REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: IN002B1760

Product name(s): Cymofil

Chemical active substance:

Cymoxanil, 450 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorisation)

Applicant: Indofil Industries (Netherlands) B.V.

Submission date: August 2022, updated January 2024

MS Finalisation date: May 2023 (initial Core Assessment)

February 2024 (Core assessment following the commenting period - 1st tour)

April 2024, updated May 20024

(Final Core assessment following the commenting period - 2 nd tour)

Version history

When	What
August 2022	Original version from applicant Indofil Industries (Netherlands) B.V. for submission to z-RMS, Poland, in the frame of the PPP Authorization according to Article 33 of Regulation (EC) No. 1107/2009.
May 2023	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.
January 2024	Applicant update following the conclusion of a new toxicity test with IN002B1760 on green alga (highlighted in turquoise).
February 2024	Core Assessment updated following the commenting period (1 sth tour) Additional information/assessments and new study for algae (subimitted on February 2024) included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through~~ and shaded.
April 2024	Core Assessment updated following the commenting period 2 nd tour). Additional information/assessments and new study for algae (submitted on February 2024) included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in purple. Information no longer relevant ~~is struck through~~ and shaded.
May 2024	Final report (National Assessment updated after the correction of Appendix 4 prepared by the Applicant) In order to facilitate tracking of changes in the Lists of data considered for national authorization (Appendix 4), amendments are highlighted in green, while not agreed use pattern is ~~struck through~~ and shaded.

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9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicological studies for the product **IN002B1760**, an fungicide containing the active substance Cymoxanil, 45 %w/w, in formulation: Wettable Granules (WDG), for use on potato. The uses on tomato and aubergine, which are grown in greenhouse, are addresses in the interzonal core dossier. Malta acts as the interzonal member state.

Cymoxanil was included in the Annex of Regulation (EU) 540/2011 (former Annex I of Directive 91/414/EEC) with Commission Directive 2008/125/EC of 19th December 2008. Where appropriate, this document refers to the agreed data of the active substance after EU review. The SANCO report for Cymoxanil (SANCO/179/08 rev.1, dated 9 July 2010) and the EFSA Conclusion on the pesticide risk assessment of cymoxanil (hereafter EFSA, 2008[1]) are considered to provide the relevant review information for cymoxanil.

The product IN002B1760 was not the representative formulation during EU review of the active substances. This dossier supports the authorisation of IN002B1760 according to art.33 of Regulation (EC) 1107/2009.

The applicant is the owner of a full and equivalent data package related to cymoxanil as demonstrated by a data matching check.

[1] European Food Safety Authority: Conclusion regarding the peer review of the pesticide risk assessment of the active substance cymoxanil. EFSA Scientific Report (2008) 167, 1-116

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of intended uses

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)	g product/ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL, AT, BE, CZ, DE, HU, IE, NL, RO, SK, SI	Potato	F	Downy Mildew (*Phythophtora infestans*)	Foliar spray	BBCH 12-95	6	5-10	a) 0.33 b) 1.98	a) 148.5 b) 891	300-500 ~~150-1000~~	7	250-330 g product/ha	A	C	A	A	A	A	A

* 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

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

The risk assessment for birds and mammals has been carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

The results of the acute risk assessments indicate acceptable risk to birds and mammals with TER values well above the trigger already at the screening step. For potatoes, the results of the long-term Tier-1 assessment showed acceptable risk for all the representative species and scenarios except for small herbivorous mammal "vole" (100% grass) and large herbivorous mammal "lagomorph" (100% non-grass herbs). Nevertheless, a higher tier risk assessment has been provided in order to demonstrate that IN002B1760 poses a low risk to these focal species when applied according to the proposed used pattern. For voles, considering, the refined DT_{50} for cymoxanil and updated deposition factors, no unacceptable risk is concluded. The same refinements have been used for the long-term risk assessment of large herbivorous mammal "lagomorph" in treated potato fields. ~~updated RUD values have been also considered.~~
Overall, all the TER_{lt} values exceed the trigger of 5, demonstrating that no unacceptable risk is expected due to the contamination of food items from spray application of IN002B1760.

No risk to birds and mammals is expected either following assumption of drinking water or via secondary poisoning.

It is possible to conclude that the exposure to cymoxanil following application of IN002B1760 according to the proposed use pattern doesn't pose unacceptable risks to birds and mammals.

During the EU evaluation of cymoxanil, no concern was identified for amphibians and reptiles. Considering that no harmonised test guidelines and risk assessment schemes are available to address this data point more thoroughly and in a consistent manner, no further action is deemed to be required.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290).

For potato, the PEC/RAC ratios, using worst-case PEC_{SW} values for cymoxanil and its relevant metabolites are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable and no mitigation measure is required.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to bees is assessed following the provisions of the 'Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC' (Anonymous 2002)[2], considering the EU agreed endpoints of the active ingredient, and the highest application rate of 148.5 g a.s./ha.

The result of the HQ calculation shows values well below the trigger, thus providing a wide safety margin to take into account any possible effect related to the formulation.

[2] Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002, 17th October 2002.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk assessment is conducted according to the ESCORT 2 Guidance Document (2000) and the Guidance Document on Terrestrial Ecotoxicology (2002).

~~Several studies on different arthropods species and with different cymoxanil formulations are available.~~ Laboratory and extended laboratory studies show that cymoxanil poses low toxicity to non-target arthropod species. Some concerns were raised in some of the available studies with *Typhlodromus pyri*, however, field data demonstrated that the adverse effect on this species is absent or transient under field conditions.

~~The quantitative risk assessment has been performed on the basis of the endpoints of Cymoxanil 45 WG which are in line with EU agreed endpoints coming from the studies with the EU representative formulation Cymoxanil 50% WP.~~ No unacceptable risk is expected for population of non-target arthropods living in the in-field and off-field areas, and no mitigation measure is required.

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

The evaluation of the risk for earthworms, other non-target soil organisms (meso- and macrofauna) and for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (2002).

~~TER_A values for acute risk assessment to earthworms are above the trigger of 10, demonstrating that no unacceptable acute risk is expected following the application of IN002B1760 according to the proposed use pattern.~~

New information on the reproductive effects on earthworms and on the reproductive effects on the standard soil-invertebrate species are available and have been taken into account. Corresponding TER_{LT} values are above the Regulation (EU) 546/2011 trigger of 5, demonstrating that no unacceptable long-term risk is expected following the application of IN002B1760 according to the proposed use pattern.

The risk to soil micro-organisms was evaluated by comparison of the maximum cymoxanil concentrations with effects $\leq$ 25% derived from laboratory tests, with the highest PEC_{soil}. The effect levels exceeded the relevant PEC_{soil} values, indicating that no unacceptable risk to soil micro-organisms is expected following the applications of IN002B1760 according to the proposed use pattern.

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

The risk assessment for non-target terrestrial plants in off-field areas is based on the "Guidance Document on Terrestrial Ecotoxicology" (2002).

No unacceptable risk to non-target terrestrial plants in off-crop areas is expected following the use of IN002B1760 according to the proposed use pattern.

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

Potential risks to terrestrial organisms have been assessed considering a broad range of species. Further testing is not deemed to be required.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the intended application use of the IN002B1760. As previously written, tomato and aubergine crops are addressed in the interzonal core dossier for uses in greenhouse.

