FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 076127 A

Product name: PROSIM

Chemical active substances:

Propamocarb hydrochloride, 400 g/L

Cymoxanil, 50 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: SHARDA Cropchem España S.L.

Submission date: October 2020

MS Finalisation date: 12/2022 ; update 03.2023

Version history

When	What
May 2021	Applicant update
December 2022	Applicant update
December 2022	The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are struck through and shaded for transparency.
January 2023	Applicant update
March 2023	ZRMS corected after commenting

Table of Contents

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU	**Potato**	F	*Phytophthora infestans*	Foliar Spray	BBCH 21-95	a) 1 b) 6	7-10	a) 2.5 b) 15	a) 1 propamocarb + 0.125 cymoxanil b) 6 propamocarb + 0.75 cymoxanil	200-400	7								

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

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

9.1.1 Overall conclusions

zRMS comments:

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.

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Regarding Propamocarb hydrochloride, according to the first-tier assessment for potato, the TERa and TERlt are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the PROSIM presents an acceptable acute and long-term risk to mammals for these uses except to vole for long-term risk assessment. After PD, FIR/bw, DT_{50}, MAF and ftwa refinement, no unacceptable risk was obtained.

Regarding Cymoxanil, according to the first-tier assessment for potato, the TER_a and TER_{lt} are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the PROSIM presents an acceptable acute and long-term risk to mammals for these uses except to vole and lagomorph for long-term risk assessment. After PD, FIR/bw, DT_{50}, MAF and ftwa refinement, no unacceptable risk was obtained.

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

• Birds:
According to the first-tier assessment for potatoes, all the TER_a and TER_{lt} values for Propamocarb and Cymoxanil are greater than the Annex VI trigger of 10 and 5, respectively, indicating that PROSIM presents no unacceptable acute and long-term risk to bords according to the intended uses on potatoes.

• Mammals:
Regarding Propamocarb hydrochloride, according to the first-tier assessment for potato, the TER_a and TER_{lt} are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the PROSIM presents an acceptable acute and long-term risk to mammals for these uses except to vole for long-term risk assessment. After PD, FIR/bw, DT_{50}, MAF and ftwa refinement, no unacceptable risk was obtained.

Regarding Cymoxanil, according to the first-tier assessment for potato, the TER_a and TER_{lt} are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the PROSIM presents an acceptable acute and long-term risk to mammals for these uses except to vole and lagomorph for long-term risk assessment. After PD, FIR/bw, DT_{50}, MAF and ftwa refinement, no unacceptable risk was obtained.

9.1.1.3 Effects on aquatic organisms (KCP 10.2)

• Propamocarb
For the intended uses on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most

sensitive group of aquatic organisms (risk for fish prolonged as characterised by an NOEC for *Lepomis macrochirus* of 6300 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

• Cymoxanil
For the intended uses potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by an NOEC for *Oncorhynchus mykiss* of 44 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-3 scenarios. Therefore, no further assessment is necessary.

Metabolites of Cymoxanil: for the intended use on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

PROSIM: for the intended use on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

9.1.1.4 Effects on bees (KCP 10.3.1)

First-tier assessments indicate that no unacceptable risk for bees exposed to PROSIM is expected according to the proposed intended uses on potato. According Reg. 284/2009 the chronic study for adult bees and chronic study for bees should be provided by the applicant.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

The PERin-field is below the rate with <50% of effects on mortality and reproduction for extended studies for the product PROSIM in potato for the representative species *Typhlodromus pyri* and *Aphidius rhopalosiphi,* and for additional species. Therefore, in-field recovery is expected.

The PERoff-field corrected is below rate with 50% effects for the product PROSIM for the representative species *Typhlodromus pyri* and *Aphidius* and for additional species, indicating no risk to non-target arthropods in vegetated off-field areas following application according to the proposed use patterns.

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

• Non-target soil meso- and macrofauna:
The chronic TER for Propamocarb hydrochloride, Cymoxanil and PROSIM are above the Annex VI trigger of 5. Therefore, it is concluded that actives and PROSIM formulation do not poses long-term risk to earthworms.

• Soil microorganisms:
Risk assessments conducted with relevant PEC_{soil} for Propamocarb and Cymoxanil in PROSIM formulation indicate a low risk to soil microorganisms when applied according to the proposed use rates.

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

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for PROSIM shows that Annex VI trigger of 5 is not exceeded, indicating that PROSIM poses a low risk to non-target plats when applied according to the proposed use rates.

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

Not relevant.

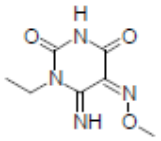
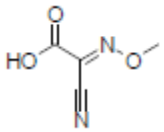
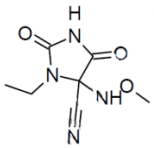
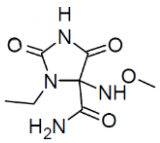
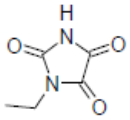
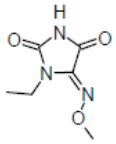
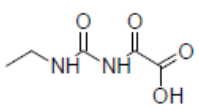
9.1.2 Grouping of intended uses for risk assessment

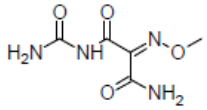
Not relevant.

9.1.3 Consideration of metabolites

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

Table 9.1-2 Metabolites of Cymoxanil

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IN-U3204 1-ethyl-6-iminodihydropyrimidine-2,4,5(3H)-trione 5-(O-methyloxime) (E-configuration)		198.5 g/mol	Soil: 24.7 Water:24.7 Sediment: 0.5 Total: 24.7	Yes, aquatic organisms
IN-W3595 Cyano(methoxyimino)acetic acid (E-configuration)		128.2 g/mol	Soil: 10.1 Water: 26.1 Sediment: 2.3 Total: 27.5	Yes, aquatic organisms
IN-JX915 3-ethyl-4-(methoxyamino)-2,5-dioxoimidazolidine-4-carbonitrile (stereomer racemate)		198.2 g/mol	Soil: 10.9 Water: 7.2 Sediment: 1.2 Total: 8.5	Yes, aquatic organisms
IN-KQ960 3-ethyl-4-(methoxyamino)-2,5-di-oxoimidazolidine-4-carboxamide (stereomer racemate)		216.2 g/mol	Soil: 6.3 Water: 13.0 Sediment: 5.5 Total: 14.3	Yes, aquatic organisms
IN-T4226 1-ethylimidazolidine-2,4,5-trione		142.1 g/mol	Soil: 1.7 Water: 11.1 Sediment: 1.0 Total: 12.0	Yes, aquatic organisms
IN-R3273 1-ethylimidazolidine-2,4,5-trione 5-(O-methyloxime) (E-configuration)		171.2 g/mol	Soil: 2.4 Water: 5.0 Sediment: 0.5 Total: 5.0	Yes, aquatic organisms
IN-KP533 {[(ethylamino)carbonyl]amino}(oxo)acetic acid		160.1 g/mol	Soil: 2.7 Water: 20.5 Sediment: 6.5 Total: 26.0	Yes, aquatic organisms

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Metabolite fraction M5 N-(aminocarbonyl)-2-(methoxymino)maloamide (E-configuration)		198.2 g/mol	Soil: 0.0 Water: 22.9 Sediment: 0.0 Total: 22.9	Yes, aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of PROSIM were not evaluated as part of the EU assessment of Propamocarb hydrochloride and Cymoxanil.

However, the provision of further data on the PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Previcur N (formulation)	Oral 14 d Acute	**LD_{50} >1842 mg a.s./kg b.w./day** LD_{50} (extrapolated) = 3477.7 mg a.s./kg b.w./day*	EFSA Scientific Report (2006) 78, 1-80 DAR of Propamocarb (2004)
	Proplant (formulation)	Oral 14 d Acute	LD_{50} >2000 mg a.s./kg b.w./day	DAR of Propamocarb (2004)
Bobwhite quail	Proplant (formulation)	Dietary 5d Shortterm	LC_{50} > 962 mg a.s./kg b.w./day	EFSA Scientific Report (2006) 78, 1-80
Bobwhite quail	Previcur N (formulation)	Reproductive 21 weeks Longterm dietary	**NOEC = 105 mg a.s./kg b.w./day**	EFSA Scientific Report (2006) 78, 1-80
Colinus virgnianus	Cymoxanil	Oral Acute	**LD_{50} > 2000 mg/kg bw**	EFSA Scientific Report (2008) 167, 1-116
Anas platyrhynchos	Cymoxanil	Dietary Short-term	LC_{50} > 260* mg/kg bw/d	
Anas platyrhynchos	Cymoxanil	Dietary Reproductive toxicity	**NOEL = 14.9 mg /kg bw/d**	

* since food consumption was reduced at dietary concentrations above and below the LC_{50} value, it is not possible to convert LC_{50} to a reliable daily dose estimate. The highest sub-LC_{50} dietary consumption that cause no significant impact on food consumption was 625 ppm, corresponding to 260 mg a.s./kg bw/day

9.2.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of PROSIM in potato

Intended use		**Potato**				
Active substance/product		Propamocarb hydrochloride				
Application rate (g/ha)		6 x 1000				
Acute toxicity (mg/kg bw)		>1842				
TER criterion		10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**		**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods	25.2	1.9		47.88	38.5
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	24.0	1.9		45.60	40.4
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.9		13.68	134.6
Reprod. toxicity (mg/kg bw/d)		105				
TER criterion		5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**

Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods	9.7	2.5 x 0.53	1	12.85	8.2
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	2.5 x 0.53	1 0.84	14.44 12.13	7.3 8.65
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	2.5 x 0.53		4.37	24.0

Active substance/product		Cymoxanil
Application rate (g/ha)		6 x 125
Acute toxicity (mg/kg bw)		> 2000
TER criterion		10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**PT**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods	25.2	1.9	1	5.99	334.2
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	24.0	1.9	1	5.70	350.9
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.9	1	1.71	1169.6

Reprod. toxicity (mg/kg bw/d)		14.9
TER criterion		5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**

Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods	9.7	2.5 x 0.53	1	1.61	9.3
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	2.5 x 0.53	1 0.84	1.81 1.51	8.3 10
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	2.5 x 0.53	1	1.81	8.3
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	2.5 x 0.53	1	0.55	27.3

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.

zRMS comments:

The acute and chronic risks to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items.

All TER_A and TER_{Lt} values exceed the relevant triggers indicating that Prosim does not pose an unacceptable risk to birds following applications according to recommended use pattern.

In addition, for BBCH 10-39 for omnivorous species lark zRMS added calculation of DDD and TER_{LT} for both active substances based on refinement PT parameter obtained from by Prosper-2017-(90 %-consumers-only-potato) Crocker et al. 1998, Finch et al. 2006, Prosser 2010) (*Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009; April 2020)* needed to mixture toxicity assessment.

Risk Assessment for combined exposure

According to the EFSA Journal (2009)[1], the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

$$LD_{50}\,(\text{mix}) = \left(\sum_{i} \frac{X\,(a.s_{i})}{LD_{50}\,(a.s_{i})} \right)^{-1}$$

With:

$X\,(a.s._i)$ = fraction of each a.s. in the mixture

$LD_{50}\,(a.s._i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

The active substance content of the formulation PROSIM addressed in this dossier is 40% Propamocarb hydrochloride and 5% Cymoxanil, making up a total of 450 g a.s./L product. According to GAP, the maximum application rate is 2.5 L product/ha, therefore, an application rate of 1125 g a.s./ha was considered in the assessment.

Table 9.2-3 shows the calculation of the predicted LD_{50} (mix) of Propamocarb hydrochloride and Cymoxanil when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Table 9.2-3: Avian LD_{50} (mix) for Propamocarb hydrochloride and Cymoxanil when combined as PROSIM (step 1 in EFSA GD 2009, Appendix B)

	Propamocarb hydrochloride	Cymoxanil
Content in the formulation PROSIM	40%	5%
Fraction in the a.s. mixture	0.8889	0.1111
LD_{50} of a.s. [mg/kg bw]	>1842	>2000
Fraction / LD_{50}	0.0005	0.0001
Sum	0.0005	
1/ sum = predicted LD_{50} (mix)	1858.31 mg mix/kg bw	

It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions of the formulated product PROSIM.

Table 9.2-4: Avian "tox per fraction" for the PROSIM (step 1 in EFSA GD 2009, Appendix B)

	Propamocarb hydrochloride	Cymoxanil	"mix"
Content in the formulation PROSIM	40%	5%	45 %
Fraction in mixture	0.8889	0.1111	1.0
LD_{50} (mg/kg bw)	>1842	>2000	1858.31
Tox per fraction	2072.25	18000.00	1858.31
Contribution to predicted toxicity	89.68 %	10.32 %	

Propamocarb hydrochloride contributes to 89.68 % to mixture toxicity, while the Cymoxanil have an impact on the predicted risk of 10.32 %, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.2-5: First-tier assessment of the acute risk for birds due to the use of PROSIM in potato

Intended use	Potato				
Active substance/product	PROSIM				
Application rate (g/ha)	6 x 1125				
LD_{50} (mix) (mg/kg bw)	1858.31				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous bird "wag-tail" ground invertebrates with interception 50% ground arthro-pods, 50% foliar arthropods	25.2	1.9	53.87	34.5
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	24.0	1.9	51.30	36.2
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.9	15.39	120.7

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.

According to reults, no unacceptable acute risk is obtained in potato acording to the proposed GAP.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

zRMS comments:

The acute comnined toxicity risk assessment for birds is considered acceptable.

zRMS added the combined long-term risk for birds assessment based on TER_{mix} approach based on the lowest TER_{LT} values.

The relevant calculations are provided below:

TER_{mix} approach for combined long-ter risk assessment.

Compound						
Propamocarb hydrochloride		**Cymoxanil**				
TER	**1/TER**	**TER**	**1/TER**	**Σ1/TER**	**$Σ1/TER^{-1}$**	**Trigger**

7.3[1)]	0.14	8.3 [1)]	0.12	0.26	**3.84**	5
8.7 [2)]	0.11	10	0.1	0.22	4.54	5

[1)] Lowest Tier 1 TER $_{LT}$ for omnivorous bird, lark ‘’

2) Refined TER_{LT} for omnivorous, lark’’ based on PT =0.84 (lark, consumers only) from Prosper-2010 Crocker et al. 1998, Finch et al. 2006, Prosser 2010) Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009; April 2020

The calculated TER_{mix} is slight below the trigger of 5 from long-term combined risk exposed to the mixture of Propamocarb hydrochloride and Cymoxanil.

zRMS is in the opinion the combined long-term risk might be considered acceptable as TER_{mix} is closed to trigger of 5 and potatoes is considered as non-attractive crop to birds.

However, this issue should be further considered at MSs level.

9.2.2.2 Higher-tier risk assessment

Not relevnat.

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

Puddle scenario

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

With a Kfoc of 718.81 (EFSA Scientific Report (2006) 78, 1-80), Propamocarb hydrochloride belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha) =	2500	Effective AR = AR x MAF		
Acute toxicity (mg/kg bw) =	>1842	quotient =	1.36	
Reprod. toxicity (mg/kg bw/d) =	105	quotient =	23.81	

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

With a K(f)oc of 43.6 (EFSA Scientific Report (2008) 167, 1-116, arithmetic mean, n = 4), Cymoxanil belongs to the group of less sorptive substances.

Effective application rate (g/ha) =	312.5	Effective AR = AR x MAF	
Acute toxicity (mg/kg bw) =	> 2000	quotient =	0.16
Reprod. toxicity (mg/kg bw/d) =	14.9	quotient =	20.97

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

zRMS comments:

As a generic approach, the EFSA Guidance Document states that no specific calculations of exposure and TER are necessary for the puddle scenario 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). The ratio is below the trigger value indicating an acceptable risk and no further consideration is needed.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Propamocarb amounts to -2.9, -1.2 and 0.67 at pH 2, 7 and 9 respectively and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of cymoxanil amounts to 0.67-0.59 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

According to the first-tier assessment for potatoes, all the TER_a and TER_{lt} values for Propamocarb and Cymoxanil are greater than the Annex VI trigger of 10 and 5, respectively, indicating that PROSIM presents no unacceptable acute and long-term risk to bords according to the intended uses on potatoes.

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 Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of PROSIM were not evaluated as part of the EU assessment of Propamocarb hydrochloride and Cymoxanil.

However, the provision of further data on the formulation PROSIM is not considered essential, because active substance toxicity data.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Proplant	Oral Acute	**LD_{50} > 1330mg a.s/kg bw**	EFSA Scientific Report (2006) 78, 1-80
Rat	Proplant	90 d Chronic	**NOAEL= 104 mg/kg bw (female)**	
Rattus norvegicus	Cymoxanil	Oral Acute	**LD_{50} = 760 mg/kg bw**	EFSA Scientific Report (2008) 167, 1-116
Rattus norvegicus	Cymoxanil	Dietary Reproductive toxicity	**NOAEL = 10.5 mg/kg bw/d**	

9.3.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for 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 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of PROSIM in potatoes

Intended use	**Potatoes**				
Active substance/product	Propamocarb				
Application rate (g/ha)	6 x 1000				
Acute toxicity (mg/kg bw)	> 1330				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	5.4	1.9	10.26	129.6
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	35.1	1.9	66.69	19.9
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	10.5	1.9	19.95	66.7
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	1.9	77.71	17.1
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	17.2	1.9	32.68	40.7
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	5.2	1.9	9.88	134.6
Reprod. toxicity (mg/kg bw/d)	104				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	2.5 x 0.53	2.52	41.3
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	14.3	2.5 x 0.53	18.95	5.5

Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	4.3	2.5 x 0.53	5.70	18.3
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	2.5 x 0.53	28.75	**3.6**
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	2.5 x 0.53	10.34 10.34 x 0.84*=8.68	10.1 12
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	2.5 x 0.53	3.05	34.1

Active substance/product	Cymoxanil
Application rate (g/ha)	6 x 125
Acute toxicity (mg/kg bw)	760
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	5.4	1.9	1.28	592.6
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	35.1	1.9	8.34	91.2
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	10.5	1.9	2.49	304.8
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	1.9	9.71	78.2
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	17.2	1.9	4.09	186.0
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	5.2	1.9	1.24	615.4

Reprod. toxicity (mg/kg bw/d)	10.5
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**

Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	2.5 x 0.53	0.31	33.4
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	14.3	2.5 x 0.53	2.37	**4.4**
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	4.3	2.5 x 0.53	0.71	14.7
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	2.5 x 0.53	3.59	**2.9**
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	2.5 x 0.53	1.29 1.29 x 0.84*=1.08	8.1 9.72
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	2.5 x 0.53		
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	2.5 x 0.53	0.38	27.6

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.

*PT=0.84 (90 th percentile) by Dreaper 2010- consumers only, Crocker et al. 1998, Finch et al. 2006, Prosser 2010) Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009; April 2020

zRMS comments:

The acute and chronic risks to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items.

TER_A values exceed the relevant triggers for both active substances indicating an acceptable risk to mammals following applications according to recommended use pattern.

TER_{LT} values exceed the relevant triggers for Propamocarb indicating an acceptable risk to mammals, except small herbivorous mammals' vole, indicating needs for further refinement.

In case of cymoxanil TER_{LT} values exceed the relevant triggers except small herbivorous mammal vole BBCH> 40 and large herbivorous mammal, indicating needs for further refinement.

zRMS in the Table above added the refinement parameter for wood mouse required for mixture toxicity assessment.

Risk Assessment for combined exposure

According to the EFSA Journal (2009)[2], the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

$$LD_{50}\,(\text{mix}) = \left(\sum_{i} \frac{X\,(a.s_i)}{LD_{50}\,(a.s_i)} \right)^{-1}$$

With:

$X\,(a.s._i)$ = fraction of each a.s. in the mixture

$LD_{50}\,(a.s._i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

The active substance content of the formulation PROSIM addressed in this dossier is 40% Propamocarb hydrochloride and 5% Cymoxanil, making up a total of 450 g a.s./L product. According to GAP, the maximum application rate is 2.5 L product/ha, therefore, an application rate of 1125 g a.s./ha was considered in the assessment.

Table 9.3-3 shows the calculation of the predicted LD_{50} (mix) of Propamocarb hydrochloride and Cymoxanil when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Table 9.3-3: Mammalian LD_{50} (mix) for Propamocarb hydrochloride and Cymoxanil when combined as PROSIM (step 1 in EFSA GD 2009, Appendix B)

	Propamocarb hydrochloride	Cymoxanil
Content in the formulation PROSIM	40%	5%
Fraction in the a.s. mixture	0.8889	0.1111
LD_{50} of a.s. [mg/kg bw]	>1330	760
Fraction / LD_{50}	0.0007	0.0001
Sum	0.0008	
1/ sum = predicted LD_{50} (mix)	1227.69 mg mix/kg bw	

It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions of the formulated product PROSIM.

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 9.3-4: Mammalian "tox per fraction" for the PROSIM (step 1 in EFSA GD 2009, Appendix B)

	Propamocarb hydrochlo-ride	Cymoxanil	"mix"
Content in the formulation PROSIM	40%	5%	45 %
Fraction in mixture	0.8889	0.1111	1.0
LD_{50} (mg/kg bw)	>1330	760	1227.69
Tox per fraction	1496.25	6840.00	1227.69
Contribution to predicted toxicity	82.05 %	17.95 %	

Propamocarb hydrochloride contributes to 82.05 % to mixture toxicity, while the Cymoxanil have an impact on the predicted risk of 17.95 %, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.3-5: First-tier assessment of the acute risk for mammals due to the use of PROSIM in potato

Intended use	Potato				
Active substance/product	PROSIM				
Application rate (g/ha)	6 x 1125				
LD_{50} (mix) (mg/kg bw)	1227.69				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling inver-tebrates with interception 100% ground arthropods	5.4	1.9	11.54	106.4
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	35.1	1.9	75.03	16.4
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	10.5	1.9	22.44	54.7
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	1.9	87.42	14.0
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse" Combination (inverte-brates without interception) 25% weeds 50% weed seeds 25% ground arthropods	17.2	1.9	36.77	33.4
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (inverte-brates without interception) 25% weeds 50% weed seeds 25% ground arthropods	5.2	1.9	11.12	110.5

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.

According to reults, no unacceptable acute risk is obtained in potato acording to the proposed GAP.

zRMS comments:

We agree with the acute combined risk assessment for mammals.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

9.3.2.2 Higher-tier risk assessment

Propamocarb hydrochloride:

In the Tier I risk assessment, unacceptable risk was obtained for next mammals for Propamocarb hydrochloride:

Potato

- Small herbivorous mammal "vole" at BBCH ≥ 40: TER_{lt} value is below the trigger of 5.

A further refinement of the long-term risk is needed. In order to refine the risk assessment, the following parameters refined below were considered.

PD

As a further refinement of the risk of vole in tomato, the PD refinement was considered. A PD refinement is commented by Netherlands[3] and a proposal of refinement is given. The refinement is based on the studies by Rinke (1991) "*Percentage of volume versus number of species: availability and intake of grasses and forbs in microtus arvalis. Folia zoological 40 (2): 143-151*" and by Lüthi, M. *et al* (2010) "*Nutritional ecology of Microtus arvalis* (*Pallas, 1779) in sown wild flower fields and quasi-natural habitats. Revue Suisse de Zoologia 117 (4): 811-828*".

In the study of Rinke (1991) the stomach content of 363 individuals (186 females and 177 males) trapped on five plots of permanent meadow in central Hessia (Germany) were analyzed. The study investigated the vole feeding preferences (mono *vs.* dicot). In the study voles showed a preference for dicots, with the majority of voles (all seasons, sexes, ages) showing > 80% dicot material in stomach contents.

Diet of common voles (%) – Rinke 1991

Season	Monocotyledons (% volume)	Dicotyledonos (% volume)	No. of voles
Spring	24	76	23
Summer	25	75	152
Autum	48	52	188

[3] Evaluation Manual for the authorization of plant protection products according to Regulation (EC) No 1107/2009 Chapter 7, version 2.2; April 2017

Total	36	64	363

In the study of Lüthi et al., 2010 the diet of the common vole in monocot and dicot dominated fields was studied. In the sown wild flower areas vegetation cover was mainly dicot (79%, 81.6% and 79% in the three fields, respectivley) and in the quasi natural habitat the cover was mainly monocots (82.5, 92.5 and 47.5%).

Diet of common voles (%) – Lüthi et al., 2010

Sown wild flower fields	**Field 1**	**Field 2**	**Field 3**	**Average**
Dicots	16.3	31.8	11.2	19.6
Monocots	43.1	36.5	53.3	44.3
Seeds	14.8	16.5	27.0	19.4
Other (roots)	25.8	15.2	8.5	16.6
Natural quasi habitat				
Dicots	17.1	6.2	9.6	11.0
Monocots	67.7	81.9	66.0	71.0
Seeds	6.6	8.4	17.0	10.7
Other (roots)	8.56	3.5	7.4	7.4

Dicot dominated fields (agricultural crops, etc): 50% non-grass herbs and 50% grass and cereals

Monocot dominated underground (grasslands, orchards, etc): 25% non-grass herbs and 75% grass and cereals.

The approach is considered appropriate for the refinement of the chronic risk assessmente for vole. Therefore, for the refinement of the risk in potato, a PD of 0.5 for non-grass herbs and 0.5 for grass and cereals will be used.

FIR/bw

For the food category grass and cereals, the FIR/bw value of 1.33, given by EFSA/2009/1438 was used. For the food category non-grass herbs, FIR/bw value was calculated. Default values given by EFSA/2009/1438 were used for the estimation of FIR and a bw value of 25 g for common vole given in EFSA/2009/1439 was used. The resulting values were: FIR = 40.433; FIR/bw = 1.62.

DT50

7 residue field trials have been conducted in Northern Europe according to *Propamocarb - Volume 3; Annex B.7, Residue Data* (Sep 2004), at different seasonal periods and according to the proposal GAP (Please, refer to point 9.2.2 from core dossier). A mean DT_{50} of 4.58 days and 90^{th} percentile of 6.17 d were obtained. The mean values of **4.58** was used for long-term refinement of vole.

TWA

In the Tier I, the default 21d twa value used is 0.53. However, since the DT_{50} is lower than 10 days, the 21d-twa value was recalculated considering the mean DT_{50} of 4.58 days and the resulting value is **0.30** that will be used in the higher-tier assessment.

MAF

Considering the half-life of 4.69days, the maximum of 6 applications with a minimum spray interval of 7 day, the refined Multiple Application Factor (MAF) was calculated to be **1.53** (MAF_m for the long-term risk assessment). The calculations were performed according to the formula of the EFSA/2009/1438.

Table 9.3-6: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of PROSIM in potato– refined parameters (*) are further described and justified in the text

Intended use	**Potato**							
Active substance/product	Propamocarb hydrochloride							
Application rate (g/ha)	6 × 1000							
Reprod. toxicity (mg/kg bw/d)	104							
TER criterion	5							
Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_m× DF* (mg/kg food)**	**MAF_m × TWA**	**PT**	**PD***	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Common vole (*Microtus arvalis*) BBCH ≥ 40	50% grass and cereals	1.33	54.2[1] × 0.3[1]	2.5 x 0.53	1.0	0.5*	14.33	5.9
	50% non-grass herbs	1.62	28.7[1] x 0.3[1]	1.53[2] x 0.30[2]	1.0	0.5*	3.20	
						Total	17.53	

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[1]According to Appendix A of EFSA/2009/1438.
[2]MAF and ftwa refinement from residue trials from *Propamocarb - Volume 3; Annex B.7, Residue Data (Sep 2004)*.

zRMS comments:

zRMS do not agree with calculation of FIR/bw provided by the applicant. Additional calculations for diets consisting of 50% of monocots and 50% of dicots is calculated by the zRMS and is presented below:

BW vole (g)	**DEE (kj)**	**RUD unit**	**PD**	**FE (kJ/g dry)**	**Moisture Fraction**	**Assimilation efficiency fraction**	**$FE_{total\ fresh}$ (kJ/g fresh weight)**	**$FIR_{total\ fresh}$ (g fresh weight/d)**	**FIR/BW**
25	65.09	Grass + cereals	0.5	17.6	0.764	0.47	1.781	36.55	1.462
		Non-grass herbs	0.5	17.8	0.881	0.76			
25	65.09	Grass + cereals	0.75	17.6	0.764	0.47	1.867	34.87	1.395
		Non-grass herbs	0.25	17.8	0.881	0.76			

Based on the calculation provided above the diet consisting of 50% monocots and 50% dicots the FIR/bw is 1.462.

