intake)

Nominal doses of the reference item: 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test),
0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test)

Actual doses of the reference item (oral test): 0.34, 0.16, 0.08 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 24 - 25 °C, relative humidity: 59 - 60%; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained from the honey bees treated with the test item were compared to those obtained from the control in both the contact and oral tests. The contact LD_{50/20/10} values of the test item were determined according to Probit Analysis (according to Finney 1971). The oral LD₅₀ was estimated and not determined by statistical procedure since the highest mortality was less than 50%. The contact and oral LD₅₀ values of the reference item were determined with binomial distribution (according to Stephan, 1977). The LD_x calculation was carried out taking into account the mortality data corrected using Abbott's formula (1925). The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1 (ToxRat® Solutions GmbH).

Dates of work: May 11th to May 14th, 2020

Results and discussion

Biological findings:

Contact Test:

Due to increasing mortality between 24 and 48 hours the contact test was prolonged for further 24 hours up to 72 hours. Dose levels of 100.0, 45.5, 20.7, 9.4, 4.3 and 1.9 µg a.s./bee led to mortality of 100.0, 93.3, 60.0, 43.3, 6.7 and 3.3% at test termination (72 hours). A 3.3% mortality was observed in the control group (water + 0.5% Adhäsit).

During the first 4 hours assessment behavioural abnormalities (e.g. moribund, affected and/or apathy) were observed in the 100.0, 45.5, 20.7 and 9.4 µg a.s./bee dose level groups. 24 hours following the application, some bees in the 45.5, 20.7 and 9.4 µg a.s./bee dose level groups were moribund, affected and/or apathetic. At the 48 hours assessment one bee in the 9.4 µg a.s./bee treated and at the 72 hours assessment two bees in the 20.7 µg a.s./bee treatment group were affected. No behavioural abnormalities were observed in the 4.3 and 1.9 µg a.s./bee dose groups.

Mortality and behavioural abnormalities of the bees in the contact toxicity test

Treatment group	After 4 h		After 24 h		After 48 h		After 72 h	
	Mortality	Behav. abnorm.	Mortality	Mortality	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0
Test item [$\mu\text{g a.s./bee}$]								
100	53.3	46.7	100.0	0.0	100.0	0.0	100.0	0.0
45.5	30.0	63.3	83.3	10.0	93.3	0.0	93.3	0.0
20.7	16.7	43.3	46.7	6.7	60.0	0.0	60.0	6.7
9.4	6.7	26.7	23.3	10.0	40.0	3.3	43.3	0.0
4.3	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0
1.9	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0
Reference item [$\mu\text{g a.s./bee}$]								
0.30	13.3	33.3	63.3	0.0	73.3	0.0	73.3	0.0
0.20	0.0	20.0	40.0	3.3	50.0	0.0	53.3	0.0
0.15	0.0	0.0	20.0	3.3	30.0	0.0	36.7	0.0
0.10	0.0	0.0	0.0	0.0	6.7	0.0	10.0	0.0

Results are averages from 3 replicates (ten bees each) for the test item, control group and the reference item groups

Test item = PRL OD75; reference item = dimethoate, control = CO₂/tap water control

Behav. abnorm. = behavioural abnormalities

Oral Test

The maximum nominal dose level of the test item (100.0 $\mu\text{g a.s./bee}$) could not be achieved, because the bees did not ingest the full volume of treated sucrose 50% w/v solution even when offered over a period of 6 hours. Actual oral doses of 76.5, 52.2, 26.7, 13.5 $\mu\text{g a.s./bee}$ resulted in mortality of 16.7, 23.3, 3.3 and 3.3% at the end of the test (48 hours after application). No mortality occurred in the 6.8 $\mu\text{g a.s./bee}$ group. In the control group (50% w/v sucrose solution), 3.3% mortality was observed.

During the 4 hours assessment moribund and/or affected bees were found in the 76.5, 52.2 and 13.5 $\mu\text{g a.s./bee}$ dose groups. After 24 hours one bee in the 26.7 $\mu\text{g a.s./bee}$ dose group was affected. 48 hours following the application, no behavioural abnormalities were found.

Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	3.3	0.0	3.3	0.0	3.3	0.0
Test item [$\mu\text{g a.s./bee}$]						
76.5	0.0	20	13.3	0.0	16.7	0.0
52.2	0.0	16.7	23.3	0.0	23.3	0.0
26.7	0.0	0.0	3.3	3.3	3.3	0.0
13.5	0.0	3.3	3.3	0.0	3.3	0.0
6.8	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [$\mu\text{g a.s./bee}$]						
0.34	0.0	90.0	100.0	0.0	100.0	0.0
0.16	0.0	6.7	53.3	0.0	80.0	3.3
0.08	0.0	0.0	3.3	0.0	10.0	0.0
0.06	0.0	0.0	0.0	0.0	0.0	0.0

Results are averages from 3 replicates (ten bees each) for the test item, control group and the reference item groups
 Test item = PRL OD75; reference item = dimethoate, control = tap water with sucrose solution
 Behav. abnorm. = behavioural abnormalities

The endpoints for the contact and oral toxicity test are shown in the table below.

Contact and oral toxicity of Prohexadione-calcium OD 75 (75 g/L) to honeybees

Test item	PRL OD75	
Test species	Honey bee <i>Apis mellifera</i> L.	
Exposure	Contact	Oral
Test duration	72 h	48 h
Dose rate [$\mu\text{g a.s./bee}$]	100.0, 45.5, 20.7, 9.4, 4.3 and 1.9	76.5, 52.2, 26.7, 13.5 and 6.8
LD ₅₀ [$\mu\text{g a.s./bee}$]	48 hours: 14.8 72 hours: 14.4	48 hours: > 76.5

Reference item

The contact and oral LD₅₀ (24 h) values of the reference item (dimethoate) were calculated to be 0.24 $\mu\text{g a.s./bee}$ and 0.16 $\mu\text{g a.s./bee}$, respectively. These values corresponded to the expected range cited in the OECD Guideline 213 (1998) and 214 (1998) and thus demonstrated the sensitivity of the test item.

Validity criteria:

The contact and oral toxicity tests were considered valid as the control mortality in each case was $\leq 10\%$ and the LD₅₀ values obtained with the reference item (dimethoate) were within the required ranges.

Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Contact Test		
	Control	≤ 10%	3.3%
	Oral Test		
	Control	≤ 10%	3.3%
LD ₅₀ of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.24 µg a.s./bee
	Oral Test		
	Dimethoate	0.10 - 0.35 µg a.s./bee	0.16 µg a.s./bee

Conclusion

The toxicity of Prohexadione-calcium OD 75 (75 g/L) was tested in an acute contact and oral toxicity test on honeybees.

The LD₅₀ (72 h) was determined to be 14.4 µg a.s./bee in the contact toxicity test. The LD₅₀ (48 h) was determined to be > 76.5 µg a.s./bee in the oral toxicity test.

Comments of zRMS:	<p>The studies were conducted to OECD guideline OECD 246 and 247 (2017) and according to the principles of GLP.</p> <p>The studies are reliable with following endpoints:</p> <p>The contact NOED value was calculated to be ≥ 63 µg a.s./bumble bee.</p> <p>The contact LD₅₀ value was estimated to be > 63 µg a.s./bumble bee.</p> <p>The oral NOED value was calculated to be ≥ 33.2 µg a.s./bumble bee.</p> <p>The contact LD₅₀ value was estimated to be > 33.2 µg a.s./bumble bee.</p> <p>Since the evaluation of the risk for bees has been performed in line with SANCO/10329/2002 rev 2 final these studies were deemed not necessary to finalize the risk assessment. Usage of this study should be considered on MS level.</p>
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Reference:	KCP 10.3.1.1/02
Title:	Prohexadione-calcium OD 75 (75 g/L): Effects (acute contact and oral) on bumblebees (<i>Bombus terrestris</i> L.) in the laboratory - Final report
Report:	Chwiesko, D.; Kowalczyk, F.; 2021; 152171105; M-761833-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 246 and 247 (2017)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive summary

The purpose of this study was to determine the acute contact and oral toxicity of prohexadione-calcium

OD 75 (75 g/L) to the bumble bee (*Bombus terrestris* L.). Mortality of bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions, 50 worker bumble bees (*Bombus terrestris*) were exposed for 48 hours to a single dose of 100 µg a.s. per bumble bee (corresponding to an analytical corrected dose of 63 µg a.s. per bumble bee) by topical application (contact limit test).

A separate batch of 30 bumble bees per treatment were exposed for 48 hours to doses of 100, 50, 25, 12.5 and 6.25 µg a.s. per bumble bee by feeding (oral dose response test). These treatment dose values resulted in mean oral doses of 42.5, 24.5, 17.9, 11.6 and 6.1 µg a.s. per bumble bee, based on the actual mean intake of the test item (analytically corrected doses are 33.2, 19.1, 14.0, 9.1 and 4.8 µg a.s. per bumble bee).

The contact test comprised a water control group (tap water containing 0.1% v/v Triton X-100). In the oral test, bumble bees were exposed to 50% w/v aqueous sucrose solution. In both tests a toxic reference item (dimethoate) was included.

The purpose of the analytical part of this study was to verify the concentrations of the active ingredient prohexadione-calcium in the single contact application solution and in the highest and lowest concentrated oral feeding solutions.

In the contact toxicity test the LD₅₀ value (48 h) of Prohexadione-calcium OD 75 (75 g/L) was >63 µg a.s./bumble bee.

The oral LD₅₀ value (48 h) of Prohexadione-calcium OD 75 (75 g/L) was > 33.2 µg a.s./bumble bee.

The study fulfils all validity criteria of current Guidelines OECD 246 (1998) and OECD 247 (2017).

Material and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Sample description:	TOX 21574-00
Type of formulation:	OD (oil dispersion)
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

Test species: Bumble bee (*Bombus terrestris* L.); female worker bees from a healthy and queen-right colony obtained from a commercial bumble bee breeding company. After collection from the hive the bumble bees were kept individually in cylindrical, latticed plastic cages. Medium-sized bumble bees were selected visually and randomly distributed to the treatment groups. Each bumble bee was weighed individually after anaesthetisation with CO₂ to prove a consistent distribution among the treatment groups. Bumble bees were acclimatised to test conditions (contact test: 21 hours 39 minutes; oral test: 43 hours 25 minutes) with *ad libitum* access to untreated 50% w/v sucrose solution.

Test design:

Acute contact toxicity of prohexadione-calcium OD 75 (75 g/L) to adult bumble bees was assessed by exposing 50 worker bumble bees (*Bombus terrestris*) to 100 µg a.s. per bumble bee (corresponding to an analytical corrected dose level 63 µg a.s. per bumble bee) dissolved in tap water containing 0.1% v/v Triton X-100 (contact limit test). Additional 50 adult bumble bees were assigned to a water control (tap water containing 0.1% v/v Triton X-100) and 30 bumble bees to the reference item (10 µg dimethoate/bumble bee) treatment group. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after treatment.

Acute oral toxicity of prohexadione-calcium OD 75 (75 g/L) to adult bumble bees was assessed by exposing 30 worker bumble bees per treatment group to 100, 50, 25, 12.5 and 6.25 µg a.s. per bumble bee in 50% w/v sucrose solution (oral dose response test). These treatment dose values resulted in mean oral doses of 42.5, 24.5, 17.9, 11.6 and 6.1 µg a.s. per bumble bee, based on the actual mean intake of the test item (analytical corrected doses are 33.2, 19.1, 14.0, 9.1 and 4.8 µg a.s. per bumble bee). In addition, 30 adult bumble bees were assigned to a water control (tap water containing 50% w/v sucrose solution) and 30 bumble bees to the reference item (4 µg dimethoate/bumble bee) treatment group. Approximately 40 µL food solution per bumblebee was provided in syringes which were weighed before and after introduction into the cages in order to determine the exact consumption. Bumble bees which did not consume at least 80% of the mean food uptake per treatment group were excluded from the evaluation. The reference item dose value resulted in the mean oral dose of 4.9 µg dimethoate/bumble bee. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after treatment.

Dose levels:

Nominal doses of the test item:	100.0 µg a.s./bumble bee (contact test), 100, 50, 25, 12.5 and 6.25 µg a.s./bumble bee (oral test)
Actual dose of the test item:	63 µg a.s./bumble bee (analytical corrected; contact test) 42.5, 24.5, 17.9, 11.6 and 6.1 µg a.s./bumble bee (actual intake oral test) 33.2, 19.1, 14.0, 9.1 and 4.8 µg a.s./bumble bee (analytical corrected; oral test)
Nominal doses of the reference item:	10 µg dimethoate/bumble bee (contact test), 4 µg dimethoate/bumble bee (oral test)
Actual doses of the reference item:	4.9 µg dimethoate/bumble bee (oral test)

Test conditions: Temperature: 24.8 – 25.0 °C, relative humidity: 48.5 – 60.1% (acute contact test), Temperature: 25.1 – 25.2 °C, relative humidity: 54.1 – 63.0% (acute oral test), photoperiod: 24 h darkness (except during observations).

Statistics: As the test item treatment groups in the contact and oral test did not show mortality above 50.0, 20.0 and 10.0%, no statistical evaluation on the LD₅₀, LD₂₀ and LD₁₀ was carried out.

The contact and oral NOED of the test item was estimated using the multiple sequential Fisher Test after Bonferroni-Holm (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

Dates of work: May 26th to May 28th, 2020 (contact limit test)
May 27th to May 29th, 2020 (oral dose response test)
September 16th to October 30th (analytical phase)

Results and discussion

Analytical findings:

The mean recoveries of the active ingredient prohexadione-calcium in the test item spiked solutions were 63% in the application solution of the contact test, and 84% and 72% in the highest and lowest oral feeding concentrations, respectively.

No residues of prohexadione-calcium were found in the control solution in the oral and contact test above the limit of detection (1.1 mg a.s./L).

Analytical results

Test system	Nominal test concentration		Recovery
	[µg a.s./bumble bee]	[g a.s./kg feeding solution]	
Contact Test	100	50	63%
Oral Test (highest concentration)	100	2.5	84%
Oral Test (lowest concentration)	6.25	0.156	72%

Biological findings:

Contact Test:

In the contact test a droplet of 2 µL containing the target dose level of 100 µg a.s./bumble bee (analytical corrected dose of 63 µg a.s./bumble bee) was applied on the dorsal thorax of each exposed bumble bee. At the end of the contact toxicity test (48 hours after application) 100 µg a.s./bumble bee led to no mortality. 2.0% mortality occurred in the water control treatment group (tap water containing 0.1% v/v Triton X-100). During the 4, 24 and 48 hours assessment not more than two affected bumble bees were observed in the test item group.