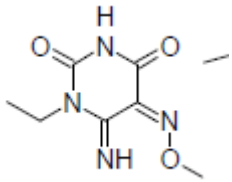
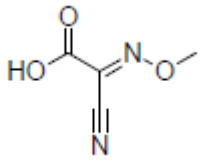
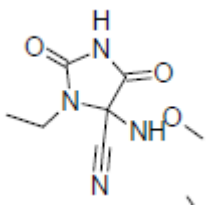
Table 9.1-2: Critical use pattern of IN002B1760

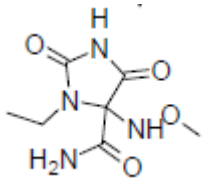
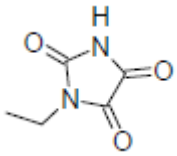
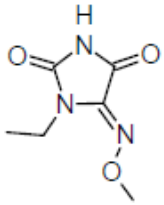
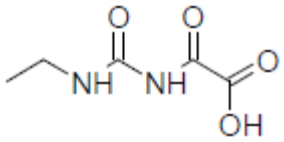
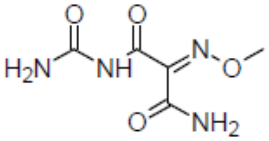
Grouping according to crop group and application pattern			
Group	**Intended uses**	**relevant use parameters for grouping**	**crop group and application pattern**
Birds and mammals (9.2 and 9.3)	Potato	Crop group according to EFSA/2009/1438	Potatoes; 6 × 148.5 g a.s./ha (5-day interval) BBCH 12-95
Aquatic organisms (9.5)	Potato	Drift values, surrogate FOCUS SW crop	Potatoes; 6 × 148.5 g a.s./ha (5-day interval) BBCH 12-95
Bees (9.6)	Potato	Application rate, crop	Potatoes; 6 × 148.5 g a.s./ha (5-day interval) BBCH 12-95
Soil meso- and macrofauna (9.8) Microbial activity (9.9)	Potato	Application rate, timining, annual/perennial crop	Potatoes; 6 × 148.5 g a.s./ha (5-day interval) BBCH 12-95
Arthropods other than bees (9.7) Non-target terrestrial plants (9.10)	Potato	Application rate, drift values	Field crops; 6 × 148.5 g a.s./ha (5-day interval) BBCH 12-95

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 IN002B1760 is indicated in the table.

Table 9.1-3 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)		198.2	Soil: max 24.7% AR by 0.33 day Water/sediment: max in water 24.7% AR after 0.13 d, max in sediment 0.5% AR after 3 d	Yes, soil and aquatic organisms
IN-W3595 Cyano(methoxyimino)acetic acid		128.1	Soil: max 10.1% AR by 1 day Water/sediment: max in water 26.1% AR after 0.25 d, max in sediment 2.3% AR after 1 d	Yes, soil and aquatic organisms
IN-JX915 3-ethyl-4-(methoxyamino)-2,5-dioxoimidazolidine-4-carbonitrile		198.2	Soil: 10.9% AR (n=1) Water/sediment: max in water 7.2% AR after 1 d, max in sediment 1.2% AR after 1 d	Yes, soil and aquatic organisms

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IN-KQ960 3-ethyl-4-(methoxyamino)-2,5-dioxoimidazolidine-4-carboxamide		216.9	Groundwater: max 6.3% AR by 3 days Water/sediment: max in water 13.0% AR after 1 d, max in sediment 5.5% AR after 30 d	Yes, aquatic organisms
IN-T4226 1-ethylimidazolidine-2,4,5-trione		142.1	Water/sediment: max in water 11.1% AR after 3 d, max in sediment 1.0% AR after 8 d	Yes, aquatic organisms
IN-R3273 1-ethylimidazolidine-2,4,5-trione 5-(Omethyloxime)		171.2	Water/sediment: max in water 5.0% AR after 3 d, max in sediment 0.5% AR after 3 d	Yes, aquatic organisms
IN-KP533 {[(ethylamino)carbonyl]amino}(oxo)acetic acid		160.1	Water/sediment: max in water 20.5% AR after 10 d, max in sediment 6.5% AR after 1 d	Yes, aquatic organisms
M5 N-(aminocarbonyl)-2-(methoxyimino)malonamide		198.2	Water/sediment: max in water 22.9% AR after 1 d, max in sediment 0.0% AR	Yes, aquatic organisms

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA Scientific Report (2008) 167, 1-116. The maximum occurrence is relevant for exposure evaluation, for more information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and $PEC_{sw/sed}$ values, considered further in the risk assessment.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of IN002B1760 were not evaluated as part of the EU assessment of cymoxanil. However, the provision of further avian toxicity data with this formulation is not considered essential since the risk to birds following the use of IN002B1760 can be adequately assessed through the risk assessment for its active substance. The risk to birds from the proposed uses of IN002B1760 will be assessed using the endpoints for cymoxanil.

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

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

Species	Substance	Exposure System	Results	Reference
Colinus virginianus Bobwhite quail	cymoxanil	Oral 1 d Acute	LD_{50} > 2000 mg a.s./kg bw	EFSA, 2008 [redacted]
Coturnix c. japonica Japanese quail	Cymoxanil 50 WP	Oral 1 d Acute	LD_{50} > 2000 mg product/kg bw	EFSA, 2008 [redacted]
Anas platyrhynchos Mallard duck	cymoxanil	Short-term 5 d	LD_{50} > 260* mg/kg bw/d	EFSA, 2008 [redacted]
Anas platyrhynchos Mallard duck	cymoxanil	Dietary Reproductive toxicity	NOAEL = 14.9 mg/kg bw/d (based on reduced egg production)	EFSA, 2008 [redacted]

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

zRMS comments:

Avian toxicity data for cymoxanil are in line with the EU agreed endpoints reported EFSA Scientific Report (2008) 167, 1-116.

9.2.1.1 Justification for new endpoints

No new endpoint has been considered.

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 table.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of IN002B1760 in potatoes

Intended use	**Potatoes**				
Active substance/product	Cymoxanil				
Application rate (g/ha)	6 × 148.5				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species (screening step)**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8	2.2	51.88	38.6

Reprod. toxicity (mg/kg bw/d)	14.9				
TER criterion	5				
Crop scenario Growth stage	**Indicator species (screening step)**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	64.8	3.0 × 0.53	15.30	**1.0**
Crop scenario Growth stage	**Generic focal species (Tier 1)**	**SV_m**	**MAF_m × TWA**	**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	3.0 × 0.53	2.29	6.51
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	3.0 × 0.53	0.78	19.12
Potatoes BBCH 10 - 19	Small insectivorous bird "wagtail" ground invertebrates without interception 50% ground arthropods, 50% foliar arthropods	11.3	3.0 × 0.53	2.67	5.58
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	10.9	3.0 × 0.53	2.57	5.79

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

The results of the acute risk assessment indicate acceptable risk to birds with TER values well above the trigger already at the screening step. The results of the long-term Tier-1 assessment show acceptable risk for all the representative species and scenarios relevant for the use in potatoes. Therefore, no further higher-tier assessment is required.

zRMS comments:

Screening step in the risk assessment

The acute screening step risk assessment for cymoxanil is validated by zRMS.
TER_A values for the exposure to cymoxanil for potatoes are above the trigger of 10, indicating acceptable risk for birds.
In case of long-term risk assessment, further calculations were needed at Tier 1.