Further zRMS using the EU agreed diet and respective FIR/bw of 1.462, as calculated above.
According to information provided in the EFSA Conclusion 2007 the DT50s values ranged from 46.44 to 1.87 days and the average was calculated as 4.69 days.

The correctness of the calculation presented in the addendum of January 2006 was questioned (e.g. DT50 calculated from average residue data instead of calculating the mean of the DT_{50}s from each individual trial, geometric mean vs. arithmetic mean).
The applicant submitted the mean value of DT_{50} = 4.58 d. It is not clear for zRMS how this value was calculated, which date were taken into account by the applicant.
No summary from the results for calculation of DT_{50} was provided and there is not kinetic evaluation of the obtained results in the current dossier Core Dossier.

Therefore, zRMS provided the own risk assessment. For the refinement, the deposition values as described in EFSA GD, Appendix E were used. Interception can only be taken be into account at later growth stages with high vegetation coverage. Interception values according FOCUS groundwater (EFSA Journal 2014;12(5):3662) were used. For the uses in potatoes at BBCH 40-89, the interception is 85 % and BBCH 90-99 the interception is 50 %.

In case of the application up to BBCH 89 instead of 91, the risk is acceptable. As only one application will be possible between BBCH 89 and 91, ZRMS considers the risk acceptable in the whole range of growth stages with high probability. However, final conclusion should be made on cMS level according to their agricultural practice.

Intended use		Potatoes						
Active substance/product								
Application rate (g/ha)		6 × 1000 BBCH						
Reprod. toxicity (mg/kg bw/d)		104						
TER criterion		5						
Focal species	**Food category, % in diet**	**PD**	**FIR/bw**	**RUD_m × DF (mg/kg food)**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Common vole (*Microtus arvalis*) Mixed diet	Monocot plants	0.5	1.462	54.2 × 0.15	2.5× 0.53	1	7.87	8.63
	Dicot plants	0.5	1.462	28.7 × 0.15	2.5 x 0.53	1	4.17	
	whole diet						12.04	

Based on refinements, acceptable risk for small herbivorous mammals exposed following the application of Propamocarb hydrochloride may be concluded.

Cymoxanil

In the Tier I risk assessment; unacceptable risk was obtained for next mammals for Cymoxanil:

Potato

- Small herbivorous mammal "vole" at BBCH ≥ 40: TER_{lt} value is below the trigger of 5.
- Large herbivorous mammal "lagomorph at BBCH 10-40: TER_{lt} value is below the trigger of 5.

A further refinement of the long-term risk is needed. In order to refine the risk assessment, the following parameters refined below were considered.

DT_{50}

According to Monograph, six unprotected residue decline trials (please, refer to B.7.61) on lettuce can be used to refine the assessment regarding the herbivorous mammals. In this context, it was shown that there is no accumulation of residue on lettuce plants due to repeated applications since the residue level before the 4th application was below the limit of detection. Hence, an estimated **DT_{50} of 2 days** seems a more appropriate and still worst-case assumption for the residue calculation in vegetation.

TWA

In the Tier I, the default 21d twa values used is 0.53. However, since the DT_{50} is lower than 10 days, the 21d-twa value was recalculated considering the mean DT_{50} of 2 days and the resulting value is **0.14** that will be used in the higher-tier assessment.

MAF

Considering the half-life of 2 days, the maximum of 6 applications with a minimum spray interval of 7 day, the refined Multiple Application Factor (MAF) was calculated to be **1.10** (MAF_m for the long-term risk assessment). The calculations were performed according to the formula of the EFSA/2009/1438.

PD and FIR/bw

Same approach for Propamocarb hydrochloride is used for Cymoxanil in case of vole. A refinement based on PD and FIR/bw was also performed by the Applicant.

Table 9.3-7: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of PROSIM in potato– refined parameters (*) are further described and justified in the text

Intended use	**Potato**
Active substance/product	Cymoxanil
Application rate (g/ha)	6 x 125
Reprod. toxicity (mg/kg bw/d)	10.5
TER criterion	5

Focal species	**Food category, % in diet**	**FIR/bw**	**RUD_m× DF*** **(mg/kg food)**	**MAF_m* × TWA***	**PT**	**PD***	**DDD_m** **(mg/kg bw/d)**	**TER_{lt}**
Rabbit (*Oryctolagus cuniculus*) BBCH 10-40	100% Non-grass Herbs	0.50	28.7[1] × 1.0[1]	1.10[2] x 0.14[2]	1.0	1.0	0.28	38.0
Common vole (*Microtus arvalis*) BBCH ≥ 40	50% grass and cereals	1.33	54.2[1] × 0.3[1]	2.5 x 0.53	1.0	0.5	1.79	5.5
	50% non-grass herbs	1.62	28.7[1] x 0.3[1]	1.10[2] x 0.14[2]	1.0	0.5	0.13	
						Total	1.93	

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

[1]According to Appendix A of EFSA/2009/1438.

[2]MAF and ftwa refinement from residue trials from *Monograph of Cymoxanil*.

zRMS comments:

zRMS do not agree with calculation of FIR/bw provided by the applicant. Additional calculations for diets consisting of 50% of monocots and 50% of dicots is calculated by the zRMS and is presented below:

BW vole (g)	**DEE (kj)**	**RUD unit**	**PD**	**FE (kJ/g dry)**	**Moisture Fraction**	**Assimilation efficiency fraction**	**$FE_{total\ fresh}$ (kJ/g fresh weight)**	**$FIR_{total\ fresh}$ (g fresh weight/d)**	**FIR/BW**
25	65.09	Grass + cereals	0.5	17.6	0.764	0.47	1.781	36.55	1.462
		Non-grass herbs	0.5	17.8	0.881	0.76			
25	65.09	Grass + cereals	0.75	17.6	0.764	0.47	1.867	34.87	1.395
		Non-grass herbs	0.25	17.8	0.881	0.76			

Based on the calculation provided above the diet consisting of 50% monocots and 50% dicots the FIR/bw is 1.462. Further zRMS using the EU agreed diet and respective FIR/bw of 1.462, as calculated above. Deposition factor of 0.3 at first stage has been used, in line with EFSA (2009). zRMS agrees with the risk assessment provided with consideration refined parameters use such as the mean DT50 of 2 days and the resulting value is 0.14 and MAFm 1.10 (for the long-term risk assessment). In addition, **DF of 0.15** (the interception is 85 %.) was used for BBCH **40-89.**

Intended use	Potatoes							
Active substance/product								
Application rate (g/ha)	6 × 125							
Reprod. toxicity (mg/kg bw/d)	10.5							
TER criterion	5							
Focal species	**Food category, % in diet**	**PD**	**FIR/bw**	**RUD_m × DF (mg/kg food)**	**MAF_m × TWA x DF**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Common vole (*Microtus arvalis*) Mixed diet	Monocot plants	0.5	1.462	54.2 × 0.15	2.5× 0.53	1	0.98	10.5
	Dicot plants	0.5	1.462	28.7 × 0.15	1.102 x 0.142	1	0.027	
	whole diet						1	

The TER_{LT} value is above trigger of 5 indicating a long-term risk assessment from exposure of cymoksanil.

Weight of evidence:

In addition, in view of the invasive behaviour of vole, in many regions voles are regarded as pest organisms (Jacob J and Tkadlec E, 2010). At high population densities, the species may strongly invade crops and is considered the most serious vertebrate pest of cultivated fields in continental Europe. In case of crop infestations, voles are usually target of large scale rodenticide applications.

Considering the above, crop specific argumentation proving that vole is not relevant could be evaluated and accepted.

The Applicant considers that the risk for small omnivorous mammals and large herbivorous mammals has been demonstrated to be acceptable, the risk assessment for "vole" is assumed to be covered by the assessment of the other mammalian species.

zRMS comments:

<u>The refined combined long-term risk assessment</u>

zRMS added the combined long-term risk assessment for mammals based on TER_{mix} approach based on the refined TER_{LT} value for each active substances.

The relevant calculations are provided below:

TER_{mix} approach for combined long-ter risk assessment to small mammals (vole and wood mouse).

Compound						
Propamocarb hydrochloride		**Cymoxanil**				
TER	**1/TER**	**TER**	**1/TER**	**Σ1/TER**	**Σ1/TER-**	**Trigger**
8.63[1)]	0.11	10.5 [1)]	0.09	0.2 [1)]	5.0 [1]	5
8.1 [2)]	0.12	10.1 [2)]	0.1	0.22 [2)]	**4.54 [2]**	5
9.72 [3)]	0.10	12.0 [3)]	0.083	0.183 [3)]	5.46 [3]	5

1) Refined TER_{LT} values for both active substances for vole
2) Tier 1 TER_{LT} for wood mouse
3) Refined parameters for wood mouse PT=0.84 (90 [th], wood mouse consumers only, Prosper-2010) Crocker et al. 1998, Finch et al. 2006, Prosser 2010) Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009; April 2020

The calculated TER_{mix} achieved the trigger of 5 for vole from long-term combined risk exposed to the mixture of Propamocarb hydrochloride and Cymoxanil indicating acceptable risk in case of mixture toxicity of small herbivorous mammal – vole for the application up to BBCH 89 instead of 95.
As only one application will be possible between BBCH 89 and BBCH 95 zRMS considers the risk acceptable in the whole range of growth stages with high probability.
However, final conclusion should be made on cMS level according to their agricultural practice.

In case of wood mouse and rabbit the combined risk assessment is considered acceptable.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a Kfoc of 718.81 (EFSA Scientific Report (2006) 78, 1-80), Propamocarb hydrochloride belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	2500	Effective AR = AR x MAF		
Acute toxicity (mg/kg bw)	=	> 1330	quotient	=	0.002
Reprod. toxicity (mg/kg bw/d)	=	104	quotient	=	0.024

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not the critical value of 3000 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

With a K(f)oc of 43.6 (EFSA Scientific Report (2008) 167, 1-116, arithmetic mean, n = 4), Cymoxanil belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	312.5	Effective AR = AR x MAF		
Acute toxicity (mg/kg bw)	=	760	quotient	=	0.411
Reprod. toxicity (mg/kg bw/d)	=	10.5	quotient	=	29.762

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not the critical value of 50 for at least one-use scenario, a quantitative risk assessment (calculation of TER values) is not necessary.

zRMS comments:

As a generic approach, the EFSA Guidance Document states that no specific calculations of exposure and TER are necessary for the puddle scenario 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). The ratio is below the trigger value indicating an acceptable risk and no further consideration is needed.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Propamocarb hydrochloride amounts to -2.9, -1.2 and 0.67 at pH 2, 7 and 9 respectively and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of cymoxanil amounts to 0.67-0.59 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

Regarding Propamocarb hydrochloride, according to the first-tier assessment for potato, the TER_a and TER_{lt} are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the PROSIM presents an acceptable acute and long-term risk to mammals for these uses except to vole for long-term risk assessment. After PD, FIR/bw, DT_{50}, MAF and ftwa refinement, no unacceptable risk was obtained.

Regarding Cymoxanil, according to the first-tier assessment for potato, the TER_a and TER_{lt} are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the PROSIM presents an acceptable acute and long-term risk to mammals for these uses except to vole and lagomorph for long-term risk assessment. After PD, FIR/bw, DT_{50}, MAF and ftwa refinement, no unacceptable risk was obtained.

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

No data available.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Propamocarb hydrochloride, Cymoxanil and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of PROSIM were not evaluated as part of the EU assessment of Propamocarb hydrochloride and Cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

However, the provision of further data on the PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Fish				
Rainbow trout *(Onchoryhynchus mykiss)*	Propamocarb hydrochloride	96 h, ss	Mortality, LC_{50} > 99 mg a.s./L	EFSA Scientific Report (2006) 78, 1-80
Bluegill Sunfish *(Lepomis macrochirus)*	Propamocarb hydrochloride	96 h, s	**Mortality, LC_{50} > 92 mg a.s./L**	
Bluegill sunfish *(Lepomis macrochirus)*	Propamocarb hydrochloride	32 d, f	**NOEC > 6.3 mg a.s./L**	
Aquatic invertebrates				
Daphnia magna	Propamocarb hydrochloride	48 h, ss	Mortality, **EC_{50} > 100 mg a.s./L**	EFSA Scientific Report (2006) 78, 1-80
Daphnia magna	Propamocarb hydrochloride	21 d, s	**NOEC = 12.3 mg a.s./L**	
Algae				
Pseudokirchneriella subcapitata	Propamocarb hydrochloride	72 h	**Growth Rate, EC_{50} > 85 mg a.s./L**	EFSA Scientific Report (2006) 78, 1-80
Higher plant				
Lemna gibba	Propamocarb hydrochloride	14 d, s	**Frond No., EC50 > 18 mg a.s./L**	EFSA Scientific Report (2006) 78, 1-80
Higher-tier studies (micro- or mesocosm studies)				
Not required.				

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 – Cymoxanil and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Lepomis macrochirus	Cymoxanil	96 h, s	**LC_{50} = 29 mg a.s./L $_{mm}$**	EFSA Scientific Report (2008) 167, 1-116
Oncorhynchus mykiss	Cymoxanil	90 d, f	**NOEC = 0.044 mg a.s./L_{mm}**	
Oncorhynchus mykiss	IN-T4226	96 h, ss	**LC_{50} > 111 mg/L_{mm}**	
Oncorhynchus mykiss	IN-KQ960	96 h, s	**LC_{50} > 120 mg/L_{n}**	
Oncorhynchus mykiss	IN-U3204	96 h, ss	**LC_{50} > 97 mg/L_{mm}**	
Oncorhynchus mykiss	IN-W3595	96 h, s	**LC_{50} > 130 mg/L_{mm}**	
Aquatic invertebrates*				
Daphnia magna	Cymoxanil	48 h, s	**LC_{50} > 27 mg a.s./L_{mm}**	EFSA Scientific Report (2008) 167, 1-116
Daphnia magna	Cymoxanil	21 d, ss	**NOEC = 0.067 mg a.s./L_{mm}**	
Daphnia magna	IN-T4226	48 h, ss	**LC_{50} > 116 mg/L_{mm}**	
Daphnia magna	IN-KQ960	48 h, s	**LC_{50} = 0.8 mg/L_{mm}**	
Daphnia magna	IN-KQ960	21 d, ss	**NOEC = 0.302 mg/L_{mm}**	
Daphnia magna	IN-U3204	48 h, ss	**LC_{50} = 100 mg/L_{mm}**	
Daphnia magna	IN-W3595	48 h, s	**LC_{50} > 126 mg/L_{mm}**	
Algae				
Anabaena flos-aquae	Cymoxanil	96 h, s	**E_rC_{50} = 0.254 mg a.s./L_{im}** E_bC_{50} = 0.122 mg a.s./L	EFSA Scientific Report (2008) 167, 1-116
Anabaena flos-aquae	IN-T4226	96 h, s	**E_rC_{50} = 35.9 mg/L_{n}** E_bC_{50} = 25.8 mg/L	
Anabaena flos-aquae	IN-W3595	96 h, s	**E_rC_{50} = 19.9 mg/L_{n}** E_bC_{50} = 12.7 mg/L	
Higher plant				
Lemna gibba	Cymoxanil	14 d, s	**E_rC_{50} > 0.7 mg a.s./L_{im}**	EFSA Scientific Report (2008) 167, 1-116
Higher-tier studies (micro- or mesocosm studies)				
Not relevant.				

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

* since no toxicity information is available for the IN-JX915, IN-R3273, IN-KP533 and fraction M5 metabolites, it was assumed that the toxicity is by a factor of 100 higher than the toxicity of the active substance.

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	PROSIM	96 h, ss	**LC_{50} = 22.34 mg/L** nom **LC_{50} = 20.74 mg/L***	KCP 10.2.1-01 Nierzedska, E. 2019, Report No. W/89/18
Daphnia magna	PROSIM	48 h, ss	**EC_{50} > 100 mg/L** nom **EC_{50} > 96.52 mg/L***	KCP 10.2.1-02 Turek, T. 2019, Report No. W/86/18
Raphidocelis subcapitata (*P. subcapitata)*	PROSIM	72 h, s	**E_rC_{50} = 90.41 mg/L** nom E_yC_{50} = 19.65 mg/L nom **E_rC_{50} = 82.82 mg/L*** E_yC_{50} = 18.00 mg/L*	KCP 102.1.-03 Turek, T. 2019, Report No. W/87/18
Lemna gibba	PROSIM	7 d, ss	Frond number **E_rC_{50} = 531.8 mg/L** nom E_yC_{50} = 192.2 mg/L nom Dry weight E_rC_{50} = 618.7 mg/L nom E_yC_{50} = 154.6 mg/L nom Frond number **E_rC_{50} = 478.51 mg/L*** E_yC_{50} = 172.94 mg/L* Dry weight E_rC_{50} = 556.71 mg/L* E_yC_{50} = 139.11 mg/L*	KCP 10.2.1-04 Turek, T. 2019, Report No. W/88/18
Higher-tier studies (micro- or mesocosm studies)				
Not relevant.				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
* Product endpoints recalculated by zRMS suggestion following the specified procedure for formulation tests with more than one active substance, when all active substances have been analytically measured; agreed and detailed in the "*Outcome of pesticides peer review meeting on recurring issues in ecotoxicology*" (EFSA Supporting publication 2019:EN-1673).

9.5.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. Studies were conducted with PROSIM and were also considered for the risk assessment.

9.5.2 Risk assessment

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

PROSIM FORMULATION

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}) for PROSIM and regulatory acceptable concentrations (RAC) for aquatic organisms are given.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PROSIM for each organism group in potato (single/multiple application)

Group		**Fish acute**	**Invertebrate acute**	**Algae**	**Lemna**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		~~22340~~ 20740	~~100000~~ 96520	~~90410~~ 82820	~~531800~~ 478510
AF		100	100	10	10
RAC (µg/L)		~~223.40~~ 207.40	~~1000~~ 965.2	~~9041~~ 8282	~~53180~~ 47851
Drift	**PEC $_{gl-max}$ (µg/L)**				
1 m	24.551/87.215	~~0.110/0.390~~ 0.118/0.421	~~0.025/0.087~~ 0.025/0.090	~~0.003/0.010~~ 0.003/0.011	~~<0.001/0.002~~ 0.001/0.002

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

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

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Propamocarb hydrochloride for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plant**
Test species		***Lepomis macrochirus***	***Lepomis macrochirus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}/E_yC_{50}	E_rC_{50}/E_yC_{50}
(µg/L)		92000	6300	100000	12300	85000	>18000
AF		100	10	100	10	10	10
RAC (µg/L)		920	630	>1000	>1230	8500	1800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	255.830 / 1530.000	0.278 / **1.663**	0.406 / **2.429**	0.256 / **1.530**	0.208 / **1.244**	0.030/0.180	0.142/0.850
Step 2							
N-Europe	26.673 / 74.037	0.029 / 0.080	0.042 / 0.118	0.027 / 0.074	0.022 / 0.060	0.003/0.009	0.015/0.041
S-Europe	46.879 / 134.192	0.051 / 0.146	0.074 / 0.213	0.047 / 0.134	0.038 / 0.109	0.006/0.006	0.026/0.026
Step 3							
D3/ditch	5.243 / 3.092	0.006/0.003	0.008/0.005	0.005/0.003	0.004/0.003	0.001/0.000	0.003/0.002
D4/pond	0.214 / 1.656	0.000/0.002	0.000/0.003	0.000/0.002	0.000/0.001	0.000/0.000	0.000/0.001
D4/stream	4.098 / 2.593	0.004/0.003	0.007/0.004	0.004/0.003	0.003/0.002	0.000/0.000	0.002/0.001
D6 1st crop/ditch	5.185 / 3.077	0.006/0.003	0.008/0.005	0.005/0.003	0.004/0.003	0.001/0.000	0.003/0.002
D6 2nd crop/ditch	5.157 / 34.68	0.006/0.038	0.008/0.055	0.005/0.035	0.004/0.028	0.001/0.004	0.003/0.019
R1/pond	1.095 / 1.694	0.001/0.002	0.002/0.003	0.001/0.002	0.001/0.001	0.000/0.000	0.001/0.001

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant
R1/stream	14.65 / 31.16	0.016/0.034	0.023/0.049	0.015/0.031	0.012/0.025	0.002/0.004	0.008/0.017
R2/stream	5.363 / 21.73	0.006/0.024	0.009/0.034	0.005/0.022	0.004/0.018	0.001/0.003	0.003/0.012
R3/stream	5.119 / 30.68	0.006/0.033	0.008/0.049	0.005/0.031	0.004/0.025	0.001/0.004	0.003/0.017

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Cymoxanil for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher plant**
Test species		***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} 29000	NOEC 44	EC_{50} 27000	NOEC 67	E_rC_{50} 254	E_rC_{50} 700
AF		100	10	100	10	10	10
RAC (µg/L)		290	4.4	270	6.7	25.4	70
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	40.918 / 40.918	0.141/0.141	**9.300/9.300**	0.152/0.152	**6.107/6.107**	**1.611/1.611**	0.585/0.585
Step 2							
N-Europe	1.149 / 0.680	0.004/0.002	0.261/0.155	0.004/0.003	0.171/0.101	0.045/0.027	0.016/0.010
S-Europe	1.149 / 0.803	0.004/0.004	0.261/0.261	0.004/0.004	0.171/0.171	0.045/0.045	0.016/0.016
Step 3							
D3/ditch	0.655 / 0.386	0.002/0.001	0.149/0.088	0.002/0.001	0.098/0.058	0.026/0.015	0.009/0.006
D4/pond	0.026 / 0.015	0.000/0.000	0.006/0.003	0.000/0.000	0.004/0.002	0.001/0.001	0.000/0.000

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Higher plant
D4/stream	0.512 / 0.312	0.002/0.001	0.116/0.071	0.002/0.001	0.076/0.047	0.020/0.012	0.007/0.004
D6 1st crop/ditch	0.648 / 0.409	0.002/0.001	0.147/0.093	0.002/0.002	0.097/0.061	0.026/0.016	0.009/0.006
D6 2nd crop/ditch	0.645 / 0.386	0.002/0.001	0.147/0.088	0.002/0.001	0.096/0.058	0.025/0.015	0.009/0.006
R1/pond	0.068 / 0.065	0.000/0.000	0.015/0.015	0.000/0.000	0.010/0.010	0.003/0.003	0.001/0.001
R1/stream	1.625 / 3.345	0.006/0.012	0.369/0.760	0.006/0.012	0.243/0.499	0.064/0.132	0.023/0.048
R2/stream	0.600 / 1.743	0.002/0.006	0.136/0.396	0.002/0.006	0.090/0.260	0.024/0.069	0.009/0.025
R3/stream	0.640 / 2.542	0.002/0.009	0.145/0.578	0.002/0.009	0.096/0.379	0.025/0.100	0.009/0.036

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

Metabolites

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-U3204 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Fish acute**	**Inverteb. acute**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***
Endpoint		LC_{50}	EC_{50}
(µg/L)		97000	100000
AF		100	100
RAC (µg/L)		970	1000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	20.129 / 20.129	0.021 / 0.021	0.020 / 0.020
Step 2			
N-Europe	0.284 / 0.168	< 0.001 / < 0.001	< 0.001 / < 0.001
S-Europe	0.284 / 0.203	< 0.001 / < 0.001	< 0.001 / < 0.001

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-W3595 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		130000	126000	19900
AF		100	100	10
RAC (µg/L)		1300	1260	1990
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	10.299 / 61.794	0.008 / 0.048	0.008 / 0.049	0.005 / 0.031
Step 2				
N-Europe	0.244 / 0.239	< 0.001 / < 0.001	< 0.001 / < 0.001	< 0.001 / < 0.001
S-Europe	0.406 / 0.418	< 0.001 /< 0.001	< 0.001 / < 0.001	< 0.001 / < 0.001

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

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-KQ960 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Fish acute**	**Inverteb. acute**	**Inverteb. prolonged**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***
Endpoint		LC_{50}	EC_{50}	NOEC
(µg/L)		120000	800	302
AF		100	100	10
RAC (µg/L)		1200	8	30.2
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	9.284 / 55.706	0.008 / 0.046	**1.161** / **6.963**	0.307 / **1.845**
Step 2				
N-Europe	0.446 / 1.101	< 0.001 / 0.001	0.056 / 0.138	0.015 / 0.036
S-Europe	0.726 / 1.738	0.001 / 0.001	0.091 / 0.217	0.024 / 0.058

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

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-T4226 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		111000	116000	35900
AF		100	100	10
RAC (µg/L)		1110	1160	3590
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	4.097 / 24.583	0.004 / 0.022	0.004 / 0.021	0.001 / 0.007
Step 2				
N-Europe	0.138 / 0.378	< 0.001 / < 0.001	< 0.001 / < 0.001	< 0.001 / < 0.001
S-Europe	0.222 / 0.706	< 0.001 / 0.001	< 0.001 / 0.001	< 0.001 / < 0.001

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

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-JX915 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Inverteb. acute**
Test species		***Daphnia magna***
Endpoint		EC_{50}

Group		**Inverteb. acute**
(µg/L)		270
AF		100
RAC (µg/L)		2.7
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**	
Step 1		
	25.149 / 25.148	**9.314 / 9.314**
Step 2		
N-Europe	0.605 / 0.380	0.224 / 0.141
S-Europe	0.605 / 0.548	0.224 / 0.203

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

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for IN-R3273 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Inverteb. acute**
Test species		***Daphnia magna***
Endpoint		EC_{50}
(µg/L)		270
AF		100
RAC (µg/L)		2.7
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**	
Step 1		
	2.576 / 15.456	0.954 / **5.724**
Step 2		
N-Europe	0.130 / 0.536	0.048 / 0.199
S-Europe	0.229 / 1.038	0.048 / 0.384

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

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) fo IN-KP533 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Inverteb. acute**
Test species		***Daphnia magna***
Endpoint		EC_{50}
(µg/L)		270
AF		100
RAC (µg/L)		2.7
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**	
Step 1		
	9.738 / 58.426	**3.607 / 21.639**

Group		**Inverteb. acute**
Step 2		
N-Europe	0.258 / 0.673	0.096 / 0.249
S-Europe	0.432 / 1.288	0.160 / 0.477

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

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fraction M5 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of PROSIM in potato

Group		**Inverteb. acute**
Test species		***Daphnia magna***
Endpoint		EC_{50}
(µg/L)		270
AF		100
RAC (µg/L)		2.7
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	
Step 1		
	9.791 / 9.791	3.626 / 3.626
Step 2		
N-Europe	0.264 / 0.161	0.098 / 0.060
S-Europe	0.264 / 0.215	0.098 / 0.080

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

~~Risk assessment based on combination of active substances~~

~~Combination toxicology is assessed for formulations containing more than one active substance, and for combinations of products, which are made according to the Instructions for Use as a tank mixture. Based on the precautionary principle, concentration addition is assumed.~~

~~For pesticides the TER (Toxicity Exposure Ratio) is used as a standard in the risk assessment. The TER must be higher than a trigger value to comply with the standards. The combination risk of formulations containing more than one active substance and for tank mixtures is calculated as follows:~~

~~When for each substance the trigger values are equal, the combined TER value can be calculated according to:~~

~~$TER_{combi} = trigger/((trigger/TER_{substance\ 1})+(trigger/TER_{substance\ 2}))$~~

~~An acceptable risk is expected when $TER_{combi} >$ trigger.~~

~~**Active susbtance / species**~~	~~**Test system**~~	~~**Endpoint (mg a.s./L)**~~
~~**Propamocarb**~~		
~~*Lepomis macrochirus*~~	~~LC_{50} 96h~~	~~>92~~
~~*Daphnia magna*~~	~~EC_{50} 48h~~	~~>100~~
~~*P. subcapitata*~~	~~EC_{50} 72h~~	~~>85~~
~~**Cymoxanil**~~		

Lepomis macrochirus	LC_{50} 96h	29
Daphnia magna	EC_{50} 48h	>27
Anabaena flos-aquae	E_rC_{50} 72h	0.254

According to the endpoints and formulations described above the risk assessment based on combination is performed below :

Aquatic organisms	**TER_{combi}***	**Trigger**
Fish	667.45	100
Aquatic invertebrates	722.30	100
Algae	163.87	10

PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*Based on worst case of PECsw for Propamocarb (0.134192 mg/L at Step 2 for SEU scenario) and Cymoxanil (0.001149 mg/L at Step 2 for SEU and NEU scenario).