Mortality and behavioural abnormalities of the bumble bees in the contact toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Mortality	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	2.0	0.0
Test item [µg a.s./bee]						
100 (63*)	0.0	4.0	0.0	2.0	0.0	2.0
Reference item [µg a.s./bee]						
10	0.0	76.7	96.7	100.0	96.7	100.0

Results are mean of 50 individuals for the test item and water control treatment group, mean of 30 individuals for the reference item treatment group

*dietary dose of the test item corrected by the dose verification value

Test item = PRL OD75; reference item = dimethoate, control = tap water containing 0.1% Triton X-100

Behav. abnorm. = behavioural abnormalities of mean of living individuals per treatment group

Oral Test

In the oral test the target dose levels of 100, 50, 25, 12.5 and 6.25 would have been achieved if exactly 40 mg treated feeding solution was consumed by each exposed bumble bee. The mean food uptake was calculated considering all replicates per treatment group. However, actual food uptake in the treatment groups ranged between 7 and 61 mg per bumble bee. Bumble bees which did not consume at least 80% of the mean food uptake per treatment group were excluded from the derivation of the end points, as well as from the calculation of the actual mean oral doses in the test and reference item treatment groups. This was done to avoid potentially overestimating the final endpoints. The actual mean oral doses following this adjustment for non-feeding bumble bees were 42.5, 24.5, 17.9, 11.6 and 6.1 µg a.s./bumble bee (analytical corrected doses of 33.2, 19.1, 14.0, 9.1 and 4.8 µg a.s./bumble bee).

For the 42.5, 24.5, 17.9, 11.6 and 6.1 test item treatment groups 18, 14, 10, 14 and 16 bumble bees were considered for the evaluation. For the water control treatment group (50% w/v sucrose solution) 26 bumble bees were considered for the evaluation.

At the end of the oral toxicity test (48 hours after application) 42.5, 24.5, 17.9, 11.6 and 6.1 µg a.s./bumble bee led to no mortality. No mortality occurred also in the water control treatment group (50%

w/v sucrose solution). No test item induced behavioural effects were observed at any time in the oral test.

Mortality and behavioural abnormalities of the bumble bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg a.s./bee]						
42.5 (33.2*)	0.0	0.0	0.0	0.0	0.0	0.0
24.5 (19.1*)	0.0	0.0	0.0	0.0	0.0	0.0
17.9 (14.0*)	0.0	0.0	0.0	0.0	0.0	0.0
11.6 (9.1*)	0.0	0.0	0.0	0.0	0.0	0.0
6.1 (4.8*)	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
4.9	0.0	100.0	80.0	100.0	100.0	-

Results are mean of individuals per treatment group, considering only those bumble bees which achieved at least 80% of the mean food uptake per treatment group (test item: 42.5 µg a.s./bumble bee: n=18, 24.5 µg a.s./bumble bee: n=14, 17.9 µg a.s./bumble bee: n=10, 11.6 µg a.s./bumble bee: n=14 and 6.1 µg a.s./bumble bee: n= 16, water control: n= 26, reference item (4.9 µg dimethoate/bumble bee): n=20)

*dietary doses of the test item corrected by mean dose verification value

Test item = PRL OD75; reference item = dimethoate, control = tap water with sucrose solution

Behav. abnorm. = behavioural abnormalities of mean of living individuals per treatment group

The endpoints for the contact and oral toxicity test are shown in the table below.

Contact and oral toxicity of Prohexadione-calcium OD 75 (75 g/L) to bumble bees

Test item	PRL OD75			
Test species	Bumble bee <i>Bombus terrestris</i> L.			
Exposure	Contact ³ (tap water containing 0.1% v/v Triton X-100)		Oral ³ (50% w/v sucrose solution) (based on recorded consumption considering bumble bees with food uptake of at least 80% of the mean uptake per treatment group ¹)	
Target dose rates [µg a.s./bumble bee]	100.0		100, 50, 25, 12.5 and 6.25	
Actual dose rates [µg a.s./bumble bee]	63		42.5, 24.5, 17.9, 11.6 and 6.1	
Analytical corrected dose rates [µg a.s./bumble bee]	63		33.2, 19.1, 14.0, 9.1 and 4.8	
Test duration	24 h	48 h	24 h	48 h
LD _{50, 20, 10} [µg a.s./bee] ^{2,5}	> 63	> 63	> 33.2	> 33.2
NOED [µg a.s./bee] ^{2,5}	≥ 63	≥ 63	≥ 33.2	≥ 33.2
LOED[µg a.s./bee] ^{2,5}	≥ 63	≥ 63	≥ 33.2	≥ 33.2

¹ For the 33.2, 19.1, 14.0, 9.1, 4.8 µg a.s./bumble bee test item treatment groups 18, 14, 10, 14 and 16 bumble bees were considered for the evaluation.

² Results obtained from test item treated groups were compared to those obtained from the water control treatment group. 26

bumble bees of the water control group were considered for the evaluation

³ No mortality was found above 50.0, 20.0 and 10.0%, thus, no statistical evaluation on the LD₅₀, LD₂₀ and LD₁₀ was carried out.

⁴ NOED/LOED determined using Fisher's Exact test after Bonferroni-Holm (pairwise comparison, one-sided greater, $\alpha = 0.05$).

⁵ Dietary concentrations and doses of the test item were corrected by mean dose verification values

Reference item

The contact target dose level of the reference item of 10 µg dimethoate/bumble bee resulted in a mortality of 96.7% (48 hours after application).

The reference item target dose level of 4 µg dimethoate/bumble bee, resulted in a mortality of 100% (48 hours after application with n = 20 individuals consuming ≥80% of offered feeding solution).

These values corresponded to the expected range cited in the OECD Guideline 246 (2017) and 247 (2017).

Validity criteria:

The contact and oral toxicity test were considered valid as the control mortality in each case was ≤ 10% and ≥50% in the reference item.

Validity criteria

Validity criteria	Recommended		Obtained
Control mortality (48 h)	Contact Test		
	Control	≤ 10%	2.0%
	Oral Test		
	Control	≤ 10%	0.0% *
Reference item mortality (48 h)	Contact Test		
	Dimethoate	≥ 50%	96.7%
	Oral Test		
	Dimethoate	≥ 50%	100% *

*Considering bumble bees with food uptake of at least 80% of the mean food uptake.

Conclusion

The toxicity of Prohexadione-calcium OD 75 (75 g/L) was tested in an acute contact and oral toxicity test on bumble bees.

The contact NOED value was calculated to be ≥ 63 µg a.s./bumble bee. The contact LD₅₀ value was estimated to be > 63 µg a.s./bumble bee.

The oral NOED value was calculated to be ≥ 33.2 µg a.s./bumble bee. The contact LD₅₀ value was estimated to be > 33.2 µg a.s./bumble bee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	<p>The study was conducted according to OECD 245 guideline and according to the principles of GLP. All the validity criteria were met.</p> <p>The study is reliable with following endpoints:</p> <p>LC₅₀ = 505 mg a.s./kg diet</p> <p>LDD₅₀ = 4.33 µg a.s./bee/day</p> <p>NOEC = 280 mg a.s./kg diet</p> <p>NOEDD = 3.34 µg a.s./bee/day</p>
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Reference:	KCP 10.3.1.2/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Honey bee (<i>Apis mellifera</i> L.) chronic oral toxicity test 10 day feeding test in the laboratory - Final report -
Report:	Kling, A.; 2021; S20-00682; M-762860-01-1
Authority registration No:	
Guideline(s):	<p>Commission Regulation (EU) No 283/2013 (Mar. 2013) and No 284/2013 (Mar. 2013) in accordance with</p> <p>Regulation (EC) No 1107/2009 (Oct. 2009)</p> <p>Directive 2003-01 (Canada/PMRA)</p> <p>US EPA OCSPP 850.SUPP</p> <p>OECD Guideline No. 245 (2017)</p>
Deviations:	The first toxic reference assessment was conducted 27 hours and 15 minutes after the start of first feeding application. All subsequent assessments were conducted at 24 hours (± 2 hours) intervals. It can be assumed that this deviation had no impact on the outcome of the study as the validity criterion of the reference item treatment group was met.
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive Summary

The objective of this study was to determine the chronic oral toxicity (LDD₅₀/LC₅₀ and NOEDD/NOEC) of Prohexadione-calcium OD 75 (75 g/L) applied on 10 consecutive days to young adults of the honeybee (*Apis mellifera* L.).

Worker honey bees (*Apis mellifera* L.) aged two days or less were orally exposed to a daily application of Prohexadione-calcium OD 75 (75 g/L) diluted in the bee food (50% w/v aqueous sucrose solution with 0.1% Xanthan and 0.5% Tween80) at nominal concentrations of 17.9, 44.8, 112, 280 and 700 mg a.s./kg feeding solution, corresponding to 0.55, 1.22, 2.37, 3.34 and 4.99 µg a.s./bee/day (actual doses) for ten consecutive days. Two control groups, an untreated control (50% w/v aqueous sucrose solution) and a thickener control (50% w/v aqueous sucrose solution with 0.1% Xanthan and 0.5% Tween80), as well a reference item (dimethoate) were included.

Mortality and behavioural abnormalities were assessed daily, except for the reference item group, as it can be assumed that moribund and affected bees of that group will die by the end of the test. The concentration of the active substances prohexadione-calcium in the feeding solutions was verified analytically.

The LDD₅₀ and LC₅₀ were determined to be 4.33 µg a.s./bee/day and 505 mg a.s./kg feeding solution, respectively. The LDD₂₀ and LC₂₀ were determined to be 3.87 µg a.s./bee/day and 392 mg a.s./kg feeding solution, respectively. The LDD₁₀ and LC₁₀ were determined to be 3.60 µg a.s./bee/day and 331 mg a.s./kg feeding solution, respectively.

The NOEDD and NOEC were determined to be 3.34 µg a.s./bee/day and 280 mg a.s./kg feeding solution, respectively.

The study fulfils all validity criteria of the current Guideline OECD 245 (2017).

Material and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
Sample description: TOX 21574
Type of OD (oil dispersion) formulation:
Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
(analysed content):
Specification no.: 102000037599
Supplier batch no.: 2020-001264
Density: 1.038 g/mL

Test species: Honeybees (*Apis mellifera* L.); newly hatched young female worker bees (max. 2 days old) from healthy, disease-free and queen-right honey bee colonies.

Test concentrations and dose levels:

Test item concentrations: 17.9, 44.8, 112, 280 and 700 mg a.s./kg feeding solution

Actual test item doses: 0.55, 1.22, 2.37, 3.34 and 4.99 µg a.s./bee/day

Reference item concentration: 0.90 mg dimethoate/kg feeding solution

Actual reference item dose (calculated based on uptake of feeding solution): 0.02 µg dimethoate/bee/day

An untreated control (C1, 50% w/v aqueous sucrose solution) and a thickener control (C2; 50% w/v aqueous sucrose solution with 0.1% Xanthan and 0.5% Tween80) were also assessed.

Each group (test item, controls and reference item) comprised 4 replicates containing 10 bees each.

Test design: In a 10-day chronic toxicity feeding test max. two days old worker honey bees (*Apis mellifera* L.) were orally exposed to a daily application of Prohexadione-calcium OD 75 (75 g/L) diluted in the bee food (50% w/v aqueous sucrose solution with 0.1% Xanthan and 0.5% Tween80).

Two days prior start of exposure, brood combs with capped cells were taken from outside hives and incubated under controlled environmental conditions in a climatic chamber in darkness. One day prior to test starting, the newly hatched worker bees were randomly collected directly from the frames, introduced into the test units and kept under test conditions until the start of the test. The acclimatisation period lasted from collection to the start of the test. During this period, bees were fed *ad libitum* with 50% w/v sucrose solution.

A fresh test item stock solution was obtained daily by mixing a defined amount of test item with a defined amount of 50% w/v aqueous sucrose solution with 0.1% Xanthan and 0.5% Tween80. Further dilution of the stock solutions to get the required concentration levels of feeding solutions were prepared using 50% (w/v) aqueous sucrose solution containing 0.1% Xanthan and 0.5% Tween80. The reference item stock solution was prepared on the day of exposure start and on two further days in the course of the test period. The reference item stock solution was stored tightly closed under cool and dark conditions (refrigerator). Further dilution of the stock solution to get the required concentration level of feeding solution were prepared using 50% (w/v) aqueous sucrose solution on the day of use.

The respective feeding solutions (test item, control and reference item) were provided *ad libitum* in a plastic syringe, which had been weighed before application. The feeders remained in the cages for about 24 h. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units.

Mortality for all the groups was assessed on a daily basis over the test period. Behavioural abnormalities were recorded at each observation interval, except for the reference item group. The reference item group was used for several studies, which were conducted in an early and a late shift. The first application (A1) was started by the early shift, the following applications took place in the late shift. Therefore, the first assessment (E1) of R was conducted 27 hours and 15 minutes after the start of feeding on the day of the first application. The following assessments of R were conducted in a 24 hours (\pm 2 hours) interval based on the second assessment. In the reference item treatment group, behavioural assessments were not conducted as it was assumed that moribund and affected bees of the reference item treatment group would die by the end of the test.

The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application.

Test conditions: Temperature: 32.1 - 32.8 °C; Relative humidity: 48.5 - 62.4% (with short-term deviations <2 h). Photoperiod: 24 h darkness (except during application and assessments).

Statistics: Results obtained from bees treated with the test item were compared to those obtained from the control group with Fisher's Exact test (one-sided greater, $\alpha = 0.05$). To estimate the $LC_{10/20/50}$ and the $LDD_{10/20/50}$, a Weibull regression was carried out. The statistical calculations were performed with the computer program ToxRat Professional 3.3.0.

Analytics: All final daily diets of the control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet. The chemical analysis was performed by using LC-MS/MS detection.

Results and discussion

Analytical results:

The measured concentrations of the test item in the feeding solutions were within $\pm 20\%$ of nominal or only marginally below (in T1 on two days; 75 and 76 % of nominal). Therefore, the concentrations of the test item in the feeding solutions were confirmed and the endpoints are based on nominal concentrations.

No residues were found in the control samples above the Limit of Quantification for prohexadione-calcium (LOQ: 0.01 mg a.s./kg) and above the Limit of Detection for prohexadione-calcium (LOD: 0.003 mg a.s./kg).

Analytical results for prohexadione-calcium

Treatment group	Nominal concentration	Recovery range	Mean recovery from target
	[mg a.s./kg diet]	[%]	[%]
Control	0	< LOD	-
Thickener Control	0	< LOD	-
Test item (PRL OD 75)	17.9	75 – 107	91
	44.8	86 – 115	96
	112	81 - 119	92
	280	88 - 119	102
	700	91 - 106	96

LOD: Limit of Detection: prohexadione-calcium = 0.003 mg a.s./kg

LOQ: Limit of Quantification: prohexadione-calcium = 0.01 mg a.s./kg diet.