Tier 1 risk assessment

The results of the long-term Tier-1 assessment for cymoxanil show acceptable risk for all the representative species and scenarios relevant for the use in potatoes.

Overall, based on calculations acceptable risk to birds from compounds active substance cymoxanil may be concluded from the intended uses of IN002B1760.

9.2.2.2 Higher-tier risk assessment

A higher-tier assessment is not required.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since IN002B1760 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a K(f)oc of 43.6 L/kg, cymoxanil belongs to the group of less sorptive substances. The assessment for the use on potatoes is shown below.

Potatoes (148.5 g a.s./ha, with 6 applications and 5 days interval):

Effective application rate (g/ha) =	445.5 (ARx MAF) ~~157.26~~		
Acute toxicity (mg/kg bw) =	2000	quotient= 0.222	~~0.079~~
Reprod. toxicity (mg/kg bw/d) =	14.9	quotient =29.9	~~10.554~~

~~The effective application rate (AReff) was calculated according to EFSA Jornal 2009; 7(12):1438, as following:~~

~~$AR_{eff} = AR \times MAF_{in} = AR \times [(1-e^{-nki})/(1-e^{-ki})]$~~

~~With:~~
~~$k = \ln(2)/DT_{50}$ (rate constant)~~
~~n= number of applications~~
~~i= application interval (d)~~
~~The DT_{50} is equal to 1.2 (geom. mean).~~
~~The trigger of the "escape clause" is met, and therefore TER calculation is not needed.~~

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 (KOC < 500 L/kg) or 3000 in the case of more sorptive substances (KOC ≥ 500 L/kg). The ratio is below the trigger value (Koc<500 L/kg) for a.s. cymoxanil indicating an acceptable risk and no further consideration is needed.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of cymoxanil was found to be 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.

No experimentally derived log P_{ow} values are available for cymoxanil metabolites. However, as exposed in the EFSA Scientific Report for Cymoxanil, these substances are considered unlikely to accumulate in fat tissue, based on mathematical models (KOWWIN, US-EPA) and on their chemical properties.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

zRMS comments:

We agree that the risk assessment for effects due to secondary poisoning is not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant

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

Not relevant.

9.2.4 Overall conclusions

The risk assessment for birds has been carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

The results of the acute risk assessment indicate acceptable risk to birds with TER values well above the trigger already at the screening step. The results of the long-term Tier-1 assessment showed acceptable risk for all the representative species and scenarios relevant for the use in potatoes.

No risk to birds is expected either following assumption of drinking water or via secondary poisoning.

It is possible to conclude that the exposure to cymoxanil following application of IN002B1760 according to the proposed use pattern doesn’t pose unacceptable risks to birds.

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

Effects on mammals of IN002B1760 were not evaluated as part of the EU assessment of cymoxanil. However, the provision of further mammalian toxicity data with this formulation is not considered essential since the risk to wild mammals following the use of IN002B1760 can be adequately assessed through the risk assessment for its active substance. The risk to birds from the proposed uses of IN002B1760 will be assessed using the endpoints for cymoxanil. For the reproductive assessment, the endpoint obtained from the long-term tox test was used, being the lowest value (10.5 mg a.s./kg bw) compared to the ratio LD_{50}/10 (96 mg a.s./kg bw).

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

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

Species	Substance	Exposure System	Results	Reference
Rattus norvegicus	cymoxanil	Oral 1 d Acute	LD_{50} = 760 mg a.s./kg bw	EFSA, 2008 [redacted]
Rattus norvegicus	Cymoxanil 50 WP	Oral 1 d Acute	LD_{50} = 1200 mg product/kg bw	EFSA, 2008 [redacted]
Rattus norvegicus	TANOS (cymoxanil 50 WG)	Oral 1 d Acute	LD_{50} (female) = 566 mg product/kg bw LD_{50} (male) = 1732 mg product/kg bw	EFSA, 2008 [redacted]
Rattus norvegicus	cymoxanil	Oral Developmental toxicity	NOEL = 10.5 mg a.s./kg bw (parental and offspring effects; no real reproductive effect)	EFSA, 2008 [redacted]

zRMS comments:

Mammalian toxicity data for cymoxanil are in line with the EU agreed endpoints reported in EFSA Scientific Report (2008) 167, 1-116.

9.3.1.1 Justification for new endpoints

No new endpoint has been considered.

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 table.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of IN002B1760 in potatoes

Intended use	**Potatoes**				
Active substance/product	cymoxanil				
Application rate (g/ha)	6 × 148.5				
Acute toxicity (mg/kg bw)	760				
TER criterion	10				
Crop scenario Growth stage	**Indicator species (screening step)**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	2.2	38.68	19.6
Reprod. toxicity (mg/kg bw/d)	10.5				
TER criterion	5				
Crop scenario Growth stage	**Indicator species (screening step)**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	48.3	3.0 × 0.53	11.40	**0.92**

Crop scenario Growth stage	**Generic focal species (Tier 1)**	**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	3.0 × 0.53	0.45	23.41
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	4.3	3.0 × 0.53	1.02	10.34
Potatoes BBCH ≥ 40	Small herbivorous mammal “vole” Grass + cereals 100% grass	21.7	3.0 × 0.53	5.12	**2.05**
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	3.0 × 0.53	0.54	19.33
Potatoes BBCH 10 - 19	Small insectivorous mammal “shrew” ground dwelling invertebrates without interception 100% ground arthropods	4.2	3.0 × 0.53	0.99	10.59
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	3.0 × 0.53	1.84	5.70
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	14.3	3.0 × 0.53	3.38	**3.11**

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

The results of the acute risk assessments indicate acceptable risk to birds with TER values well above the trigger at the screening step.
The results of the reproductive Tier 1 assessment show that TER values for the generic focal species small herbivorous mammal “vole” (100% grass) and large herbivorous mammal “lagomorph” (100% non-grass herbs) for the use in potatoes are below the trigger of 5 and further refinement are needed.

zRMS comments:

Screening step in the risk assessment

The acute screening step risk assessment for cymoxanil is validated by zRMS.
TER_A values for the exposure to cymoxanil for potatoes are above the trigger of 10, indicating acceptable risk for mammals.

In case of long-term risk assessment, further calculations are needed at Tier 1.

Tier 1 risk assessment

The results of the long-term Tier-1 assessment for cymoxanil show an unacceptable risk for vole at BBCH>40
And for large herbivorous mammal "lagomorph".

Therefore, further refinement was provided in the Point 9.3.2.2.

9.3.2.2 Higher-tier risk assessment

Small herbivorous mammal

The first-tier risk assessment after the application of IN005B1760 indicates a long-term risk of cymoxanil for the generic specie Common vole. Thus, a refined risk assessment for long-term exposure of mammals must be performed. A refinement of the reproductive dietary risk assessment was considered. For this refinement, the calculation below is considered.

Toxicity Exposure Ratio (TER) = Toxicity endpoint/ Daily Dietary Dose (DDD)

DDD = (FIR/bw) x PT x PD x RUD x DF x Application rate x MAF x TWA

Where:

- FIR/bw= Food intake rate/body weight
- PT = Fraction of diet obtained in the treated area (related to foraging time)
- PD = Composition of diet obtained from the treated area
- RUD = Residue per unit dose
- DF = Deposition factor
- MAF = Multiple application factor if more than one application
- TWA = Time weighted average factor (only for reproductive risk assessment)

A more realistic values for the multiple application factor (MAF) and the time weighted average factor TWA were considered instead of the default ones provided by EFSA document. As stated in Cymoxanil DAR, an estimated DT_{50} of 2 days can be considered an appropriate and still worst-case assumption for the residue calculation in vegetation. Therefore, MAF and TWA have been re-calculated using the "moving time-window approach" as described in Appendix H of EFSA/2009/1438. The rrefinement presented below is based on deposition/interception values according to Appendix A of EFSA/2009/1438.
The result of the refined risk assessment for small herbivorous mammal is reported in the following table.