Acceptable risk for combination is expected for PROSIM since TER_{combi} > trigger for each organisms.

Risk assessment based on combination of active substances according to AGD 2013 – Updated March 2021

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substance present in the formulation. In addition, as demonstrated in the short-term studies here above there are no indications for interactions of the active substances (no synergisms or additional toxicity occurs due to the co-formulants) given that the formulation does not cause an (unexpected) increased toxicity compared to the active substances. An evaluation of the risk posed by the intact formulation is therefore relevant only for the acute/short-term assessment. The long-term risk was assessed considering data for the active substances in the formulation and no chronic combined risk assessment has been performed.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA.

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\ CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ FLUPEN}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, *i.e.* if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Active susbtance / species	**Test system**	**Endpoint (mg a.s./L)**
Propamocarb		
Lepomis macrochirus	LC_{50} 96h	>92
Daphnia magna	EC_{50} 48h	>100
P. subcapitata	EC_{50} 72h	>85
Lemna gibba	Frond No., EC_{50} 14 d, s	> 18
Cymoxanil		
Lepomis macrochirus	LC_{50} 96h	29
Daphnia magna	EC_{50} 48h	>27
Anabaena flos-aquae	E_rC_{50} 72h	0.254
Lemna gibba	E_rC_{50} 14 d, s	> 0.7

The calculated MDR values are between 0.2 and 5 for *Daphnia magna* (see Table 9.5-15), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for aquatic invertebrates. No synergisms or additional toxicity occurs due to the co-formulants for these species. However, antagonistic (less than additive) mixture toxicity is indicated for algae and *Lemna* since MDR is <0.2. The apparent antagonism for fish (toxicity of the formulation lower than expected) can be explained by the fact that endpoints for individual active substances are "higher than" values.

Table 9.5-15: Summary of results obtained in the studies with the formulated product PROSIM and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of PROSIM (LC_{50} PROSIM or EC_{50} PROSIM) (mg/L)	Measured toxicity of PROSIM (converted to be a.i. based) (LC_{50} PROSIM or EC_{50} PROSIM) (mg a.s./L)	Calculated mixture toxicity[a] LC_{50} mix-CA or EC_{50} mix-CA	Model deviation ratio (MDR = EC_{50} mix-CA / EC_{50} PROSIM)
Fish	LC_{50}, acute, 96 h	22.34	9.452	74.111	7.841
D. magna	EC_{50}, acute, 48 h	100	42.309	76.899	1.818
Algae	E_rC_{50}, 72 h	90.41	38.252	2.233	0.058
Lemna gibba	E_rC_{50}, 14 d	531.8	225.000	4.805	0.021

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Propamocarb hydrochloride (400 g/L) and Cymoxanil (50 g/L) within the formulation.

The calculated factors fall outside 0.8-1.2 for algae (see Table 9.5-16), indicating that the mixture composition in the formulation study giving the measured mixture toxicity is not similar to the mixture composition at the PEC_{mix} for these organisms.

Table 9.5-16: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PROSIM) LC_{50} mix-CA or EC_{50} mix-CA	Calculated mixture toxicity (a.s. in PEC_{mix})[b] LC_{50} mix-CA or EC_{50} mix-CA at lower exposure tier	Factors (EC_{50} mix-CA (a.s. in PROSIM)/EC_{50} mix-CA (a.s. in PEC_{mix})) at lower exposure tier
Fish	LC_{50}, acute, 96 h	74.111	74.111	0.960
D. magna	EC_{50}, acute, 48 h	76.899	76.899	0.952
Algae	E_rC_{50}, 72 h	2.233	2.232	0.797
Lemna gibba	E_rC_{50}, 14 d	4.805	4.805	0.847

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Propamocarb hydrochloride (400 g/L) and Cymoxanil (50 g/L) within the formulation.

[b] The mixture toxicity of the formulation was calculated based on the mixture composition at the PEC_{mix} for Propamocarb hydrochloride (0.034680 mg/L at Step 3 for D6 2nd crop ditch scenario) and Cymoxanil (0.003345 mg/L at Step 3 for R1 stream scenario).

With regard to the mixture risk assessment EFSA further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'. Regarding PROSIM, Cymoxanil is clearly driving the chronic risk for algae, the studies performed with the formulated product reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – comes above 90 % from the toxicity per fraction of a single a.s. (TUi). Regarding fish, *Daphnia* and *Lemna*, no active substance is clearly driving the chronic risk. The studies performed with the formulated product do not reflect the toxicity of one particular active substance, as the formulation toxicity

- endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-17).

Table 9.5-17: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC50 / EC50 [mg/L]		
		Calculated mixture toxicity (a.s. in PROSIM) LC50 mix-CA or EC50 mix-CA	Calculated toxicity per fraction of PROSIM (based on each a.s.) (1/TUi)a	Deviation from mixture toxicity (1-ECx mix-CA x (1/ECx mix-CA - TUi)) [%]
Fish	LC50, acute, 96 h	74.111	Propamocarb hydrochloride: 103.500 Cymoxanil: 261.000	Propamocarb hydrochloride: 71.60% Cymoxanil: 28.40%
D. magna	EC50, acute, 48 h	76.899	Propamocarb hydrochloride: 112.500 Cymoxanil: 243.000	Propamocarb hydrochloride: 68.35% Cymoxanil: 31.65%
Algae	ErC50, static, 72 h	2.233	Propamocarb hydrochloride: 95.625 Cymoxanil: 2.280	Propamocarb hydrochloride: 2.33% **Cymoxanil: 97.67%**
Lemna gibba	ErC50, 14 d	4.805	Propamocarb hydrochloride: 20.250 Cymoxanil: 6.300	Propamocarb hydrochloride: 2.0% Cymoxanil: 76.27%

[a] – TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ PROSIM}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on nominal contents of Propamocarb hydrochloride (400 g/L) and Cymoxanil (50 g/L) within the formulation.

Table 9.5-18: Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (triggervalue) decisive for the specific endpoint/exposure scenario combination from EFSA AGD in potato for fish and *Daphnia*

Exposure	Lower exposure tier	
	Propamocarb hydrochloride	Cymoxanil
Exposure tier (FOCUS step)	Step 3 (D6 2nd crop ditch scenario)	Step 3 (R1 stream scenario)
PECsw [mg a.s./L]	0.034680	0.003345
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.038025	
Aquatic organisms	Fish	*Daphnia*
ETRmix = PECmix/ECx PROSIM	0.004	0.001
Trigger	0.01	

Table 9.5-19: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in potato for *Lemna gibba*

Exposure	Lower exposure tier	
	Propamocarb hydrochloride	Cymoxanil
Exposure tier (FOCUS step)	Step 3 (D6 2nd crop ditch scenario)	Step 3 (R1 stream scenario)
PECsw [mg a.s./L]	0.034680	0.003345
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.038025	
Calculated mixture toxicity (a.s. in PECmix) (ECx mix, CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	5.671	
ETRmix = PECmix/ECx PROSIM	0.007	
Trigger	0.10	

The risk assessment is conducted by taking into account FOCUS PECsw values for Propamocarb hydrochloride and Cymoxanil (Step 3). No unacceptable risk to all organisms are expected from the exposure to the combined active substances following proposed uses of the product.

These conditions are assessed following a step-wise approach. A detailed description of this approach is presented below:

Fish

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.);	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2

9	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECx-PROSIM) by means of the model deviation ratio (MDR = ECxmix CA/ECx-PROSIM).	MDR > 5	Please refer to table 9.5-15	Go to 10
10	Carefully recheck the apparent synergism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of heterogeneous input data (a.s.) and of co-formulants ignored in the CA calculation. Does the apparent synergism remain?	Yes		Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPROSIM) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECx-PROSIM with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix CA (a.s. in product)/ECx mix CA (a.s. in PECmix) is 0.8-1.2	Please refer to table 9.5-16	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix} < trigger	Please refer to table 9.5-18	Low risk

Daphnia

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.);	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2

2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECx PROSIM) by means of the model deviation ratio (MDR = ECxmix CA/ECx PROSIM).	MDR = 0.2–5 (CA approximately holds for the mixture)	Please refer to table 9.5-15	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPROSIM) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECx PROSIM with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix CA (a.s. in product)/ECx mix CA (a.s. in PECmix) is 0.8–1.2	Please refer to table 9.5-16	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix} < trigger	Please refer to table 9.5-18	Low risk

Algae

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.);	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECx PROSIM) by means of the model deviation ratio (MDR = ECxmix CA/ECx PROSIM).	MDR <0.2 (Antagonistic (less than additive) mixture toxicity)	Please refer to table 9.5-15	Go to 9

9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	No (measured mixture toxicity plausible	Some explanations could explain the apparent antagonism between measured mixture toxicity data regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation: - The use of endpoints from different species (*P. subcapitata* and *Anabaena flos aquae*).	Go to 3
9	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPROSIM) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECx PROSIM with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix CA (a.s. in product)/ECx mix CA (a.s. in PECmix) is <0.8 or >1.2	Please refer to table 9.5-16	Go to 5
5	Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECx PPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?	Deviation from mixture toxicity = 1- ECx mix CA x (1/ECx mix CA TUi) [%] >=90% for Cymoxanil	Please refer to table 9.5-17	Go to 6
6	Conduct a RA based on single substance toxicity data (ECx a.s.) for the identified 'driver' of mixture toxicity, with the exposure toxicity ratio (ETRa.s.) being defined as the PECa.s. divided by the measured ECx a.s. and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	**Covered by active substance assessment**		

Lemna gibba

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.);	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECx PROSIM) by means of the model deviation ratio (MDR = ECxmix CA/ECx PROSIM).	MDR <0.2 (Antagonistic (less than additive) mixture toxicity)	Please refer to table 9.5-15	Go to 9
9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	Yes (measured mixture toxicity not plausible)		Go to 8
8	Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8	If ETR_{mix} < trigger	Please refer to table 9.5-19	Low risk

Risk assessment based on combination of active substances according to AGD 2013 – Updated December 2022 - Product endpoints recalculated by zRMS suggestion following the "Outcome of pesticides peer review meeting on recurring issues in ecotoxicology" (EFSA Supporting publication 2019:EN-1673).

The calculated MDR values are between 0.2 and 5 for *Daphnia magna* (see Table 9.5-20), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for aquatic invertebrates. No synergisms or additional toxicity occurs due to the co-formulants for these species. However, antagonistic (less than additive) mixture toxicity is indicated for algae and *Lemna* since MDR is <0.2. The apparent antagonism for fish (toxicity of the formulation lower than expected) can be explained by the fact that endpoints for individual active substances are "higher than" values.

Table 9.5-20: Summary of results obtained in the studies with the formulated product PROSIM and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of PROSIM (LC_{50} PROSIM or EC_{50} PROSIM) (mg/L)	Measured toxicity of PROSIM (converted to be a.i. based) (LC_{50} PROSIM or EC_{50} PROSIM) (mg a.s./L)	Calculated mixture toxicity[a] LC_{50} mix-CA or EC_{50} mix-CA	Model deviation ratio (MDR = EC_{50} mix-CA / EC_{50} PROSIM)
Fish	LC_{50}, acute, 96 h	20.74	8.775	74.111	8.446
D. magna	EC_{50}, acute, 48 h	96.52	40.837	76.899	1.883
Algae	E_rC_{50}, 72 h	82.82	35.040	2.233	0.064
Lemna gibba	E_rC_{50}, 14 d	478.51	202.453	4.805	0.024

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Propamocarb hydrochloride (400 g/L) and Cymoxanil (50 g/L) within the formulation.

The calculated factors fall outside 0.8-1.2 for algae (see Table 9.5-21), indicating that the mixture composition in the formulation study giving the measured mixture toxicity is not similar to the mixture composition at the PEC_{mix} for these organisms.

Table 9.5-21: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PROSIM) LC_{50} mix-CA or EC_{50} mix-CA	Calculated mixture toxicity (a.s. in PEC_{mix})[b] LC_{50} mix-CA or EC_{50} mix-CA at lower exposure tier	Factors (EC_{50} mix-CA (a.s. in PROSIM)/EC_{50} mix-CA (a.s. in PEC_{mix})) at lower exposure tier
Fish	LC_{50}, acute, 96 h	74.111	77.239	0.960
D. magna	EC_{50}, acute, 48 h	76.899	80.786	0.952
Algae	E_rC_{50}, 72 h	2.233	2.801	0.797
Lemna gibba	E_rC_{50}, 14 d	4.805	5.671	0.847

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Propamocarb hydrochloride (400 g/L) and Cymoxanil (50 g/L) within the formulation.

[b] The mixture toxicity of the formulation was calculated based on the mixture composition at the PEC_{mix} for Propamocarb hydrochloride (0.034680 mg/L at Step 3 for D6 2nd crop ditch scenario) and Cymoxanil (0.003345 mg/L at Step 3 for R1 stream scenario).

With regard to the mixture risk assessment EFSA further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'. Regarding PROSIM, Cymoxanil is clearly driving the chronic risk for algae, the studies performed with the formulated product reflect the toxicity of

one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – comes above 90 % from the toxicity per fraction of a single a.s. (TUi). Regarding fish, *Daphnia* and *Lemna*, no active substance is clearly driving the chronic risk The studies performed with the formulated product do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-22).

Table 9.5-22: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC50 / EC50 [mg/L]		
		Calculated mixture toxicity (a.s. in PROSIM) LC50 mix-CA or EC50 mix-CA	Calculated toxicity per fraction of PROSIM (based on each a.s.) (1/TUi)a	Deviation from mixture toxicity (1-ECx mix-CA x (1/ECx mix-CA - TUi)) [%]
Fish	LC50, acute, 96 h	74.111	Propamocarb hydrochloride: 103.500 Cymoxanil: 261.000	Propamocarb hydrochloride: 71.6% Cymoxanil: 28.4%
D. magna	EC50, acute, 48 h	76.899	Propamocarb hydrochloride: 112.500 Cymoxanil: 243.000	Propamocarb hydrochloride: 68.4% Cymoxanil: 31.7%
Algae	ErC50, static, 72 h	2.233	Propamocarb hydrochloride: 95.625 Cymoxanil: 2.286	Propamocarb hydrochloride: 2.3% **Cymoxanil: 97.7%**
Lemna gibba	ErC50, 14 d	4.805	Propamocarb hydrochloride: 20.250 Cymoxanil: 6.300	Propamocarb hydrochloride: 23.7% Cymoxanil: 76.3%

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ PROSIM}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on nominal contents of Propamocarb hydrochloride (400 g/L) and Cymoxanil (50 g/L) within the formulation.

Table 9.5-23: Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (triggervalue) decisive for the specific endpoint/exposure scenario combination from EFSA AGD in potato for fish

Exposure	**Lower exposure tier**	
	Propamocarb hydrochloride	**Cymoxanil**
Exposure tier (FOCUS step)	Step 3 (D6 2nd crop ditch scenario)	Step 3 (R1 stream scenario)
PECsw [mg a.s./L]	0.034680	0.003345
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.038025	
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	8.775	
ETRmix = PECmix/ECx PROSIM	0.004	
Trigger	0.01	

Table 9.5-24: Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (triggervalue) decisive for the specific endpoint/exposure scenario combination from EFSA AGD in potato for *Daphnia*

Exposure	Lower exposure tier	
	Propamocarb hydrochloride	Cymoxanil
Exposure tier (FOCUS step)	Step 3 (D6 2nd crop ditch scenario)	Step 3 (R1 stream scenario)
PECsw [mg a.s./L]	0.034680	0.003345
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.038025	
Toxicity of the product (a.s. based) (ECx PPP) [mg a.s./L]	40.837	
ETRmix = PECmix/ECx PROSIM	0.001	
Trigger	0.01	

Table 9.5-25: Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (triggervalue) decisive for the specific endpoint/exposure scenario combination from EFSA AGD in potato for *Lemna*

Exposure	Lower exposure tier	
	Propamocarb hydrochloride	Cymoxanil
Exposure tier (FOCUS step)	Step 3 (D6 2nd crop ditch scenario)	Step 3 (R1 stream scenario)
PECsw [mg a.s./L]	0.034680	0.003345
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.038025	
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	5.671	
ETRmix = PECmix/ECx PROSIM	0.0067	
Trigger	0.1	

The risk assessment is conducted by taking into account FOCUS PECsw values for Propamocarb hydrochloride and Cymoxanil (Step 3). No unacceptable risk to all organisms are expected from the exposure to the combined active substances following proposed uses of the product.

These conditions are assessed following a step-wise approach. A detailed description of this approach is presented below:

Fish

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.):	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPROSIM) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPROSIM).	MDR > 5	Please refer to table 9.5-20	Go to 10
10	Carefully recheck the apparent synergism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of heterogeneous input data (a.s.) and of co-formulants ignored in the CA calculation. Does the apparent synergism remain?	Yes		Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPROSIM) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPROSIM with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is 0.8-1.2	Please refer to table 9.5-21	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix} < trigger	Please refer to table 9.5-23	**Low risk**

Daphnia

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.):	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPROSIM) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPROSIM).	MDR = 0.2–5 (CA approximately holds for the mixture)	Please refer to table 9.5-20	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPROSIM) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPROSIM with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is 0.8-1.2	Please refer to table 9.5-21	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If ETR_{mix} < trigger	Please refer to table 9.5-24	**Low risk**

Algae

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.):	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPROSIM) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPROSIM).	MDR <0.2 (Antagonistic (less than additive) mixture toxicity)	Please refer to table 9.5-20	Go to 9
9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	No (measured mixture toxicity plausible	Some explanations could explain the apparent antagonism between measured mixture toxicity data regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation: - The use of endpoints from different species (*P. subcapitata* and *Anabaena flos-aquae*).	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPROSIM) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPROSIM with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is <0.8 or >1.2	Please refer to table 9.5-21	Go to 5
5	Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECx PPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?	Deviation from mixture toxicity = 1-ECx mix-CA x (1/ECx mix-CA-TUi) [%] >=90% for Cymoxanil	Please refer to table 9.5-22	Go to 6

6	Conduct a RA based on single-substance toxicity data (ECx a.s.) for the identified 'driver' of mixture toxicity, with the exposure-toxicity ratio (ETRa.s.) being defined as the PECa.s. divided by the measured ECx a.s. and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	**Covered by active substance assessment**

Lemna gibba

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxPROSIM) and a.s. (ECxa.s.):	Please refer to tables 9.5-1, 9.5-2 and 9.5-3	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxPROSIM) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxPROSIM) by means of the model deviation ratio (MDR = ECxmix-CA/ECxPROSIM).	MDR <0.2 (Antagonistic (less than additive) mixture toxicity)	Please refer to table 9.5-20	Go to 9
9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	Yes (measured mixture toxicity not plausible)		Go to 8
8	Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8	If ETR_{mix} < trigger	Please refer to table 9.5-25	**Low risk**

zRMS comment:

The risk assessment for aquatic organism for both active substance and their metabolites is validated by zRMS.

Propamocarb
For the intended uses on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by a NOEC for *Lepomis macrochirus* of 6300 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

•Cymoxanil
For the intended uses potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by a NOEC for *Oncorhynchus mykiss* of 44 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-3 scenarios. Therefore, no further assessment is necessary.

Metabolites of Cymoxanil: for the intended use on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

PROSIM: for the intended use on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

The mixture toxicity assessment is considered acceptable.

9.5.3 Overall conclusions

• Propamocarb
For the intended uses on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by an NOEC for *Lepomis macrochirus* of 6300 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-2 scenarios. Therefore, no further assessment is necessary.

• Cymoxanil
For the intended uses potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by an NOEC for *Oncorhynchus mykiss* of 44 µg/L in connection with an assessment factor of 10) in all FOCUS Steps 1-3 scenarios. Therefore, no further assessment is necessary.

Metabolites of Cymoxanil: for the intended use on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

PROSIM: for the intended use on potato, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms. Therefore, no further assessment is necessary.

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 Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of PROSIM were not evaluated as part of the EU assessment of Propamocarb hydrochloride and Cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

However, the provision of further data on PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Propamocarb hydrochloride	Oral	**LD_{50} > 84 µg a.s./bee**	EFSA Scientific Report (2006) 78, 1-80
Apis mellifera	Propamocarb hydrochloride	Contact	**LD_{50} > 100 µg a.s./bee**	
Apis mellifera	Cymoxanil	Oral	**LD_{50} > 85.29 µg a.s./bee**	EFSA Scientific Report (2008) 167, 1-116
Apis mellifera	Cymoxanil	Contact	**LD_{50} > 100 µg a.s./bee**	
Apis mellifera	PROSIM	Oral	**LD_{50} > 400 µg/bee**	KCP 10.3.1.1.1 Parma, P. 2018, Report No. B/27/17
Apis mellifera	PROSIM	Contact	**LD_{50} > 400 µg/bee**	KCP 10.3.1.1.2 Parma, P. 2018, Report No. B/28/17
Apis mellifera	Propamocarb Hydrochloride 40% + Cymoxanil 5% SC	Chronic, 10d	LDD_{50} = 86.59 µg/bee/day **NOEDD = 44.4 µg a.s./bee/day**	KCP 10.3.1.2 S Rajeshwari. 2022 9038/2021
Apis mellifera	Propamocarb Hydrochloride 40% + Cymoxanil 5% SC	Larvae, repeated exposure, 22 d	NOED = **27.7 µg/larva**	KCP10.3.1.3 S Rajeshwari. 2022 9037/2021
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

9.6.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. Studies were conducted with PROSIM and were also considered for the risk assessment.

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of PROSIM in potato

Intended use	Potato		
Active substance	Propamocarb		
Application rate (g/ha)	6 x 1000		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 84	1000	<11.90

Contact toxicity	> 100		<10.00
Active substance **Application rate (g/ha)**	Cymoxanil 6 x 125		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 85.29	125	<1.47
Contact toxicity	> 100		<1.25
Product **Application rate (g/ha)**	PROSIM 6 x 2659 (Based on density value of 1.0636 g/cm^3)		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 400	2659	<6.65
Contact toxicity	> 400		<6.65

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

zRMS comment:

The acute risk assessment for bees presented in Table 9.6 - 2 is agreed by the zRMS.

Overall, acceptable risk to bees may be concluded from the intended uses of Prosim.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level.

~~The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final. However, according to EU Regulation 284/2008 the chronic studies to bees should be provided for the product as for adult bees as well as for larvae.~~

The assessment on the base EPPO was accepted, indicating that the proposed uses of PROSIM pose an acceptable chronic risk to bees.

There are an endpoints from a chronic studies on bees and the endpoints has been assessed by adapting the EPPO 2010 scheme. The EPPO 2010 scheme does not recommend a chronic assessment for adults for foliar spray applications. However, an approach is proposed as an assessment refinement for seed coatings/soil treatments (point 7 on the scheme), and this approach was adapted to provide a worst-case assessment for foliar sprays.

A worst-case of potential exposure via residues in pollen and nectar can be estimated based on the generic worst-case value of 1 mg a.s./kg proposed in the EPPO 2010 (note 6 of EPPO 2010). This value is based on a data base of measured values from aerial plant parts as a surrogate for pollen and nectar.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from Rortais *et al*., 2005, as proposed in the EPPO 2010 scheme have been used to estimate the consumption by bee foragers: 898.8 mg sugar/bee for seven days (worst-case for nectar foragers). Considering the maximum amount of sugar an adult bee consumes per day (128 mg/bee/day) and the amount of sugar in nectar of 15% (worst-case sugar content based on the available scientific literature (Maccagnani *et al*., 2003; Monzon *et al*., 2004; Nicolson, 2009), adults consume an amount of nectar of 853 mg/bee/day (thus will be exposed to 0.853 µg a.s./bee/day). Relevant calculations are presented below:

- 1 kg (= 1000000 mg) of plant matrix contains 1 mg of a.s. (= 1000 µg a.s.) → 1 mg plant matrix (=nectar) contains 0.001 µg a.s.

- Consumption of 128 mg sugar/bee/day and 15% sugar content in nectar → 853 mg/bee/day → 853 x 0.001 µg a.s. = 0.853 µg a.s.

In addition, according to Rortais *et al*. (2005) honeybees might consume several milligrams of pollen per day. As a worst case scenario, the nurses pollen consumption was considered, which might be up to 65 mg of pollen in 10 days, which corresponds to 6.5 mg pollen/bee/day (0.0065 µg pollen/bee/day).
Tier 1 calculations based on consumptions of both nectar and pollen are presented below

Table 9.6-3: Risk assessment of the chronic risk for adult bees due to the use of PROSIM

Test design	NOEDD (lab.) (µg/bee)	Consumption (µg a.s./bee)		TER criterion: TER≥ 1
		Nectar	Pollen	
Foraging bees	44.4	0.853	0.0065	51.66

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

Table 9.6-4: Risk assessment of the chronic risk for larvae bees due to the use of PROSIM

Test design	NOED (lab.) (µg/bee)	Consumption (µg a.s./bee)		TER criterion: TER≥ 1
		Nectar	Pollen	
Larvae	27.7	0.0792	0.00108	345.04

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to bees. It is clear that with a TER values calculated above there is a safety margin, indicating that the proposed uses of PROSIM pose an acceptable chronic risk to bees.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

First-tier assessments indicate that no unacceptable risk for bees exposed to PROSIM is expected according to the proposed intended uses on potato.