Biological results:

Summary of mean mortality and toxicity of Prohexadione-calcium OD 75 (75 g/L) to adult honeybees after 10 days of chronic exposure:

10-day chronic oral toxicity test with Prohexadione-calcium OD 75 (75 g/L) to young honeybees

Treatment group	Daily dose	Concentration	At day 10
	Consumed A		Mean mortality
	[µg a.s./bee/day]		[%]
Control	-	-	2.5
Thickener control	-	-	0.0
Test item (PRL OD 75)	0.55	17.9	0.0
	1.22	44.8	2.5
	2.37	112	0.0
	3.34	280	5.0
	4.99	700	95.0 ^B
Reference Item	[µg a.s./bee/day] ^c	[mg a.s./kg diet] ^c	
	0.02	0.90	100
Endpoints			10 d
Test item doses	LDD ₅₀ [µg a.s./bee/day] (95 % C.I.) ^D		4.33 (4.02 - 4.53)
	LDD ₂₀ [µg a.s./bee/day] (95 % C.I.) ^D		3.87 (3.42 - 4.14)
	LDD ₁₀ [µg a.s./bee/day] (95 % C.I.) ^D		3.60 (3.07 - 3.91)
	LOEDD [µg a.s./bee/day]		4.99
	NOEDD [µg a.s./bee/day]		3.34
Test item concentrations	LC ₅₀ [mg a.s./kg feeding solution] (95 % C.I.) ^D		505 (425 / 561)
	LC ₂₀ [mg a.s./kg feeding solution] (95 % C.I.) ^D		392 (293 / 456)
	LC ₁₀ [mg a.s./kg feeding solution] (95 % C.I.) ^D		331 (228 / 401)
	LOEC [mg a.s./kg feeding solution]		700
	NOEC [mg a.s./kg feeding solution]		280

Results are mean values of 4 replicates, containing 10 bees each.

C.I.: Confidence interval

^A Actual dose per bee per day; dose measured based on consumed feeding solution

^B Significantly increased compared to control (Fisher's Exact Binomial Test; one sided greater, $\alpha = 0.05$)

^C Based on the analysed content of dimethoate

^D Estimated using Weibull analysis using linear maximum likelihood regression

Two bees were observed as affected on day 3 at the concentration of 112 mg a.s./kg. Two affected bees were observed on day 3 and one single bee was observed as affected on day 7 at 280 mg a.s./ kg. Few affected and moribund bees were observed on several assessment dates at the concentration of 700 mg a.s./ kg.

The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application. Taking into account the actual food uptake, the bees consumed doses of 0.55, 1.22, 2.37, 3.34 and 4.99 µg a.s./bee/day, which caused mortalities of 0.0, 2.5, 0.0, 5.0 and 95.0% respectively, after 10 days.

In the test item group, the food consumption ranged between 7.1 and 30.6 mg feeding solution per bee per day. A decreasing consumption of feeding solution was observed for increasing concentrations of test item. In the control and thickener control the food consumption was 33.5 and 36.5 mg feeding solution

per bee per day, respectively.

Reference item

The reference item (dimethoate) was administered in one dosage of 0.02 µg a.s./bee/day (actual average intake based analysed content of dimethoate) which caused a continuously increasing mortality leading to 100% mortality at day 10.

Validity criteria:

All validity criteria of the current OECD Guideline 245 (2017) were met in this study.

Validity criteria

Validity criteria according to OECD GD 245 (2017)	Recommended		Obtained
Mortality after 10 days of exposure	Control	≤ 15%	2.5%
	Thickener control	≤ 15%	0.0%
Mortality after 10 days of exposure	Dimethoate	≥ 50%	100%

Conclusion

The chronic oral toxicity of Prohexadione-calcium OD 75 (75 g/L) was tested on young adult honeybees (*Apis mellifera* L.) in a 10-day feeding study under laboratory conditions.

The LDD₅₀ and LC₅₀ were determined to be 4.33 µg a.s./bee/day and 505 mg a.s./kg feeding solution, respectively. The LDD₂₀ and LC₂₀ were determined to be 3.87 µg a.s./bee/day and 392 mg a.s./kg feeding solution, respectively. The LDD₁₀ and LC₁₀ were determined to be 3.60 µg a.s./bee/day and 331 mg a.s./kg feeding solution, respectively.

The NOEDD and NOEC were determined to be 3.34 µg a.s./bee/day and 280 mg a.s./kg feeding solution, respectively.

A 2.3.1.3 KCP 10.3.1.3 Effects on honeybee development and other honeybee life stages

Comments of zRMS:	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: NOEC (emergence) = 19.2 mg a.s./kg diet NOED (emergence) = 2.96 µg a.s./larva
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Reference:	KCP 10.3.1.3/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Honey bee (<i>Apis mellifera</i> L.) 22 day larval toxicity test (repeated exposure) - Final report -
Report:	Kling, A.; 2021; S20-00850; M-762844-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 (Oct. 2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document 239 (2016)
Deviations:	Yes, but acceptable. For the toxic reference item group(s) mortality but no other observations were assessed. No emergence boxes were used from day 15 onwards to enable the assignment of each emerged bee to the respective replicate. Temperature target: 34 - 35 °C, but not below 23 °C or above 40 °C. Actual: 31.9 to 35.6 °C with deviations (≥ 2 hours). However, these deviations are considered unlikely to have made any discernible impact on the test performance as the validity criteria were fulfilled.
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive Summary

The objective of this study was to determine the effects of Prohexadione-calcium OD 75 (75 g/L) on the emergence of adult honey bees, *Apis mellifera* L., from repeated feeding exposure in a 22 day laboratory test and to determine the cumulative mortalities during the larval phase and the pupation phase as well as the adult emergence rate. The test item was administered to honeybee larvae from day 3 until day 6 at a constant concentration in the diet according to their growth, within a range of five increasing doses spaced by a factor of 2.5. Larvae were exposed to 5 concentrations of Prohexadione-calcium OD 75 (75 g/L) via the larval diet on 4 consecutive days (D3 to D6). No additional feeding of the larvae took place after D6. The chronic toxicity of the test item was determined at nominal doses of 7.68, 19.2, 48.0, 120 and 300 mg a.s./kg feeding solution, corresponding to 1.18, 2.96, 7.39, 18.5 and 46.2 µg a.s./larva/developmental period, respectively. Cumulative mortalities of honeybee larvae treated with the test item were assessed daily from D4 to D8. Cumulative mortalities during the pupation phase were assessed on D15. Cumulative mortality and adult emergence rate were assessed on D22. The presence of uneaten food was qualitatively recorded on day 8. A reference item (dimethoate tech. at a cumulative dose of 7.39 µg a.s./larva/developmental period) and an untreated control were included in the experimental design.

In the analytical phase of the study, the concentration of prohexadione-calcium was determined in the larval diet of each day of the exposure period.

Since the mean recoveries per treatment group were within the range of 80 to 120% of the nominal concentrations, further evaluations were done with nominal concentrations.

The EC₁₀ for adult emergence on day 22 was determined to be 34.3 mg a.s./kg diet, equivalent to an ED₁₀ of 5.28 µg a.s./larva per developmental period. The EC₂₀ for adult emergence on day 22 was determined to be 55.3 mg a.s./kg diet, equivalent to an ED₂₀ of 8.52 µg a.s./larva per developmental period. The EC₅₀ for adult emergence on day 22 was determined as 114 mg a.s./kg diet, equivalent to an ED₅₀ of 17.6 µg a.s./larva per developmental period.

The LOEC for adult emergence on day 22 was determined to be 48.0 mg a.s./kg diet, equivalent to a LOED of 7.39 µg a.s./larva per developmental period. The NOEC for adult emergence on day 22 was determined to be 19.2 mg a.s./kg diet, equivalent to a NOED of 2.96 µg a.s./larva per developmental period.

The study fulfils all validity criteria of the current Guideline OECD 239 (2016).

Material and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
Sample description: TOX 21574-00
Type of OD (oil dispersion) formulation:
Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
(analysed content):
Specification no.: 102000037599
Supplier batch no.: 2020-001264
Density: 1.038 g/mL

Test species: Honey bees (*Apis mellifera* L.); synchronized first instar larvae from three different healthy, disease-free and queen-right honey bee colonies.

Test concentrations and dose levels:

Test item concentrations: 7.68, 19.2, 48.0, 120 and 300 mg a.s./kg feeding solution, equivalent to 1.18, 2.96, 7.39, 18.5 and 46.2 µg a.s./larva/developmental period

Reference item concentration: 48 mg dimethoate/kg feeding solution, equivalent to 7.39 µg dimethoate/larva/developmental period

An untreated control (C, 50% w/v aqueous sucrose solution) was also assessed.

Each group (test item, controls and reference item) comprised 3 replicates containing 16 larvae each.

Test design: In a 22-day repeated exposure laboratory test with first instar honey bee larvae (*Apis mellifera* L.) cumulative mortalities during the larval phase and the pupation phase as well as the adult emergence rate were determined.

From day 3 until day 6 Prohexadione-calcium OD 75 (75 g/L) was administered daily to the larvae in the diet in a range of increasing concentrations, which remained constant during the application period.

The larval diet was prepared with deionized, autoclaved water using the following ingredients: Diet A: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose; Diet B: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose; Diet C: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose. The volume of water/solvent/test item solution/reference item solution added during the preparation of treated Diet B and Diet C was considered for the preparation of the larval diet (i.e. 10% v/v of water was subtracted for the preparation, considering a density of the diet of 1.1 g/cm³). During the larval stage each live larva was fed once a day from day 1 to day 6 (except on day 2) with a standardized amount of artificial diet. On day 1 each larva was fed with 20 µL of untreated diet A, on day 3 with 20 µL of treated diet B, on day 4 with 30 µL of treated diet C, on day 5 with 40 µL of treated diet C and on day 6 with 50 µL of treated diet C.

The respective feeding solutions (test item, control and reference item) were provided *ad libitum*. Treated diet was dropped next to the larva to prevent drowning and to avoid damaging the larva. Treatments started with the control group followed by the test item groups (with increasing concentrations) and lastly the reference item group.

Assessments of larval mortality were done on D4, D5, D6, D7 and D8. Additionally, other observations such as small body size or unconsumed food on D8 were noted. Pupal mortality was assessed on D15 and D22 and emergence of adults was evaluated on D22. The presence of uneaten food was qualitatively recorded on day 8.

In the analytical phase of the study, the concentration of prohexadione-calcium was determined in the larval diet of each day of the exposure period.

A reference item (dimethoate tech. at a cumulative dose of 7.39 µg a.s./larva/developmental period) and an untreated control were included in the experimental design.

Test conditions: Temperature: 31.9 - 35.6 °C with some deviations ≥ 2 h, which are considered unlikely to have made any discernible impact on the test performance as the validity criteria were fulfilled; Relative humidity: day 1 to 8: 56.2-100%, day 9 to 15: 39.1 – 86.0%, day 15 to 22: 28.4-63.2%; Photoperiod: 24 h darkness (except during application and assessments).

Statistics: Results obtained from bees treated with the test item were compared to those obtained from the control group with Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) or Cochran-Armitage test with Rao-Scott adjustment (one sided greater, $\alpha = 0.05$). To estimate the $EC_{10/2050}$ and the $ED_{10/2050}$, a Weibull regression was carried out. The statistical calculations were performed with the computer program ToxRat Professional 3.3.0.

Analytics: All final diets of the control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet. The chemical analysis was performed by using LC-MS/MS detection.

Results and discussion

Analytical results:

The measured concentrations of the test item in the feeding solutions were within $\pm 20\%$ of nominal. Therefore, the concentrations of the test item in the feeding solutions were confirmed and the endpoints are based on nominal concentrations.

No residues were found in the control samples above the Limit of Detection for prohexadione-calcium (LOD: 0.003 mg a.s./kg) for prohexadione-calcium.

Analytical results for prohexadione-calcium

Treatment group	Nominal concentration	Recovery range	Mean recovery from target
	[mg a.s./kg diet]	[%]	[%]
Control	0	< LOD	-
Test item (PRL OD 75)	7.68	99 – 108	102
	19.2	99 - 104	101
	48.0	93 - 106	98
	120	99 - 107	103
	300	93 – 99	96

LOD: Limit of Detection: prohexadione-calcium = 0.003 mg a.s./kg

Biological results:

During the assessments of mortality and emergence no other test item related observations such as deviating sizes, appearances and malformations of the test organisms were made. On day 8, uneaten food was observed in the test item groups of 120 and 300 mg a.s./kg diet and in the reference item group.

Summary of mean mortality and effects on emergence of Prohexadione-calcium OD 75 (75 g/L) to adult honeybees after 22 days of repeated exposure:

Mortality and other observations of larvae and adult emergence in the repeated exposure toxicity test with Prohexadione-calcium OD 75 (75 g/L)

Treatment group	Cumulative dose per developmental period ^A	Concentration	At day 8		At day 22	
			Larval mortality		Adult Emergence	
	[µg a.s./larva per developmental period] ^B	[mg a.s./kg diet] ^B	Actual [%]	Corrected [%] ^D	Actual [%]	Corrected [%] ^{D, E}
Control	-	-	4.2	-	87.5	-
Test item (PRL OD 75)	1.18	7.68	4.2	0.0	89.6	-2.4
	2.96	19.2	8.3	4.3	81.3	7.1
	7.39	48.0	6.3	2.2	70.8**	19.1
	18.5	120	12.5	8.7	47.9**	45.3
	46.2	300	91.7*	91.3	2.1**	97.6
Reference Item	[µg a.s./larva/developmental period] ^c	[mg a.s./kg diet] ^c				
	7.39	48.0	72.9	71.7	-	-
Endpoints				22 d		
Test item doses	ED ₅₀ [µg a.s./bee/developmental period] (95 % C.I.) ^F			17.6 (14.6 – 20.8)		
	ED ₂₀ [µg a.s./bee/ developmental period] (95 % C.I.) ^F			8.52 (6.24 – 10.6)		
	ED ₁₀ [µg a.s./bee/ developmental period] (95 % C.I.) ^F			5.28 (3.45 – 7.04)		
	LOED [µg a.s./bee/ developmental period]			7.39		
	NOED [µg a.s./bee/ developmental period]			2.96		
Test item concentrations	EC ₅₀ [mg a.s./kg diet] (95 % C.I.) ^F			114 (94.6 – 135)		
	EC ₂₀ [mg a.s./kg diet] (95 % C.I.) ^F			55.3 (40.5 – 69.0)		
	EC ₁₀ [mg a.s./kg diet] (95 % C.I.) ^F			34.3 (22.4 – 45.7)		
	LOEC [mg a.s./kg diet]			48.0		
	NOEC [mg a.s./kg diet]			19.2		

Results are mean values of 3 replicates, containing 16 larvae each.

C.I.: Confidence interval

^A based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm³

^B based on the analysed content of active substance (a.s.)