Table 9.3-6. Higher Tier assessment of the reproductive risk for mammals for cymoxanil due the use of IN002B1760 in potatoes.

Active substance / product				-Cymoxanil ~~Difenoconazole~~						
Reproductive toxicity - LD_{50} (mg/kg bw)				-10.5- ~~17.3~~						
TER Criterion				5						
Application rate (g/kg)				6 x 148.5						
Growth stage	**Generic focal species**	**FIR/bw**	**PT**	**PD**	**RUD_m**	**DF**	**MAF~~A~~$_m$**	**TWA**	**DDD (mg a.s./ kg bw/d)**	**TER**
BBCH ≥ 40	Small herbivorous mammal "vole"	1.33	1.0	1 100 % grass (grass +cereals)	54.2	0.3	1.215	0.49	1.91	5.49

FIR/bw: Food intake rate/body weight; PT: fraction of diet obtained in treated area; PD = Composition of diet obtained from the treated are; RUD = residue per unit dose; DF = Deposition factor; 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.
* FOCUS, 2014 (ground water assessment)

Based on the higher risk assessment step, TER value for the long-term risk resulting from an exposure of small herbivorous mammal 'vole' to cymoxanil is above the trigger of 5 for potatoes. Therefore, acceptable risk can be demonstrated for small herbivorous mammals and it therefore seems highly unlikely that population abundances of small herbivorous mammals such as the vole will be affected on a long-term scale.

Large herbivorous mammals

The long-term risk assessment for the large herbivorous mammal "lagomorph" feeding in treated potato fields is refined considering the quick dissipation of cymoxanil (estimated DT_{50} of 2 days in vegetation).

Table 9.3- 7 ~~3~~: Refined long-term risk assessment for large herbivorous mammals due to the use of IN002B1760 in potato

Intended use				Potatoes						
Active substance/product				cymoxanil						
Application rate (g/ha)				6 x 148.5						
Number of application (interval)				6 (5 days)						
Reprod. toxicity (mg/kg bw/d)				10.5						
TER criterion				5						
Growth stage	**Generic focal species**	**FIR/bw**	**PT**	**PD**	**RUD_m**	**DF**	**MAF_{A-m}**	**TWA**	**DDD (mg a.s./ kg bw/d)**	**TER**
BBCH 10-40	Rabbit (*Oryctolagus cuniculus*)	0.5	1 100 % non-grass herbs	1	28.7	1	1.215	0.49	1.27	8.28

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.

Since the obtained TER value is above the trigger of 5 for the identified focal species, it is possible to conclude that IN002B1760 does not pose unacceptable long-term risks to large herbivorous mammals when applied according to the proposed use pattern.

zRMS comments:

zRMS amended the risk assessment for vole at BBCH>40. Therefore, additional calculations are performed for diets consisting of 50% of monocots and 50% of dicots for proposed use in potatoes based on the diet of voles from Ctgb Manual 2017 for this species for arable crops (dicot dominate).

The relevant zRMS's calculations of FIR/bw are provided below:

Table 9.3-7. FIR/bw calculations for vole

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			

Based on the calculations provided above the diet consisting of 50% monocots and 50% dicots the FIR/bw is 1.462. Therefore, zRMS provided the own risk assessment for vole at BBCH> 40. 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 to 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 addition, based on DT_{50} of 2 days MAF and twa parameters are refined.

In case of the application up to **BBCH 89 instead of 95,** the risk is acceptable. As only one application will be possible between BBCH 89 and 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.

Table 9.3-8. The long -term risk assessment based on refined parameters for vole at BBCH>40.

Intended use	Potatoes							
Active substance/product								
Application rate (g/ha)	6 × 148.5 with 5 days interval							
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**	**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	3.0× 0.53	1	1.40	
	Dicot plants	0.5	1.462	28.7 × 0.15	1.215 x 0.49	1	0.28	
	whole diet							6.25

Large herbivorous mammals

The risk assessment for Large herbivorous mammals based on a more realistic values for the multiple application factor (MAF) and the time weighted average factor TWA were considered instead of the default ones provided by EFSA document. As stated in Cymoxanil DAR, an estimated DT_{50} of 2 days can be considered an appropriate. Therefore, MAF and TWA have been re-calculated using the "moving time-window approach" as described in Appendix H of EFSA/2009/1438.

Based on those calculations provided above the risk is considered as 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 K(f)oc of 43.6 L/kg, cymoxanil belongs to the group of less sorptive substances.

Potatoes (148.5 g a.s./ha, with 6 applications and 5 days interval):

Effective application rate (g/ha) =	455.5 ~~157.26~~	
Acute toxicity (mg/kg bw) =	760	quotient = 0.6 ~~0.207~~
Reprod. toxicity (mg/kg bw/d) =	10.5	quotient = 43.4 ~~14.977~~

~~The trigger of the "escape clause" is met. Therefore, no TER calculations are needed.~~

~~The effective application rate (AReff) was calculated according to EFSA Jornal 2009; 7(12):1438, as following:~~

$$AR_{eff} = AR \times MAF_{in} = AR \times [(1-e^{-nki})/(1-e^{-ki})]$$

~~With:~~
~~k= ln(2)/DT_{50} (rate constant)~~
~~n= number of applications~~
~~i= application interval (d)~~

~~The DT_{50} is equal to 1.2 (geom. mean).~~
~~The trigger of the "escape clause" is met, and therefore TER calculation is not needed.~~

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 (KOC < 500 L/kg) or 3000 in the case of more sorptive substances (KOC ≥ 500 L/kg). The ratio is below the trigger value (Koc<500 L/kg) for a.s. – cymoxanil, indicating an acceptable risk and no further consideration is needed.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of cymoxanil was found to be 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.
No experimentally derived log P_{ow} values are available for cymoxanil metabolites. However, as exposed in the *EFSA Scientific Report for Cymoxanil*, these substances are considered unlikely to accumulate in fat tissue, based on mathematical models (KOWWIN, US-EPA) and on their chemical properties.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The risk assessment for mammals has been carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

The results of the acute risk assessments indicate acceptable risk to mammals with TER values well above the trigger already at the screening step. The results of the reproductive Tier 1 assessment show that TER values for the focal species small herbivorous mammal “vole” (100% grass) and large herbivorous mammal “lagomorph” (100% non-grass herbs) for the use in potatoes are below the trigger of 5 and further refinement are needed.

Based on the refined assessment step of reproductive dietary risk assessment, the calculated TER values for the long-term risk resulting from the cymoxanil exposure in the evaluated scenarios achieve the acceptability criteria TER ≥ 5 for long-term effects.

No risk to mammals is expected either following assumption of drinking water or via secondary poisoning.

It is possible to conclude that the exposure to cymoxanil following application of IN002B1760 according to the proposed use pattern doesn’t pose unacceptable risks to mammals.