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 of Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of PROSIM were not evaluated as part of the EU assessment of Propamocarb and Cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

However, the provision of further data on the PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Aphidius rhopalosiphi	Previcur N	Laboratory test glass plates (2D)	LD/EC_{50} = 500 g/ha LOEL = 500 g/ha NOEL = 170 g/ha	EFSA Scientific Report (2006) 78, 1-80
Aphidius rhopalosiphi	Previcur N	Extended laboraty (barley)	LD/EC_{50} > 4315 g/ha LOEL > 4315 g/ha NOEL = 4315 g/ha	
Diaeretiella rapae	Previcur N	Laboratory test glass plates (2D)	LD/EC_{50} > 2190 g/ha LOEL > 2190 g/ha NOEL < 2190 g/ha	
Trichogramma caoeciae	Previcur N	Laboratory test glass plates (2D)	LD/EC_{50} = 790 g/ha LOEL = 790 g/ha	
Typhlodromus pyri	Previcur N	Laboratory test glass plates (2D)	LD/EC_{50} > 360 g/ha LOEL > 360 g/ha NOEL = 360 g/ha	
Typhlodromus pyri	Previcur N	Extended laboraty (lettuce)	LD/EC_{50} > 3 x 1450 g/ha LOEL > 3 x 1450 g/ha NOEL = 3 x 1450 g/ha	
Aleochara bilineata	Previcur N	Laboratory test (sand)	LD/EC_{50} > 9690 g/ha LOEL > 9690 g/ha NOEL = 9690 g/ha	
Poecilus cupreus	Previcur N	Laboratory test (sand)	LD/EC_{50} > 9690 g/ha LOEL > 9690 g/ha NOEL = 9690 g/ha	
Chrysoperla carnea	Previcur N	Laboratory test glass plates (2D)	LD/EC_{50} > 1080 g/ha LOEL > 1080 g/ha NOEL < 1080 g/ha	
Chrysoperla carnea	Previcur N	Extended laboraty (lettuce)	LD/EC_{50} > 3 x 1453 g/ha LOEL > 3 x 1453 g/ha NOEL = 3 x 1453g/ha	

Species	Substance	Exposure System	Results	Reference
Coccinella septempunctata	Previcur N	Laboratory test glass plates (2D)	LD/EC_{50} > 1920 g/ha LOEL > 1920 g/ha NOEL = 1920 g/ha	
Aphidius rhopalosiphi	Proplant	Laboratory test glass plates (2D)	At 1.083* kg a.s./ha: Mortality: 32.6% Fertility: -72.4%	
Aphidius rhopalosiphi	Proplant	Extended laboraty (barley)	At 3.450 kg a.s./ha: Mortality: 9.1% Fertility: -23.9%	
Typhlodromus pyri	Proplant	Laboratory test glass plates (2D)	At 1.083* kg a.s./ha: Mortality: -1.1% Fertility: 21.1%	
Coccinella septempunctata	Proplant	Laboratory test glass plates (2D)	At 1.083* kg a.s./ha: Mortality: -3.5% Fertility: 19.0%	
Chrysoperla carnea	Proplant	Laboratory test glass plates (2D)	At 1.083* kg a.s./ha: Mortality: -7.2% Fertility: 10.04%	
Poecilus cupreus	Proplant	Laboratory test glass plates (2D)	At 108.3 kg a.s./ha: Mortality: 3.6% Food consumption: -4.4%	
Pardosa sp.	Proplant	Extended laboraty	At 108.3 kg a.s./ha: Mortality: 0.0% Food consumption: -7.5%	
Field or semi-field tests				
Not required.				

* no MAF is taken into account, the dose in the test is equivalent to a singe application only

Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods - Cymoxanil

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri	Cymoxanil/F 50 WP	Laboratory test glass plates (2D)	LR_{50} > 480 g/ha	EFSA Scientific Report (2008) 167, 1-116
Aphidius rhopalosiphi	Cymoxanil/F 50 WP	Laboratory test glass plates (2D)	LR_{50} > 480 g/ha	
Aphidius rhopalosiphi (adults)	Cymoxanil/F 20 SC	Extended lab (leaves) 48h + 10 d	At 264 g as/ha: Mortality: 0% Fecundity: 34% At 3 x 264 g as/ha: Mortality: 33% Fecundity: 35% At 6 x 264 g as/ha: Mortality: 25% Fecundity: 23%	
Aphidius colemani (adults)	Cymoxanil/F 50 WG	Semi-field (potato leaves), 12 d	At 179 g as/ha: Parasitation: -7% At 3 x 179 g as/ha:	

Species	Substance	Exposure System	Results	Reference
			Parasitation: 25/26% At 6 x 179 g as/ha: Parasitation: 5/52% At 9 x 179 g as/ha: Parasitation: -43%	
Trichogramma cacoeciae (adults)	Cymoxanil/F 5 WG	Extended lab (grapes leaves) 8 d	At 120 g as/ha: Reproduction: -11.7% At 3 x 120 g as/ha: Reproduction: -50.5% At 6 x 120 g as/ha: Reproduction: 13.1% At 9 x 120 g as/ha: Reproduction: 8.1% At 12 x 120 g as/ha: Reproduction: -5.6%	
Typhlodromus pyri (protonymphs)	Cymoxanil/F 20 SC	Laboratory test glass plates (2D)	At 132 g as/ha: Mortality: 98.9% Reproduction: 100%	
Typhlodromus pyri (protonymphs)	Cymoxanil/F 20 SC	Extended lab (leaves) 14 d	At 120 g as/ha: Mortality: 22.5% Benef. Capacity: 24.8% At 3 x 120 g as/ha: Mortality: 40% Benef. Capacity: 28.6% At 6 x 120 g as/ha: Mortality: 37.5% Benef. Capacity: 27.5% At 9 x 120 g as/ha: Mortality: 23.9% Benef. Capacity: 23.9% At 12 x 120 g as/ha: Mortality: 21.2% Benef. Capacity: 16.5%	
Typhlodromus pyri (protonymphs)	Cymoxanil/F 20 SC	Extended lab (leaves) 14 d	At 1-6 x 159 g as/ha: Mortality: 25.3% Benef. Capacity: 65-89%	
Typhlodromus pyri (protonymphs)	Cymoxanil/F 5 WG	Extended lab (leaves) 14 d	At 1-6 x 134 g as/ha: Mortality: 34.7% Benef. Capacity: 85-100% At 1-6 x 268 g as/ha: Mortality:42.4% Benef. Capacity: 100%	
Chrysoperla carnea (larvae)	Cymoxanil/F 50 WP	Laboratory test glass plates (2D)	At 480 g as/ha: Mortality: 7.1% Fecunditty: 9%	
Chrysoperla carnea (larvae)	Cymoxanil/F 20 SC	Laboratory test glass plates (2D)	At 132 g as/ha: Mortality: -7.3% Fecunditty: 17.3%	
Chrysoperla carnea (larvae)	Cymoxanil/F 50 WG	Laboratory test glass plates (2D)	At 186 g as/ha: Mortality: -8.4%	

Species	Substance	Exposure System	Results	Reference
			Fecunditty: 4.1%	
Chrysoperla carnea (larvae)	Cymoxanil/F 50 WG	Extended lab (leaves) 10 d	At 179 g as/ha: Mortality: 23% At 3 x 179 g as/ha: Mortality: 0% At 6 x 179 g as/ha: Mortality: 13%	
Episyrphus balteatus (larvae to adults)	Cymoxanil/F 50 WG	Laboratory test glass plates (2D)	At 186 g as/ha: Mortality: 1.2% Fecundity: 33.5%	
Episyrphus balteatus (larvae to adults)	Cymoxanil/F 20 SC	Extended lab (leaves) 3 x	At 289 g as/ha: Mortality: 1.1% Fecundity: 33.4% At 3 x 289 g as/ha: Mortality: -3.4% Fecundity: 26.3% At 6 x 289 g as/ha: Mortality: -0.3% Fecundity: 23.2%	
Episyrphus balteatus (larvae to adults)	Cymoxanil/F 50 WG	Extended lab (leaves) 4 w	At 3 x 186 g as/ha: Mortality: 0% Fecundity: 69% At 6 x 186 g as/ha: Mortality: 7% Fecundity: 50%	
Episyrphus balteatus (larvae to adults)	Cymoxanil/F 50 WG	Semi-field (buck-wheat plants), 14 d	At 181 g as/ha: Mortality: -1.3% Reproduction: -58.1% At 363 g as/ha: Mortality: 2.8% Reproduction: -94.6%	
Poecilus cupreus (adults)	Cymoxanil/F 50 WP	Laboratory test glass plates (2D)	At 480 g as/ha: Mortality: 0%	
Poecilus cupreus (adults)	Cymoxanil/F 20 SC	Laboratory test glass plates (2D)	At 132 g as/ha: Mortality: 0%	
Poecilus cupreus (adults)	Cymoxanil/F 50 WG	Laboratory test glass plates (2D)	At 186 g as/ha: Mortality: 0%	
Poecilus cupreus (adults)	Cymoxanil/F 50 WG	Extended lab (soil), 7 d	At 186 g as/ha: Mortality: 0% At 4 x 186 g as/ha: Mortality: 0% At 6 x 186 g as/ha: Mortality: 0% At 9 x 186 g as/ha: Mortality: 0%	
Poecilus cupreus (adults)	Cymoxanil/F 20 SC	Semi-field (silty sand soil) 7 d	At 289 g as/ha: Mortality: 11.3% At 3 x 289 g as/ha: Mortality: 18.9% At 6 x 289 g as/ha: Mortality: 7.5%	

Species	Substance	Exposure System	Results	Reference
Aleochara bilineata (larvae to adults)	Cymoxanil/F 50 WG	Laboratory test quartz sand	At 186 g as/ga: Mortality: 7.5% Parasitic capacity: 2.6%	
Field or semi-field tests				
Typhlodromus pyri: treatment of 6 x 120 – 168 g a.s./ha (Cymoxanil/F 52.5 WG), vineyard: transient reductions in abundance (mites + eggs), no long-term effect *Typhlodromus pyri*: treatment of 5 x 240 or 4 x 480 g a.s./ha (Cymoxanil/F 50 WP), vineyard: no adverse effects on abundance (mites + eggs), no long-term effect				

Table 9.7-3: Endpoints and effect values relevant for the risk assessment for non-target arthropods – PROSIM

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri	PROSIM	Extended lab. on rosa leaves (2D)	**LR_{50} > 20 L/ha** **ER_{50} > 20 L/ha**	KCP 10.3.2.2-01 Parma, P. 2018, Report No. B/30/17
Aphidius rhopalosiphi	PROSIM	Extended lab. on barley seedlings (3D)	LR_{50} > 20 L/ha **ER_{50} = 8.9 L/ha**	KCP 10.3.2.2-02 Parma, P. 2018, Report No. B/29/17
Coccinella septempunctata (L.)	PROSIM	Laboratory test	**LR_{50} = 20.47 L/ha**	KCP 10.3.2.1-01 Angayarkanni, V. 2021 8273/2020
C. carnea	PROSIM	Laboratory test	**LR_{50} = 20.05 L/ha**	KCP 10.3.2.1-02 Murali, K. 2021 8274/2020

9.7.1.1 Justification for new endpoints

Studies were conducted with PROSIM and were also considered 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 9.7-4: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of PROSIM in potato

Intended use	**Potato**
Product	PROSIM
Application rate (L f.p./ha)	6 x 2.5
MAF	3.2 (foliar)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Coccinella septempunctata (L.)	20.47	8.00	0.39
C. carnea	20.05		0.40
Test species Higher-tier	**Rate with ≤ 50 % effect* (L f.p./ha)**	**$PER_{in\text{-}field}$ (L f.p./ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	> 20	8.00	yes
Aphidius rhopalosiphi	8.9		yes
~~**Intended use**~~	~~**Potato**~~		
~~**Product**~~	~~PROSIM~~		
~~**Application rate (L f.p./ha)**~~	~~6 × 2.5 L f.p./ha (6 × 1.0** L f.p./ha)~~		
~~**MAF**~~	~~4.6 (soil)~~		
~~**Test species Tier I**~~	~~**LR_{50} (lab.) (g/ha)**~~	~~**$PER_{in\text{-}field}$ (g/ha)**~~	~~**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**~~
~~*Coccinella septempunctata* (L.)~~	~~20.47~~	~~4.6~~	~~0.22~~
~~*C. carnea*~~	~~20.05~~		~~0.57~~
~~**Test species Higher-tier**~~	~~**Rate with ≤ 50 % effect* (L f.p./ha)**~~	~~**$PER_{in\text{-}field}$ (L f.p./ha)**~~	~~**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**~~
~~*Typhlodromus pyri*~~	~~> 20~~	~~4.6~~	~~yes~~
~~*Aphidius rhopalosiphi*~~	~~8.9~~		~~yes~~

~~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 LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.~~

~~**rate with a 60% of interception at BBCH 21-79. According to the interception values of. EFSA Journal 2014;12(5):3662[4]~~

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of PROSIM in potato

Intended use	**Potato**				
Product	PROSIM				
Application rate (L f.p./ha)	6 x 2.5				
MAF	3.2 (foliar)				
vdf	~~10 (2D)~~ , 5 (2D) 1 (3D)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Coccinella septempunctata (L.)	20.47	0.0164	~~0.01~~ 0.026	10	~~0.003~~ 0.012
C. carnea	20.05				0.007

[4] EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil

Test species Higher-tier	**Rate with ≤ 50 % effect* (L f.p./ha)**	**Drift rate**	**$PER_{off-field}$ (L f.p./ha)**	**CF**	**corr. $PER_{off-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	> 20	0.0164	~~0.01~~ 0.026	5	yes
Aphidius rhopalosiphi	8.9		0.13	5	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

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

zRMS comments:

The in- field exposure to the formulated product is validated by the zRMS.
As a worst case the VDF of 5 has been considered, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure. It should be, however, noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further. Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by the zRMS.

Based on calculations performed with consideration of the Tier I laboratory data acceptable off-field risk to non-target arthropods from the intended uses of Prosim may be concluded with no need for risk mitigation measures.

Overall, no unacceptable effects for NTA are expected following application of Prosim.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The PERin-field is below the rate with <50% of effects on mortality and reproduction for extended studies for the product PROSIM in potato for the representative species *Typhlodromus pyri* and *Aphidius rhopalosiphi*, and for additional species. Therefore, in-field recovery is expected.

The PERoff-field corrected is below rate with 50% effects for the product PROSIM for the representative species *Typhlodromus pyri* and *Aphidius* and for additional species, indicating no risk to non-target arthropods in vegetated off-field areas following application according to the proposed use patterns.

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 of Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of PROSIM were not evaluated as part of the EU assessment of Propamocarb and Cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

However, the provision of further data on the PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~Proplant~~	~~Mixed into substrate 14 d, acute 10% Sohagnum peat, 20% kaolin clay, 69% industrial sand and 1% calcium carbonate~~	~~LC_{50} > 660 mg a.s./kg dry soil~~	EFSA Scientific Report (2006)78, 1-80
Eisenia fetida	Propamocarb hydrochloride	Mixed into substrate 28 d, chronic	**NOEC = 362 mg a.s./kg dry soil**	
Field studies				
Not required.				
Litter bag test				
Not required.				

Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) - Cymoxanil

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~Cymoxanil~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg dw~~	EFSA Scientific Report (2008) 167, 1-116
~~*Eisenia fetida*~~	~~Cyoxanil 50 WP~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 505 mg/kg dw~~	
~~*Eisenia fetida*~~	~~Cymoxanil/Famoxadone 52.5 WP~~	~~Mixed into substrate 14 d, acute~~	~~LC_{50} = 297 mg/kg dw~~	

Species	Substance	Exposure System	Results	Reference
		~~10 % peat content~~		
Eisenia fetida	Cymoxanil/Famoxadone 50 WG	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC = 6.6 mg/kg dw**	
Field studies				
Not required.				
Litter bag test				
Not required.				

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – PROSIM

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	PROSIM	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC ≥1000 mg/kg dw (reproduction) (equivalent to ≥368.7 mg Propamocarb hydrochloride/kg dw soil + ≥46.1 mg Cymoxanil/kg dw soil)**	KCP 10.4.1.1 Wróbel, A. 2020 Report No. G/128/18
Folsomia candida	PROSIM	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 560 mg/kg dw (reproduction) (equivalent to 206.5 mg Propamocarb hydrochloride/kg dw soil + 25.8 mg Cymoxanil/kg dw soil)**	KCP 10.4.2.1-01 Wrobel, A. 2019, Report No. G/130/18
Hypoaspis aculeifer	PROSIM	Mixed into substrate chronic 5 % peat content	**NOEC = 308.64 mg/kg dw (reproduction) (equivalent to 113.79 mg Propamocarb hydrochloride/kg dw soil + 14.22 mg Cymoxanil/kg dw soil)**	KCP 10.4.2.1-02 Angayarkanni, V. 2020, Report No. 6101/2019

9.8.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. Studies were conducted with PROSIM and were also considered for the risk assessment.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for Propamocarb hydrochloride.

Table 9.8-4: 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 PROSIM in potato

Intended use	**Potato**		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Propamocarb hydrochloride	362	3.094	117.00
Cymoxanil	6.6	0.135	48.89
PROSIM	1000	8.509	117.52
PROSIM[1]	≥368.7	3.094	≥119.17
PROSIM[2]	≥46.1	0.135	≥341.48
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)**
PROSIM (*Folsomia candida*)	560	8.509	65.81
PROSIM[1] (*Folsomia candida*)	206.5	3.094	66.74
PROSIM[2] (*Folsomia candida*)	25.8	0.135	191.11
PROSIM (*Hypoaspis aculeifer*)	308.64	8.509	36.27
PROSIM[1] (*Hypoaspis aculeifer*)	113.79	3.094	36.78
PROSIM[2] (*Hypoaspis aculeifer*)	14.22	0.135	105.33

TER values shown in bold fall below the relevant trigger.
[1]Risk assessment based on an endpoint expressed as mg Propamocarb hydrochloride/kg dw from PROSIM study.
[2]Risk assessment based on an endpoint expressed as mg Cymoxanil/kg dw from PROSIM study.

zRMS comments:

Based on calculations in the Table above, acceptable risk to earthworms can be concluded, from active substances their metabolites and formulation product.

Overall no unacceptable effects for earthworm and soil macro-organism are expected following application of Prosim.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The chronic TER for Propamocarb hydrochloride, Cymoxanil and PROSIM are above the Annex VI trigger of 5. Therefore, it is concluded that actives and PROSIM formulation do not poses long-term risk to earthworms.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of PROSIM were not evaluated as part of the EU assessment of Propamocarb hydrochloride and Cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

However, the provision of further data on PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Previcur N	28 d, aerobic Loamy sand / loamy silt	**No adverse effects up to 28.9 mg a.s./kg dw soil**	EFSA Scientific Report (2006) 78, 1-80
C-mineralisation	Previcur N	28 d, aerobic Loamy sand / loamy silt	**No adverse effects up to 28.9 mg a.s./kg dw soil**	
N-mineralisation	Cymoxanil	28 d, aerobic	- 15.5 % effect at day 28 at **1.6 mg a.s./kg d.w.soil** (1.2 kg a.s./ha)	EFSA Scientific Report (2008) 167, 1-116
C-mineralisation	Cymoxanil	14 d, aerobic	- 8.4 % effect at day 28 at **1.6 mg a.s./kg d.w.soil** (1.2 kg a.s./ha)	

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	PROSIM	28 d, aerobic	+ 12.8 % effect at day 28 at 108.6 mg f.p./kg dw soil (equivalent to 40.2 mg Propamocarb hydrochloride/kg dw soil + 4.8 mg Cymoxanil/kg dw soil) **+6.3% effect at day 28 at 181.0 mg f.p./kg dw soil (equivalent to 67.0 mg Propamocarb hydrochloride/kg dw soil + 8.0 mg Cymoxanil/kg dw soil)**	KCP 10.5.1 Wrobel, A. 2020, Report No. G/127/18
C-mineralisation	PROSIM	28 d, aerobic	- 0.4 % effect at day 28 at 108.6 mg f.p./kg dw soil (equivalent to 40.2 mg Propamocarb hydrochloride/kg dw soil + 4.8 mg Cymoxanil/kg dw soil) **-5.6 % effect at day 28 at 181.0 mg f.p./kg dw soil (equivalent to 67.0 mg Propamocarb hydrochloride/kg dw soil + 8.0 mg Cymoxanil/kg dw soil)**	KCP 10.5.2 Wrobel, A. 2020, Report No. G/126/18

9.9.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints. Studies were conducted with PROSIM and were also considered for the risk assessment.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of PROSIM in potato

Intended use	Potato		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**

Propamocarb hydrochloride	28.90 (at 28 d)	3.094	yes
Cymoxanil	1.60 (at 28 d)	0.135	yes
PROSIM	181 (at 28 d)	8.509	yes
PROSIM[1]	67.00 (at 28 d)	3.094	yes
PROSIM[2]	8.00 (at 28 d)	0.135	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Propamocarb hydrochloride	28.9 (at 28 d)	3.094	yes
Cymoxanil	1.6 (at 28 d)	0.135	yes
PROSIM	181 (at 28 d)	8.509	yes
PROSIM[1]	67.00 (at 28 d)	3.094	yes
PROSIM[2]	8.00 (at 28 d)	0.135	yes

[1]Risk assessment based on an endpoint expressed as mg Propamocarb hydrochloride/kg dw from PROSIM study.
[2]Risk assessment based on an endpoint expressed as mg Cymoxanil/kg dw from PROSIM study.

zRMS comments:

The risk assessment presented in Table 9.9-2 above is in general agreed by the zRMS with PEC_{soil} values agreed in the course of evaluation in area of Section 8.

Overall, no unacceptable effects on soil microbial activity are expected following application of Prosim.

9.9.3 Overall conclusions

Risk assessments conducted with relevant PEC_{soil} for Propamocarb hydrochloride and Cymoxanil in PROSIM formulation indicate a low risk to soil microorganisms when applied according to the proposed use rates.

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 Propamocarb hydrochloride and Cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of PROSIM were not evaluated as part of the EU assessment of Propamocarb hydrochloride and Cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

However, the provision of further data on PROSIM is not considered essential, because active substance toxicity data can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Corn m Oats m Onion m Wheat m Carrots d Cucumber d Lettuce d Radish d Soybean d Tomato d	Previcur N	Seedling emergence and vegetative vigour	ER_{50} germination > 9.18 kg as/ha	EFSA Scientific Report (2006) 78, 1-80
Cucumber d (1) Wheat m (2)	Previcur N	Seedling emergence	(1) ER_{50} emergence > 27.54 kg a.s/ha (2) ER_{50} emergence > 82.62 kg a.s/ha	
Lettuce Potatoe	Cymoxanil 50 WP	14 d Vegetative vigour	ER_{50} >240 g	EFSA Scientific Report (2008) 167, 1-116
Pisum sativum d *Brassica oleracea var. capitata* d *Daucus carota* d *Helianthus annuus* d *Allium cepa* m *Triticum aestivum* m	PROSIM	21 d Seedling emergence	**ER_{50} >15000 mL f.p./ha**	KCP 10.6.2-01 Wróbel, A., 2020 Report nº G/132/18
Pisum sativum d *Brassica oleracea var. capitata* d *Daucus carota* d *Helianthus annuus* d *Allium cepa* m *Triticum aestivum* m	PROSIM	21 d Vegetative vigour	**ER_{50} >15000 mL f.p./ha**	KCP 10.6.2-02 Wróbel, A., 2020 Report nº G/129/18

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Studies were conducted with PROSIM and were also considered for the risk assessment.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology",

(SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of PROSIM in potato

Intended use	**Potato**			
Product	PROSIM			
Application rate (L f.p./ha)	6 x 2.5			
MAF	3.2 (foliar)			
Test species	**ER_{50} (L f.p./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L f.p./ha)**	**TER criterion: TER ≥ 5**
Pisum sativum [d] *Brassica oleracea var. capitata* [d] *Daucus carota* [d] *Helianthus annuus* [d] *Allium cepa* [m] *Triticum aestivum* [m]	15	0.0161	0.13	116.5

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

zRMS comments:

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. According to the risk assessment provided with formulation Prosim with most sensitive endpoints ER_{50} of 15 L product/ha, an acceptable risk to non-target plants was indicated for the representative use of Prosim.

No risk mitigation measures are required.

9.10.2.3 Effects on non-target aquatic plants

9.10.2.4 Higher-tier risk assessment

Not relevant.

9.10.2.5 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for PROSIM shows that Annex VI trigger of 5 is not exceeded, indicating that PROSIM poses a low risk to non-target plats when applied according to the proposed use rates.

After the application of the test item at the rates between 185.2 and 15000.0 mL/ha the phytotoxic symptoms were not observed for any of the tested plant species.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	PROSIM
Common name	Propamocarb 40% + Cymoxanil 5% SC
Classification and proposal labelling	
With regard to ecotoxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes (s), categories: Aquatic Chronic 2 Code(s) for hazard pictogram(s): GHS09 Signal word: - Hazard statement(s): H411 Precautionary statement: P273, P391, P501

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1-01	xxxxx	2019	Propamocarb 40% + Cymoxanil 5% SC: Rainbow trout, acute toxicity test Xxxxx GLP/Unpublished	Y	SHARDA Cropchem Limited
KCP 10.2.1-02	Tina Turek	2019	Propamocarb 40% + Cymoxanil 5% SC: *Daphnia magna*, acute immobilisation test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. W/86/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-03	Tina Turek	2019	Propamocarb 40% + Cymoxanil 5% SC: *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) growth inhibition test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. W/87/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.2.1-04	Tina Turek	2019	Propamocarb 40% + Cymoxanil 5% SC: Lemna gibba CPCC 310, growth inhibition test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. W/88/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.1.1	Pawel Parma	2018	Propamocarb 40% + Cymoxanil 5% SC: Honeybees (*Apis mellifera* L.), acute oral toxicity test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. B/27/17 GLP/Unpublished	N	SHARDA Cropchem Limited

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 10.3.1.1.2	Pawel Parma	2018	Propamocarb 40% + Cymoxanil 5% SC: Honeybees (*Apis mellifera* L.), acute contact toxicity test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. B/28/17 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.2	S.Rajeshwari	2022	Chronic Oral Toxicity Study of Propamocarb Hydrochloride 40% + Cymoxanil 5% SC on adult honey bee (*Apis mellifera*) Bioscience Research Foundation Report No. 9038/2021 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.1.3	S.Rajeshwari	2022	Effect of Propamocarb Hydrochloride 40% + Cymoxanil 5% SC on larvae of honey bee, *Apis mellifera (L.)* following repeated exposure Bioscience Research Foundation Report No. 9037/2021 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.2.1-01	Angayarkanni, V.	2021	A laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on the ladybird beetle, *Coccinella septempunctata* (L.) Bioscience Research Foundation Report No. 8273/2020 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.2.1-02	Murali, K.	2021	A laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on larvae of the green Lacewing *Chrysoperla carnea* L. (Neuroptera: Chrysopidae) Bioscience Research Foundation Report No. 8273/2020 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.3.2.2-01	Pawel Parma	2018	An extended laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on the predatory mite, *Typhlodromus pyri* (Sch.) Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. B/30/17 GLP/Unpublished	N	SHARDA Cropchem Limited

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 10.3.2.2-02	Pawel Parma	2018	An extended laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani – Perez) Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. B/29/17 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.4.1.1	Anna Wróbel	2020	Propamocarb 40% + Cymoxanil 5% SC: Earthworm Reproduction Test (*Eisenia andrei*) Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. G/128/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.4.2.1-01	Anna Wróbel	2019	Propamocarb 40% + Cymoxanil 5% SC: Collembolan (*Folsomia candida*) Reproduction test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. G/130/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.4.2.1-02	V. Angayarkanni	2020	Effect of Propamocarb 40% + Cymoxanil 5% SC on the reproductive output of the predatory soil mite Hypoaspis (Geolaelaps) aculeifer Canestrini (Acari: Laelapidae) in artificial soil Bioscience Research Foundation Report No. ~~G/130/18~~ 6101/2019 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.5-01	Anna Wróbel	2020	Propamocarb 40% + Cymoxanil 5% SC: Soil microorganisms Nitrogen transformation test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. G/127/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.5-02	Anna Wróbel	2020	Propamocarb 40% + Cymoxanil 5% SC: Soil microorganisms Carbon transformation test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. G/126/18 GLP/Unpublished	N	SHARDA Cropchem Limited

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 10.6.2-01	Anna Wróbel	2020	Propamocarb 40% + Cymoxanil 5% SC: Terrestrial Plant Test : Seedling emergence and Seedling Growth Test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. G/132/18 GLP/Unpublished	N	SHARDA Cropchem Limited
KCP 10.6.2-02	Anna Wróbel	2020	Propamocarb 40% + Cymoxanil 5% SC: Terrestrial Plant Test : Vegetative Vigour Test Institute of Industrial Organic Chemistry, Branch Pszczyna Report No. G/129/18 GLP/Unpublished	N	SHARDA Cropchem Limited

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

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

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of zRMS:	The study is conducted according to OECD 203 GD. All validity criteria were met. • the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used); • dissolved oxygen concentrations were within the range of 90 – 99% of air saturation value (obligatory above 60% of air saturation value). The determined concentrations of the propamocarb in fresh samples were in the range of 91.59 –101.17% of the nominal concentration. The determined concentrations of the cymoxanil in fresh samples were in the range of 80.27 – 96.48% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined concentrations of the propamocarb in spent samples were in the range of 89.86 –100.49% of the nominal concentration. The determined concentrations of cymoxanil in spent samples were in the range of 30.40 – 64.41% of the nominal concentration. Therefore, concentrations of propamocarb were stable under the test conditions, whereas the concentration of cymoxanil were unstable under test conditions.