^C based on the analysed content of dimethoate

^D cumulative mortality corrected for corresponding control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947)

^E negative values indicate a higher emergence compared to the control group

^F Weibull analysis using linear maximum likelihood regression

* significantly increased compared to control (Cochran-Armitage test; one sided greater, $\alpha = 0.05$)

** significantly increased compared to control (Cochran-Armitage test with Rao-Scott adjustment; one sided greater, $\alpha = 0.05$)

Reference item

The reference item (dimethoate) was administered in one dosage of 7.39 µg a.s./larva/developmental period which caused a continuously increasing mortality leading to 71.7% corrected larval mortality at day 8.

Validity criteria:

All validity criteria of the current OECD Guideline 239 (2016) were met in this study.

Validity criteria

Validity criteria according to OECD GD 239 (2016)	Recommended	Obtained
Control larval mortality day 8	$\leq 15\%$	4.2%
Control emergence day 22	$\geq 70\%$	87.5%
Reference item mortality day 8	$\geq 50\%$	72.9%

Conclusion

In a repeated exposure larval toxicity test with Prohexadione-calcium OD 75 (75 g/L) and a duration of 22 days the LOEC for adult emergence on day 22 was determined to be 48.0 mg a.s./kg diet, equivalent to a LOED of 7.39 µg a.s./larva per developmental period. The NOEC for adult emergence on day 22 was determined to be 19.2 mg a.s./kg diet, equivalent to a NOED of 2.96 µg a.s./larva per developmental period.

The EC₁₀ for adult emergence on day 22 was determined to be 34.3 mg a.s./kg diet, equivalent to an ED₁₀ of 5.28 µg a.s./larva per developmental period. The EC₂₀ for adult emergence on day 22 was determined to be 55.3 mg a.s./kg diet, equivalent to an ED₂₀ of 8.52 µg a.s./larva per developmental period. The EC₅₀ for adult emergence on day 22 was determined as 114 mg a.s./kg diet, equivalent to an ED₅₀ of 17.6 µg a.s./larva per developmental period.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional study submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No additional study submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No additional study submitted.

A 2.3.2 KCP 10.3.2. Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1. Standard laboratory testing for non-target arthropods

Comments of zRMS:	<p>The study follows the guideline specified by Blumel S. et al., 2000 and according to the principles of GLP.</p> <p>In the definitive test all the validity criteria were met. The study is reliable for risk assessment purposes with following endpoints:</p> <p>LR₅₀ = 80.7 g a.s./ha</p>
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Reference:	KCP 10.3.2.1/01
Title:	Amendment no. 01: Toxicity to the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) using a laboratory test; prohexadione-calcium OD 75 (75 g/L)
Report:	Waibel, J.; 2021; CW20/019; M-761029-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable BLÜMEL ET AL. (2000) CANDOLFI ET AL. (2001)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Objective

The aim of the study was to determine the toxicity of Prohexadione-calcium OD 75 (75 g/L) applied onto glass plates to the predatory mite *Typhlodromus pyri*.

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

The test item was applied on glass plates at rates of 18, 32, 57, 101 and 180 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 206 L/ha). The effects of the test item on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 3.3 g a.s./ha in 200 L deionised water/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 100 predatory mites, protonymphs not older than 24 h at study start (5 replicates with 20 individuals per test group), was assessed 4 and 7 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

The reproduction performance of the surviving mites in the control and those test item rates which showed a corrected mortality <50% was then evaluated on day 7, 8, 11 and 14 after application by counting the total number of offspring (eggs and larvae) produced.

The climatic test conditions during the study were 24.0 - 25.0 °C temperature and 60 - 73% relative humidity. The light / dark cycle was 16 : 8 h with a light intensity range of 275 - 937 lux.

The LR₅₀ value (lethal rate causing 50% mortality) was calculated by using the linear regression model Probit. The computer programs Microsoft Excel (2010) with Visual Basic for Application (VBA) and SAS (version 9.4) were used to perform the calculations

Results and discussion

In this laboratory test the effects of Prohexadione-calcium OD 75 (75 g/L) residues on the survival and reproduction of the predatory mite *Typhlodromus pyri* were determined at 18, 32, 57, 101 and 180 g a.s./ha, applied to glass plates.

The mortality / escaping rate in the control exposure units up to day 7 after treatment was 9.0%. A statistically significant mortality was found in the higher rates of 57, 101 and 180 g a.s./ha (Fisher's Exact test, one-sided, $\alpha = 0.05$). At the lower test item rates of 18 and 32 g a.s./ha, a corrected mortality of 1.1% and 6.6% has been observed, respectively. At the test item rates of 57, 101 and 180 g a.s./ha the corrected mortality was 24.2%, 65.9% and 100.0%, respectively.

Reproduction was assessed only for the three lowest rates of Prohexadione-calcium OD 75 (75 g/L), 18, 32 and 57 g a.s./ha. The reproduction was reduced by 5.0% at the 18 g a.s./ha rate and by 13.0% at the 32 g a.s./ha rate. At the test item rate of 57 g a.s./ha the reduction was 23.4%. Only the rate of 57 g a.s./ha showed statistical significance in reduction compared to the control (Dunnett's test, one-sided, $\alpha = 0.05$).

A summary of the effects observed in this study is given on the next page.

Test item:		Prohexadione-calcium OD 75 (75 g/L)					
Test organism:		<i>Typhlodromus pyri</i>					
Exposure on:		Glass plates					
		Mortality after 48 h [%]			Reproduction		
Treatment	g a.s./ha	Uncorr.	Corr.	P-Value (*)	Rate (mummies per female)	Reduction relative to control [%]	P-Value (#)
Control	0	9.0	-	-	8.6	-	-
Test item	18	10.0	1.1	0.500 n.sign.	8.2	5.0	0.534 n.sign.
Test item	32	15.0	6.6	0.276 n.sign.	7.5	13.0	0.185 n.sign.
Test item	57	31.0	24.2	<0.001 sign.	6.6	23.4	0.024 sign.
Test item	101	69.0	65.9	<0.001 sign.	n.a.	n.a.	n.a.
Test item	180	100.0	100.0	<0.001 sign.	n.a.	n.a.	n.a.
Reference item	3.3	100.0	100.0	-	n.a.	n.a.	-
LR₅₀: 80.7 g a.s./ha ; 95% Confidence Interval: (71.7 – 88.4); calculated with Probit analysis ER ₅₀ : >57 g a.s./ha * Fisher's Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm # ANOVA, Dunnett's test (one-sided, $\alpha = 0.05$) n.a. not assessed sign. Significant							

Validity

All validity criteria of the laboratory method using glass plates (Blümel et al., 2000) were fulfilled.

	Validity criteria	Finding
MortEsc.-rate in the control group on day 7	≤20%	9.0%

Corrected mortality in the reference item group on day 7	≥50%	100.0%
Cumulative mean number of eggs per female in the control group (from day 7 to day 14)	≥4	8.6

Conclusion

The LR₅₀ was calculated to be 80.7 g a.s./ha. The NOER for mortality was 32 g a.s./ha.
The ER₅₀ was estimated to be >57 g a.s./ha. The NOER for reproduction was 32 g a.s./ha.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

Comments of zRMS:	<p>The study follows the guideline specified by Mead-Briggs et al. (2000) and according to the principles of GLP.</p> <p>In the definitive test all the validity criteria were met. The study is reliable for risk assessment purposes with following endpoints:</p> <p>LR₅₀ = 26.1 g a.s./ha</p>
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Reference:	KCP 10.3.2.1/02
Title:	Amendment no. 01: Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) using a laboratory test; prohexadione-calcium OD 75 (75 g/L)
Report:	Waibel, J.; 2021; CW20/020; M-761030-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable MEAD-BRIGGS ET AL. (2000) CANDOLFI ET AL. (2001)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Objective

The aim of the study was to determine the toxicity of Prohexadione-calcium OD 75 (75 g/L) applied onto glass plates to the parasitoid wasp *Aphidius rhopalosiphi*.

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

The test item was applied on glass plates at rates of 18, 32, 57, 101 and 180 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 198 L/ha). The effects of the test item on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a

The LR₅₀ value (lethal rate causing 50% mortality) was calculated by using the Trimmed Spearman-Kärber test. The computer programs Microsoft Excel (2010) with Visual Basic for Application (VBA) and SAS (version 9.4) were used to perform the calculations.

Reproduction was assessed only for the lowest rate of Prohexadione-calcium OD 75 (75 g/L), 18 g a.s./ha. The reduction in reproductive success relative to the control at the 18 g a.s./ha rate was 40.3% which was statistically significant (Wilcoxon test; one-sided, $\alpha = 0.05$).

Test item:		Prohexadione-calcium OD 75 (75 g/L)					
Test organism:		<i>Aphidius rhopalosiphi</i>					
Exposure on:		Glass plates					
		Mortality after 48 h [%]			Reproduction		
Treatment	g a.s./ha	Uncorr.	Corr.	P-Value (*)	Rate (mummies per female)	Reduction relative to control [%]	P-Value (#)
Control	0	3.3	-	-	28.3	-	-
Test item	18	15.0	12.1	0.027 sign.	16.9	40.3	0.005 sign.
Test item	32	71.7	70.7	<0.001 sign.	n.a.	-	-
Test item	57	96.7	96.6	<0.001 sign.	n.a.	-	-
Test item	101	98.3	98.3	<0.001 sign.	n.a.	-	-
Test item	180	100.0	100.0	<0.001 sign.	n.a.	-	-
Reference item	0.03	56.7	55.2	n.a.	-	-	-

LR₅₀: 26.1 g a.s/ha; 95% Confidence Interval: (23.8 – 28.7); calculated with Trimmed Spearman-Kärber test (Trim: 29.3%)

ER₅₀: >18 g a.s./ha

* Fisher`s Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm

Wilcoxon test (one-sided, $\alpha = 0.05$)

n.a. not assessed sign. Significant

Validity

All validity criteria of the laboratory method using glass plates (Mead-Briggs et al., 2000) were fulfilled.

	Validity criteria	Finding
Mortality in control treatment	$\leq 13\%$	3.3%
Corrected mortality in reference item treatment	$\geq 50\%$	55.2%
Mean number of mummies per surviving female wasp in control treatment	≥ 5	28.3
Number of surviving female wasps in control treatment producing zero values for reproduction	≤ 2	2

Conclusion

The LR₅₀ was calculated to be 26.1 g a.s./ha. The NOER for mortality was <18 g a.s./ha. The ER₅₀ was estimated to be >18 g a.s./ha. The NOER for reproduction was <18 g a.s./ha.
 The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

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

Comments of zRMS:	<p>The study follows the guideline specified by Blumel S. et al., 2000 and according to the principles of GLP.</p> <p>In the definitive test all the validity criteria were met. The study is reliable for risk assessment purposes with following endpoints:</p> <p>LR₅₀: > 180 g a.s./ha</p> <p>ER₅₀: > 180 g a.s./ha</p>
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Reference:	KCP 10.3.2.2/01
Title:	Amendment no. 01: Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using an extended laboratory test on bean; prohexadione-calcium OD 75 (75 g/L)
Report:	Waibel, J.; 2021; CW20/034; M-762531-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable BLÜMEL ET AL. (2000) modified CANDOLFI ET AL. (2001)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)

Type of formulation: OD (oil dispersion)
Sample description: TOX 21574-00
Active substance: prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
(analysed content):
Specification no.: 102000037599
Supplier batch no.: 2020-001264
Density: 1.038 g/mL

The test item was applied onto detached bean leaves (*Phaseolus vulgaris*) at rates of 18, 32, 57, 101 and 180 g a.s./ha using a calibrated laboratory track sprayer (mean measured application rate: 205 L/ha). The effects of the test item on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 12 g a.s./ha in 200 L deionised water/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 100 predatory mites, protonymphs not older than 24 h at study start (5 replicates with 20 individuals per test group), was assessed 4, 7 after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed. On day 7 after application the surviving mites were transferred on untreated open exposure units (glass plates) and the reproduction rate of surviving mites was then evaluated from day 7 until day 14 after treatment by counting the total number of offspring (eggs and larvae) produced.

The climatic test conditions during the study were 24.0 - 25.0 °C temperature and 60 - 74% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 809 - 1179 lux. The computer programs Microsoft Excel (2019) with Visual Basic for Application (VBA) and SAS (Version 9.4) were used to perform the calculations.

Results and discussion

In this extended laboratory test the effects of Prohexadione-calcium OD 75 (75 g/L) residues on the survival and reproduction of the predatory mite *Typhlodromus pyri* were determined at the rates of 18, 32, 57, 101 and 180 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

The mortality / escaping rate in the control exposure units up to day 7 after application was 19.0%. In the highest test item rate of 180 g a.s./ha a statistically significantly different mortality compared to the control was found (Fisher's Exact test, one-sided, $\alpha = 0.05$). At the lower test item rates of 18, 32, 57 and 101 g a.s./ha the corrected mortality was below 1.5%. The corrected mortality for the 180 g a.s./ha rate was 23.5%.

Reproduction was assessed for all rates of Prohexadione-calcium OD 75 (75 g/L), 18, 32, 57, 101 and 180 g a.s./ha. The reduction of the test item rates of 18, 32, 57 and 101 g a.s./ha was below 14.5%. The reproduction was reduced by 34.5% at the highest rate of 180 g a.s./ha. All tested test item rates showed no statistical significance in reduction compared to the control (Wilcoxon test, one-sided, $\alpha = 0.05$, corrected level $\alpha/m=0.010$ ($m = 5$)).

A summary of the effects observed in this study is given below.

Test item:		Prohexadione-calcium OD 75 (75 g/L)					
Test organism:		<i>Typhlodromus pyri</i>					
Exposure on:		Detached bean leaves					
		Mortality after 48 h [%]			Reproduction		
Treatment	g a.s./ha	Un-corrected	Corrected	P-Value (*)	Rate (eggs per female)	Reduction r relative to control [%]	P-Value (#)

Test item:		Prohexadione-calcium OD 75 (75 g/L)					
Test organism:		<i>Typhlodromus pyri</i>					
Exposure on:		Detached bean leaves					
		Mortality after 48 h [%]			Reproduction		
Treatment	g a.s./ha	Un-corrected	Corrected	P-Value (*)	Rate (eggs per female)	Reduction r relative to control [%]	P-Value (#)
Control	0	19.0	-	-	8.7	-	-
Test item	18	3.0	-19.8	1.000 n.sign.	8.0	7.6	0.105 n.sign.
Test item	32	8.0	-13.6	1.000 n.sign.	9.0	-3.1	0.735 n.sign.
Test item	57	9.0	-12.3	1.000 n.sign.	7.5	14.1	0.072 n.sign.
Test item	101	20.0	1.2	1.000 n.sign.	8.1	6.5	0.148 n.sign.
Test item	180	38.0	23.5	0.011 sign.	5.7	34.5	0.018 n.sign.
Reference item	12	100.0	100.0	n.a.	n.a.	n.a.	-
LR₅₀: >180 g a.s./ha ER₅₀: >180 g a.s./ha * Fisher`s Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm # Wilcoxon test, one-sided, $\alpha = 0.05$, corrected level $\alpha/m=0.010$ ($m = 5$) n.a. not assessed n.sign. not significant sign. significant							

Validity

All validity criteria of the laboratory method using glass plates (Blümel et al., 2000) were fulfilled.