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

During the EU evaluation of cymoxanil, no concern was identified for amphibians and reptiles. Considering that no harmonised test guidelines and risk assessment schemes are available to address this data point more thoroughly and in a consistent manner, no further action is deemed to be required.

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 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 IN002B1760 were not evaluated as part of the EU assessment of cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. A new toxicity test carried out with IN002B1760 on the green alga *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) has been included within this section and considered for risk assessment purposes. According to the available information on the acute toxicity of cymoxanil to aquatic organisms, green algae are expected to be the most sensitive aquatic organism group. The new study is listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Acute toxicity to fish				
Lepomis macrochirus	cymoxanil	96 h, s	LC_{50} = **29 mg a.s./L** mm	EFSA, 2008 [redacted]
Oncorhynchus mykiss	IN-U3204	96 h, s	LC_{50} > **97 mg met./L** mm	EFSA, 2008 [redacted]
Oncorhynchus mykiss	IN-W3595	96 h, s	LC_{50} > **130 mg met./L** mm	EFSA, 2008 [redacted]
Oncorhynchus mykiss	IN-KQ960	96 h, s	LC_{50} > **120 mgmet./L** mm	EFSA, 2008 [redacted]
Oncorhynchus mykiss	IN-T4226	96 h, s	LC_{50} > **111 mg met./L** mm	EFSA, 2008 [redacted]
Oncorhynchus mykiss	Cymoxanil 50 WP	96 h, ss	LC_{50} = 120 mg prod./L nom (equivalent to 60.6 mg a.s./ha)	EFSA, 2008 [redacted]
Oncorhynchus mykiss	TANOS	96 h, f	LC_{50} = 0.0287 mg prod./L nom	EFSA, 2008 [redacted]
Long-term toxicity to fish				
Oncorhynchus mykiss	cymoxanil	90 d (ELS), f	NOAEC = **0.044 mg a.s./L** mm	EFSA, 2008 [redacted]
Acute toxicity to aquatic invertebrates				
Daphnia magna	cymoxanil	48 h, s	EC_{50} = **27 mg a.s./L** mm	EFSA, 2008 Baer (1993c) DAR IIA, 8.2.4
Daphnia magna	IN-U3204	48 h, ss	EC_{50} = **100 mg met./L** mm	EFSA, 2008 Samel (2002c) DAR IIA, 8.2.4
Daphnia magna	IN-W3595	48 h, s	EC_{50} > **126 mg met./L** mm	EFSA, 2008 Boeri et al. (2002d) DAR IIA, 8.2.4
Daphnia magna	IN-KQ960	48 h, s	EC_{50} = **0.8 mg met./L** mm	EFSA, 2008 Samel (2002d) DAR IIA, 8.2.4
Daphnia magna	IN-T4226	48 h, ss	EC_{50} > **116 mg met./L** mm	EFSA, 2008 Boeri et al. (2002c) DAR IIA, 8.2.4
Daphnia magna	Cymoxanil 50 WP	48 h, ss	EC_{50} > 200 mg prod./L nom (equivalent to > 101 mg a.s./ha)	EFSA, 2008 Migchielsen (1999) DAR IIIA, 10.2.1
Daphnia magna	TANOS	48 h, f	EC_{50} = 0.0555 mg prod./L nom	EFSA, 2008 Brown (1997b) DAR IIIA, 10.2.1
Long-term toxicity to aquatic invertebrates				

Species	Substance	Exposure System	Results	Reference
Daphnia magna	cymoxanil	21 d, ss	NOEC = **0.067 mg a.s./L** $_{mm}$	EFSA, 2008 Baer (1993d) DAR IIA, 8.2.5
Daphnia magna	IN-KQ960	21 d, ss	NOEC = 0.302 mg met./L $_{mm}$	EFSA, 2008 Samel (2003) DAR IIA, 8.2.5
Toxicity to green 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 $_{im}$	EFSA, 2008 Hughes et al. (1996a) DAR IIA, 8.2.6
Anabaena flos-aquae	IN-W3595	96 h, s	E_rC_{50} = 19.9 mg met./L $_{nom}$ E_bC_{50} = **12.7** mg met./L $_{nom}$	EFSA, 2008 Sloman (2001b) DAR IIA, 8.2.6
Anabaena flos-aquae	IN-T4226	96 h, s	E_rC_{50} = 35.9 mg met./L $_{nom}$ E_bC_{50} = **25.8** mg met./L $_{nom}$	EFSA, 2008 Sloman (2001a) DAR IIA, 8.2.6
Pseudokirchneriella subcapitata	Cymoxanil 50 WP	72 h, s	E_rC_{50} = 4.72 mg prod./L $_{twa}$ (equivalent to E_rC_{50}> 0.41 mg a.s./ha) E_bC_{50} = 0.11 mg a.s./L $_{twa}$	EFSA, 2008 Bogers (1999b) DAR IIIA 10.2.1
Pseudokirchneriella subcapitata	DPX-KX007	72 h, s	E_rC_{50} = 11.0 mg prod./L $_{nom}$ (equivalent to > 0.41 mg a.s./ha) E_bC_{50} = 4.2 mg prod./L $_{nom}$	EFSA, 2008 Leva et al. (1997) DAR IIIA 10.2.1
Toxicity to aquatic plants				
Lemna gibba	cymoxanil	14 d, s	E_rC_{50} > **0.7** mg a.s./L $_{im}$ E_bC_{50} > 0.7 mg a.s./L $_{im}$	EFSA, 2008 Leva et al. (1996) DAR IIA, 8.2.8
Higher-tier studies (micro- or mesocosm studies)				
-				

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – IN002B1760

Species	Substance	Exposure System	Results	Reference
~~*Danio rerio*~~	~~Moximate 505 WP~~	~~96 h, *ss*~~	~~LC_{50} = 30.3 mg prod./L $_{nom}$ (0.4 mg a.s./ L $_{nom}$)~~	[redacted] ~~(2009a)~~ ~~KCP 10.2.1/01~~
~~*Daphnia magna*~~	~~Moximate 505 WP~~	~~48 h, *ss*~~	~~EC_{50} = 56.2 mg prod./L $_{nom}$ (2.3 mg a.s./ L $_{nom}$)~~	~~Neri, M.C. (2009b),~~ ~~Doc. No. 822-004,~~ ~~KCP 10.2.1/02~~
~~*Pseudokirchneriella subcapitata*~~	~~Moximate 505 WP~~	~~72 h, *s*~~	~~E_yC_{50} = 18.3 mg prod./L $_{nom}$ (0.1 mg a.s./L)~~ ~~E_rC_{50} = 16.8 mg prod./L $_{twa}$ (0.117 mg a.s./L)~~	~~Neri, M.C. (2009c),~~ ~~Doc. No. 823-004,~~ ~~KCP 10.2.1/03~~
Raphidocelis subcapitata (formerly known as *Pseudokirchneriella subcapitata*)	IN002B1760	72 h, s	E_rC_{50} = 15.93 mg prod./L $_{nom}$ (equivalent to = 7.22 mg a.s./L) E_yC_{50} = 4.02 mg prod./L $_{nom}$ (equivalent to = 1.82 mg a.s./L)	Venkanna, B. (2024), Report No.: 23/0177, KCP 10.2.1/04

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

The following table compares the toxicity between active substance and Cymoxanil 450 g/kg to the most aquatic sensitive species expressed in terms of the toxicity of the active substance.