The concentration of cymoksanil is not 80-120% of nominal therefore the endpoints for product should be recalculated according to: EFSA 2019: Outcome of pesticide Peer Review Meeting on General recurring issues in Ecotoxicology, Supporting publication 2019:EN-1673.

The applicant's recalculations are provided below:

Expressing endpoints from Tier 1 tests and formulation tests (with one or more active substances) for unstable substances - Procedure for formulation tests with more than one active substance (according to "Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology" [EFSA Supporting publication 2019:EN-1673].

Case 1: All active substances have been analytically measured
Option B (faster to calculate but associated with more uncertainties) is carried out as follows:

(1) For each active substance, calculate the geometric mean concentration between the start and end of the test for each tested concentration level; calculate the recovery rates at each tested concentration (geomean compared with nominal or initial measured).

Propamocarb calculations

[Nom. Product]	4.4	9.7	21	47	103	227	500
[Nom. Propamocarb]	1.622	3.576	7.742	17.327	37.972	83.686	184.33
start	1.611	3.55	7.348	16900	37.804	82.79	168.835
24 spent	1.63	3.414	7.193	16.455	34.121	81.8	176.393
24 fresh	1.634	3.618	7.156	15.916	36.794	80.518	
48 spent	1.613	3.413	7.259	16.822	34.754	80.299	
48 fresh	1.641	3.602	7.789	17.113	37.476	84.548	
72 spent	1.619	3.396	7.355	16.946	35.294	80.328	
72 fresh	1.622	3.58	7.649	17.378	37.943	84.163	
termination	1.59	3.309	7.268	16.826	34.993	83.338	
Calc. Geomean	**1.620**	**3.484**	**7.374**	**16.789**	**36.119**	**82.207**	**172.573**
Recovery rates	**99.877**	**97.427**	**95.247**	**96.895**	**95.120**	**98.233**	**93.622**

Cymoxanil calculations

[Nom. Product]	4.4	9.7	21	47	103	227	500
[Nom. Cymoxanil]	0.202	0.446	0.966	2.16	4.74	10.44	23
start	0.181	0.386	0.861	2.04	4.366	9.745	21.427
24 spent	0.0663	0.145	0.298	0.904	1.591	4.972	12.559
24 fresh	0.169	0.358	0.797	1.768	3.828	8.536	
48 spent	0.0614	0.166	0.352	0.916	2.906	6.376	
48 fresh	0.173	0.395	0.884	2.084	4.381	9.836	
72 spent	0.0779	0.19	0.417	1.191	1.944	6.017	
72 fresh	0.17	0.372	0.836	1.822	4.084	9.234	
termination	0.0687	0.193	0.39	1.039	3.053	5.172	
Calc. Geomean	**0.109**	**0.255**	**0.552**	**1.391**	**3.085**	**7.228**	**16.404**
Report Geomean	0.109	0.256	0.554	1.396	3.105	7.236	16.404
Recovery rates	**53.849**	**57.193**	**57.169**	**64.408**	**65.086**	**69.238**	**71.323**

(2) For each active substance, calculate the mean recovery rate and standard deviation, by considering the recovery rates for each concentration level as in (1).

Propamocarb			Cymoxanil		
Nominal	Geomean	Recovery	Nominal	Geomean	Recovery
1.622	**1.620**	99.88	0.202	0.109	53.85
3.576	**3.484**	97.43	0.446	0.255	57.19
7.742	**7.374**	95.25	0.966	0.552	57.17
17.327	**16.789**	96.90	2.16	1.391	64.41
37.972	**36.119**	95.12	4.74	3.085	65.09
83.686	**82.207**	98.23	10.44	7.228	69.24
184.33	**172.573**	93.62	23	16.404	71.32
	mean	**96.63**		mean	**62.61**
	SD	2.12		SD	6.64

(3) For each active substance, recalculate the mean measured concentration, based on the mean recovery rate.

(4) Sum up the new calculated concentration levels for the active substance to derive the mean 'sum of active substance' concentration levels.

Propamocarb		Cymoxanil		Product		
Nominal	Recovered	Nominal	Recovered	Nominal	Recalc.	Factor
1.622	1.567	0.202	0.126	1.824	1.694	
3.576	3.455	0.446	0.279	4.022	3.735	
7.742	7.481	0.966	0.605	8.708	8.086	
17.327	16.743	2.16	1.352	19.487	18.095	
37.972	36.692	4.74	2.968	42.712	39.660	
83.686	80.866	10.44	6.536	94.126	87.402	
184.33	178.118	23	14.400	207.33	192.518	**0.9286**

(5) Recalculate the endpoint based on the recovery rates of the 'sum of active substances.

Endpoint recalculation	
Product endpoint	22.34 mg/L
Factor	0.9286
Corrected endpoint	**20.74 mg/L**

Reference:	KCP 10.2.1-01
Report	Propamocarb 40% + Cymoxanil 5% SC: Rainbow trout, acute toxicity test, Ewa Nierzedska, 2019, report No. W/89/18
Guidelines:	Yes, OECD guideline No. 203
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test items: Propamocarb 40% + Cymoxanil 5% SC, batch number: SCL - 64932; the content of propamocarb HCl is 400 g/l, the content of cymoxanil is 50 g/l, manufacture date: January 9, 2018; expiry date: January 8, 2020

Test organism: Rainbow trout (*Oncorhynchus mykiss* Walb.), age: approximately 4.5 months, average weight: 0.87 g ± 0.26 g, average length: 4.21 cm ± 0.27 cm, supplier: 'The Culture of Salmonidae Fish in Zawoja', Poland.

Test design: Semi-static system with daily renewal (96 h of exposure), one replicate of each test item concentration and control, seven fish in each aquarium, the ratio of fish weight per volume (10 L) was 0.61 g/L.

Nominal test item concentrations: 500, 227, 103, 47, 21, 9.7, 4.4 mg/L plus the control

Nominal concentration of Cymoxanil: 23.0, 10.44, 4.74, 2.16, 0.966, 0.446, 0.202 mg/L plus the control

Nominal concentrations of Propamocarb: 184.330, 83.686, 37.972, 17.327, 7.742, 3.576, 1.622 mg/L plus the control

The geometric means of determined concentration of Cymoxanil: 16.404, 7.236, 3.105, 1.396, 0.554, 0.256, 0.109 mg/L plus the control.

Test conditions: Temperature of water: 13.8 – 15.1°C; pH of the control: 7.51 – 7.70; dissolved oxygen concentration in the test item concentration and the control: 90 – 99% ASV; lighting daily cycle: 16 h light: 8 h dark; no feeding; constant aeration

Chemical determinations: The concentrations of cymoxanil were determined with a validated liquid chromatographic method with DAD detection. The concentrations of propamocarb were chemically determined using a validated liquid chromatographic method with MS/MS detection

Statistics: Probit method calculations and analysis by the Fisher Exact Binomial Test with Bonferroni Correction, the Bartlett test Procedure on Variance Homogeneity, Student-t test for Homogeneous Variances with Bonferroni- Holm Adjustment, Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment

Endpoints values: LC50, LOEC and NOEC

Results and conclusions

The endpoint values determined on the basis of the nominal test item concentrations and mortality of fish are given below:
The LC_{50} value after 96 h of exposure is 22.34 mg/L (with 95% confidence limit: 10.02 – 42.58).
The LOEC/96 h value is 9.70 mg/L.
The NOEC/96 h value is 4.40 mg/L.
The endpoint values determined on the basis of the nominal of concentrations of propamocarb:
The LC_{50}/96 h value is 8.236 mg/L (with 95% confidence limit: 3.695 – 15.696).
The LOEC/96 h value is 3.576 mg/L.
The NOEC/96 h value is 1.622 mg/L.
The endpoint values determined on the basis of the nominal of concentrations of cymoxanil:
The LC_{50}/96 h value is 1.027 mg/L (with 95% confidence limit: 0.461 – 1.958).
The LOEC/96 h value is 0.446 mg/L.
The NOEC/96 h value is 0.202 mg/L.

The endpoint values determined on the basis of the geometric means of determined concentrations of cymoxanil:
The LC_{50}/96 h value is 0.612 mg/L (with 95% confidence limit: 0.260 – 1.217).
The LOEC/96 h value is 0.256 mg/L.
The NOEC/96 h value is 0.109 mg/L.

Comments of zRMS:

The study is conducted according to OECD 202 GD.

All validity criteria were met.

- the immobilisation of *Daphnia magna* in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 8.4 – 9.0 mg/L(criterion: not less than 3 mg/L).

In fresh samples, the determined concentration of propamocarb was 101.5% of the nominal concentration and the determined concentration of cymoxanil was 92.7 and 81.4% of the nominal concentration.
The results confirm that the test item concentration was prepared correctly.
In spent samples, the determined concentration of propamocarb was 100.0 and 101.9% of the nominal concentration and the determined concentration of cymoxanil was 37.2 and 43.6% of the nominal concentration. Therefore, the concentration of propamocarb was stable under test conditions, whereas the concentration of cymoxanil was not stable under test conditions.

The concentration of cymoksanil is not 80-120% of nominal therefore the endpoints for product should be recalculated according to: EFSA 2019: Outcome of pesticide Peer Review Meeting on General recurring issues in Ecotoxicology, Supporting publication 2019:EN-1673.

The applicant's recalculations are provided below:

Propamocarb 40% + Cymoxanil 5% SC *Daphnia magna, acute immobilisation test* (W/86/18)

(1) For each active substance, calculate the geometric mean concentration between the start and end of the test for each tested concentration level; calculate the recovery rates at each tested concentration (geomean compared with nominal or initial measured).

Propamocarb calculations	
[Nom. Product]	100
[Nom. Propamocarb]	36.866
start	37.406
24 spent	36.864
24 fresh	37.401
termination	37.549
Calc. Geomean	**37.304**
Recovery rates	**101.188**

Cymoxanil calculations	
[Nom. Product]	100
[Nom. Cymoxanil]	4.608
start	4.271
24 spent	1.715
24 fresh	3.749
termination	2.009
Calc. Geomean	**2.725**
Report Geomean	2.725
Recovery rates	**59.14388**

(2) For each active substance, calculate the mean recovery rate and standard deviation, by considering the recovery rates for each concentration level as in (1).

Nominal	Geomean	Recovery
36.866	**37.304**	101.19
	mean	**101.19**

Nominal	Geomean	Recovery
4.608	2.725	59.14
	mean	**59.14**

(3) For each active substance, recalculate the mean measured concentration, based on the mean recovery rate.
(4) Sum up the new calculated concentration levels for the active substance to derive the mean 'sum of active substance' concentration levels.

Propamocarb		Cymoxanil		Product		
Nominal	Recovered	Nominal	Recovered	Nominal	Recalc.	Factor
36.866	37.304	4.608	2.725	41.474	40.029	0.9652

(5) Recalculate the endpoint based on the recovery rates of the 'sum of active substances'.

Endpoint recalculation	
Product endpoint	100 mg/L
Factor	0.9652
Corrected endpoint	**96.52 mg/L**

Reference:	KCP 10.2.1-02
Report	Propamocarb 40% + Cymoxanil 5% SC: *Daphnia magna*, acute immobilisation test, Tina Turek, 2019, report No. W/86/18
Guidelines:	Yes, OECD guideline No. 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: Propamocarb 40% + Cymoxanil 5% SC; batch no. SCL – 64932; content of propamocarb HCl: 400 g/L; content of cymoxanil: 50 g/L; density at 20°C: 1.085 g/ml; manufacturing date: 9th January, 2018; expiry date: 8th January, 2020

Test organisms: *Daphnia magna* Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology

Test design: Semi-static test with renewal after 24 h of exposure (exposure: 48 h); four replicates per the test item concentration and the control, five *Daphnia magna* in each replicate

Nominal test item concentrations: 100 mg/L plus the control

Nominal concentrations of Propamocarb: 36.866 mg/L plus the control

Nominal concentration of Cymoxanil: 4.608 mg/L plus the control

Geometric means of concentrations of Cymoxanil: 2.725 mg/L plus the control

Test conditions: Temperature: 18.8 – 20.0ºC; pH of the control: 7.30 – 7.61; dissolved oxygen concentration in the control: 8.8 – 9.0 mg/L; daily cycle: 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7

Chemical determinations: The concentration of propamocarb was determined using a validated liquid chromatographic method with MS/MS detection and the concentration of cymoxanil was determined using a validated liquid chromatographic method with DAD detection

Endpoints values: EC_{50}/48 h.

Results and conclusions

The endpoint values determined based on nominal test item concentrations:
The EC_{50}/48 h is higher than 100 mg/L.

The endpoint values determined based on nominal concentrations of propamocarb:
The EC_{50}/48 h is higher than 36.866 mg/L.
The endpoint values determined based on nominal concentrations of cymoxanil:
The EC_{50}/48 h is higher than 4.608 mg/L.
The endpoint values determined based on geometric means of determined concentrations of cymoxanil:
The EC_{50}/48 h is higher than 2.725 mg/L.

Comments of zRMS:	The study is conducted according to OECD 202 GD. All validity criteria were met. • The biomass in the control increased by a factor of 145.5 within the 72-hour test period (criterion at least a 16-fold growth), • the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 2.2% (criterion: it must not exceed 7%) • the mean coefficient of variation for the section-by-section growth rate in the control culture was 23.1% (criterion: it must not exceed 35%). At exposure initiation, the determined concentrations of propamocarb were in the range of 95.3 – 101.7% of the nominal concentration and the determined concentrations of cymoxanil were in the range of 82.2 – 98.3% of the nominal concentration. After 24 h of exposure, the determined concentrations of propamocarb were in the range of 97.6 – 101.6% of the nominal concentration. In the test item concentration of 2.56 mg/L, the analysed concentration of cymoxanil was below LoD. In the test item concentrations in the range of 6.4 – 250 mg/L, the determined concentrations of cymoxanil were in the range of 21.1 – 88.1% of the nominal concentration. After 48 h of exposure, the determined concentrations of propamocarb were in the range of 99.8 – 101.6% of the nominal concentration. In the test item concentrations of 16, 6.4 and 2.56 mg/L, the analysed concentrations of cymoxanil were below LoD. In the test item concentrations in the range of 40 – 250 mg/L, the determined concentrations of cymoxanil were in the range of 12.0 – 58.5% of the nominal concentration. At exposure termination, the determined concentrations of propamocarb were in the range of 100.0 – 103.2% of the nominal concentration. In the test item concentrations of 16, 6.4 and 2.56 mg/L, the analyzed concentrations of cymoxanil were below LoD. In the test item concentrations in the range of 40 – 250 mg/L, the determined concentrations of cymoxanil were in the range of 2.4 – 18.7% of the nominal concentration. Therefore, the concentrations of propamocarb were stable under test conditions, whereas the concentrations of cymoxanil were not stable under test conditions. The concentration of cymoksanil is not 80-120% of nominal therefore the endpoints for product should be recalculated according to: EFSA 2019: Outcome of pesticide Peer Review Meeting on General recurring issues in Ecotoxicology , Supporting publication 2019:EN-1673.

The applicant calculation is provided below:

Propamocarb 40% + Cymoxanil 5% SC
Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) Growth inhibition test
(W/87/18)

(1) For each active substance, calculate the geometric mean concentration between the start and end of the test for each tested concentration level; calculate the recovery rates at each tested concentration (geomean compared with nominal or initial measured).

Propamocarb calculations

[Nom. Product]	2.56	6.4	16	40	100	250
[Nom. Propamocarb]	0.944	2.359	5.899	14.746	36.866	92.165
start	0.943	2.354	5.62	14.996	37.254	92.324
24	0.921	2.338	5.975	14.845	36.875	93.621
48	0.945	2.367	5.904	14.846	37.444	92.012
72	0.944	2.411	5.907	14.949	38.037	92.41
Calc. Geomean	**0.936**	**2.362**	**5.880**	**14.888**	**37.319**	**92.664**
Recovery rates	**99.153**	**100.127**	**99.678**	**100.963**	**101.229**	**100.541**

Cymoxanil calculations

[Nom. Product]	2.56	6.4	16	40	100	250
[Nom. Cymoxanil]	0.118	0.294	0.74	1.84	4.6	11.5
start	0.116	0.259	0.608	1.807	4.518	10.855
24	0.0001	0.062	0.165	0.974	3.311	10.134
48	0.0001	0.0001	0.0001	0.269	1.757	6.725
72	0.0001	0.0001	0.0001	0.105	0.468	2.155
Calc. Geomean	**0.0003**	**0.0032**	**0.005**	**0.485**	**2.038**	**6.908**
Recovery rates	**0.254**	**1.088**	**0.676**	**26.359**	**44.304**	**60.070**

(2) For each active substance, calculate the mean recovery rate and standard deviation, by considering the recovery rates for each concentration level as in (1).

Propamocarb			**Cymoxanil**		
Nominal	Geomean	Recovery	Nominal	Geomean	Recovery
0.944	0.936	99.15	0.118	0.0003	0.25
2.359	2.362	100.13	0.294	0.0032	1.09
5.899	5.88	99.68	0.74	0.005	0.68
14.746	14.888	100.96	1.84	0.485	26.36
36.866	37.319	101.23	4.6	2.038	44.30
92.165	92.664	100.54	11.5	6.908	60.07
	mean	**100.28**		mean	**22.13**
	SD	0.79		SD	25.81

(3) For each active substance, recalculate the mean measured concentration, based on the mean recovery rate.
(4) Sum up the new calculated concentration levels for the active substance to derive the mean 'sum of active substance' concentration levels.

Propamocarb	Cymoxanil	Product

Nominal	Recovered	Nominal	Recovered	Nominal	Recalc.	Factor
0.944	0.947	0.118	0.026	1.062	0.973	
2.359	2.366	0.294	0.065	2.653	2.431	
5.899	5.916	0.74	0.164	6.639	6.079	
14.746	14.787	1.84	0.407	16.586	15.194	
36.866	36.969	4.6	1.018	41.466	37.987	
92.165	92.423	11.5	2.545	103.665	94.968	0.916

(5) Recalculate the endpoint based on the recovery rates of the 'sum of active substances.

Endpoint recalculation	ErC50	EyC50
Product endpoint	90.41 mg/L	19.65 mg/L
Factor	0.916	0.916
Corrected endpoint	**82.82 mg/L**	**18.00 mg/L**

Reference:	KCP 10.2.1-03
Report	Propamocarb 40% + Cymoxanil 5% SC: *Raphidocelis subcapitata* SAG 61.81 (formerly *Pseudokirchneriella subcapitata*) growth inhibition test, Tina Turek, 2019, report No. W/87/18
Guidelines:	Yes, OECD guideline No. 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: Propamocarb 40% + Cymoxanil 5% SC; batch no. SCL – 64932; content of propamocarb HCl: 400 g/L; content of cymoxanil: 50 g/L; density at 20°C: 1.085 g/ml; manufacturing date: 9th January, 2018; expiry date: 8th January, 2020

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

Test design: 72 hours of exposure; three replicates for each test item concentration and six for the control; a background for the control and each test item concentration; initial algal cell density: 1 x 10^4 cells/mL

Nominal test item concentrations: 250 (with pH adjustment), 250, 100, 40, 16, 6.4, 2.56 (without pH adjustment) mg/L plus the control

Nominal concentrations of Propamocarb: 92.165 (with pH adjustment), 92.165, 36.866, 14.746, 5.899, 2.359, 0.944 (without pH adjustment) mg/L plus the control

Nominal concentrations of Cymoxanil: 11.5 (with pH adjustment), 11.5, 4.60, 1.84, 0.74, 0.294, 0.118 (without pH adjustment) mg/L plus the control

Geometric means of determined concentrations of Cymoxanil: 2.01(with pH adjustment), 6.91, 2.04, 0.485, 0.0050, 0.0032, 0.0003 (without pH adjustment) mg/L plus the control

Test conditions: Temperature: 21.8 – 22.3ºC; pH of the control: 7.54 – 8.86; mean light intensity: 7200 – 7398 lux; constant illumination and shaking; medium: AAP.

Chemical determinations: The concentration of propamocarb was determined using a validated liquid chromatographic method with MS/MS detection and the concentration of cymoxanil was determined using a validated liquid chromatographic method with DAD detection

Statistics: Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential test procedure, Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm.

Endpoints values: E_rC_{50}, E_yC_{50}, LOEC and NOEC.

Results and conclusions

The endpoint values based on the nominal test item concentrations:
The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the E_rC_{50}/72 h value is 90.41 mg/L (95% confidence interval: 85.06 – 96.23).
The LOEC/72 h value for growth rate is 16 mg/L.
The NOEC/72 h value for growth rate is 6.4 mg/L.
The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the E_yC_{50}/72 h value is 19.65 mg/L (95% confidence interval: 17.96 – 21.50).
The LOEC/72 h value for yield is 6.4 mg/L.
The NOEC/72 h value for yield is 2.56 mg/L.

The endpoint values based on the nominal concentrations of propamocarb:
The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the E_rC_{50}/72 h value is 33.332 mg/L (95% confidence interval: 31.360 – 35.474).
The LOEC/72 h value for growth rate is 5.899 mg/L.
The NOEC/72 h value for growth rate is 2.359 mg/L.
The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the E_yC_{50}/72 h value is 7.244 mg/L (95% confidence interval: 6.622 – 7.925).
The LOEC/72 h value for yield is 2.359 mg/L.
The NOEC/72 h value for yield is 0.944 mg/L.

The endpoint values based on the nominal concentrations of cymoxanil:
The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the E_rC_{50}/72 h value is 4.159 mg/L (95% confidence interval:3.913 – 4.426).
The LOEC/72 h value for growth rate is 0.736 mg/L.
The NOEC/72 h value for growth rate is 0.294 mg/L.
The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata)*, i.e. the E_yC_{50}/72 h value is 0.904 mg/L (95% confidence interval: 0.826 – 0.989).
The LOEC/72 h value for yield is 0.294 mg/L.

The NOEC/72 h value for yield is 0.118 mg/L.

The endpoint values based on geometric means of determined concentrations of cymoxanil:
The concentration causing a 50% inhibition of the growth rate of *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*), i.e. the E_rC_{50}/72 h value is 1.6139 mg/L (95% confidence interval: 1.4590 – 1.7815).
The LOEC/72 h value for growth rate is 0.0050 mg/L.
The NOEC/72 h value for growth rate is 0.0032 mg/L.
The concentration causing a 50% inhibition of yield of *Raphidocelis subcapitata* (formerly P*seudokirchneriella subcapitata)*, i.e. the E_yC_{50}/72 h value is 0.0293 mg/L (95% confidence interval: 0.0225 – 0.0386).
The LOEC/72 h value for yield is 0.0032 mg/L.
The NOEC/72 h value for yield is 0.0003 mg/L.

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- The doubling time of frond number in the control was 1.5 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 23.1).
- The average specific growth rate in the control between day 0 and day 7 was 0.448 d-1 (minimum requirement: higher than 0.275 d-1).

The concentration of cymoksanil is not 80-120% of nominal therefore the endpoints for product should be recalculated according to: EFSA 2019: Outcome of pesticide Peer Review Meeting on General recurring issues in Ecotoxicology , Supporting publication 2019:EN-1673.

Propamocarb 40% + Cymoxanil 5% SC *Lemna gibba CPCC 310, Growth inhibition test* (W/88/18)

(1) For each active substance, calculate the geometric mean concentration between the start and end of the test for each tested concentration level; calculate the recovery rates at each tested concentration (geomean compared with nominal or initial measured).

Propamocarb calculations

	62.5	125	250	500	1000
	23.041	46.083	92.165	184.33	368.66
start	23.85	46.372	93.39	192.299	382.648
day 1 spent	23.761	45.386	88.128	179.251	361.447
day 1 fresh	23.46	46.418	88.235	180.842	362.567
day 2 spent	23.699	46.752	92.756	181.126	365.148
day 2 fresh	22.892	46.699	94.406	186.627	373.307
day 3 spent	22.585	45.805	92.08	185.765	369.963
day 3 fresh	23.345	46.834	92.495	187.888	372.999
day 4 spent	22.928	46.148	91.594	148.899	374.999
day 4 fresh	23.111	47.401	92.756	183.447	369.961
day 5 spent	23.497	47.195	93.455	186.086	373.028
day 5 fresh	23.286	46.612	93.418	185.477	374.344
day 6 spent	23.578	46.866	94.482	189.963	375.301
day 6 fresh	23.384	47.209	92.74	189.883	376.675
day 7 spent	21.616	43.507	88.56	179.378	360.055
Calc. Geomean	**23.207**	**46.362**	**92.011**	**182.326**	**370.836**
Recovery rates	**100.719**	**100.605**	**99.833**	**98.913**	**100.590**

Cymoxanil calculations

	62.5	125	250	500	1000
	2.875	5.75	11.5	23	46
start	2.654	5.502	11.275	21.523	40.752
day 1 spent	0.0001	0.0001	0.0001	0.667	5.507
day 1 fresh	3.103	6.098	11.666	22.106	41.618
day 2 spent	0.0001	0.0001	0.0001	0.513	3.227
day 2 fresh	2.753	5.399	11.008	21.137	40.555
day 3 spent	0.0001	0.0001	0.097	0.241	1.121
day 3 fresh	2.971	5.812	11.713	22.218	41.786
day 4 spent	0.0001	0.0001	0.0001	0.522	5.285
day 4 fresh	2.824	5.835	11.839	22.17	41.927
day 5 spent	0.0001	0.0001	0.0001	0.804	6.449
day 5 fresh	3.032	6.007	12.059	22.566	42.497
day 6 spent	0.0001	0.0001	0.0001	0.259	4.131
day 6 fresh	2.691	5.431	11.048	22.31	43.22
day 7 spent	0.0001	0.0001	0.0001	0.0001	4.205
Calc. Geomean	0.017	0.024	0.055	1.735	12.624
Report Geomean	**0.017**	**0.024**	**0.18**	**2.79**	**13**
Recovery rates	**0.591304**	**0.417391**	**1.565217**	**12.13043**	**28.26087**

(2) For each active substance, calculate the mean recovery rate and standard deviation, by considering the recovery rates for each concentration level as in (1).

Propamocarb

Nominal	Geomean	Recovery
23.041	**23.207**	100.72
46.083	**46.362**	100.60
92.165	**92.011**	99.83
184.33	**182.326**	98.91
368.66	**370.836**	100.59
	mean	**100.13**
	SD	0.77

Cymoxanil

Nominal	Geomean	Recovery
2.875	**0.017**	0.59
5.75	**0.024**	0.42
11.5	**0.18**	1.57
23	**2.79**	12.13
46	**13**	28.26
	mean	**8.59**
	SD	12.04

(3) For each active substance, recalculate the mean measured concentration, based on the mean recovery rate.

(4) Sum up the new calculated concentration levels for the active substance to derive the mean 'sum of active substance' concentration levels.