	Validity criteria	Finding
MortEsc.-rate in the control group on day 7	$\leq 20\%$	19.0%
Corrected mortality in the reference item group on day 7	$\geq 50\%$	100.0%
Cumulative mean number of eggs per female in the control group (from day 7 to day 14)	≥ 4	8.7

Conclusion

The LR₅₀ was calculated to be > 180 g a.s./ha. The NOER for mortality was 101 g a.s./ha.
The ER₅₀ was estimated to be > 180 g a.s./ha. The NOER for reproduction was ≥ 180 g a.s./ha.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

Comments of zRMS:	<p>The study follows the guideline specified by Mead-Briggs et al. (2000) and according to the principles of GLP.</p> <p>In the definitive test all the validity criteria were met. The study is reliable for risk assessment purposes with following endpoints:</p> <p>LR₅₀: > 180 g a.s./ha</p> <p>ER₅₀: > 180 g a.s./ha</p>
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Reference:	KCP 10.3.2.2/02
Title:	Amendment no. 01: Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) using an extended laboratory test on barley; prohexadione-calcium OD 75 (75 g/L)
Report:	Waibel, J.; 2021; CW20/035; M-762532-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable MEAD-BRIGGS ET AL. (2010) CANDOLFI ET AL. (2001)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

The test item was applied on barley seedlings (*Hordeum vulgare*) at rates of 18, 32, 57, 101 and 180 g a.s./ha using a calibrated laboratory track sprayer (mean measured application rate: 406 L/ha). The effects of the test item on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 3.5 g a.s./ha in 400 L deionised water/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 30 female wasps, not older than 48 h at study start (6 replicates with 5 individuals per test group), was assessed 2, 24 and 48 h after exposure.

Repellency of the test item was assessed during the initial 3 h after the release of the females. Five separate observations were made at 30-minute intervals starting 15 - 30 minutes after the introduction of all wasps. An additional repellency assessment for the control and the test item rates of 57, 101 and 180 g a.s./ha was conducted 24 h and 48 h after the release of the wasps into the exposure units.

From the water control and all test item rates of Prohexadione-calcium OD 75 (75 g/L), 20 impartially chosen females per treatment were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 h. The number of mummies (parasitized aphids in which wasp pupae subsequently develop) was assessed 11 days later.

The climatic test conditions during the study were 19.0 - 22.0 °C temperature and 64 - 81% relative

humidity. The light / dark cycle was 16:8 h with a light intensity range of 505 - 733 lux in the mortality phase, 2570 - 4320 lux in the parasitisation phase and 11120 - 16660 lux in the reproduction phase of the study.

The computer programs Microsoft Excel (2019) with Visual Basic for Application (VBA) and SAS (Version 9.4) were used to perform the calculations.

Results and discussion

In this extended laboratory test the effects of Prohexadione-calcium OD 75 (75 g/L) residues on the survival and reproduction of the parasitoid wasp *Aphidius rhopalosiphi* were determined at the rates of 18, 32, 57, 101 and 180 g a.s./ha applied to barley seedlings (*Hordeum vulgare*).

After 48 h of the study 3.3% of the wasps were found dead in the control group. In the groups treated with 18, 32, 57, 101 and 180 g a.s./ha 10.0%, 0.0%, 3.3%, 6.7% and 3.3% of the wasps were dead, respectively. No statistically significant mortality was found in all test rates (Fisher's Exact test (one-sided, $\alpha = 0.05$)). The corrected mortality in all test item rates was below 7.0%.

Repellent effects of the test item (settling of the wasps on plants < 30%) were observed in the first 3 hours and after 24 h and 48 h after the introduction of the wasps into the exposure units at the higher test item rates of 57, 101 and 180 g a.s./ha.

Reproduction was assessed for all rates of Prohexadione-calcium OD 75 (75 g/L), 18, 32, 57, 101 and 180 g a.s./ha. There was no statistically significant reduction in reproductive success relative to the control at all test item rates (Wilcoxon test, one-sided, $\alpha = 0.05$, corrected level $\alpha/m=0.010$ ($m = 5$)). The reduction in the rate of 101 g a.s./ha was 26.2%. The three lowest rates of 18, 32 and 57 g a.s./ha as well as the highest rate of 180 g a.s./ha showed no reduction of reproduction.

A summary of the effects observed in this study is given below.

Test item:		Prohexadione-calcium OD 75 (75 g/L)						
Test organism:		<i>Aphidius rhopalosiphi</i>						
Exposure on:		Barley seedlings						
		Mortality after 48 h [%]			Reproduction			Repellency (first 3 h)
Treatment	g a.s./ha	Uncorr.	Corr.	P-Value(*)	Rate (mummies per female)	Reduction relative to control [%]	P-Value (#)	% Wasps on plant P-Value (##)
Control	0	3.3	-	-	28.1	-	-	50.7
Test item	18	10.0	6.9	1.000 n.sign.	30.4	-8.0	0.789 n.sign.	39.1 0.421 n.sign.
Test item	32	0.0	-3.4	1.000 n.sign.	37.4	-32.9	0.965 n.sign.	36.7 0.308 n.sign.
Test item	57	3.3	0.0	1.000 n.sign.	33.0	-17.2	0.895 n.sign.	23.2 0.011 sign.
Test item	101	6.7	3.4	1.000 n.sign.	20.7	26.2	0.057 n.sign.	29.7 0.094 n.sign.
Test item	180	3.3	0.0	1.000 n.sign.	31.2	-10.9	0.763 n.sign.	22.2 0.008 sign.
Reference item	3.5	86.7	86.2	-	n.a.	n.a.	-	38.0

Test item:		Prohexadione-calcium OD 75 (75 g/L)						
Test organism:		<i>Aphidius rhopalosiphi</i>						
Exposure on:		Barley seedlings						
		Mortality after 48 h [%]			Reproduction			Repellency (first 3 h)
Treatment	g a.s./ha	Uncorr.	Corr.	P-Value(*)	Rate (mummies per female)	Reduction relative to control [%]	P-Value (#)	% Wasps on plant P-Value (##)
LR₅₀: > 180 g a.s./ha ER₅₀: > 180 g a.s./ha * Fisher`s Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm # Wilcoxon test, one-sided, $\alpha = 0.05$, corrected level $\alpha/m=0.010$ ($m = 5$) ## Dunnett`s test (one-sided, $\alpha = 0.05$) n.a. not assessed n.sign. not significant sign. Significant								

Validity

All validity criteria of the laboratory method using glass plates (Mead-Briggs et al., 2010) were fulfilled.

	Validity criteria	Finding
Mortality in control treatment	$\leq 10\%$	3.3%
Corrected mortality in reference item treatment	$> 50\%$	86.2%
Mean number of mummies per surviving female wasp in control treatment	≥ 5	28.1
Number of surviving female wasps in control treatment producing zero values for reproduction	≤ 2	1

Conclusion

The LR₅₀ was calculated to be > 180 g a.s./ha. The NOER for mortality was ≥ 180 g a.s./ha.
The ER₅₀ was estimated to be > 180 g a.s./ha. The NOER for reproduction was ≥ 180 g a.s./ha.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

Comments of zRMS:	The effects of PRL OD 75 on the ladybird beetle, <i>Coccinella septempunctata</i> were evaluated in an extended laboratory study with the respective guideline (Schmuck et al. 2000) and according to the principles of GLP. No deviations were noted during the study and the study fulfilled all the validity criteria. Following endpoint is accepted and may be used in the risk assessment: LR ₅₀ > 180 g a.s./ha
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Reference:	KCP 10.3.2.2/03
Title:	Amendment no. 01: Toxicity to the ladybird beetle <i>Coccinella septempunctata</i> (Coleoptera: Coccinellidae) using an extended laboratory test on bean; prohexadione-calcium OD 75 (75 g/L)
Report:	Waibel, J.; 2021; CW20/041; M-763934-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable SCHMUCK ET AL. (2000) modified CANDOLFI ET AL. (2001)
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

The test item was applied to detached bean leaves (*Phaseolus vulgaris*) at rates of 18, 32, 57, 101 and 180 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 206 L/ha). The effects of the test item on the ladybird beetle *Coccinella septempunctata* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 12 g a.s./ha in 200 L deionised water/ha was included.

The preimaginal mortality of 40 larvae (4 days old at study start) per test group, was assessed till the hatch of the imagines up to 16 days.

The reproduction assessment of the surviving hatched adults started one week after the first eggs in the control could be observed. The number of fertile eggs laid per viable female was recorded over a period of two weeks. This reproduction assessment was done for the control and all test item rates.

The climatic test conditions during the study were 24.0 - 25.5 °C temperature and 66 - 85% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1250 - 6471 lux during the study.

The computer programs Microsoft Excel (2019) with Visual Basic for Application (VBA) and SAS (version 9.4) were used to perform the calculations.

Results and discussion

In this extended laboratory study, the effects of Prohexadione-calcium OD 75 (75 g/L) residues on the survival and reproduction of the ladybird beetle *Coccinella septempunctata* were determined at the rates of 18, 32, 57, 101 and 180 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

The mortality of all test item rates was not statistically significantly different compared to the control (Fisher's Exact test (one-sided, $\alpha = 0.05$)). At the test item rates of 18, 32, 57, 101 and 180 g a.s./ha, the corrected preimaginal mortality was below 3%.

Reproduction was assessed for all rates of Prohexadione-calcium OD 75 (75 g/L). The mean number of fertile eggs per female and day was 16.4 in the control treatment and 12.1, 13.3, 11.5, 14.7 and 11.7 in the test item rates of 18, 32, 57, 101 and 180 g a.s./ha, respectively. Since the reproductive performance was

within the range of the historical data base for control beetles (≥ 2 fertile eggs per female and day, Schmuck et al. 2000) this parameter is considered as not affected at all test item rates.

A summary of the effects observed in this study is given below.

Test item:		Prohexadione-calcium OD 75 (75 g/L)			
Test organism:		<i>Coccinella septempunctata</i>			
Exposure on:		Detached bean leaves			
		Preimaginal mortality [%]			Reproduction
Treatment	g a.s./ha	Uncorrected	Corrected (*)	P-Value (**)	Fertile eggs per female and day
Control	0	10.0	-	-	16.4
Test item	18	0.0	-11.1	1.000 n.sign.	12.1
Test item	32	2.5	-8.3	1.000 n.sign.	13.3
Test item	57	12.5	2.8	1.000 n.sign.	11.5
Test item	101	12.5	2.8	1.000 n.sign.	14.7
Test item	180	5.0	-5.6	1.000 n.sign.	11.7
Reference item	12	100.0	100.0	n.a.	n.a.
LR₅₀: > 180 g a.s./ha * Corrected mortality according to Schneider-Orelli (1947) ** Fisher`s Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm n.a. not assessed n.sign. not significant					

Validity

All validity criteria of the laboratory method using glass plates (Schmuck et al., 2000) were fulfilled.

Criteria	Validity criteria	Finding
Preimaginal mortality in water control	$\leq 30\%$	10.0%
Preimaginal mortality reference item	$\geq 40\%$	100.0%
Mean number of fertile eggs per female and day in water control	≥ 2	16.4

Conclusion

The LR₅₀ was estimated to be > 180 g a.s./ha. The NOER for mortality was ≥ 180 g a.s./ha.
 The reproductive performance was not affected up to and including the test item rate of 180 g a.s./ha.
 The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

Comments of zRMS:	The effects of PRL OD 75 on the lacewing <i>Chrysoperla carnea</i> in an extended laboratory study was performed in line with the respective guideline (Vogt et al. 2000) and according to the principles of GLP. Since the study fulfilled all the validity criteria, minor deviation (temperature) had no negative impact on the outcome of the study. Following endpoint is accepted and may be used in the risk assessment: LR ₅₀ > 180 g a.s./ha
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Reference:	KCP 10.3.2.2/04
Title:	Toxicity to the green lacewing <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae) using an extended laboratory test on bean; prohexadione-calcium OD 75 (75 g/L)
Report:	Waibel, J.; 2021; CW20/040; M-762533-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable VOGT ET AL. (2000) modified CANDOLFI ET AL. (2001)
Deviations:	During mortality phase the temperature increased up to 29.0 °C for the duration of 2 h. This had no negative impact on the outcome of the study since all validity criteria were met.
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Type of formulation:	OD (oil dispersion)
Sample description:	TOX 21574-00
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

The test item was applied to detached bean leaves (*Phaseolus vulgaris*) at rates of 18, 32, 57, 101 and 180 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 203 L/ha). The effects of the test item on the green lacewing *Chrysoperla carnea* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 36 g a.s./ha in 200 L deionised water/ha was included.

The mortality of 40 larvae (per test group), 2 days old and in the 1st larval instar at study start, was assessed till the hatch of the imagines up to 21 days. The fertility and fecundity of the surviving hatched adults were then evaluated over the period of one week.

The climatic test conditions during the study were 23.0 - 27.0 °C temperature and 62 - 75% relative humidity. During mortality phase the temperature increased up to 29.0 °C for the duration of 2 h. This had no negative impact on the outcome of the study since all validity criteria were met. The light / dark cycle was 16:8 h with a light intensity range of 1274 - 4166 lux during the study.

The computer programs Microsoft Excel (2019) with Visual Basic for Application (VBA) and SAS (version 9.4) were used to perform the calculations.

Results and discussions

In this extended laboratory study, the effects of Prohexadione-calcium OD 75 (75 g/L) residues on the survival and reproduction of the green lacewing *Chrysoperla carnea* were determined at the rates of 18, 32, 57, 101 and 180 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

All test item rates had no influence on the preimaginal mortality. No significantly different mortality compared to the control was found (Fisher's Exact test; one-sided, $\alpha = 0.05$).

Reproduction was assessed for all rates of Prohexadione-calcium OD 75 (75 g/L), 18, 32, 57, 101 and 180 g a.s./ha. There were no adverse effects of the test item on the reproductive performance. The mean

number of eggs/female/day was above the lower limit given as validity criterion for the glass plate method (mean number of eggs/female/day: ≥ 15 , mean hatching rate: $\geq 70\%$) according to the historical database of the ring testing group (Vogt et al., 2000).

A summary of the effects observed in this study is given below.