Species	Cymoxanil (a.s)	IN002B1760
Fish	29 mg a.s./L $_{nom}$	~~0.4 mg a.s./L $_{nom}$~~, not available
Aquatic Invertebrates	27 mg a.s./L $_{nom}$	~~2.3 mg a.s./L$_{nom}$~~ not available
Algae	E_bC_{50}= 0.122 mg a.s./L $_{mm}$ E_rC_{50} = 0.254 mg a.s./L	~~0.117 mg a.s./L~~ E_yC_{50}=1.82 mg a.s./L E_rC_{50}=7.22 ~~15.93~~ mg a.s./L

The formulation Moximate 505 WP is more toxic to fish, aquatic invertebrates and algae than on it's own as an active substance. ~~Therefore, the risk assessment conducted for the formulation in the following sections covers the risk to these species for the Cymoxanil active substance.~~

~~No study on aquatic organisms were performed with IN002B1760. However,~~ The applicant owns an acute toxicity test on fish, *daphnia*, and the alga *Pseudokirchneriella subcapitata* carried out with the product Moximate 505 WP (Mancozeb 465 g/kg and Cymoxanil 40 g/kg, wettable powder formulation). This product has a worse ecotoxicological profile, nevertheless, the result of the studies confirms a low acute toxicity to aquatic organisms.
The new study on green alga carried out with IN002B1760 (KCP 10.2.1/04) shows that the formulation does not increase the toxicity of the active ingredient. Moreover, it has to be noted that IN002B1760 shows a lower toxicity to the green algae Raphidocelis subcapitata compared to the EU reference product Cymoxanil 50 WP.

Overall, is has been demonstrated that the risk assessment to aquatic organisms for IN002B1760 can be adequately assessed using the EU agreed endpoint of cymoxanil.

zRMS comments:

No studies for fish and aquatic invertebrates were submitted with IN002B1760. Instead of this the Applicant provided the studies for fish, *daphnia*, and the alga Pseudokirchneriella subcapitata carried out with the product Moximate 505 WP (Mancozeb 465 g/kg and Cymoxanil 40 g/kg, wettable powder formulation).
This approach is not validated by zRMS due to IN002B1760 is a solo formulation and generally aquatic studies should be provided with formulation on concern or similar.
In the same time, it should be taken into account the requirements outlined in Section 10 of PART A of the Annex to Regulation 284/2013.

10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes.

Circumstances in which required

Testing shall be performed where:

(a) the acute toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance; or

(b) the intended use includes direct application on water;

(c) extrapolation on the basis of available data for a similar plant protection product is not possible.

The active substance data are available for all group of aquatic organisms.
Based ~~on comparison of~~ the results presented for active substance ~~and solo formulation 50 WP (505 g s.a./kg)~~ the algae is the one of the most sensitive organism for both a.s. and representative formulation ~~and indicated similar toxicity to this aquatic organism (E_rC_{50} for a.s.=0.122 mg a.s./L and E_rC_{50}=0.41 mg a.s./L in case of formulation 50 WP).~~
In case of aquatic plants, the study for representative formulation is not available.
Therefore, for the most sensitive organism - algae the new formulation study by Venkanna, B. (2024) with IN002B1760 was submitted during commenting period process.
~~Therefore, the results indicated that toxicity mainly comes from that the active substance cymoxanil.~~
~~The formulation IN002B1760 contains cymoxanil in amount of 450 g /kg as the only active ingredient, and formulants (mainly filler and dispersing agents) are not expected to display toxicity to aquatic organism.~~

~~In zRMS's opinion based on all available data~~

Based on the results from the study on algae with formulation IN002B1760 with E_yC_{50} of 1.82 mg a.s./L and E_rC_{50} of 7.22~~77~~ mg a.s./L values it can be concluded that formulation does not increase the toxicity of the active substance to algae. The formulation study for Pseudokirchinella sp. appears more than 28 times less toxic than the a.s. for algae species Anabaena flos-aquae.
It has to be also noted that IN002B1760 shows a lower toxicity to the green algae Raphidocelis subcapitata compared to the EU reference product Cymoxanil 50 WP.
No study for green algae for technical a.s. is available at EU level.
However, it should be indicated that at EU level the study for the active substance for species Anabaena flos-aquae was considered in the risk assessment. The same approach was chosen by zRMS in the current dossier.
Taking into account that the risk for the a.s for the lowest endpoint for algae Anabaena flos-aquae passed the trigger value with STEP 2 it can be concluded that risk assessment for a.s for algae covers the risk assessment for formulation IN002B1760 to this organism.

9.5.1.1 Justification for new endpoints

No new endpoint has been considered.

9.5.2 Risk assessment

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

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

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

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cymoxanil for each organism group based on FOCUS Steps 1 and 2 calculations for the use of IN002B1760 in potatoes

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Algae**	**Aquatic plants**
Test species		***Lepomis macro-chirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Anabaena flos-aquae***	***Lemna gibba***
End-point (µg/L)		LC_{50} 29000	NOEC 44	EC_{50} 27000	NOEC 67	E_bC_{50} 122	E_rC_{50} 254	E_rC_{50} 700
AF		100	10	100	10	10	10	10
RAC (µg/L)		290	4.4	270	6.7	12.2	25.4	70
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	48.15	0.166	**10.943**	0.178	**7.187**	**3.947**	**1.89**	0.688
Step 2								
N-Europe (Mar-May)	1.37	0.005	0.311	0.005	0.204	0.112	0.054	0.020
N-Europe (Jun-Sep)	1.37	0.005	0.311	0.005	0.204	0.112	0.054	0.020
~~N-Europe (Oct-Feb)~~	~~2.09~~	~~0.007~~	~~0.475~~	~~0.008~~	~~0.312~~	~~0.171~~		~~0.030~~
S-Europe (Mar-May)	1.67	0.006	0.380	0.006	0.249	0.137	0.064	0.024
S-Europe (Jun-Sep)	1.37	0.005	0.311	0.005	0.204	0.112	0.054	0.020
~~S-Europe (Oct-Feb)~~	~~1.67~~	~~0.006~~	~~0.380~~	~~0.006~~	~~0.249~~	~~0.137~~		~~0.024~~

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

Relevant metabolites:

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite IN-U3204 based on FOCUS Steps 1 calculations in potatoes

Group		**Fish acute**	**Inverteb. acute**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***
Endpoint (µg/L)		LC_{50} 97000	EC_{50} 100000
AF		100	100
RAC (µg/L)		970	1000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	23.91	0.025	0.024

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 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite IN-W3595 based on FOCUS Steps 1 calculations in potatoes

Group		**Fish acute**	**Inverteb. acute**	**Algae**	
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***	
Endpoint (µg/L)		LC_{50} 130000	EC_{50} 126000	E_bC_{50} 12700	E_rC_{50} 19900
AF		100	100	10	10
RAC (µg/L)		1300	1260	1270	1990
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	72.76	0.056	0.058	0.057	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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite IN-KQ960 based on FOCUS Steps 1 and 2 calculations in potatoes