Propamocarb		Cymoxanil		Product		
Nominal	Recovered	Nominal	Recovered	Nominal	Recalc.	Factor
23.041	23.071	2.875	0.247	25.916	23.318	
46.083	46.143	5.75	0.494	51.833	46.637	
92.165	92.285	11.5	0.988	103.665	93.273	
184.33	184.570	23	1.976	207.33	186.545	
368.66	369.139	46	3.951	414.66	373.091	0.8

(5) Recalculate the endpoint based on the recovery rates of the 'sum of active substances'.

	Frond number		Dry weight	
Endpoint recalculation	ErC50	EyC50	ErC50	EyC50
Product endpoint	531.8 mg/L	192.2 mg/L	618.7 mg/L	154.6 m

	Factor	0.8998	0.8998	0.8998	0.8998
	Corrected endpoint	**478.51 mg/L**	**172.94 mg/L**	**556.71 mg/L**	**139.11 mg/L**

Reference:	KCP 10.2.1-04
Report	Propamocarb 40% + Cymoxanil 5% SC: *Lemna gibba* CPCC 310, growth inhibition test, Tina Turek, 2019, report No. W/88/18
Guidelines:	Yes, OECD guideline No. 221
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: Propamocarb 40% + Cymoxanil 5% SC; batch no. SCL- 64932; content of propamocarb HCl: 400 g/l; content of cymoxanil: 50 g/l; density at 20°C: 1.085 g/ml; manufacturing date: 9th January, 2018; expiry date: 8th January, 2020.

Test organisms: The freshwater aquatic plant, Lemna gibba CPCC 310 cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology; the plants were obtained from the Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada

Test design: Semi-static system with daily renewals; 7 days of exposure; three replicates for each test item concentration and six replicates for control

Nominal test item concentrations: 1000, 500, 250, 125, 62.5 mg/L plus the control

Nominal concentrations of Propamocarb: 368.66, 184.33, 92.165, 46.083, 23.041 mg/L plus the control

Nominal concentration of Cymoxanil: 46, 23, 11.5, 5.75, 2.875 mg/L plus the control.

Geometric means of determined concentrations of Cymoxanil: 13, 2.79, 0.18, 0.024, 0.017 mg/L plus the control

Test conditions: 20X AAP nutrient solution, pH of the control: 7.39 – 8.94, mean light intensity: 8564 – 8608 lux, constant illumination, glass crystallizers containing 150 mL of a given test item concentration or control; initial frond number: 9, i.e. 3 plants per 3 fronds; temperature: 22.9 – 23.3ºC.

Chemical determinations: The concentration of propamocarb was determined using a validated liquid chromatographic method with MS/MS detection and the concentration of cymoxanil was determined using a validated liquid chromatographic method with DAD detection

Statistics: Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Step-dwon Jonckheere-Terpstra test procedure

Endpoints values: E_rC_{50}, E_rC_{20}, E_rC_{10}, E_yC_{50}, E_yC_{20}, E_yC_{10}, LOEC and NOEC, based on frond number and dry weight

Results and conclusions

The endpoint values based on the nominal test item concentrations:
Endpoints based on the frond number:
The E_rC_{50}/7 d value is 531.8 mg/L (95% confidence interval 486.2 – 585.9).
The E_rC_{20}/7 d value is 168.6 mg/L (95% confidence interval 145.0 – 191.1).
The E_rC_{10}/7 d value is 92.5 mg/L (95% confidence interval 74.2 – 110.5).
For growth rate NOEC/7 d value is 62.5 mg/L, whereas the LOEC/7 d value is 125 mg/L.
The E_yC_{50}/7 d value is 192.2 mg/L (95% confidence interval 181.2 – 203.9).
The E_yC_{20}/7 d value is 96.0 mg/L (95% confidence interval 86.8 – 104.6).
The E_yC_{10}/7 d value is 66.8 mg/L (95% confidence interval 58.3 – 74.8).
For yield the NOEC/7 d value is 62.5 mg/L, whereas the LOEC/7 d value is 125 mg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 618.7 mg/L (95% confidence interval 576.6 – 667.6).
The E_rC_{20}/7 d value is 172.1 mg/L (95% confidence interval 154.6 – 189.0).
The E_rC_{10}/7 d value is 88.1 mg/L (95% confidence interval 75.1 – 101.1).
For growth rate NOEC/7 d value is 62.5 mg/L, whereas the LOEC/7 d value is 125 mg/L.
The E_yC_{50}/7 d value is 154.6 mg/L (95% confidence interval 149.5 – 159.9).
The E_yC_{20}/7 d value is 69.4 mg/L (95% confidence interval 65.4 – 73.3).
The E_yC_{10}/7 d value is lower than 62.5 mg/L.
For yield the NOEC/7 d value is 62.5 mg/L, whereas the LOEC/7 d value is 125 mg/L.

The endpoint values based on the nominal concentrations of propamocarb:
Endpoints based on the frond number:
The E_rC_{50}/7 d value is 196.052 mg/L (95% confidence interval 179.245 – 215.998).
The E_rC_{20}/7 d value is 62.144 mg/L (95% confidence interval 53.446 – 70.465).
The E_rC_{10}/7 d value is 34.087 mg/L (95% confidence interval 27.356 – 40.723).
For growth rate NOEC/7 d value is 23.041 mg/L, whereas the LOEC/7 d value is 46.083 mg/L.
The E_yC_{50}/7 d value is 70.856 mg/L (95% confidence interval 66.796 – 75.152).
The E_yC_{20}/7 d value is 35.381 mg/L (95% confidence interval 31.987 – 38.558).
The E_yC_{10}/7 d value is 24.610 mg/L (95% confidence interval 21.480 – 27.570).
For yield the NOEC/7 d value is 23.041 mg/L, whereas the LOEC/7 d value is 46.083 mg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 228.075 mg/L (95% confidence interval 212.570 – 246.104).
The E_rC_{20}/7 d value is 63.435 mg/L (95% confidence interval 57.002 – 69.683).
The E_rC_{10}/7 d value is 32.496 mg/L (95% confidence interval 27.686 – 37.283).
For growth rate NOEC/7 d value is 23.041 mg/L, whereas the LOEC/7 d value is 46.083 mg/L.
The E_yC_{50}/7 d value is 57.012 mg/L (95% confidence interval 55.116 – 58.959).
The E_yC_{20}/7 d value is 25.584 mg/L (95% confidence interval 24.119 – 27.006).
The E_yC_{10}/7 d value is lower than 23.041 mg/L.
For yield the NOEC/7 d value is 23.041 mg/L, whereas the LOEC/7 d value is 46.083 mg/L.

The endpoint values based on the nominal concentrations of cymoxanil:
Endpoints based on the frond number:
The E_rC_{50}/7 d value is 24.463 mg/L (95% confidence interval 22.365 – 26.951).
The E_rC_{20}/7 d value is 7.754 mg/L (95% confidence interval 6.669 – 8.792).
The E_rC_{10}/7 d value is 4.253 mg/L (95% confidence interval 3.413 – 5.081).
For growth rate NOEC/7 d value is 2.875 mg/L, whereas the LOEC/7 d value is 5.75 mg/L.
The E_yC_{50}/7 d value is 8.841 mg/L (95% confidence interval 8.335 – 9.377).

The E_yC_{20}/7 d value is 4.415 mg/L (95% confidence interval 3.991 – 4.811).
The E_yC_{10}/7 d value is 3.071 mg/L (95% confidence interval 2.680 – 3.440).
For yield the NOEC/7 d value is 2.875 mg/L, whereas the LOEC/7 d value is 5.75 mg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 28.458 mg/L (95% confidence interval 26.524 – 30.708).
The E_rC_{20}/7 d value is 7.915 mg/L (95% confidence interval 7.112 – 8.695).
The E_rC_{10}/7 d value is 4.055 mg/L (95% confidence interval 3.455 – 4.652).
For growth rate NOEC/7 d value is 2.875 mg/L, whereas the LOEC/7 d value is 5.75 mg/L.
The E_yC_{50}/7 d value is 7.114 mg/L (95% confidence interval 6.877 – 7.357).
The E_yC_{20}/7 d value is 3.192 mg/L (95% confidence interval 3.009 – 3.370).
The E_yC_{10}/7 d value is lower than 2.875 mg/L.
For yield the NOEC/7 d value is 2.875 mg/L, whereas the LOEC/7 d value is 5.75 mg/L.

The endpoint values based on the geometric means of determined concentrations of cymoxanil:
Endpoints based on the frond number:
The E_rC_{50}/7 d value is 2.479 mg/L (95% confidence interval 1.912 – 3.278).
The E_rC_{20}/7 d value is 0.091 mg/L (95% confidence interval 0.060 – 0.129).
The E_rC_{10}/7 d value is lower than 0.017 mg/L.
For growth rate NOEC/7 d value is 0.017 mg/L, whereas the LOEC/7 d value is 0.024 mg/L.
The E_yC_{50}/7 d value is 0.100 mg/L (95% confidence interval 0.086 – 0.116).
The E_yC_{20}/7 d value is 0.021 mg/L (95% confidence interval 0.017 – 0.025).
The E_yC_{10}/7 d value is lower than 0.017 mg/L.
For yield the NOEC/7 d value is 0.017 mg/L, whereas the LOEC/7 d value is 0.024 mg/L.

Endpoints based on the dry weight:
The E_rC_{50}/7 d value is 3.871 mg/L (95% confidence interval 3.154 – 4.827).
The E_rC_{20}/7 d value is 0.095 mg/L (95% confidence interval 0.071 – 0.124).
The E_rC_{10}/7 d value is lower than 0.017 mg/L.
For growth rate NOEC/7 d value is 0.017 mg/L, whereas the LOEC/7 d value is 0.024 mg/L.
The E_yC_{50}/7 d value is 0.063 mg/L (95% confidence interval 0.058 – 0.068).
The E_yC_{20}/7 d value is lower than 0.017 mg/L.
The E_yC_{10}/7 d value is lower than 0.017 mg/L.
For yield the NOEC/7 d value is 0.017 mg/L, whereas the LOEC/7 d value is 0.024 mg/L.

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met.

	• the average mortality for the total number of controls was 0.0% at the end of the experiment (criterion: it must not exceed 10%). • 24h LD_{50} of the reference item (dimethoate) was 0.11 µg a.i./bee (criterion:0.10 - 0.35 µg a.i./bee). 48 h LD_{50}>400 µg/bee (>147.5 µg Propacamocarb/honeybee + 18.4 µg Cymoxanil/bee)

Reference:	KCP 10.3.1.1.1
Report	Propamocarb 40% + Cymoxanil 5% SC: Honeybees (*Apis mellifera* L.), acute oral toxicity test, Pawel Parma, 2018, report No. B/27/17
Guidelines:	Yes, OECD guideline No. 213 and EU Method C.16
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Propamocarb 40% + Cymoxanil 5% SC, content: 400 g/L of Propamocarb HCl and 50 g/L of Cymoxanil, batch number: SCL-30891 production date: February 19, 2016 expiry date: February 18, 2018
Biological test system	the honeybee. *Apis mellifera* L. strain: carnica source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna age: approximately 3 weeks
Test design	- test item: exposure time: 48 hours number of doses: 5 doses and a control number of replicates: 3 replicates number of bees: 10 bees/replicate - reference item: exposure time: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate
Test item doses	25.0, 50.0, 100.0, 200.0 and 400.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses	0.03, 0.06, and 0.12 µg a.i./bee
Test conditions	temperature: 25.5 – 26°C, relative air humidity: 63 – 64% place: a dark room
Endpoints	- honeybee mortality after 48 hours of exposure - LD_{50}/24h and LD_{50}/48h - the LD_{50}/24h of the reference item (dimethoate)

Statistical method regression analysis using the log-probit method

Results and discussions

Dose		Nb. of tested bees [no.]	Mortality after 48 h		LD_{50} after 48 h	
			Total			
µg/bee	µg ai/bee		**[no.]**	**[%]**	**µg/bee**	µg ai/bee
0.0 (control)		30	**0**	**0.0**	Above 400.0	Above 147.5[a] + 18.4[b]
25.0	9.2[a] + 1.2[b]	30	**0**	**0.0**		
50.0	18.4[a] + 2.3[b]	30	**0**	**0.0**		
100.0	36.9[a] + 4.6[b]	30	**0**	**0.0**		
200.0	73.7[a] + 9.2[b]	30	**0**	**0.0**		
400.0	147.5[a] + 18.4[b]	30	**0**	**0.0**		

a: Propamocarb
b: Cymoxanil

Conclusion

The median lethal doses (LD_{50}/24h and LD_{50}/48h) are higher than the maximum used dose, i.e. 400 µg test item/honeybee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met. • the average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%), • the LD50/24 h of the reference item (dimethoate) was 0.27 µg a.i./bee (criterion: 0.1 - 0.3 µg a.i./bee). LD_{50}/48 h contact > 400.0 µg product /honeybee (>147.5 µg Propacamocarb/honeybee + 18.4 µg Cymoxanil/ honeybee)

Reference:	KCP 10.3.1.1.2
Report	Propamocarb 40% + Cymoxanil 5% SC: Honeybees (*Apis mellifera* L.), acute contact toxicity test, Pawel Parma, 2018, report No. B/28/17
Guidelines:	Yes, OECD guideline No. 214 and EU Method C.17
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: Propamocarb 40% + Cymoxanil 5% SC,
content: 400 g/L of Propamocarb HCl and 50 g/L of Cymoxanil,
batch number: SCL-30891
production date: February 19, 2016
expiry date: February 18, 2018

Biological system: the honeybee, *Apis mellifera* L.
strain: carnica
source: an apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna
age: approximately 3 weeks

Test design: - test item:
exposure time: 48 hours
number of doses: 4 doses and a control
number of replicates: 3 replicates
number of bees: 10 bees/replicate
- reference item:
exposure time: 24 hours
number of doses: 3 doses
number of replicates: 3 replicates
number of bees: 10 bees/replicate

Test item doses: 25.0, 50.0, 100.0, 200.0 and 400.0 µg test item/bee and a control (0.0 µg/bee)

Reference item doses: 0.1, 0.2 and 0.4 µg a.i./bee

Test conditions: temperature: 24 – 25°C, relative air humidity: 59 – 65%,
place: a dark room

Endpoints: - honeybee mortality after 48 hours of exposure
- the LD_{50}/24h and LD_{50}/48h of the test item
- the LD_{50}/24h of the reference item (dimethoate)

Statistical method: Regression analysis using the log-probit method

Results and discussions

Dose		**Nb. of tested bees**	**Mortality after 48 h**		**LD_{50} after 48 h**	
			Total			
µg/bee	µg ai/bee	**[no.]**	**[no.]**	**[%]**	**µg/bee**	µg ai/bee
0.0 (control)		30	**0**	**0.0**	Above 400.0	Above 147.5[a] + 18.4[b]
25.0	9.2[a] + 1.2[b]	30	**0**	**0.0**		
50.0	18.4[a] + 2.3[b]	30	**0**	**0.0**		
100.0	36.9[a] + 4.6[b]	30	**0**	**0.0**		
200.0	73.7[a] + 9.2[b]	30	**0**	**0.0**		
400.0	147.5[a] + 18.4[b]	30	**0**	**0.0**		

a: Propamocarb
b: Cymoxanil

Conclusion

The median lethal doses (LD_{50}/24 h and LD_{50}/48 h contact) are higher than the highest dose used in the test, i.e. 400.0 µg/honeybee [>(147.5 µg Propacamocarb/honeybee + 18.4 µg Cymoxanil/ honeybee~~ha~~)].

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study is considered valid. All validity criteria were met (mortality in the control was 5.0% after 10 days of exposure)

	LC_{50} = 4328.99 mg s.a./kg LDD_{50} = 86.59 µg s. a./bee/day NOEC = 2222.2 mg/kg NOEDD = 44.4 µg s.a./bee/day

Reference Report KCP 10.3.1.2
Chronic Oral Toxicity Study of Propamocarb Hydrochloride 40% + Cymoxanil 5% SC on adult honey bee (*Apis mellifera*).
S. Rajeshwari. 2022, 9038/2021. Bioscience Research Foundation

Guideline(s): OECD Guideline No. 245

Deviations: Yes, Draft report was taken on July 2022 not in December 2021 and this didn't affect the output of the study

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item:

Description:	Propamocarb Hydrochloride 40% + Cymoxanil 5% SC
Production batch:	SCL-50382
A.i. content:	propamocarb hydrochloride – 400 g/L; Cymoxanil – 50 g/L

Test system:

Species:	*Apis mellifera*
Strain:	carnica
Age:	freshly emerged worker honeybees from the same queen-right colony
Source:	bee hive maintained at BRF test facility
Acclimation period:	24h
Diet:	50% solution of sucrose in water (w/v)

Experimental conditions:

Temperature:	31.5 – 34.6°C
Humidity:	54 – 68%
Light and photoperiod:	24h darkness (except during observations).
Loading:	3 replicates per dose, 10 bees per replicate
Test procedure:	Each group of bees was fed with 1 g of a 50% sucrose solution containing the reference item or the test item for 10 days.

Experimental period: 10d

Test design and treatment

Cages (8.5 x 4.5 x 5.5 cm) made of stainless steel with the front removable transparent part and the bottom of the cage consist of perforated steel, which guarantees sufficient air supply. During acclimatisation the bees were fed ad libitum with 50% sucrose solution.
In total, 7 treatment groups were set up: 5 doses of the test item (19.8, 29.6, 44.4, 66.7 and 100.00 µg/bee/day), one untreated control groups and 1 dose of the reference item with 3 replicates per dose and 10 insects per replicate.

Food consumption (mg/bee/day) in each study group was determined by weighing the feeders with a sucrose solution and dividing the amount of food by the number of surviving bees in the previous observation time. The doses of the test item (µg/bee/day) consumed by the bees were calculated directly from treated 50% sucrose solution consumption, the concentrations of the test item, and the density of the solutions at each concentration.
Mortality results were analyzed using the probit method, in order to determine the LDD_{50}, LC_{50}, NOEDD and NOEC values. The statistical analysis of the data on mortality was conducted using the NCSS 2000 software.

Results
The results are summarized below.

Concentration		**Consumed concentration**		**Number of tested bees [no]**	**Mortality**		**LC_{50}**	**LDD_{50}**
[mg/kg]	**[µg/bee/day] [µg/30 mg/day]**	**[mg/kg]**	**[µg/bee/day] [µg/30 mg/day] [a]**		**Total**			
					No.	**[%]**	**[mg/kg]**	**[µg/bee/day]**
Propamocarb Hydrochloride 40% + Cymoxanil 5% SC								
0.0 (Control)				30	0	0	4328.99 ± 223.79	86.59 ± 4.48
987.7	19.8	987.7	26.7	30	0	0		
1481.5	29.6	1481.5	37.4	30	1	3.33		
2222.2	44.4	2222.2	49.9	30	3	10.00		
3333.3	66.7	3333.3	65.8	30	10	33.33		
5000	100.0	5000	87.7	30	18	60.00		
NOEC					2222.2 [mg/kg]			
NOEDD					44.4 [µg/bee/day]			
Concentration		**Consumed concentration**		**Dimethoate**				
[mg a.i./kg]	**[µg/bee/day] [µg/30 mg/day]**	**[mg/kg]**	**[µg/bee/day] [µg/30 mg/day]**					
0.8	0.016	0.8	0.015	30	26	86.66	not determined	

Conclusion
The validity criterion concerning mortality was met, because mortality in the control was 5.0% after 10 days of exposure [1].

The percentages of mortality of the honeybees exposed to the test item, Pendimethalin Technical at the concentrations of 987.7, 1481.5, 2222.2, 3333.3 and 5000 mg/kg (19.8, 29.6, 44.4, 66.7 and 100.00 µg/bee/day) were 0.0, 3.33, 10.0, 33.33 and 60.0%, respectively.

On the basis of the obtained mortality results the LDD_{50} value, is 86.59 µg/bee/day. The LC_{50} is 4328.99 mg/kg, the NOEC is 2222.2 mg/kg and NOEDD is 44.4 µg/bee/day were determined.

The validity criterion concerning mortality of the honeybees exposed to the reference item, dimethoate was met, because mortality was 86.66% after 10 days of exposure. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

Average consumption of a 50% sucrose solution in the control group was 28.22 mg/bee/day. Average consumption in the groups treated with the test item at the concentrations of 987.7, 1481.5, 2222.2, 3333.3 and 5000 mg/kg (19.8, 29.6, 44.4, 66.7 and 100.00 µg/bee/day) were 27.03, 25.25, 22.48, 19.75 and 17.54 mg/bee/day, respectively.

Average consumption of a 50% sucrose solution containing the reference item at the concentration of 0.016

µg/bee (0.8 mg/kg) was 18.47 mg/bee/day.

In all study groups average consumption of a 50% sucrose solution was 23.38 mg/bee/day. On the basis of average consumption of a 50% sucrose solution in the study groups, it may be concluded that each bee treated with the test item at the concentration of 19.8, 29.6, 44.4, 66.7 and 100.00 µg/30 mg/day of Propamocarb Hydrochloride 40% + Cymoxanil 5% SC ingested 26.7, 37.4, 49.9, 65.8 and 87.7 µg of the test item/day. The ingested concentrations were 987.7, 1481.5, 2222.2, 3333.3 and 5000 mg/kg, respectively.

Each insect from the group fed with a 50% sucrose solution containing the reference item at the concentration of 0.016 µg/30 mg of emulsion ingested 0.015 µg of dimethoate/day (0.8 mg/kg).

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

Comments of z RMS:	The study is considered valid. All validity criteria were met. Values based on nominal Dose ED_{10} = 8.65 µg/larva ED_{20} =18.30 µg/larva ED_{50} = 76.70 µg/larva NOED = 27.7 µg/larva Values based on nominal concentration mg/kg food s ED_{10} =56.13 mg/kg food ED_{20} =118.82 mg/kg food ED_{50} = 498.83 mg/kg food NOED = 180.1 mg/kg food
Reference Report	KCP 10.3.1.3 Effect of Propamocarb Hydrochloride 40% + Cymoxanil 5% SC on larvae of honey bee, *Apis mellifera* (L.) following repeated exposure. S. Rajeshwari, 2022, 9037/2021. Bioscience Research Foundation
Guideline(s):	OECD (2016), Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Environment Monograph, Series on Testing and Assessment No. 239,
Deviations:	Yes, Draft report was taken on July 2022 not in December 2021 and this didn't affect the output of the study
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

Description:	Propamocarb Hydrochloride 40% + Cymoxanil 5% SC
Production batch:	SCL-50382
A.i. content:	propamocarb hydrochloride – 400 g/L; Cymoxanil – 50 g/L

Test system:

Species: *Apis mellifera*
Strain: *carnica*
Age: one day old larvae
Source: Bee hive maintained at BRF test facility
Acclimation period: 3 days
Diet: 50% aqueous sugar solution and 50% royal jelly

Experimental conditions:

Temperature: 34.2 – 34.9°C
Humidity: Day 1 – Day 3: 91 - 97%
Day 3 – Day 8: 94 – 96%
Day 8 – Day 15: 86 – 96%
Light and photoperiod: 24h darkness (except during observations).
Loading: 3 replicates per dose, 12 larvae per replicate
Test procedure: On 4 successive days (day 3 to day 6) the larvae were repeatedly exposed to Propamocarb Hydrochloride 40% + Cymoxanil 5% SC diluted in the larval food.

Experimental period: 22 days

Test design and treatment

Polystyrene grafting cells in 48-well cell culture plates. During 4 successive days the larvae were repeatedly exposed to Propamocarb Hydrochloride 40% + Cymoxanil 5% SC diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications no additional feedings of the larvae took place.
In total, 7 treatment groups were set up: 5 doses of the test item (7.7, 14.6, 27.7, 52.6 and 100 µg/larva), one untreated control group and 1 dose of the reference item with 3 replicates per dose and 12 larvae per replicate.
Assessments of cumulated larval mortality were done on days 4, 5, 6, 7 and 8. Additionally, other observations such as small body size or large quantities of remaining food on day 8 were noted. Pupal mortality was assessed at day 15 and emergence of adults was evaluated at day 22.
Descriptive statistics;
NOED/NOEC was determined by one-way ANOVA test using Graph Pad Prism 8.2. ED/$EC_{10/20/50}$ values were determined probit analysis using NCSS-2000.

Results

The results are summarized below.

Toxicity of Propamocarb Hydrochloride 40% + Cymoxanil 5% SC to larvae of *Apis mellifera* L.

Treatment group	Dose [µg/larva]	Concentration [mg/kg food]	On day 8: Larval mortality Day 3 – Day 8 [%] abs.	On day 8: Mean OO [%]	On day 22: Pupal mortality Day 8 – Day 22 [%] abs.	On day 22: Total mortality Day 3 – Day 22 [%] abs.	On day 22: Adult emergence rate [%] abs.
Control	-	-	8.33	0.0	0.00	16.67	83.33
Test item	7.70	49.9	16.67	0.0	2.78	25.00	75.00
	14.6	94.8	16.67	0.0	8.33	30.56	69.44
	27.70	180.1	22.22	0.0	2.78	38.89	61.11
	52.60	342.1	27.78	0.0	11.11	47.22	52.78
	100.00	650.0	50.00	0.0	8.33	66.67	33.33
Reference item	7.6	48.0	52.78	0.0	25.00	77.78	11.11
Treatment		Endpoint: Successful adult emergence				Up to day 22	

Test item doses	ED_{50} [µg/larva] [2] (95% CL)	76.70 ± 11.23
	ED_{20} [µg/larva] [2] (95% CL)	18.30 ± 2.39
	ED_{10} [µg/larva] [2] (95% CL)	8.65 ± 1.72
	NOED [µg a.i./larva] [1]	27.7
Test item concentrations	EC_{50} [mg/kg food] [2] (95% CL)	498.83 ± 73.15
	EC_{20} [mg/kg food] [2] (95% CL)	118.82 ± 15.51
	EC_{10} [mg/kg food] [2] (95% CL)	56.13 ± 11.19
	NOEC [mg/kg food] [1]	180.1

Results are averages based on 3 replicates, containing 12 larvae each;
OO: Other observations (e.g. remaining food)
[1] one-way ANOVA
[2] probit analysis

On D8, larval mortalities of 8.33% were observed in control. Pupal mortality (between D8 and D22) was 0.0% in the control. The control group showed a total mortality of 16.67%, at D22. In the test item groups larval mortalities at D8 ranged between 16.67 and 50.00%. Pupal mortalities ranged between 2.78 and 11.11% in the test item treatment groups. Total mortalities at D22 ranged between 25.00 and 66.67%. Mortality in the reference was above 50% across all replicates on D8, being 52.78%.

On D8, none of all remaining larvae treated with test item showed remaining food or other observations such as a smaller body size.

In the final assessment at D22, adult emergence rates of 83.33% were determined for the honey bees in the control group. In the test item groups the adult honey bees emerged at rates 75.0, 69.44, 61.11, 52.78 and 33.33% following an application of 7.7, 14.6, 27.7, 52.6 and 100 µg/larva, respectively, during the larval stages.

The determined concentrations of propamocarb hydrochloride collected at each exposure were in the range between 99.1% and 101.9% of the nominal concentration. No test item was detected in the control specimen.

Because control mortality was ≤ 15% on D8 (8.33%), cumulated mortality in the reference item dose of 7.6 µg a.i./larva was ≥ 50% on D8 (52.78%) and adult emergence in the control was ≥ 70% on D22 (83.33%), the study can be regarded as valid.