Test item:		Prohexadione-calcium OD 75 (75 g/L)				
Test organism:		<i>Chrysoperla carnea</i>				
Exposure on:		Detached bean leaves				
		Mortality [%]			Reproduction	
Treatment	g a.s./ha	Uncorrected	Corrected (*)	P-Value (**)	Eggs per female and day	Fertility [hatching rate in %]
Control	0	17.5	-	-	31.8	90.7
Test item	18	5.0	-15.2	1.000 n.sign.	40.1	82.5
Test item	32	12.5	-6.1	1.000 n.sign.	35.4	83.1
Test item	57	7.5	-12.1	1.000 n.sign.	37.5	88.0
Test item	101	12.5	-6.1	1.000 n.sign.	35.5	84.0
Test item	180	7.5	-12.1	1.000 n.sign.	39.3	81.4
Reference item	36	95.0	93.9	-	n.a.	n.a.
LR₅₀: > 180 g a.s./ha * Corrected mortality according to Schneider-Orelli (1947) ** Fisher's Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm n.a. not assessed n.sign. not significant						

Validity

All validity criteria of the laboratory method using glass plates (Vogt et al., 2000) were fulfilled.

	Validity criteria	Finding
Mortality in control treatment	$\leq 20\%$	17.5%
Mortality in reference item treatment	$\geq 50\%$	95.0%
Mean number of eggs per female and day (fecundity) in water control	≥ 15	31.8
Mean hatching rate of the eggs (fertility) in water control	$\geq 70\%$	90.7%

Conclusion

The LR₅₀ was estimated to be > 180 g a.s./ha. The NOER for mortality was ≥ 180 g a.s./ha. The reproductive performance was not affected up to and including the test item rate of 180 g a.s./ha. The figures obtained fulfil the validity criteria of the laboratory method for the exposure on glass plates.

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

No additional study submitted.

A 2.3.2.4 KCP 10.3.2.4. Field studies with non-target arthropods

No additional study submitted.

A 2.3.2.5 KCP 10.3.2.5. Other routes of exposure for non-target arthropods

No additional study submitted.

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	<p>The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviations were noted during the study.</p> <p>All the validity criteria were met according to OECD Guideline No. 222.</p> <p>Overall, the study is considered acceptable with following endpoint: NOEC \geq 1000 mg test item/kg dry weight artificial soil</p>
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Reference:	KCP 10.4.1.1/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Effects on survival, growth and reproduction of the earthworm <i>Eisenia fetida</i> tested in artificial soil
Report:	Büttner, G.; 2021; E 312 05562-4; M-763276-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC, Regulation (EC) No. 1107/2009, US EPA OCSPP Not Applicable, ISO 11268-2: 1998 (E), OECD 222: July 29, 2016
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
 Sample description: TOX 21574-00
 Type of OD (oil dispersion) formulation:
 Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
 (analysed content):
 Specification no.: 102000037599
 Supplier batch no.: 2020-001264
 Density: 1.038 g/mL

Adult *Eisenia fetida* 10-11 months old, 8 x 10 earthworms for the control group and 4 x 10 animals per test concentration of the treatment groups with weight between 0.30 and 0.50 g, were exposed to control and treatment. Nominal test concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were mixed into the artificial soil containing 75% quartz sand, 20% kaolin clay and 10% sphagnum peat. During the study they were fed with animal manure. A temperature of 20 ± 2 °C and a light regime of 400 – 800 lux, 16 h light and 8 h dark during the conduct of the study were applied. The artificial soil was prepared according to the guideline with the following constituents. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

Toxic standard: 1.25, 2.5 and 5.0 mg a.s./kg soil d.w. of Carbendazim EC 360 G; control: untreated, solvent control: none.

Dates of work: July 31, 2020 – October 01, 2020

Results and discussions

Effects on mortality and biomass change of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table.

Test item	Prohexadione-calcium OD 75 (75 g/L)		
Test object	<i>Eisenia fetida</i>		
Exposure	Artificial soil		
	Mortality	Biomass change	Reproduction
	[mg test item/kg d.w.]		
NOEC	≥1000	≥1000	≥1000
LOEC	>1000	>1000	>1000
EC ₁₀ (95% confidence limits)	-	-	-
EC ₂₀ (95% confidence limits)	-	-	-

Prohexadione-calcium OD 75 (75 g/L)									
[mg test item/kg d.w.]									
	Control	18	32	56	100	178	316	562	1000
<i>Mortality of adult worms after 4 weeks</i>									
Mortality (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)</i>									
Mean (%)	19.46	23.68	22.48	25.14	30.68	28.37	22.58	25.93	23.01
<i>Number of juveniles per replicate after 8 weeks</i>									
Mean	242.8	244.5	229.5	237.0	241.5	244.8	246.8	234.8	226.5
CV (%)	5.1	8.1	12.3	7.9	15.0	8.8	15.7	12.7	12.4
<i>Reproduction compared to control (%)</i>									
% to control	--	100.7	94.5	97.6	99.5	100.8	101.6	96.7	93.3

No statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater) and for biomass (William's t-test, two-sided, $\alpha = 0.05$) and reproduction (Williams-t-test, $\alpha = 0.05$, one-sided smaller)
CV (%) = Coefficient of variation

Validity criteria (for control group)

Adult mortality:	≤ 10% (being 0.0% after 4 weeks)
Number of juveniles per replicate:	≥ 30 (being 231 to 270)
Coefficient of variation of reproduction:	≤ 30% (being 5.1%)

In separate study (Rg-R-Ref 34/20; NON-GLP) the reference item Carbendazim EC 360 G mixed into the artificial soil, showed a statistically significant adverse effect on the biomass of the adult earthworms at 5.0 mg a.s./kg dry weight soil and a statistically significant reduction of the number of juveniles per test vessel in the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil. Therefore, the observed effects assure a high sensitivity of the test system.

Conclusion

Prohexadione-calcium OD 75 (75 g/L) showed no statistically significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to and including 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested. The test item caused no statistically significant effects on adult

biomass of the earthworm *Eisenia fetida* up to and including 1000 mg test item/kg soil dry weight. No statistically significant differences concerning the number of juveniles relative to the control were observed in the test item concentrations up to and including 1000 mg test item/kg dry weight artificial soil. Due to the lack of a concentration-response relationship no reliable EC₁₀/EC₂₀ calculation is possible. Therefore, no EC₁₀/EC₂₀ value can be reported. The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil d.w., and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil d.w.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No additional study submitted.

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	<p>The study was conducted to OECD guideline 232 and according to the principles of GLP.</p> <p>Minor deviation (temperature) to the guideline was noted.</p> <p>Since in the definitive test all the validity criteria were met the study is reliable and suitable for the risk assessment with following endpoints:</p> <p>NOECadult mortality: ≥ 1000 mg test item/kg dry weight artificial soil</p> <p>LOECadult mortality: > 1000 mg test item/kg dry weight artificial soil</p> <p>NOECreproduction: 562 mg test item/kg dry weight artificial soil</p> <p>LOECreproduction: 1000 mg test item/kg dry weight artificial soil</p> <p>EC₁₀-reproduction: 649 mg test item/kg dry weight artificial soil</p> <p>EC₂₀-reproduction: 807 mg test item/kg dry weight artificial soil</p>
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Reference:	KCP 10.4.2.1/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Influence on mortality and reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil
Report:	Richter, A.; 2021; E 314 05536-7; M-762156-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 232 US EPA OCSPP Not Applicable
Deviations:	<p>Minor deviation. For the duration of approx. 12 hours, the allowed temperature (climatic chamber) was slightly exceeded by max. 0.3 °C. It is expected that this minimal deviation had no influence on the study</p> <p>None</p> <p>pH from the soil charge was in other studies</p>
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
 Sample description: TOX 21574-00
 Type of OD (oil dispersion) formulation:
 Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
 (analysed content):
 Specification no.: 102000037599
 Supplier batch no.: 2020-001264
 Density: 1.038 g/mL

10 Collembola (9-12 days old) were exposed to 10, 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight of soil containing 75% quartz sand, 20% kaolin clay and 5% sphagnum peat, at 18.0 – 22.0 °C and a photoperiod: light : dark = 16 h : 8 h (400-800 lux) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg dry weight artificial soil; control: untreated, solvent control: none.

Dates of work: May 27, 2020 – July 20, 2020

Results and discussion

Test item	Prohexadione-calcium OD 75 (75 g/L)				
Test object	<i>Folsomia candida</i>				
Exposure	Artificial soil				
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles per test vessel ± standard deviation	CV	Reproduction (% of control)	Significance (*)
Control	5.0	565.8 ± 109.6	19.4	-	
18	0.0	542.8 ± 52.6	9.7	95.9	-
32	2.5	572.5 ± 59.9	10.5	101.2	-
56	5.0	594.5 ± 90.2	15.2	105.1	-
100	2.5	502.8 ± 120.0	23.9	88.9	-
178	2.5	654.8 ± 71.4	10.9	115.7	-
316	2.5	550.5 ± 47.9	8.7	97.3	-
562	2.5	534.8 ± 76.5	14.3	94.5	-
1000	0.0	366.3 ± 68.9	18.8	64.7	*
				Mortality	Reproduction
NOEC _{reproduction} (mg test item/kg soil dry weight)				≥1000	562
LOEC _{reproduction} (mg test item/kg soil dry weight)				>1000	1000
					Reproduction
EC ₁₀ (mg test item/kg soil dry weight) ¹⁾				n.d.	649
95% confidence limits				(n.d. – n.d.)	(553-770)
EC ₂₀ (mg test item/kg soil dry weight) ¹⁾				n.d.	807
95% confidence limits				(n.d. – n.d.)	(738-878)

The calculations were performed with unrounded values

CV: Coefficient of Variation

¹⁾ 3-Parameter analysis

(*) = (Williams t-test one-sided-smaller, $\alpha = 0.05$, + = significant, - = not significant)

Percent reproduction: $(R_t / R_c) * 100\%$

R_t = mean number of juveniles observed in the treated groups

R_c = mean number of juveniles observed in the control group

In a separate study (Coll-Ref-37/20, Richter, Andreas, February 2020), the EC₅₀ (reproduction) of the reference item boric acid was calculated to be 127 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

Extraction Efficiency

The most recent non-GLP-test (Coll-EE-34/20, January 2020) shows an extraction efficiency of 99% on average combined for adults and juveniles. The extraction efficiency for juveniles was 99%. In line with the requirements of OECD 232 (July 2016) the extraction efficiency of juvenile *Folsomia candida* with this method was greater than 95%.

Validity criteria (for the control group):

	Recommended	Obtained
Mean adult mortality	≤ 20%	5.0%
Mean number of juvenile per replicate	≥ 100	566
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	19.4%

Conclusion

The test item Prohexadione-calcium OD 75 (75 g/L) showed no statistically significantly adverse effects on adult mortality of the collembolans *Folsomia candida* in artificial soil up to and including 1000 mg test item/kg dry weight artificial soil.

The test item caused a significant reduction of reproduction of the collembolan *Folsomia candida* in artificial soil at 1000 mg test item/kg soil dry weight. Therefore, the No-Observed-Effect-Concentration (NOEC) was determined to be 562 mg test item/kg dry weight artificial soil and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 1000 mg test item/kg dry weight artificial soil. The EC₁₀ and EC₂₀ values for reproduction were calculated to be 649 mg test item/kg soil dry weight (95% confidence limits: 553-770) and 807 mg test item/kg soil dry weight (95% confidence limits: 738-878), respectively (3-Parameter analysis).

Comments of zRMS:	<p>The study on influence on mortality and reproduction of the soil mite species <i>Hypoaspis aculeifer</i> tested in artificial soil was performed in line with requirements of OECD 226 and according to the principles of GLP. No deviations occurred during the study.</p> <p>Since in the definitive test all the validity criteria were met according the study is reliable and suitable for the risk assessment with following endpoints:</p> <p>NOEC_{reproduction}: ≥1000 mg product/kg dry weight artificial soil</p>
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Reference:	KCP 10.4.2.1/02
Title:	Prohexadione-calcium OD 75 G (75 g/L): Influence on mortality and reproduction of the soil mite species <i>Hypoaspis aculeifer</i> tested in artificial soil
Report:	Richter, A.; 2020; E 428 05529-5; M-687925-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 226 (2016) US EPA OCSPP Not Applicable
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
 Sample description: TOX 21574-00
 Type of OD (oil dispersion) formulation:
 Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
 (analysed content):
 Specification no.: 102000037599
 Supplier batch no.: 2020-001264
 Density: 1.038 g/mL

10 adult, fertilized female *Hypoaspis aculeifer* were exposed to 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight (d.w.) of soil containing 75% fine quartz sand, 20% Kaolin clay, 5% Sphagnum peat and calcium carbonate (CaCO₃), at 20 ± 2°C and a photoperiod: light: dark = 16 h : 8 h (400 - 800 lux) and were fed at 3, 7 and 10 days with nematodes (*Panagrellus redivivus*). Mortality and reproduction were determined after 14 days of exposure. Reference item (Dimethoate): 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg soil d.w.; control: untreated, solvent control: none.

Results and discussion

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item	Prohexadione-calcium OD 75 G (75 g/L)	
Test object	<i>Hypoaspis aculeifer</i>	
Exposure	Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 1000	≥1000
LOEC	> 1000	>1000
EC ₁₀	-	n.d.
(95 % confidence limits)		
EC ₂₀	-	n.d.
(95 % confidence limits)		

n.d. = not determined (see observations)

Endpoint	Control	Treatment (mg test item/kg soil d.w.)								group
		18	32	56	100	178	316	562	1000	
Mortality of soil mites after 14 days (%)	5.0	0	2.5	5.0	2.5	2.5	2.5	2.5	0.0	
Mean number of juveniles ± standard deviation after 14 days	394.3 ± 32.8	421.8 ± 36.2	419.8 ± 10.6	426.0 ± 19.9	412.8 ± 21.7	412.0 ± 16.4	412.5 ± 9.5	409.8 ± 23.1	378.5 ± 9.6	
CV (%)	8.3	8.6	2.5	4.7	5.3	4.0	2.3	5.6	2.5	
Reproduction (% of control)	N/A	107.0	106.5	108.1	104.7	104.5	104.6	103.9	96.0	

Not statistically significantly different compared to the control (Fisher's Exact Binomial with Bonferroni Correction, $\alpha = 0.05$, one-sided greater (mortality) and Williams t-test, one sided smaller, $\alpha = 0.05$ (reproduction))

In a separate study (HR-Ref-29/20, February 12, 2020), the EC₅₀ (reproduction) of the reference item Dimethoate was calculated to be 6.1 mg a.s./kg soil d.w.

The results of the reference test demonstrate the sensitivity of the test system.

Extraction Efficiency

The most recent non-GLP-test (HR-EE-32/20, conducted on January 2020) shows an extraction efficiency of 94%. In line to OECD 226 (from July 29, 2016) the extraction efficiency of *Hypoaspis aculeifer* with this method was greater than 90%.