Group		**Fish acute**	**Inverteb. acute**	**Inverteb. prolonged**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg/L)		LC_{50} 120000	EC_{50} 800	NOEC 302
AF		100	100	10
RAC (µg/L)		1200	8	30.2
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	66.15	0.055	**8.269**	**2.190**
Step 2				
N-Europe (Mar-May)	2.11 ~~0.85~~	0.00175 ~~0.001~~	0.26 ~~0.106~~	0.07 ~~0.028~~
N-Europe (Jun-Sep)	2.11 ~~0.85~~	0.00175 ~~0.001~~	0.26 ~~0.106~~	0.07 ~~0.028~~
~~N-Europe (Oct-Feb)~~	~~1.24~~	~~0.001~~	~~0.155~~	~~0.041~~
S-Europe (Mar-May)	3.64 ~~1.11~~	0.0030 ~~0.001~~	0.45 ~~0.139~~	0.12 ~~0.037~~

S-Europe (Jun-Sep)	2.88 ~~0.98~~	~~0.001~~	~~0.123~~	~~0.032~~
~~S-Europe (Oct-Feb)~~	~~1.11~~	~~0.001~~	~~0.139~~	~~0.037~~

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 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite IN-T4226 based on FOCUS Steps 1 calculations in potatoes

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint **(µg/L)**		LC_{50} 111000	EC_{50} 116000	EC_{50} 25800
AF		100	100	10
RAC (µg/L)		1110	1160	2580
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	29.20	0.026	0.025	0.011

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

No acute studies on fish are available for metabolites IN-JX915, IN-R3273, IN-KP533, and the metabolite fraction M5. However, as stated in the *EFSA Scientific report for Cymoxanil*, in an additional static acute study on the toxicity of cymoxanil to fish, metabolites arising from the mainly abiotic degradation of the parent compound under test conditions were identified and quantified (in parallel test solutions without fish). Moreover, the PECsw values estimated for the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 were several orders of magnitude lower than the concentrations at which no fish died in the static test. Therefore, the risk to fish from these metabolites was considered to be low.

Long-term/chronic tests with metabolites are required only if the metabolites are more acutely toxic than the active substance. As above exposed, the available toxicity tests on fish indicate lower acute toxicity of the metabolites compared to the parent compound. It is therefore possible to conclude that the application of the product IN002B1760 according to the proposed uses, doesn't pose long-term risk to fish.

No studies on *Daphnia* are available for metabolites IN-JX915, IN-R3273, IN-KP533, and the metabolite fraction M5. However, as stated in the EFSA Scientific report for Cymoxanil, if the toxicity of these metabolites to daphnids were higher than the toxicity of the active substance by a factor of 100, TER values would still be above the respective Annex VI trigger, indicating a low acute risk to invertebrates.

The available acute toxicity tests on *Daphnia* indicate that Cymoxanil metabolites are less toxic than the parent compound, with the exception of metabolite IN-KQ960. A chronic toxicity test was therefore carried out for this metabolite, and the related risk to daphnids was assessed. Following an extreme worst-case approach, despite to the quick degradation of the a.s. Cymoxanil, the initial (maximum) PECsw was used in the long-term risk assessment for daphnids. As reported in the table above, the resulting TER values for Cymoxanil and IN-KQ960 are above the trigger value, indicating acceptable long-term risk to aquatic invertebrates.

No studies on algae are available for metabolites IN-U3204, IN-KQ960, IN-JX915, IN-R3273, IN-KP533, and the metabolite fraction M5. However, as stated in the ESA Scientific report for Cymoxanil, and accepted by member state experts at PRAPeR 48, all studies with algae were performed under stat-

ic conditions at relatively high pH levels. At alkaline pH levels the degradation of Cymoxanil is mainly driven by abiotic processes (basically hydrolysis). Hence from fate and behaviour information on Cymoxanil it is reasonable to assume that the metabolites INKQ960, IN-U3204, IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 have been present in the test solutions of the algae studies with Cymoxanil to a sufficient extent to have influenced the outcome of the studies. The risk to aquatic algae from exposure to these metabolites was therefore considered to be low, as it was covered by the assessment of the parent substance.

zRMS comments:

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 FOCUS Steps 2 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.

9.5.3 Overall conclusions

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290).

For all the intended uses, the PEC/RAC ratios, using worst-case PEC_{SW} values for cymoxanil and its relevant metabolites are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable and no mitigation measure is required.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation IN002B1760 which was performed in line with the EU agreed methodology.

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

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of IN002B1760 were not evaluated as part of the EU assessment of cymoxanil. New data submitted with this application and is summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	cymoxanil	Adult, acute oral	**LD_{50} > 85.3 µg/bee**	EFSA, 2008 Schur, A. (1999) DAR IIA, 8.3.1.1
Apis mellifera	cymoxanil	Adult, acute contact	**LD_{50} > 100 µg/bee**	
~~*Apis mellifera*~~	~~cymoxanil~~	~~Adult, acute oral~~	~~LD_{50} > 106.6 µg/bee~~	~~Schmitzer, S. (2007)~~ ~~Report No. 36571035~~ ~~KCP 10.3.1.1/01~~
~~*Apis mellifera*~~	~~cymoxanil~~	~~Adult, acute contact~~	~~LD_{50} > 100 µg/bee~~	
~~*Apis mellifera*~~	~~Moximate 505 WP~~	~~Adult, acute oral~~	~~LD_{50} > 600 µg prod./bee~~	~~Colli, M. (2009a)~~ ~~Doc. No. 832-004~~ ~~KCP 10.3.1.1/02~~
~~*Apis mellifera*~~	~~Moximate 505 WP~~	~~Adult, acute contact~~	~~LD_{50} > 300 µg prod./bee~~	
Apis mellifera	IN002B1760	Adult, chronic oral. 10-d feeding study	LDD_{50} = 10.7 µg prod./bee/day (corresponding to 5.1 µg a.s./bee/day)	Ponti, B. (2021) Report No. CH–0257/2021 KCP 10.3.1.2/01
Apis mellifera	IN002B1760	Larval mortality, Adult emergence. 22 d-oral, repeated exposure	NOED = 3.80 µg prod./larvae (corresponding to 1.79 µg a.s./larvae)	Noè, F. (2022) Report No. CH–0258/2021 KCP 10.3.1.3/01
Higher-tier studies (tunnel test, field studies)				
-				

zRMS comments:

Acute bee toxicity endpoints cymoxanil provided in Tables 9.6-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 167, 1-116.
Studies on acute effects of the formulated product to bees IN002B1760 were not submitted.
The acute studies for the a.s-cymoxanil was considered appropriate to use in the risk assessment.
It is noted that in order to fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on chronic and larvae toxicity were performed with the formulated product IN002B1760.

9.6.1.1 Justification for new endpoints

New data requirements, introduced by the Regulation (EU) 284/2013, related to the chronic toxicity to bees and the effects on other bee life stage are covered by the studies carried out with formulation IN002B1760, submitted within this application.

Moreover, an additional acute toxicity study carried out with the cymoxanil technical of the applicant source of active substance is available. The result is very similar to the EU agreed endpoint. The study was submitted and evaluated for the Annex II matching dossier owned by the applicant.