Conclusion
In a repeated exposure larval toxicity study with Propamocarb Hydrochloride 40% + Cymoxanil 5% SC, the ED_{50} (successful adult emergence up to D22) was calculated to be 76.70 µg/larva, which is equivalent to an EC_{50} of 498.83 mg/kg food.
The ED_{10} and ED_{20} (D22) was determined to be 8.65 and 18.30 µg product/larva, respectively, which is equivalent to an EC_{10} and EC_{20} (D22) of 56.13 and 118.82 mg product/kg food, respectively.
The respective NOED was 27.7 µg/larva and the corresponding NOEC was 180.1 mg/kg food.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

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

Comments of zRMS: The study is considered valid.

All validity criteria were met.

- the was no mortality of the larvae in the control group (criterion: less than ≤ 30%)
- the average mortality of the larvae in reference group was 90.00% (criterion: ≥ 40%)
- the average number of viable eggs laid by the adult control ladybirds per day was 3.5 (criterion: ≥2 fertile eggs per viable female per day)

Agreed endpoints:

Study group (application rate) (L/ha)	Mortality		Reproduction					
	Total (%)	Corrected* (%)	Mean number of eggs laid/day	Eggs hatched/day		Mean number of eggs laid/viable female/day	Mean number of viable eggs laid/viable female/day	Fecundity reduction** [%]
				Mean [No]	Mean [%]			
Control	0	-	115.14	110.57	96.0	3.5	3.4	-
Propamocarb 40% + Cymoxanil 5% SC								
8.88	10.0	-	106.29	100.57	94.6	3.3	3.1	8.82
13.33	27.5	-	75.86	65.43	86.3	3.0	2.6	23.53
20.00	47.5	-	35.64	31.21	87.5	2.0	1.7	50.00
30.00	70.0	-	-#	-#	-#	-#	-#	-#
50.00	92.5	-	-#	-#	-#	-#	-#	-#
$LR50_{mortality}$	**20.47 L/ha** ($8.19^a + 1.02^b$ kg a.i./ha)		**$ER50_{fecundity}$**			**20.14 L/ha** ($8.06^a + 1.00^b$ g a.i./ha)		
$NOER_{mortality}$	**< 8.88 L/ha** ($3.55^a + 0.44^b$ kg a.i./ha)		**$NOER_{fecundity}$**			**< 8.88 L/ha** ($3.55^a + 0.44^b$ g a.i./ha)		

Reference: KCP 10.3.2.1-01

Report: "A laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on the ladybird beetle, *Coccinella septempuncata* (L.)".
Angayarkanni V., Study No.: 8273/2020, 2021
Bioscence research foundation

Guideline(s): ESCORT 1, ESCORT 2

Deviations: NO

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

Summary

The laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on mortality and fecundity of the seven-spotted ladybird, *Coccinella septempuncata L.* (Coleoptera: Coccinellidae) was carried out based on Sponsor recommended rates for the test item as the definitive test, i.e. 8.88, 13.33, 20.00, 30.00 and 50.00 L/ha. Four days old larvae of *Coccinella septempuncata* were exposed to the test item applied to bean leaf disc as substrate. The duration of the study was 49 days. The total pre-imaginal mortality of *C. septempuncata* during the 21-day exposure phase was assessed three times per week and larval mortality, pupation as well as adult hatching were recorded, Any behavioural abnormalities of the larvae and abnormal appearance of the larvae, pupae or adults were also noted. The assessment of the reproductive performance started one week after the control beetles started lay eggs. Over a period of two weeks, all eggs laid were collected daily (except on weekends) and checked for fertility (larvae hatch). The mean number of eggs laid per female per day was determined by dividing the total number of eggs laid within each treatment group by the mean number of viable females in the treatment group. In addition, the percentage of fertile eggs was assessed from the larval hatch. Pre-imaginal mortality after 21 days of exposure and fecundity of females over a period two weeks were the endpoints. To verify the sensitivity of the biological test system and the precision of the test procedure, the insecticide, TAFGOR (30% dimetheoate, w/w) was used as a reference item. The rate of the reference item was 5.0 mL/ha (1.5 g dimetheoate/ha). The control group was treated with distilled water.

Material and methods

Test item:	Propamocarb 40% + Cymoxanil 5% SC Batch number: SCL – 52486 Content: Propamocarb: 400 g/L Cymoxanil: 50 g/L Production date: 13th May, 2019 Expiry date: 12th May, 2021
Test system:	Species: the seven spotted ladybird beetle, *Coccinella septempuncata* (L.), Coleoptera, *Coccinellidae* Stage: of three to five days old Source: BRF Insectary
Test design:	Test duration: 49 days Number of treatments: 7 (5 treatments, 1 control, 1 reference item) Number of replicates: 4 Number of organisms/replicate: 10
Test condition:	Temperature: 20.8 - 21.4°C (exposure phase) 20.5 – 21.2 (reproduction phase) Relative humidity: 68 – 75% (exposure phase) 65 – 72% (reproduction phase) Light and photoperiod: 16 h light: 8 h dark (985 - 1150 lux)
Test concentrations:	8.88, 13.33, 20.00, 30.00 and 50.00 L/ha
Statistical analysis:	The endpoint values for mortality and fecundity were determined by using a Probit analysis in NCSS (Number Cruncher Statistical System) and one-way ANOVA using Graphad Prism 8.0. The means and standard deviations were calculated using validated Excel sheets.
Validity criteria:	- the was no mortality of the larvae in the control group (criterion: less than ≤ 30%) - the average mortality of the larvae in reference gropu was 90.00% (criterion: ≥ 40%)

- the average number of viable eggs laid by the adult control ladybirds per day was 3.5 (criterion: ≥2 fertile eggs per viable female per day)

Findings:

The endpoint values showing the impact of the test item on mortality and fecundity on *Coccinella septempuncata*

Study group (application rate) (L/ha)	Mortality		Reproduction					
	Total (%)	Corrected* (%)	Mean number of eggs laid/day	Eggs hatched/day		Mean number of eggs laid/viable female/day	Mean number of viable eggs laid/viable female/day	Fecundity reduction** [%]
				Mean [No]	Mean [%]			
Control	0	-	115.14	110.57	96.0	3.5	3.4	-
Propamocarb 40% + Cymoxanil 5% SC								
8.88	10.0	-	106.29	100.57	94.6	3.3	3.1	8.82
13.33	27.5	-	75.86	65.43	86.3	3.0	2.6	23.53
20.00	47.5	-	35.64	31.21	87.5	2.0	1.7	50.00
30.00	70.0	-	-#	-#	-#	-#	-#	-#
50.00	92.5	-	-#	-#	-#	-#	-#	-#
$LR50_{mortality}$	**20.47 L/ha** (8.19^a + 1.02^b kg a.i./ha)		**$ER50_{fecundity}$**	**20.14 L/ha** (8.06^a + 1.00^b g a.i./ha)				
$NOER_{mortality}$	**< 8.88 L/ha** (3.55^a + 0.44^b kg a.i./ha)		**$NOER_{fecundity}$**	**< 8.88 L/ha** (3.55^a + 0.44^b g a.i./ha)				
Reference item – TAFGOR (DIMETHOATE 30% EC)								
5.0 mL/ha	90	-	-					

*: Mortality corrected according to Abbott's formula:
Corrected mortality [%] = ((Mt – Mc) ÷ (100 – Mc)) × 100; Mt = Mortality treated, Mc = Mortality control
a – Propamocarb
b - Cymoxanil
**: based on the mean number of eggs laid/viable female/day obtained for treatments in relation to the control group
#: reproduction phase was not performed due to mortality higher than 50% in comparison with the control group
+: statistically significant differences at $p < 0.05$

Comments of zRMS: The study is considered valid. All validity criteria were met.

Agreed endpoints:

Study group (application rate) (L/ha)	Mortality		Reproduction			
	Total (%)	Corrected# (%)	Fecundity (No)	Fecundity reduction (%)	Fertility (%)	Fertility reduction (%)
Control						
0.0	10.00	-	37.35	-	97.87	-
Propamocarb 40% + Cymoxanil 5% SC						
8.88	23.33	14.81	32.95	11.78+	97.58	0.29
13.33	33.33	25.93	28.35	24.10+	96.94	0.95
20	50.00+	44.44	18.65	50.07+	94.31	3.63+
30	73.33+	70.37	-*	-*	-*	-*
45	93.33+	92.59	-*	-*	-*	-*
Endpoints	**$LR50_{mortality}$**		**20.05 L/ha** (8.02^a + 1.00^b Kg a.s./ha)	**$ER50_{fecundity}$**		**20.38 L/ha** (8.15^a + 1.02^b + Kg a.s./ha)
	$NOER_{mortality}$		**13.33 L/ha** (8.00^a + 1.00^b kg a.s./ha)	**$NOER_{fecundity}$**		**<8.88 L/ha** <(3.55^a + 0.44^b Kg a.s./ha)

	Reference item – TAFGOR (DIMETHOATE 30% EC)			
	0.65	100	100	-
	#: Mortality corrected according to Abbott's formula Corrected mortality [%] = ((Mt – Mc) / (100 – Mc)) x 100; Mt = Mortality treated, Mc = Mortality control +: statistically significant difference between the control and the treatment group at $p < 0.05$ a: Propamocarb, b; Cymoxanil			

Reference:	KCP 10.3.2.1 - 02
Report	"A laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on larvae of the green Lacewing *Chrysoperla carnea* L. (Neuroptera: Chrysopidae)". Mr. K. Murali, 2021, 8274/2020. Bioscience Research Foundation
Guideline(s):	ESCORT 1 (Barrett K.L. *et al.*, 1994) ESCORT 2 (Candolfi M.P. *et al.*, 2001) Guidelines developed by the IOBC/WPRS (Candolfi M. P. *et al.*, 2001)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Propamocarb 40% + Cymoxanil 5% SC; Batch Number SCL-52486; active substance content: Propamocarb: 400 g/L; Cymoxanil: 50 g/L
Test species:	*Chrysoperla carnea* (L.), Neuroptera, *Chrysopidae* from the BFR insectary. The larvae used in the study were 2 – 3 days old.
Diet:	Honeybee pollen
Study design:	Number of replicates: 30 replicates for mortality, 10 replicates for reproduction Number of larvae: 1/replicate Test duration: until pupation The test item was applied with a laboratory track sprayer on bean plants at seven application rates. TAFGOR (Dimethoate 30%) was used as reference item whereas deionised water was used as control. After treatment, the treated leaves were transferred to a reproduction unit.
Application rates:	Control, 8.88, 13.33, 20, 30 and 45 L of the test item/ha (3.55, 5.33, 8, 12 and 18 Kg Propamocarb/ha and 0.44, 0.67, 1, 1.5 and 2.25 Kg Cymoxanil/ha)
Test conditions:	Temperature: 23.9 – 25.5 ºC; humidity: 63.6 – 79.5%; lighting: 16 h light : 8 h dark; light intensity: 1205 – 1641 lux
Statistical analysis:	LR_{50} and NOER for mortality and ER_{50} and NOER for reproduction were determined by using a Probit analysis in NCSS (Number Cruncher Statistical System) and one-way ANOVA using Graphpad Prism 8.0. The means and standard deviations were calculated using validated Excel sheets.
Endpoints:	LR_{50}, NOER ER_{50}, NOER

Results and Conclusions

The effects of Propamocarb 40% + Cymoxanil 5% SC on mortality and fecundity of *Chrysoperla carnea* in the extended laboratory test are summarized below:

Study group (application rate) (L/ha)	Mortality		Reproduction			
	Total (%)	Corrected# (%)	Fecundity (No)	Fecundity reduction (%)	Fertility (%)	Fertility reduction (%)
Control						
0.0	10.00	-	37.35	-	97.87	-
Propamocarb 40% + Cymoxanil 5% SC						
8.88	23.33	14.81	32.95	11.78+	97.58	0.29
13.33	33.33	25.93	28.35	24.10+	96.94	0.95
20	50.00+	44.44	18.65	50.07+	94.31	3.63+
30	73.33+	70.37	-*	-*	-*	-*
45	93.33+	92.59	-*	-*	-*	-*
Endpoints	**$LR50_{mortality}$**		**20.05 L/ha** (8.02[a] + 1.00[b] Kg a.s./ha)	**$ER50_{fecundity}$**		**20.38 L/ha** (8.15[a] + 1.02[b] + Kg a.s./ha)
	$NOER_{mortality}$		**13.33 L/ha** (8.00[a] + 1.00[b] kg a.s./ha)	**$NOER_{fecundity}$**		**<8.88 L/ha** <(3.55[a] + 0.44[b] Kg a.s./ha)
Reference item – TAFGOR (DIMETHOATE 30% EC)						
0.65	100	100	-			

#: Mortality corrected according to Abbott's formula
Corrected mortality [%] = ((Mt – Mc) / (100 – Mc)) x 100; Mt = Mortality treated, Mc = Mortality control
+: statistically significant difference between the control and the treatment group at $p < 0.05$
a: Propamocarb, b; Cymoxanil

The validity criterion for mortality was met, because mortality of the control group after 10 days of expoure was 10.00% (criterion: ≤20%).
There were statistically significant differences in mortality between group treated with Propamocarb 40% + Cymoxanil 5% SC at the rate of 20, 30 and 45L/ha and the control group (one-way ANOVA, p< 0.05).
For the reference item Tafgor (Dimethoate 30% EC, w/w), the corrected mortality of *C.carnea* after exposure at the rate of 0.65 L/ha was 100%, hence the criterion (>50%) specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate.

The validity criterion for fecundity was met, because the mean number of eggs per female per day in the control group was 37.35 (criterion: ≥15)
There were statistically significant difference in fecundity between group treated with the test item at rates of 8.88, 13.33 and 20L/ha and the control group (one-way ANOVA, p< 0.05).
The validity criterion for fecundity was met, because the mean hatching rate in the control group was 97.87% (criterion: ≥70%).
There was statistically significant difference in fertility between group treated with the test item at the rate of 20 L/ha and the control group (one-way ANOVA, p< 0.05).

On the basis of the obtained results, it can be concluded that Propamocarb 40% + Cymoxanil 5% SC had adverse effects on mortality and fecundity of *C. carnea* at rates of 20, 30and 45 L/ha and at rates of 8.88, 13.33 and 20L/ha, respectively.

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

Comments of zRMS:	The study is considered valid. All validity criteria were met. • mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%), • mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 78.3% on day 7 of exposure (criterion: a minimum of 50%), • the mean number of eggs per female in the control group was 4.2 (required: ≥ 4 eggs per female). **Agreed endpoints:**

Study group [app. rate]		Parameters (endpoints)	
		Mortality	Reproduction

Test item			Total	LR_{50}		Mean number of eggs/female (Rr) [no.]	Reproduction reduction (Pr) [%]	ER_{50}	
[L/ha]	[kg ai/ha]		[%]	[L/ha]	[kg ai/ha]			[L/ha]	[kg ai/ha]
Control (0.0)			0.0	-		4.2	-	-	
2.5	1.0[a] + 0.13[b]		11.7[+]	> 20.0	> (8.0[a] + 1.0[b])	4.8	-13.3*	> 20.0	> (8.0[a] + 1.0[b])
5.0	2.0[a] + 0.25[b]		6.7[+]			4.4	-4.3*		
10.0	4.0[a] + 5.0[b]		21.7[+]			3.7	13.0		
20.0	8.0[a] + 1.0[b]		35.0[+]			4.5	-6.2*		
		$NOER_{mortality}$		< 2.5	< (1.0[a] + 0.13[b)]	$NOER_{reproduction}$		≥ 20.0	≥ (8.0[a] + 1.0[b])
Reference item			Bi 58 Top 400 EC						
[mL/ha]	[g ai/ha]								
9.0	3.6		78.3	-					

+: statistically significant differences
a: Propamocarb content
b: Cymoxanil content
*: the negative values indicate that the mean reproduction rate in the treated group is higher than in the control

Reference:	KCP 10.3.2.2-01
Report	An extended laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on the predatory mite, *Typhlodromus pyri* (Sch.), Pawel Parma, 2018, report No. B/30/17
Guidelines:	Yes, according to the ESCORT 1 and the ESCORT 2 guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: name: Propamocarb 40% + Cymoxanil 5% SC; content propamocarb HCl: 400 g/L and cymoxanil: 50 g/L; batch number: SCL - 64932; manufacturing date: 9th January 2018; expiry date: 8th January 2020

Biological test system: the predatory mite, *Typhlodromus pyri* (Sch.) (Acari: *Phytoseiidae*)
- age: 24-hour-old protonymphs
- source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from the commercial supplier Katz Biotech (Germany) and renewed by culture from BiasLabs (Great Britain)

Experiment design: – 6 study groups:
– a control group (0.0 L/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 2.5 L/ha (1.0 kg Propamocarb/ha + 0.13 kg Cymoxanil/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 5.0 L/ha (2.0 kg Propamocarb/ha + 0.25 kg Cymoxanil/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 10.0 L/ha (4.0 kg Propamocarb/ha + 0.5 kg Cymoxanil/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 20.0 L/ha (8.0 kg Propamocarb/ha + 1.0 kg Cymoxanil/ha)
– Bi 58 Top 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha)
number of replicates: 3; number of mites in each replicate: 20

Test conditions:- temperature: 24 – 26°C
- relative air humidity: 62 – 75%
- photoperiod (light intensity): 16 h light (922 lux) : 8 h dark

Statistical analysis: regression analysis using the log-probit method, Step-down Rao-Scott- Cochran-Armitage Test Procedure, Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity, Williams Multiple Sequantial t-test Procedure

Endpoints: – mite mortality after 7 days of the treatment
– LR_{50} and $NOER_{mortality}$
– reproduction reduction (Pr) after 14 days of the treatment
– ER_{50} and $NOER_{reproduction}$

Results and discussions

Study group [app. rate]		**Parameters (endpoints)**						
		Mortality			**Reproduction**			
Test item		**Total**	**LR_{50}**		**Mean number of eggs/female (Rr) [no.]**	**Reproduction reduction (Pr) [%]**	**ER_{50}**	
[L/ha]	[kg ai/ha]	**[%]**	[L/ha]	[kg ai/ha]			[L/ha]	[kg ai/ha]
Control (0.0)		**0.0**	-		**4.2**	-	-	
2.5	$1.0^a + 0.13^b$	**11.7**$^+$	**> 20.0**	> ($8.0^a + 1.0^b$)	**4.8**	**-13.3***	**> 20.0**	> ($8.0^a + 1.0^b$)
5.0	$2.0^a + 0.25^b$	**6.7**$^+$			**4.4**	**-4.3***		
10.0	$4.0^a + 5.0^b$	**21.7**$^+$			**3.7**	**13.0**		
20.0	$8.0^a + 1.0^b$	**35.0**$^+$			**4.5**	**-6.2***		
$NOER_{mortality}$			**< 2.5**	< ($1.0^a + 0.13^{b)}$	**$NOER_{reproduction}$**		**≥ 20.0**	≥ ($8.0^a + 1.0^b$)
Reference item		**Bi 58 Top 400 EC**						
[mL/ha]	[g ai/ha]							
9.0	3.6	**78.3**	-					

+: statistically significant differences
a: Propamocarb content
b: Cymoxanil content
*: the negative values indicate that the mean reproduction rate in the treated group is higher than in the control

Conclusion

On the basis of the obtained results it can be concluded that Propamocarb 40% + Cymoxanil 5% SC at the rate of 2.5, 5.0, 10.0 and 20.0 L/ha has adverse effect on mortality of the mites.
The test item at the rates of 2.5, 5.0, 10.0 and 20.0 L/ha has no adverse effect on reproduction of the mites.

Comments of zRMS:	The study is considered valid. All validity criteria were met.

- after 48 hours mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
- after 48 hours mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 73.3% (criterion: a minimum of 50%),
- all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- the mean number of mummies per female in the control group was 34.1 (criterion: a minimum of 5.0 mummies/female),
- all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

Agreed endpoints:

Study group		**Parameter (endpoint)**						
		Mortality			**Fecundity**			
Test item			**LR_{50}**				**ER_{50}**	
L/ha	kg ai/ha	**To-tal [%]**	L/ha	kg ai/ha	**Mean no. of mum-mies/fe-male**	**Fe-cun-dity re-duc-tion Pr [%]**	L/ha	kg ai/ha
Control (0.0)		0.0	-		31.4	-	-	
2.5	1.0[a] + 0.13[b]	0.0	> **20.0**	> (8.0[a] + 1.0[b])	26.5[+]	22.3	**8.9** (1.3-56.8)*	3.6[a] (0.5-22.7)[a] 0.5[b] (0.1-2.8)[b]
5.0	2.0[a] + 0.25[b]	0.0			19.5[+]	43.0		
10.0	4.0[a] + 0.5	0.0			16.1[+]	52.9		
20.0	8.0[a] + 1.0[b]	0.0			12.5[+]	63.3		
$NOER_{mortality}$			**≥ 20.0**	≥ (8.0[a] + 1.0[b])	**$NOER_{fecundity}$**		**< 2.5**	< (1.0[a] + 0.13[b])
Reference item		Mortality after 48 h						
mL/ha	g ai/ha							
5.0	2.0	73.3						

+: statistically significant differences
a: Propamocarb content
b: Cymoxanil content
*: 95% confidence intervals

Reference:	KCP 10.3.2.2-02
Report	An extended laboratory test for evaluating the effects of Propamocarb 40% + Cymoxanil 5% SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani – Perez), Pawel Parma, 2018, report No. B/29/17
Guidelines:	Yes, according to the ESCOTR 1 and the ESCORT 2 guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint initiative
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item: name: Propamocarb 40% + Cymoxanil 5% SC; content propamocarb HCl: 400 g/L and cymoxanil: 50 g/L; batch number: SCL - 64932; manufacturing date: 9th January 2018; expiry date: 8th January 2020

Biological test system: the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: *Braconidae, Aphiidinae*
- age: adult females (24 - 48 hours after emerging from mummies)
- source: a laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was obtained from Katz Biotech AG (Baruth, Germany)

Experimental design: 6 test groups:
– a control group (0.0 L/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 2.5 L/ha (1.0 kg Propamocarb/ha + 0.13 kg Cymozanil/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 5.0 L/ha (2.0 kg Propamocarb/ha + 0.25 kg Cymozanil/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 10.0 L/ha (4.0 kg Propamocarb/ha + 0.5 kg Cymozanil/ha)
– Propamocarb 40% + Cymoxanil 5% SC at the rate of 20.0 L/ha (8.0 kg Propamocarb/ha + 1.0 kg Cymozanil/ha)
– Danadim 400 EC at the rate of 5.0 mL/ha (2.0 g a.i./ha)
number of replicates: 6 replicates/group
number of females: 5 females/replicate

Test conditions: - temperature: 18.5 - 21°C
- relative air humidity: 70 - 82%
- photoperiod: 16 hours light (mortality assessment and oviposition: 2220 lx; fecundity assessment: 5010 lx): 8 hours dark

Statistical analysis: Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity, Williams Multiple Sequential t-test procedure, Kruskall-Wallis test

Endpoints: – wasp mortality after 48 hours of exposure
– determination of the LR_{50} and the $NOER_{mortality}$
– reduction in fecundity (Pr) of surviving female wasps exposed to Propamocarb 40% + Cymoxanil 5% SC, recorded 12 days after the oviposition period
– determination of the ER_{50} and the $NOER_{fecundity}$

Results and discussions

Study group		**Parameter (endpoint)**						
		Mortality			**Fecundity**			
Test item		**Total [%]**	**LR_{50}**		**Mean no. of mummies/female**	**Fecundity reduction Pr [%]**	**ER_{50}**	
L/ha	kg ai/ha		L/ha	kg ai/ha			L/ha	kg ai/ha
Control (0.0)		0.0	-		31.4	-	-	
2.5	$1.0^a + 0.13^b$	0.0	**> 20.0**	> $(8.0^a + 1.0^b)$	26.5^+	22.3	**8.9** (1.3-56.8)*	3.6^a (0.5-22.7)^a 0.5^b (0.1-2.8)^b
5.0	$2.0^a + 0.25^b$	0.0			19.5^+	43.0		
10.0	$4.0^a + 0.5$	0.0			16.1^+	52.9		
20.0	$8.0^a + 1.0^b$	0.0			12.5^+	63.3		
$NOER_{mortality}$			**≥ 20.0**	≥ $(8.0^a + 1.0^b)$	**$NOER_{fecundity}$**		**< 2.5**	< $(1.0^a + 0.13^b)$
Reference item		Mortality after 48 h						
mL/ha	g ai/ha							

5.0	2.0	73.3

+: statistically significant differences
a: Propamocarb content
b: Cymoxanil content
*: 95% confidence intervals

Conclusion

On the basis of the obtained results it can be concluded that Propamocarb 40% + Cymoxanil 5% SC at the rates of 2.5, 5.0, 10.0 and 20.0 L/ha has no adverse effect on mortality. However, the test item at the rates of 2.5, 5.0, 10.0 and 20.0 L/ha has adverse effect on fecundity of the wasps

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:

The study is considered valid. All validity criteria were met.

- each replicate produced 81 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 25.5% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 6.3% (Criterion: ≤ 10%).

Agreed endpoints:

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of propamocarb HCl/kg dry weight of artificial soil]	Value [mg of cymoxanil/kg dry weight of artificial soil]
EC_{10}	>1000	>368.7	>46.1
EC_{20}	>1000	>368.7	>46.1
EC_{50}	>1000	>368.7	>46.1
NOEC (reproduction)	≥1000	≥368.7	≥46.1
LOEC (reproduction)	>1000	>368.7	>46.1

Reference	KCP 10.4.1.1
Report	"Propamocarb 40% + Cymoxanil 5% SC: Earthworm Reproduction Test (Eisenia andrei)l" Anna Wróbel (2020) Study code: G/128/18. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s)	OECD Guideline No. 222 (2016)
Deviations	Deviation from the Study Plan: The study finished in July 2020, not in September 2019 as it had been planned. The deviation did not affect the study results.
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item	Propamocarb 40% + Cymoxanil 5% SC: SCL – 64932, active substance: propamocarb HCl 400 g/L and cymoxanil 50g/L
Artificial soil	10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
Test organism	Earthworm, *Eisenia andrei* obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Toxicology
Test design	Test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicates
Concentration of the test item	control, 10, 18, 32, 56, 100, 180, 320, 560, and 1000 mg/kg dry weight of the artificial soil
Test conditions	temperature: 18.0 – 21.0°C; pH at the beginning of the experiment: 5.56 – 5.62; pH at the end of the experiment: 5.52 – 5.60; soil moisture content at the beginning of the experiment: 25.4– 26.9% (47.5 – 50.3% of the maximum water holding capacity); soil moisture content at the end of the experiment: 25.5 – 28.4% (47.8 – 53.2% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 615 – 638 lux light intensity at the end of the experiment: 620 – 645 lux
Statistical analysis	EC_{10}, EC_{20}, EC_{50}, LC_{50} – probit analysis using linear max. likelihood regression, NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Williams Multiple Sequential t-test Procedure NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction LOEC: a values suggested by the ToxRat Professional 2.10 statistical computer software.
Endpoints	EC_{10}, EC_{20}, EC_{50}, NOEC, LOEC LC_{50}, NOEC, LOEC

Results and discussions

On the basis of the results, it was concluded that after 4 weeks, at the control group the mortality of adult earthworms was noticed, and it was equal to 6.3%. At concentrations ranging from 10.0 to 1000 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was ranging from 0.0 to 7.5%.

The concentration of the test item causing 50% mortality of the adult earthworms (LC_{50}) is above 1000.0 mg of the test item/kg dry weight of artificial soil (368.7 mg of propamocarb HCl + 46.1 mg of cymoxanil/kg dry weight of artificial soil).

After 4 weeks of the experiment, the treated living earthworms did not exhibit any changes in appearance and behaviour.

After the application of the test item at the concentrations ranging from 10.0 to 1000.0 mg of the test item/kg dry weight of artificial soil, the body weight decrease was between 0.8 and 29.2%. As for the control group, the body weight decrease was equal to 8.2%.