Validity criteria (for the control group)

	Recommended		Obtained	
Mean mortality of adult females		≤ 20%		5.0%
Mean number of juveniles per replicate		≥ 50		394.3
Coefficient of variation (mean number of juveniles per replicate)		≤ 30%		8.3%

Conclusion

The test item Prohexadione-calcium OD 75 G (75 g/L) showed no statistically significantly adverse effects on adult mortality of the predatory mite *Hypoaspis aculeifer* in artificial soil at all tested concentrations. Furthermore, the test item showed no statistically significantly adverse effects on reproduction.

Therefore, the No-Observed-Effect-Concentration (NOEC) and Lowest-Observed-Effect-Concentration (LOEC) for mortality were determined to be ≥1000 and >1000 mg test item/kg soil d.w., respectively.

The NOEC and LOEC for reproduction were determined to be ≥1000 and >1000 mg test item/kg soil d.w., respectively.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No additional study submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The study was performed in line with requirements of OECD 216 and according to the principles of GLP.</p> <p>The soil nitrate formation rates were below the 25% (difference to control) trigger value given by the OECD 216 guideline.</p> <p>Based on the results of this study, it is concluded that PRL OD 75 had no significant impact on soil microorganisms (nitrogen transformation) when applied at test item concentrations up to 16.61 mg formulation/kg dw soil</p>
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Reference:	KCP 10.5/01
Title:	Prohexadione-calcium OD 75 (75 g/L): Effects on the activity of soil microflora (nitrogen transformation test)
Report:	Schulz, L.: 2020; 20 48 SMN 0038; M-758535-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP Not Applicable
Deviations:	None
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Materials and methods

Prohexadione-calcium OD 75 (75 g/L), [Short name: Prohexadione-calcium OD 75 (75 g/L)], BCS-code: BCS - BC44231, Supplier batch No.: 2020-001264, Specification No.: 102000037599, Sample description: TOX21574-00, analytical findings: 7.57% w/w (78.54 g/L) prohexadione-calcium (KUH-833), density (20 °C): 1.038 g/mL, water solubility: dispersible.

A loamy sand soil (DIN 4220) was exposed for 28 days to 1.66 mg test item/kg soil dry weight and 16.61 mg test item/kg soil dry weight. Application rates were equivalent to 1.20 L product/ha and 12.0 L product/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5%). NH₄-nitrogen, NO₃⁻ and NO₂-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

The coefficients of variation in the control (NO₃-N) were maximum 2.4% and thus fulfilled the demanded range (≤15%).

Dates of work: September 10, 2020 - October 08, 2020

Results and discussion

The findings are summarised in the table below. Values are given as mg NO₃-N/kg soil d.w.. The test item Prohexadione-calcium OD 75 (75 g/L) caused a temporary stimulation of the daily nitrate rate at the tested concentration of 16.61 mg test item/kg dry soil at time interval 7-14 days after application.

However, no adverse effects of Prohexadione-calcium OD 75 (75 g/L) on nitrogen transformation in soil could be observed at both tested concentrations (1.66 mg test item and 16.61 mg test item/kg dry soil) at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +11.5% (test concentration 1.66 mg test item/kg dry soil) and -7.4% (test concentration 16.61 mg test item/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Effects on nitrogen transformation in soil after treatment with Prohexadione-calcium OD 75 (75g/L)

Time Interval (days)	Control			1.66 mg test item/kg soil dry weight equivalent to 1.20 L test item/ha				16.61 mg test item/kg soil dry weight equivalent to 12.0 L test item/ha			
	Nitrate-N ¹⁾			Nitrate-N ¹⁾			% difference to control	Nitrate-N ¹⁾			% difference to control
0-7	3.74	±	0.26	3.49	±	0.16	-6.8 n.s.	3.76	±	0.51	+0.6 n.s.
7-14	1.48	±	0.13	1.61	±	0.52	+9.0 n.s.	1.90	±	0.59	+28.0 n.s.
14-28	1.12	±	0.19	1.25	±	0.44	+11.5 n.s.	1.04	±	0.08	-7.4 n.s.

The calculations were performed with unrounded values

¹⁾ Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p > 0.05)

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +59.9%, +216.3% and +238.5% at 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Validity criteria

The coefficients of variation in the control for NO₃-N were maximum 2.4% and thus fulfilled the demanded range (≤ 15%).

In the most recent test with the toxic standard (conducted from 2020-01-07 to 2020-02-04), Dinoterb caused an effect of +59.9%, +216.3% and +238.5% (required ≥25%) on the nitrogen transformation in a field soil at the tested concentrations of 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system.

Conclusion

Prohexadione-calcium OD 75 (75 g/L) caused no adverse effects (difference to control <25%, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 16.61 mg test item/kg soil dry weight, which are equivalent to application rates up to 12.0 L product/ha.

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

Comments of zRMS:	<p>The study on the Effects on the seedling emergence and growth of ten non-target terrestrial plant species was performed in line with requirements of OECD 208 and according to the principles of GLP.</p> <p>The validity criteria for the study were fulfilled. No adverse effects above the 50% effect level were observed on emergence, survival, plant height and shoot dry weight of the species tested at the test item rate of 90 g a.s./ha. The only statistically significant effect was found for the shoot dry weight reductions of <i>Lactuca sativa</i> (12.7%) compared to the control plants.</p>
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Reference:	KCP 10.6.1/01
Title:	Effects on the seedling emergence and growth of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L)
Report:	Köhler, P.; 2020; SE20/022; M-691185-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4100 (2012) OECD 208 (2006)
Deviations:	No deviations from the current guideline except for light intensity. The guideline recommends $350 \pm 50 \mu\text{mol}/\text{m}^2/\text{s}$, measured values were $140 - 735 \mu\text{mol}/\text{m}^2/\text{s}$. This deviation had no influence on the reliability of the study and endpoints.
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Executive summary

The objective of this study was to evaluate the potential effects of Prohexadione-calcium OD 75 (75 g/L) on the seedling emergence and growth of ten species of non-target terrestrial plants, following a pre-emergence application of the product to the soil surface. A total of ten species were tested, 6 dicotyledonous and 4 monocotyledonous species representing 8 different plant families. The test was conducted as a limit test. The single rate of 90 g a.s./ha of Prohexadione-calcium OD 75 (75 g/L) and a control solution (deionized water) were applied once at test initiation to the soil surface using a calibrated laboratory track sprayer.

The validity criteria for the study were fulfilled. No adverse effects above the 50% effect level were observed on emergence, survival, plant height and shoot dry weight of the species tested at the test item rate of 90 g a.s./ha. The only statistically significant effect was found for the shoot dry weight reductions of *Lactuca sativa* (12.7%) compared to the control plants.

Material and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
Sample description: TOX 21574-00
Type of OD (oil dispersion) formulation:
Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
(analysed content):
Specification no.: 102000037599
Supplier batch no.: 2020-001264
Density: 1.038 g/mL

A total of ten species, 6 dicotyledonous and 4 monocotyledonous species from 8 plant families, were tested in this seedling emergence and growth test. The plant species used in this study are representative of a wide range of plant families and were chosen because they are readily cultivated test organisms and widely used in research. The germination rate of the seeds used in this study, observed in annual germination tests, was $\geq 70\%$. The seeds were sown one day prior to application of the test item to the soil surface in commercial non/porous 15 cm diameter plastic pots. The seeds were sown on the day of the application (filled with approx. 1.2 L soil). The soil used was a sandy loam.

Planting density was 2 or 4 seeds per pot with 10 or 5 replicate pots, respectively, for a total of 20 seeds per treatment level. The study was conducted as a limit test with a single test item application rate and a deionised water control.

The application solutions were transported to the application site immediately before treatment. The single rate of 90 g a.s./ha of Prohexadione-calcium OD 75 (75 g/L) and a control solution (deionized water) were applied once at test initiation to the soil surface using a calibrated laboratory track sprayer at a volume rate of 200 L/ha.

List of species tested:

Species name	EPPO CODE	Common name
<i>Beta vulgaris</i>	BEAVA	Sugar beet
<i>Brassica rapa</i>	BRSRR	Turnip
<i>Cucumis sativus</i>	CUMSA	Cucumber
<i>Glycine max</i>	GLXMA	Soybean
<i>Lactuca sativa</i>	LACSC	Butterhead lettuce
<i>Solanum lycopersicum</i>	LYPES	Tomato
<i>Allium cepa</i>	ALLCE	Onion
<i>Avena sativa</i>	AVESA	Oat
<i>Lolium perenne</i>	LOLPE	Ryegrass
<i>Zea mays</i>	ZEAMA	Corn

After application, the pots with seeds were transferred back to the greenhouse and placed on the tables in a randomized design with all pots of one species arranged together in a species plot. During the course of the experimental study part the pots of each plant species were rearranged within each species plot. Following application, the pots with plants were maintained under greenhouse conditions and natural

daylight was supplemented by artificial lighting. The temperature was 18°C to 26°C during the light period (16 h) and during the dark period (8 h) of the light-dark cycle. The relative humidity was 71 to 78%.

The control pots of each species were observed daily for the number of seedlings emerged until 50% of the seedlings had emerged (= day 0). Assessments were made individually for each species on this day (= day 0) and 7, 14 and 21 days post-emergence of 50% of the control seedlings. On day 0, 7 and 14, only plant emergence, survival and visual injury were recorded.

Final assessments were made for emergence, plant survival, visual injury, plant growth stage, plant height and shoot dry weight 21 days post emergence of 50% of the control seedlings.

Statistical analysis of emergence, survival, plant height and shoot dry weight data was carried out with the Mann-Whitney-U-Test (one sided smaller; $p \leq 0.05$), included in ToxRat statistics (ToxRat; version 3.3.0).

Results and discussion

Validity criteria:

All species in this study met the validity criteria of at least 70% for seedling emergence and of at least 90% for survival in the controls. In accordance with OECD guideline (OECD 208) and US EPA guideline (OCSPP 850.4100), there was no visible phytotoxicity, and normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions were kept identical for all plants of a species over the study duration. The pots used for all species of this study were prepared in the same manner using the same soil.

Analytical findings:

The analysis of prohexadione-calcium content in the initial test item stock solution revealed measured concentrations of 99.9% of nominal.

Biological findings:

The following table summarises percent inhibition of emergence, survival, shoot dry weight and shoot length as calculated for the final assessment (21 days after 50% emergence of the control seedlings). In addition, ratings of phytotoxicity and growth stage (BBCH) are provided for all species tested.

Effects of 90 g a.s./ha of Prohexadione-calcium OD 75 (75 g/L) in the seedling emergence test

Plant Species	Observations at the test item rate of 90 g a.s./ha					BBCH control min - max	BBCH treated min - max
	Emergence (% inhibition) N	Survival (% inhibition) N	Shoot dry weight (% inhibition) N	Plant height (% inhibition)	Phyto-toxicity (%)		
<i>Beta vulgaris</i>	-5.3	0.0	-0.2	3.7	0.0	16-17	16-17
<i>Brassica rapa</i>	5.0	0.0	8.1	2.9	0.0	16-19	16-19
<i>Cucumis sativus</i>	0.0	0.0	-5.1	5.3	0.0	12-15	11-16
<i>Glycine max</i>	5.6	0.0	-2.4	-1.1	0.0	22-25	21-25
<i>Lactuca sativa</i>	-5.6	0.0	12.7	-2.0	1.0 a	41-41	41-41
<i>Solanum lycopersicum</i>	0.0	0.0	-4.6	-3.5	0.0	15-16	15-16
<i>Allium cepa</i>	-14.3	-7.7	8.2	3.8	0.0	12-13	12-13
<i>Avena sativa</i>	0.0	0.0	-0.5	0.9	0.0	31-31	31-31
<i>Lolium perenne</i>	15.0	0.0	-2.5	-6.3	0.0	22-26	12-27
<i>Zea mays</i>	-5.6	0.0	-14.1	-6.2	0.0	14-15	14-15

^N A negative value indicates an increase compared to the control

Bold figures are statistically significant (Pairwise Mann-Whitney-U-test, one sided smaller; $p \leq 0.05$).

Codes for phytotoxic symptoms:

a: change of colour (e.g. chlorosis, reddening, bleaching)

Conclusion

In a seedling emergence and growth limit test, Prohexadione-calcium OD 75 (75 g/L) was tested under greenhouse conditions for effects on seedling emergence, survival, growth, plant height and shoot dry weight of ten non-target terrestrial plant species, following a pre-emergence application of the test item to the soil surface.

No adverse effects above the 50% effect level at the test item rate of 90 g a.s./ha were observed on emergence, survival, plant height and shoot dry weight of the species tested.

The only statistically significant effect was found for the shoot dry weight reductions of *Lactuca sativa* (12.7%) compared to the control plants.

Assessment and conclusion by applicant:

This Tier 1 seedling emergence and growth study in which the effect of Prohexadione-calcium OD 75 (75 g/L) on ten non-target terrestrial plant species was tested under greenhouse conditions resulted in no adverse effects on emergence, survival, shoot length and shoot dry weight above the 50% effect level at the test item rate of 90 g a.s./ha.

Comments of zRMS:	<p>The study on the Effects on the vegetative vigor of ten non-target terrestrial plant species was performed in line with requirements of OECD 227 and according to the principles of GLP.</p> <p>The validity criteria for the study were fulfilled.</p> <p>This Tier 1 vegetative vigour and growth study in which the effect of Prohexadione-calcium OD 75 (75 g/L) on ten non-target terrestrial plant species was tested under greenhouse conditions resulted in a plant height reduction of 60.5% in <i>Cucumis sativus</i> at the test item rate of 90 g a.s./ha. The effects in all other tested species were below the 50% effect level.</p>
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Reference:	KCP 10.6.1/02
Title:	Amendment no. 01: Effects on the vegetative vigor of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L)
Report:	Köhler, P.; 2020; VV20/021; M-691184-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150(2012) OECD 227 (2006)
Deviations:	No deviations from the current guideline except for light intensity. The guideline recommends $350 \pm 50 \mu\text{mol/m}^2/\text{s}$, measured values were $110 - 728 \mu\text{mol/m}^2/\text{s}$. This deviation had no influence on the reliability of the study and endpoints.
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Executive summary

The objective of this study was to evaluate the potential effects of Prohexadione-calcium OD 75 (75 g/L) on the vegetative vigour of ten non-target terrestrial plant species, following a post-emergence application of the test item onto the foliage of plants at the 2-4 leaf stage. A total of ten species were tested, 6 dicotyledonous and 4 monocotyledonous species from 8 plant families. Planting density included 2 or 4 plants per pot with 10 or 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated at the 2-4 leaf stage. The test was conducted as a limit test. The single rate of 90 g a.s./ha of Prohexadione-calcium OD 75 (75 g/L) and the control (deionized water) were applied once at test initiation.