After the application of the test item at the concentrations ranging from 10.0 to 1000.0 mg of the test item/kg dry weight of the artificial soil, the mean number of juveniles was between 68 – 94 per replicate. The mean number of juveniles in the control group was equal to 81 per replicate.

After 8 weeks of the experiment, it was concluded that Propamocarb 40% + Cymoxanil 5% SC had no statistically significant impact on reproduction of the earthworms at concentrations between 10- 1000.0 mg of the test item/kg dry weight of artificial soil.

The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC_{10}) is above to 1000 mg of the test item/kg dry weight of artificial soil (>368.7 mg of propamocarb HCl + > 46.1 mg of cymoxanil/kg dry weight of artificial soil).
The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC_{20}) is above to 1000 mg of the test item/kg dry weight of artificial soil (>368.7 mg of propamocarb HCl + > 46.1 mg of cymoxanil/kg dry weight of artificial soil).
The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC_{50}) is above to 1000 mg of the test item/kg dry weight of artificial soil (>368.7 mg of propamocarb HCl + > 46.1 mg of cymoxanil/kg dry weight of artificial soil).
The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is equal to 1000 mg of the test item/kg dry weight of artificial soil (368.7 mg of propamocarb HCl + 46.1 mg of cymoxanil/kg dry weight of artificial soil).
The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is above to 1000 mg of the test item/kg dry weight of artificial soil (>368.7 mg of propamocarb HCl + > 46.1 mg of cymoxanil/kg dry weight of artificial soil).
After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

Validity criteria

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

- each replicate produced 81 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 25.5% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 6.3% (criterion: ≤ 10%).

Conclusion

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

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of propamocarb HCl/kg dry weight of artificial soil]	Value [mg of cymoxanil/kg dry weight of artificial soil]
EC_{10}	>1000	>368.7	>46.1
EC_{20}	>1000	>368.7	>46.1
EC_{50}	>1000	>368.7	>46.1
NOEC (reproduction)	≥1000	≥368.7	≥46.1
LOEC (reproduction)	>1000	>368.7	>46.1
LC_{50}	>1000	>368.7	>46.1
NOEC (survival)	≥1000	≥368.7	≥46.1
LOEC (survival)	>1000	>368.7	>46.1

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS: The study is considered valid. All validity criteria were met.

- mean adult mortality: 17.5% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 951 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 21.6% (criterion: ≤ 30%).

Endpoints	Value [mg of the test item/kg dry weight of the artificial soil]	Value [mg Propamocarb HCl/ kg dry weight of the artificial soil]	Value [mg Cymoxanil/ kg dry weight of the artificial soil]
EC_{10}	263.7 (71.9 → 1000*)	97.2 (26.5 → 368.7*)	12.2 (3.3 → 46.1*)
EC_{20}	> 1000	> 368.7	> 46.1
EC_{50}	> 1000	> 368.7	> 46.1
NOEC	560	206.5	25.8
LOEC	1000	368.7	46.1

* Value obtained above the tested concentrations range

Reference: KCP 10.4.2.1-01

Report Propamocarb 40% + Cymoxanil 5% SC: Collembolan (*Folsomia candida*) Reproduction test, Anna Wrobel, 2019, report No. G/130/18

Guidelines: Yes, OECD guidelines No. 232

Deviations: Yes, at the end of the test, the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD guideline.
Physiological or pathological symptoms or distinct changes in behavior were not described. However, the deviations did not affect the study results.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Propamocarb 40% +Cymoxanil 5% SC
Batch No SCL-64932
Active substance Propamocarb HCL 400 g/L
Cymoxanil 50 g/L

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

Test organisms: the collembolan, *Folsomia candida* obtained from a standard laboratory culture ot the Lukasiewicz Research Network – institute of Industrial Organic Chemistry, Branch Pszczyna, Laboratory of Soil Toxicology

Test design test duration: 28 days

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

Concentration of the test item: control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg of the test item/kg dry weight of artificial soil

Test conditions:
temperature: 19.0 – 21.0 °C
pH at the beginning of the test: 5.52-5.56
pH at the end of the test: 5.63-5.73
Soil moisture content at the beginning of the test: 13.7 – 15.2% (43.3-48.1% of the maximum water holding capacity)
Soil moisture content at the end of the test: 12.6-14.0% (40.1-44.3% of the maximum water holding capacity)
Lighting: 16 h light and 8 h dark
Light intensity at the beginning of the experiment: 422-517 lux
Light intensity at the end of the experiment 440-520 lux

Statistical analysis:
EC_{10}, EC_{20} and EC_{50} – Weibull analysis using simple linear regression
LC_{10}, LC_{20} and LC_{50} – probit analysis using linear max. likelihood regression
NOEC:
- Shapiro-Wilk's test on normal distribution
- Barlett's test procedure on variance homogeneity
- Williams multiple sequential t-test procedure (reproduction)

LOEC: a value suggested by the ToxRat Professional 2.10 statistical computer software
- Fisher's Exact binomial Test with Bonferroni Correction (survival)

Endpoints: EC_{10}, EC_{20}, EC_{50}, NOEC, LOEC
LC_{10} ; LC_{20} ; LC_{50} ; NOEC

Results and conclusions

Mortality at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil ranged from 12.5 to 20.0%. As for the control group, it was equal to 17.5%
The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table below:

Endpoints	**Value [mg of the test item/kg dry weight of the artificial soil]**	**Value [mg Propamocarb HCl/ kg dry weight of the artificial soil]**	**Value [mg Cymoxanil/ kg dry weight of the artificial soil]**
LC_{10}	> 1000	> 368.7	> 46.1
LC_{20}	> 1000	> 368.7	> 46.1
LC_{50}	> 1000	> 368.7	> 46.1
NOEC	≥ 1000	≥ 368.7	≥ 46.1
LOEC	> 1000	> 368.7	> 46.1

After the exposure of the adult collembolans to the test item at the concentration ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 615 and 981 per replicate. As for the control group, the mean number of juveniles was equal to 951 per replicate.
The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the table below:

Endpoints	**Value [mg of the test item/kg dry weight of the artificial soil]**	**Value [mg Propamocarb HCl/ kg dry weight of the artificial soil]**	**Value [mg Cymoxanil/ kg dry weight of the artificial soil]**
EC_{10}	263.7 (71.9 → 1000*)	97.2 (26.5 → 368.7*)	12.2 (3.3 → 46.1*)

EC_{20}	> 1000	> 368.7	> 46.1
EC_{50}	> 1000	> 368.7	> 46.1
NOEC	560	206.5	25.8
LOEC	1000	368.7	46.1

* value obtained above the tested concentrations range

Comments of zRMS: The study is considered valid. All validity criteria were met.

- mean adult mortality: 2.5% (criterion: ≤ 20%),
- the mean number of juveniles per replicate at the end of the test: 126.88 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation for the number of juveniles: 2.29(criterion: ≤ 30%).

Agreed endpoints:

Endpoint	Value [mg test item/kg dw soil]	Value [mg as/kg dw soil]
EC_{10}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
EC_{20}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
EC_{50}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
NOEC	**308.64** (n.d.)	**113.79[a] + 14.22[b]** (n.d.)
LOEC	**555.56** (n.d.)	**204.81[a] + 25.60[b]** (n.d.)

a: Propamocarb
b: Cymoxanil
n.b.: not determined

Reference: KCP 10.4.2.1-02

Report Effect of Propamocarb 40% + cymoxanil 5% SC on the reproductive output of the predatory soil mite *Hypoaspis (Geolaelaps) aculeifer Canestrini* (Acari: Laelapidae) in artificial soil, V. Angayarkanni, 2020, report No. 6101/2019

Guidelines: Yes, OECD guideline No. 226

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Propamocarb 40% + cymoxanil 5% SC
Batch No. SCL-52486
Propamocarb: 400 g/L
Cymoxanil: 50 g/L

Test organisms: *Hypoaspis* (Geolaelaps) *aculeifer* Canestrini (Acari, Laelapidae)
Source: BRF Insectary
Adult females from the in-house culture were transferred to new rearing units filled with a plaster of Paris/charcoal mixture. Synchronization units with 100 females in each, were

prepared and food was added. After a period of 3 days of egg-laying, females were removed from the synchronization containers. The eggs and later on the emerged mites, were kept in rearing units. During their development the mites were fed with cheese mites (*Tyrophagus putrescentiae*) 2 to 3 times a week. The developed females were introduced into test units 33 days after the start of the egg-laying period for synchronisation.

Test design: Dose-response test
Duration: 14 days
Number of treatment group: 1 Control group
10 test item group, i.e. 5.04, 9.07, 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg/kg dry weight of the artificial soil.
Test organisms per treatment: 40 (80 for the control group)
Replicates per treatment group: 4 each containing 10 test organisms (8 each containing 10 test organisms for the control group), 2 additional replicates/treatment groups (without test organisms) for the determination of pH and water content at the beginning and at the end of the exposure phase. 6 additional replicates/treatment group for analysis purposes (without organisms)

The study groups were as follows:

Groups	**Concentration of the test item (mg/kg d.w. soil)**	**Concentration of Propamocarb[a] (mg/kg d.w. soil)**	**Concentration of Cymoxanil[b] (mg/kg d.w. soil)**
Control	0.0	0.0	0.0
T1	5.04	1.86	0.23
T2	9.07	3.35	0.42
T3	16.33	6.02	0.75
T4	29.40	10.84	1.35
T5	52.92	19.51	2.44
T6	95.26	35.12	4.39
T7	171.47	63.21	7.90
T8	308.64	113.79	14.22
T9	555.56	204.81	25.60
T10	1000	368.66	46.08

a: based on the content of Propamocarb in the test item, i.e. 400 g/L
b: based on the content of Cymoxanil in the test item, i.e. 50 g/L
Density of the test item: 1.085 g/mL

Results and conclusions

Mortality at the concentrations ranging from 5.04 to 10000 mg/kg dry weight of the artificial soil ranged from 0.0 to 12.5%. As for the control group, it was 2.5%.

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC_{50} is > 1000 mg/kg dry weight of the artificial soil, i.e. > (368.66 mg Propamocarb and 46.08 mg Cymoxanil/kg dry weight of the artificial soil).

The endpoints values showing the impact of the test item on the survival of adult *Hypoaspis aculeifer* are presented in the table below:

Endpoint	**Value [mg test item/kg dw soil]**	**Value [mg as/kg dw soil]**
LC_{10}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
LC_{20}	**> 1000**	**> 368.66[a] + 46.08[b]**

	(n.d.)	(n.d.)
LC_{50}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
NOEC	**555.56** (n.d.)	**204.81[a] + 25.60[b]** (n.d.)
LOEC	**1000** (n.d.)	**368.66[a] + 46.08[b]** (n.d.)

a: Propamocarb
b: Cymoxanil
n.b.: not determined

After the exposure of *Hypoaspis aculeifer* to the test item at the concentration ranging from 5.04 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 113.00 and 126.75 per replicate. As for the control group, the mean number of juveniles was equal to 126.88 per replicate.

The endpoint values showing the impact of the test item on reproductive output of *Hypoaspis aculeifer* are presented in the table below:

Endpoint	**Value [mg test item/kg dw soil]**	**Value [mg as/kg dw soil]**
EC_{10}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
EC_{20}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
EC_{50}	**> 1000** (n.d.)	**> 368.66[a] + 46.08[b]** (n.d.)
NOEC	**308.64** (n.d.)	**113.79[a] + 14.22[b]** (n.d.)
LOEC	**555.56** (n.d.)	**204.81[a] + 25.60[b]** (n.d.)

a: Propamocarb
b: Cymoxanil
n.b.: not determined

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered valid. All validity criteria were met. The coefficients of variation (CV) in the control group were 1.5, 5.3, 4.6 and 3.0 %, after 0, 7, 14, and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%. On the basis of the results, it was concluded that Propamocarb 40% + Cymoxanil 5% SC at the concentration corresponding to the PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil), upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Reference: KCP 10.5-01

Report	Propamocarb 40% + Cymoxanil 5% SC: Soil microorganisms Nitrogen transformation test, Anna Wrobel, 2020, report No. G/127/18
Guidelines:	Yes, OECD guideline No. 216/EU Method C.21
Deviations:	Yes, the soil extraction should be conducted at 150 rpm for 60 min, however in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which slowed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer. The PER was calculated assuming 1 cm of the soil depth according to German conditions for the substances with the mobility in soil Kfoc > 500 mL/g. Thus, the applied soil depth is a deviation from OECD guideline where PEC is calculated by using 5 cm of the soil depth. The substance chosen should have a favourable carbon to nitrogen ratio. In this study a C/N ratio is lower than the one mentioned in OECD guideline; however it is not a validity criterion and a critical point in this study and it has no influence on the obtained results during the test.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material: Propamocarb 40% + Cymoxanil 5% SC
SCL- 64932

Active substance: Propamocarb HCl – 400 g/L
Cymoxanil – 50 g/L

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

Test design: Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 28 days

Concentrations of the test item: control, PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil), upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil).

Test conditions: temperature: 18.0 – 20.0°C,
soil moisture: 40.0% – 46.7% of the maximum water holding capacity incubation in darkness

Endpoints: The concentration of nitrate [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation
The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 - 7, 0 – 14, 0 – 28 days.
Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 - 7, 0 – 14, 0 – 28 days

Statistical analysis: - Shapiro-Wilk's test on Normal Distribution

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

Results and discussions

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentration corresponding to the PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil), upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil) did not exceed 25% on 28 day of analysis.

Conclusion

On the basis of the results, it was concluded that Propamocarb 40% + Cymoxanil 5% SC at the concentration corresponding to the PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil), upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Reference:	KCP 10.5-02
Report	Propamocarb 40% + Cymoxanil 5% SC: soil microorganisms, Carbon transformation test, Anna Wrobel, 2020, report No. G/126/18
Guidelines:	Yes, OECD guideline No. 217 / EU method C.22
Deviations:	Yes, the PEC was calculated assuming 1 cm of the soil depth according to the German conditions for the active substances with the mobility in soil Kfoc > 500 mL/g. However, the PEC should be calculated by using 5 cm of the soil depth.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material:	Propamocarb 40% + Cymoxanil 5% SC SCL- 64932 Content of Propamocarb HCl: 400 g/L Content of Cymoxanil: 50 g/L
Soil:	Agricultural soil taken from the area belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna
Test design:	Three portions of soil weighing 1500 g each: one control group and two groups containing the test item. Every portion was divided into three replicates weighing 500 g each. Test duration: 28 days
Concentrations of the test item:	control, PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil), upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil).

Test conditions: temperature: 18.0 – 20.0°C,
soil moisture: 40.4% – 42.1% of the maximum water holding capacity, incubation in darkness

Statistical analysis: In order to determine significance in the soil respiration rate of differences between the control and the treated groups, Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity and Williams Multiple Sequential t-test Procedure were used

Endpoints: The mean respiration rate in the treated soil samples was compared with that in the control, and the percent deviation of the treated from the control was calculated after 0, 7, 14, and 28 days of incubation.

Results and discussions

The difference in the soil respiration rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil) and upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil) did not exceed 25% on 28 day of analysis.

Conclusion

On the basis of the results, it was concluded that Propamocarb 40% + Cymoxanil 5% SC at the concentrations corresponding to PEC: 108.6 mg test item/kg dry soil (i.e. 40.2 mg of propamocarb HCl/ kg dry soil + 4.8 mg of cymoxanil /kg dry soil) and upper PEC: 181.0 mg the test item/kg dry soil (i.e. 67.0 mg of propamocarb HCl/ kg dry soil + 8.0 mg of cymoxanil /kg dry soil), did not have any long-term adverse effects on the process of carbon transformation in aerobic surface soils.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study is considered valid. All validity criteria were met. • the seedling emergence in the control (validity criterion: at least 70%) was as follows: 100.0% – pea,

100.0% – cabbage,
95.0% – carrot,
100.0% – sunflower,
75.0% – onion,
95.0% – wheat,

- the mean survival of the emerged control seedlings was 100% for sunflower, pea, cabbage, onion and oats (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

Propamocarb 40% + Cymoxanil 5% SC: ER_{50}, and NOER values.

Endpoint value		Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Sunflower *Helianthus annuus*	Onion *Allium cepa*	Wheat *Triticum aestivum*
Plant number at the end of the experiment							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
	g/ha[a]	>6000	>6000	>6000	>6000	>6000	>6000
	g/ha[b]	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
	g/ha[a]	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
	g/ha[b]	≥750	≥750	≥750	≥750	≥750	≥750
Shoot length (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
	g/ha[a]	>6000	>6000	>6000	>6000	>6000	>6000
	g/ha[b]	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
	g/ha[a]	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
	g/ha[b]	≥750	≥750	≥750	≥750	≥750	≥750
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
	g/ha[a]	>6000	>6000	>6000	>6000	>6000	>6000
	g/ha[b]	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
	g/ha[a]	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
	g/ha[b]	≥750	≥750	≥750	≥750	≥750	≥750

a: Propamocarb HCl

b: cymoxanil

Request to the applicant:
The visual phototoxicity effects was not reported in the study. Therefore, applicant should add this information during commenting period.

Reference: KCP 10.6.2-01

Report: "Propamocarb 40% + Cymoxanil 5% SC. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test".
Anna Wróbel., G/132/18, 2020
Institute of Industrial Organic Chemistry, Branch Pszcyna

Guideline(s): OECD No. 208 (2006)

Deviations: Deviations from OECD Guideline No. 208:
According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 50.90 and 152.1 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the

	light intensity was suitable for plant growing. Deviation from the study plan: The study was finished in May 2020 and not in September/October 2019 as it had been planned.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Summary

The study, aimed at evaluating the effect of Propamocarb 40% + Cymoxanil 5% SC on seedling emergence and seedling growth of 6 terrestrial plants, was conducted on 4 dicotyledonous and 2 monocotyledonous species. The test item was sprayed onto the soil surface. For each species, five application rates were used. There was also a concurrent control group. Seeds of the test plant species were sown in plastic pots 3 (sunflower, pea, cabbage) or 5 (carrot, onion, wheat) seeds/pot). The experiment was conducted in a special room. Suitable environmental conditions for each test species were provided. During the experiment, the plants were observed for emergence (every day and then every 2 – 3 days) and visual phytotoxicity (after 7 and 14 days). The experiment finished 14 days after the emergence of 50% of the control seedlings. At the end of the experiment, the number of surviving plants was determined. Next, the plants were cut down, measured, dried to a constant weight at 60ºC, and weighed.

The results concerning the emergence, the shoot length, and the dry weight were statistically analyzed in order to determine the ER_{10}, ER_{25}, ER_{50}, and NOER.

Material and methods

Test item:	Propamocarb 40% + Cymoxanil 5% SC Batch number: SCL-64932 Production date: January 09, 2018 Expiry date: January 08, 2020
Test species::	sunflower (*Helianthus annuus*) pea (*Pisum sativum*), cabbage (*Brassica oleracea var. capitata*), carrot (*Daucus carota*), onion (*Allium cepa*), wheat (*Triticum aestivum*).
Test design:	Number of rates: 5 application rates + control Number of replicates: 4 (carrot, onion, wheat) or 7 (sunflower, cabbage, pea) Number of seeds: 20 (carrot, onion, wheat) or 21 (sunflower, cabbage, pea) The total number of plants per application rate: 20 (carrot, onion, wheat) or 21 (sunflower, cabbage, pea) Test termination: 14 days after the emergence of 50% of the control seedlings
Test duration:	14 days after 50 % emergence of the control seedlings.
Application rates:	a control, 15000, 5000, 1666.7, 555.6, 185.2 mL test item/ha (i.e. 6000 + 750, 2000 + 250, 666.7 + 83.3, 222.2 + 27.8, 74.1 + 9.3 g of propamocarb HCl + cymoxanil/ha) volume of deionized water used to prepare the highest rate corresponded 300 L water/ha
Soil:	sandy loam

Endpoints: ER_{10}, ER_{25}, ER_{50}, NOER

Test conditions: Temperature: 18.3 – 27.9°C
Humidity: 47.1 – 87.6%
Photoperiod – 16h day:8h night
Light intensity: 50.9 – 152.1 µE/m^2/s
Carbon dioxide concentration: 367– 387 ppm

Statistical analysis: ER_{10}, ER_{25}, ER_{50} – probit analysis,
NOER:
In order to determine the NOER values for the emergence the following statistical tests were used:
Fisher's Exact Binomial Test with Bonferroni Correction.
In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) and for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used:
- Shapiro-Wilk's Test on Normal Distribution,
- Levene's Test on Variance Homogeneity (with Residuals),
- Willimas Mulitiple Sequential t-test Procedure or Dunnett's Mulitiple t-test Procedure.

Validity criteria: - the seedling emergence in the control (validity criterion: at least 70%) was as follows:
100.0% – pea,
100.0% – cabbage,
95.0% – carrot,
100.0% – sunflower,
75.0% – onion,
95.0% – wheat,
- the mean survival of the emerged control seedlings was 100% for sunflower, pea, cabbage, onion and oats (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic symptoms
- environmental conditions for all plants belonging to the same species were identical.

Findings

Propamocarb 40% + Cymoxanil 5% SC: ER_{50}, and NOER values.

Endpoint value		**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Wheat** *Triticum aestivum*
Plant number at the end of the experiment							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
	g/ha[a]	>6000	>6000	>6000	>6000	>6000	>6000
	g/ha[b]	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
	g/ha[a]	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
	g/ha[b]	≥750	≥750	≥750	≥750	≥750	≥750
Shoot length (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
	g/ha[a]	>6000	>6000	>6000	>6000	>6000	>6000
	g/ha[b]	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
	g/ha[a]	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000

	g/ha[b]	≥750	≥750	≥750	≥750	≥750	≥750
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
	g/ha[a]	>6000	>6000	>6000	>6000	>6000	>6000
	g/ha[b]	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
	g/ha[a]	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
	g/ha[b]	≥750	≥750	≥750	≥750	≥750	≥750

a: Propamocarb HCl

b: cymoxanil

Comments of zRMS:

The study is considered valid. All validity criteria were met.

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

97.6 – 100.0 – pea,
92.9 – 97.6 – cabbage,
92.5 – 95.0 – carrot,
95.2 – 97.6– sunflower,
92.5 – 95.0 – onion,
85.0 – 95.0 – wheat,

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

Agreed endpoints:

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below:

Endpoint value		**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Wheat** *Triticum aestivum*
Plant number							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
NOER	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
Shoot length (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of propamocarb HCl/ha for all test species are given below.

Endpoint value		**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Wheat** *Triticum aestivum*
Plant number							
ER_{50}	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
NOER	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
Shoot length (plants without roots)							
ER_{50}	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
NOER	mL/ha	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
Plant dry weight (plants without roots)							

ER_{50}	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
NOER	mL/ha	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of cymoxanil/ha for all test species are given below.

Endpoint value		**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Wheat** *Triticum aestivum*
Plant number							
ER_{50}	mL/ha	>750	>750	>750	>750	>750	>750
NOER	mL/ha	>750	>750	>750	>750	>750	>750
Shoot length (plants without roots)							
ER_{50}	mL/ha	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥750	≥750	≥750	≥750	≥750	≥750
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥750	≥750	≥750	≥750	≥750	≥750

Request to the applicant:
The visual phototoxicity effects was not reported in the study. Therefore, applicant should add this information during commenting period.

Reference: KCP 10.6.2-02

Report "Propamocarb 40% + Cymoxanil 5% SC: Terrestial Plant Test: Vegetative Vigour Test". Anna Wróbel, 2020, Report number G/129/18. Institute of Industrial Organic Chemistry, Branch Pszczyna.

Guideline(s): OECD Guideline No. 227 (2006)

Deviations: Deviation from OECD Guideline No. 227:
According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 85.7 and 189.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
Deviation from the study plan:
According to SOP/G/70 and the Study Plan, the light intensity should be 50 – 150 µE/m2/s. During the experiment the light intensity was between 85.7 and 189.2 µE/m2/s.
Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
All above mentioned deviations did not affect the results of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item:	Propamocarb 40% + Cymoxanil 5% SC; Batch Number SCL-64932; active substance: Propamocarb HCl – 400 g/L, Cymoxanil – 50 g/L
Test species:	pea (Pisum sativum), cabbage (Brassica oleracea var. capitata), carrot (Daucus carota), sunflower (Helianthus annuus), onion (Allium cepa), wheat (Triticum aestivum).
Soil:	Sandy loam soil containing 1.2% organic carbon
Study design:	number of rates: 5 + control; number of replicates/rate: 4 (carrot, onion, wheat) or 7 (sunflower, cabbage, pea). The total number of plants per application rate – 20 (carrot, onion, wheat) or 21 (cabbage, pea, sunflower) Test termination: 21 days after the spraying
Application rates:	a control, 185.2, 555.6, 1666.7, 5000, 15000 mL test item/ha (i.e. 74.1 + 9.3, 222.2 + 27.8, 666.7 +83.3, 2000 + 250, 6000 + 750 g of propamocarb HCl + cymoxanil/ha) volume of deionized water used to prepare the highest rate corresponded 300 L water/ha
Test conditions:	temperature: 19.8 – 26.7°C, humidity: 45.5 – 88.4%, lighting: 16 h light : 8 h dark; light intensity: 85.7 – 189.2 µE/m2/s; carbon dioxide concentration: 353 – 387 ppm
Statistical analysis:	ER10, ER25, ER50 –probit analysis, NOER: In order to determine the NOER value for the plant number at the end of the experiment any computations had been perfomed because of no change in mortality of plants. In order to determine the NOER values for the shoot length at the end of the experiment (shoots cut down above the ground) and for the plant weight at the end of the experiment (shoots cut down above the ground), the following statistical tests were used: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure
Endpoints:	ER_{10}, ER_{25}, ER_{50} and NOER

Results and Conclusions

The test item, i.e. Propamocarb 40% + Cymoxanil 5% SC applied at rates ranging from 185.2 to 15000 mL test item/ha had no influence on the plant number, shoot length and shoot dry weight of the tested plant species at the end of the experiment.

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below:

Endpoint value		**Pea** *Pisum sativum*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Sunflower** *Helianthus annuus*	**Onion** *Allium cepa*	**Wheat** *Triticum aestivum*
Plant number							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
NOER	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
Shoot length (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>15000	>15000	>15000	>15000	>15000	>15000
NOER	mL/ha	≥15000	≥15000	≥15000	≥15000	≥15000	≥15000

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of propamocarb HCl/ha for all test species are given below.

Endpoint value		Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Sunflower *Helianthus annuus*	Onion *Allium cepa*	Wheat *Triticum aestivum*
Plant number							
ER_{50}	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
NOER	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
Shoot length (plants without roots)							
ER_{50}	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
NOER	mL/ha	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>6000	>6000	>6000	>6000	>6000	>6000
NOER	mL/ha	≥6000	≥6000	≥6000	≥6000	≥6000	≥6000

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of cymoxanil/ha for all test species are given below.

Endpoint value		Pea *Pisum sativum*	Cabbage *Brassica oleracea var. capitata*	Carrot *Daucus carota*	Sunflower *Helianthus annuus*	Onion *Allium cepa*	Wheat *Triticum aestivum*
Plant number							
ER_{50}	mL/ha	>750	>750	>750	>750	>750	>750
NOER	mL/ha	>750	>750	>750	>750	>750	>750
Shoot length (plants without roots)							
ER_{50}	mL/ha	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥750	≥750	≥750	≥750	≥750	≥750
Plant dry weight (plants without roots)							
ER_{50}	mL/ha	>750	>750	>750	>750	>750	>750
NOER	mL/ha	≥750	≥750	≥750	≥750	≥750	≥750

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

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

A 2.8 KCP 10.8 Monitoring data