The validity criteria for the study were fulfilled. No adverse effects on survival and shoot dry weight above the 50% effect level were observed. There were no effects above the 50% effect level observed on shoot dry weight for the species tested, however, the dry weight reductions of *Beta vulgaris*, *Brassica rapa*, *Lactuca sativa*, *Solanum lycopersicum*, *Allium cepa*, *Avena sativa* and for *Lolium perenne* were found to be statistically significant compared to the control plants. The shoot length inhibition of *Cucumis sativus* plants treated with Prohexadione-calcium OD 75 (75 g/L) compared to the control plants was 60.5% and statistically significant. The plant height reductions of *Beta vulgaris*, *Brassica rapa*, *Avena sativa*, *Lolium perenne* and for *Zea mays* were found to be statistically significant compared to the control plants, but below 50%. Minor growth stage retardation was observed for *Beta vulgaris*, *Glycine max*, *Lactuca sativa*, *Allium cepa*, *Lolium perenne* and for *Zea mays* at the final assessment. Slight visual injury was observed in a few cases for *Brassica rapa*, *Cucumis sativus*, *Avena sativa* and *Zea mays* at the final assessment.

Material and methods

Test item: Prohexadione-calcium OD 75 (75 g/L)
 Synonym: none
 Sample description: TOX 21574-00
 Type of OD (oil dispersion) formulation:
 Active substance prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
 (analysed content):
 Specification no.: 102000037599
 Supplier batch no.: 2020-001264
 Density: 1.038 g/mL

A total of ten species, 6 dicotyledonous and 4 monocotyledonous species from 8 plant families were tested in this vegetative vigour test. The plant species used in this study are representative of a wide range of plant families and were chosen because they are readily cultivated test organisms and widely used in research. The germination rate of the seeds used in this study, observed in annual germination tests, was $\geq 70\%$. The plants were grown in a greenhouse in commercial non-porous 15 cm plastic pots (filled with approx. 1.2 L soil). The used soil was a sandy loam.

Planting density included 2 or 4 plants per pot with 10 or 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated at the 2-4 leaf stage. The test was conducted as a limit test with a single test item application rate and a water control.

The application solutions were prepared in the laboratory and transported to the application site immediately before application.

The single rate 90 g a.s./ha of Prohexadione-calcium OD 75 (75 g/L) and the control (deionized water) were applied once at test initiation to the soil and aboveground portions of the plants using a calibrated laboratory track sprayer at a volume rate of 200 L/ha.

List of species tested:

Species name	EPPO CODE	Common name
<i>Beta vulgaris</i>	BEAVA	Sugar beet
<i>Brassica rapa</i>	BRSRR	Turnip
<i>Cucumis sativus</i>	CUMSA	Cucumber
<i>Glycine max</i>	GLXMA	Soybean
<i>Lactuca sativa</i>	LACSC	Butterhead lettuce
<i>Solanum lycopersicum</i>	LYPES	Tomato
<i>Allium cepa</i>	ALLCE	Onion
<i>Avena sativa</i>	AVESA	Oat
<i>Lolium perenne</i>	LOLPE	Ryegrass
<i>Zea mays</i>	ZEAMA	Corn

After application, the pots with plants were transferred back to the greenhouse and placed on the tables in a randomized design with all pots of one species arranged together in a species plot. During the course of the experimental study part, the pots of each plant species were rearranged within each species plot.

Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The temperature was 18 °C to 26 °C during the light

period (16 h) and during the dark period (8 h) of the light-dark cycle. The relative humidity was 71 to 78%.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded.

Final assessments (on day 21 after application) were made for plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight.

Statistical analysis of survival, shoot length and shoot dry weight data was carried out with the Mann-Whitney-U-Test (one sided smaller; $p \leq 0.05$), included in ToxRat statistics.

Results and discussion

Validity criteria:

The emergence rate of the sown seeds in this study was $\geq 70\%$. All plant species in this study met the validity criterion of at least 90% for survival in the controls. In accordance with US EPA guideline (OCSPP 850.4150) and OECD guideline (OECD 227), there was no visible phytotoxicity, and normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled in equal manner with the same soil.

Analytical findings:

The analysis of prohexadione-calcium content in the test item solution revealed measured concentrations of 99.9% of nominal.

Biological findings:

The following table summarizes percent inhibition of survival, shoot dry weight and shoot length as calculated for the final assessment (21 days after application). In addition, ratings of phytotoxicity and growth stage (BBCH) are provided for all species tested.

Effects of 90 g a.s./ha of Prohexadione-calcium OD 75 (75 g/L) in the vegetative vigour test

Plant Species	Observations at the test item rate of 90 g a.s./ha				BBCH control min - max	BBCH treated min - max
	Survival (% inhibition)	Shoot dry weight (% inhibition) ^N	Shoot length (% inhibition) ^N	Phyto-toxicity (%)		
<i>Beta vulgaris</i>	0.0	36.8	29.9	0.0	19-19	17-19
<i>Brassica rapa</i>	0.0	12.0	8.3	16.0 bc	42-42	42-42
<i>Cucumis sativus</i>	0.0	37.1	60.5	2.0 ab	53-66	53-66
<i>Glycine max</i>	0.0	5.4	4.9	0.0	55-59	51-59
<i>Lactuca sativa</i>	0.0	21.9	-3.7	0.0	44-46	44-45
<i>Solanum lycopersicum</i>	0.0	6.7	-7.1	0.0	51-51	51-51
<i>Allium cepa</i>	0.0	21.5	5.5	0.0	15-41	14-41
<i>Avena sativa</i>	0.0	16.4	20.7	2.0 b	43-43	43-43
<i>Lolium perenne</i>	0.0	22.8	20.1	0.0	19-19	18-19

<i>Zea mays</i>	0.0	10.3	14.5	1.0 ab	31-31	15-31
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^N A negative value indicates an increase compared to the control

Bold figures of the inhibitions are statistically significant (Pairwise Mann-Whitney-U-test, one sided smaller; $p \leq 0.05$).

Codes for phytotoxic symptoms:

- a: change of color (e.g. chlorosis, reddening, bleaching)
- b: necrosis (e.g. dry brown shoot tissue, parts of the plant are dead)
- c: deformation (e.g. leaf curl, abnormal leaf shape, abnormal plant habitus, wilting)

Conclusion

This Tier 1 vegetative vigour and growth study in which the effect of Prohexadione-calcium OD 75 (75 g/L) on ten non-target terrestrial plant species was tested under greenhouse conditions resulted in no adverse effects on survival at the test item rate of 90 g a.s./ha. There were no effects above the 50% effect level observed on shoot dry weight for the species tested, however, the shoot dry weight reductions of *Beta vulgaris*, *Brassica rapa*, *Lactuca sativa*, *Solanum lycopersicum*, *Allium cepa*, *Avena sativa* and for *Lolium perenne* were found to be statistically significant compared to the control plants. The plant height reduction of *Cucumis sativus* plants treated with Prohexadione-calcium OD 75 (75 g/L) compared to the control plants was 60.5% and statistically significant. The plant height reductions of *Beta vulgaris*, *Brassica rapa*, *Avena sativa*, *Lolium perenne* and for *Zea mays* were found to be statistically significant compared to the control plants, but below 50%. Minor growth stage retardation was observed for *Beta vulgaris*, *Glycine max*, *Lactuca sativa*, *Allium cepa*, *Lolium perenne* and for *Zea mays* at the final assessment. Slight visual injury was observed in a few cases for *Brassica rapa*, *Cucumis sativus*, *Avena sativa* and *Zea mays* at the final assessment.

Assessment and conclusion by applicant:

This Tier 1 vegetative vigour and growth study in which the effect of Prohexadione-calcium OD 75 (75 g/L) on ten non-target terrestrial plant species was tested under greenhouse conditions resulted in a plant height reduction of **60.5** ~~69.5~~% in *Cucumis sativus* at the test item rate of 90 g a.s./ha. The effects in all other tested species were below the 50% effect level.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	<p>The study on the Effects on the vegetative vigor of ten non-target terrestrial plant species was performed in line with requirements of OECD 227 and according to the principles of GLP.</p> <p>The validity criteria for the study were fulfilled.</p> <p>Lowest ER₅₀ = 48.8 g a.s./ha was found for plant height of <i>Cucumis sativus</i></p>
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Reference:	KCP 10.6.2/01
Title:	Effects on the vegetative vigor of the non-target terrestrial plant species <i>Cucumis sativus</i> (Tier 2); prohexadione-calcium OD 75 (75 g/L)
Report:	Köhler, P.; 2020; VV20/039; M-758398-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150(2012) OECD 227 (2006)
Deviations:	No deviations from the current guideline except for light intensity. The guideline recommends $350 \pm 50 \mu\text{mol/m}^2/\text{s}$, measured values were $303.4 - 679.1 \mu\text{mol/m}^2/\text{s}$. This deviation had no influence on the reliability of the study and endpoints.
GLP/GEP:	yes
Acceptability:	YES
Duplication (if vertebrate study):	no

Executive summary

The objective of this study was to evaluate the potential effects of prohexadione-calcium OD 75 (75 g/L) on the vegetative vigour of the non-target terrestrial plant species *Cucumis sativus* following a post-emergence application of the product onto the foliage and above-ground portions of plants. The selected plant species had shown relevant effects in a preceding limit test and was therefore investigated in this rate-response study.

Planting density included 2 plants per pot with 10 replicate pots, respectively, for a total of 20 plants per treatment level. The plants were treated at the 2-4 leaf stage. The test was conducted as a rate response test with five application rates. Serial dilutions of Prohexadione-calcium OD 75 (75 g/L) at 5, 10.3, 21.2, 43.7 and 90 g a.s./ha and the control (deionized water) were applied once at test initiation. The validity criteria for the study were fulfilled. The lowest ER₅₀ of 48.8 g a.s./ha was found for plant height.

Material and methods

Test item:	Prohexadione-calcium OD 75 (75 g/L)
Synonym:	none
Sample description:	TOX 21574-00
Type of formulation:	OD (oil dispersion)
Active substance (analysed content):	prohexadione-calcium (KUH-833): 7.57% w/w (78.54 g/L)
Specification no.:	102000037599
Supplier batch no.:	2020-001264
Density:	1.038 g/mL

One dicotyledonous crop species of one plant family was tested in this vegetative vigour test. The plant species *Cucumis sativus* (Cucumber, EPPO code: CUMSA) was chosen as it had shown relevant effects in a preceding limit test and was therefore investigated in this rate-response study. The plants were grown in a greenhouse in commercial non-porous 15 cm plastic pots (filled with approx. 1.2 L soil). The used soil was a sandy loam.

Planting density included 2 plants per pot with 10 replicate pots, for a total of 20 plants per treatment level. The plant species were treated at the 2-4 leaf stage. The test was conducted as a dose response test with 5 test item application rates and a water control.

The application solutions were prepared in the laboratory and transported to the application site immediately before application.

The serial dilutions of Prohexadione-calcium OD 75 (75 g/L) at 5, 10.3, 21.2, 43.7 and 90 g a.s./ha and

the control (deionized water) were applied once at test initiation to the soil and aboveground portions of the plants using a calibrated laboratory track sprayer at a volume rate of 200 L/ha (205 L/ha mean measured).

After application, the pots with plants were transferred back to the greenhouse and placed on the tables in a randomized design with all pots of one species arranged together in a species plot. During the course of the experimental study part, the pots of each plant species were rearranged within each species plot. Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The temperature was 17 °C to 26 °C during the light period (16 h) and during the dark period (8 h) of the light-dark cycle. The relative humidity was 49 to 91% during light and dark period.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded.

Final assessments (on day 21 after application) were made for plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight.

ER₂₅ and ER₅₀ values (effect rate causing 25% and 50% effect) with the corresponding 95% confidence limits for quantal endpoints (survival) were estimated using linear regression models (ToxRat; version 3.3.0). ER₂₅ and ER₅₀ values for continuous endpoints (plant height and shoot dry weight) with the corresponding 95% confidence limits were estimated using non-linear regression models implemented in Microsoft Excel (2010) with Visual Basic for Application (VBA).

NOER and LOER values for survival were determined using hypothesis tests (ToxRat; version 3.3.0) and for plant height and shoot dry weight NOER and LOER values were determined using hypothesis tests implemented in Microsoft Excel (2010) with Visual Basic for Application (VBA)) with $\alpha = 0.05$.

Results and discussion

Validity criteria:

The emergence rate of the sown seeds in this study was 93% and thus met the validity criterion of $\geq 70\%$. The validity criterion of at least 90% for survival in the controls was met (actual: 100%). In accordance with US EPA guideline (OCSPP 850.4150) and OECD guideline (OECD 227), there was no visible phytotoxicity, and normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology.

Analytical findings:

The analysis of prohexadione-calcium content in the test item solution revealed measured concentrations of 98% of nominal.

Biological findings:

The following table summarizes percent inhibition of survival, shoot dry weight and shoot length as calculated for the final assessment (21 days after application). In addition, ratings of phytotoxicity and growth stage (BBCH) are provided.

Effects of Prohexadione-calcium OD 75 (75 g/L) in the vegetative vigour test on *Cucumis sativus*

Test item rates (g a.s./ha)	Survival (% inhibition)	Shoot dry weight (% inhibition) N	Plant height (% inhibition) N	Phyto- toxicity (%)	BBCH min - max
Control	-	-	-	0.0	66-69
5	0	7.8	21.61	0.0	65-69

10.3	0	10.3	30.02	1.0 a	65-69
21.2	0	8.1	43.69	4.0 a	64-69
43.7	0	2.7	43.98	7.0 a	63-69
90	0	8.0	60.28	11.0 a	63-69
	Survival [g a.s./ha]	Shoot dry weight [g a.s./ha]	Plant height [g a.s./ha]		
NOER	90	90	<5		
ER ₂₅	>90	>90	6.1 (c.l. 2.1-10.0)		
ER ₅₀	>90	>90	48.8 (c.l. 32.4-65.1)		

^N A negative value indicates an increase compared to the control

Bold figures of the inhibitions are statistically significant (estimated with Weibull model).

Codes for phytotoxic symptoms:

a: change of color (e.g. chlorosis, reddening, bleaching)

Conclusion

In this Tier 2 vegetative vigour and growth study, Prohexadione-calcium OD 75 (75 g/L) was tested under greenhouse conditions for effects on the survival, growth and shoot dry weight of the non-target terrestrial plant species *Cucumis sativus*, following a post-emergence application of the test item the foliage of plants at the 2-4 leaf stage. All validity criteria were met. The lowest ER₅₀ was found for plant height at 48.8 g a.s./ha.

Assessment and conclusion by applicant:

In this Tier 2 vegetative vigour and growth study, the lowest ER₅₀ = 48.8 g a.s./ha was found for plant height of *Cucumis sativus*

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

No additional studies are submitted.

A 2.6.4 KCP 10.6.4. Semi-field and field tests on non-target plants

No additional studies are submitted.

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

No additional studies are submitted.

A 2.8 KCP 10.8 Monitoring data

No additional studies are submitted.