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 Summary for the results of the risk assessments for bees

1st tier contact - Tomato			
scenario	BBCH	Honeybee	
		HQ	trigger
treated crop	< 50	0.0	42
treated crop	≥ 50	0.0	42
weeds	< 50	8.9	42
weeds	≥ 50	2.7	42
field margin	< 50	0.2	42
field margin	≥ 50	0.2	42

1st tier contact - potatoes			
scenario	BBCH	Honeybee	
		HQ	trigger
treated crop	< 40	0.0	42
treated crop	≥ 40	0.0	42
weeds	< 40	7.4	42
weeds	≥ 40	2.2	42
field margin	< 40	0.2	42
field margin	≥ 40	0.2	42

1st Tier Oral - Tomato				
category	scenario	BBCH	Honeybee	
			ETR	trigger
acute	treated crop	< 10	0.01	0.2
acute	treated crop	≥ 70	0.00	0.2
acute	treated crop	10 - 69	0.08	0.2
acute	weeds	< 10	0.04	0.2
acute	weeds	≥ 70	0.04	0.2
acute	weeds	10 - 69	0.04	0.2
acute	field margin	< 10	0.00	0.2
acute	field margin	≥ 70	0.00	0.2
acute	field margin	10 - 69	0.00	0.2
acute	adjacent crop	< 10	0.00	0.2
acute	adjacent crop	≥ 70	0.00	0.2
acute	adjacent crop	10 - 69	0.00	0.2
acute	next crop	< 10	0.01	0.2
acute	next crop	≥ 70	0.01	0.2
acute	next crop	10 - 69	0.01	0.2

1st Tier Oral - Potatoes				
category	scenario	BBCH	Honeybee	
			ETR	trigger
acute	treated crop	< 10	0.01	0.2
acute	treated crop	≥ 70	0.00	0.2
acute	treated crop	10 - 69	0.07	0.2
acute	weeds	< 10	0.03	0.2
acute	weeds	≥ 70	0.03	0.2
acute	weeds	10 - 69	0.03	0.2
acute	field margin	< 10	0.00	0.2
acute	field margin	≥ 70	0.00	0.2
acute	field margin	10 - 69	0.00	0.2
acute	adjacent crop	< 10	0.00	0.2
acute	adjacent crop	≥ 70	0.00	0.2
acute	adjacent crop	10 - 69	0.00	0.2
acute	next crop	< 10	0.01	0.2
acute	next crop	≥ 70	0.01	0.2
acute	next crop	10 - 69	0.01	0.2

According to the EFSA bee tool, all the values calculated are below the trigger, and therefore the formulation does not pose a risk to bees.

Table 9.6- 1: First-tier assessment of the risk for bees due to the use of IN002B1760.

Intended use	Potato		
Active substance	cymoxanil		
Application rate (g/ha)	6 × 148.5		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 85.3	148.5	< 1.74
Contact toxicity	> 100		< 1.49

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

No acute oral toxicity test on bees was performed with IN002B1760. This formulated product contains cymoxanil as the only active ingredient, and formulants (mainly filler and dispersing agents) which are not expected to display any toxicity to non-target organisms. It is therefore reasonable to expect that any adverse effect possibly caused by IN002B1760 to honeybees is to be ascribed to the active ingredient cymoxanil. In addition, the applicant owns an acute toxicity study with bees carried out with the product Moximate 505 WP (Mancozeb 465 g/kg and Cymoxanil 40 g/kg, wettable powder formulation). This product has a worse ecotoxicological profile, nevertheless, the result of the study confirms a low acute toxicity to bees.

The EU agreed endpoints of the active ingredient were therefore used to assess the risk to honeybees. The result of such assessment gives HQ values well below the trigger (see above), thus providing a wide safety margin to take into account any possible effect related to the formulation.

Chronic risk assessment:

Screening step risk assessment

The acute and chronic risks to adult honey bees and honey bee larvae bees from the use of IN002B1760 were assessed using the maximum single application rates and the respective 'hazard quotients' (HQs) and 'exposure toxicity ratios' (ETRs).

Table 9.6-2. Screening step risk assessment

Test	**Endpoint µg a.s./bee**	**Calculation factor**	**ETR**	**Trigger**	**Risk acceptable?**
Cereals, BBCH 12-95 maximum application dose 148.5 g a.s./ha					
Oral route of exposure					
Acute oral	85.3	7.6	0.01	0.2	Yes
Acute contact	100	1	1.5	42	Yes
Honey bee, chronic	5.1	7.6 / 10.6	0.221	0.03	**No**
Honey bee, larvae	1.79	4.4 / 6.1	0.37	0.2	**No**

HQ/ETR values in bold are above the trigger value

Considering the proposed uses of at a maximum application rate of 0.1485 kg a.s./ha a potential risk of formulation is indicated following the chronic exposure of adults and for honey bee larvae at this stage of testing.
Therefore, 1st tier oral risk assessments were carried out (see Table below).

1st tier, oral risk assessment

In the screening step, potential risk was indicated for adult honey bees following the chronic exposure as well as for honey bee larvae. In the following, a crop and life stage-specific (adult/larvae) risk assessment is carried out, which is a first step of refinement. On the one hand, this takes into account crop dependent exposure factors (Ef), and on the other hand it considers SV values, which depend on default values for pollen and nectar consumption, sugar content in nectar, residues (RUDs) in pollen and nectar as well as crop attractiveness (see table below). It is noted that 1st tier risk assessment scheme in EFSA (2013) allows for distinguishing between particular BBCH stages of

the crop in question. Therefore, it was decided by the zRMS to perform separate risk assessment for particular stages at which. will be applied to potatoes.

Table 9.6-3. 1st tier oral risk assessment for honey bees (chronic and larvae).

Crop (Crop group according to EFSA tool)	Endpoint	ETR (oral exposure scenario)					Trigger
		Treated crop	Weeds	Field margin	Adjacent crop	Next crop	
Maximum single application rate: 0.1458 kg product/ha, BBCH 40-69							
Potato	adult, chronic	0.019	0.018	0.001	0.000	0.011	0.03
	larvae	0.01	0.05	0.00	0.00	0.03	0.2
Maximum single application rate: 0.1485 kg product/ha, BBCH >70							
Potato	adult, chronic	0.00	0.018	0.001	0.000	0.011	0.03
	larvae	0.00	0.05	0.00	0.00	0.03	0.2
Maximum single application rate: 0.1485 kg product/ha, BBCH 10-39							
Potato	adult, chronic	0.019	**0.061**	0.001	0.000	0.011	0.03
	larvae	0.01	0.16	0.00	0.00	0.03	0.2

Based on provided above calculations for application to potato an acceptable chronic risk could be concluded only for larvae ~~adult~~ bees. In the same time an unacceptable chronic risk for adult bees ~~bee larvae~~ was identified for ~~all scenarios.~~ exposure via flowering at BBCH 10-39. Further-consideration of refinement risk should be considered at MS level.
Risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

zRMS comment:

Acute risk assessment:

The acute risk assessment for bees presented in Table 9.6-1 is validated by the zRMS.
It should be noted that no acute studies for formulation are not available.
However, we agree that as the product IN002B1760 contains cymoxanil in amount of 450 g /L as the only active ingredient, and formulants (mainly filler and dispersing agents) are not expected to display toxicity to bees.
Therefore, the risk assessment based on the a.s. studies are considered sufficient in this case.

Chronic risk assessment to bees:

The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final.
Due to the fact that the chronic tests are available for adult bee and larvae, the screening step and Tier 1 risk assessment in line with EFSA (2013 it has been performed and checked by the zRMS below, using endpoints from submitted studies for formulation and for active substance.

All steps for the chronic risk assessment, i.e. the screening step, 1st and 2nd oral tier calculations were checked by zRMS in the Tables above using the corresponding EFSA Bee calculator Tool (Bee-Tool v.3) provided by EFSA.
For completeness acute risk was also presented also.

Based on calculations provided according to EFSA GD, 2013 the chronic risk to larvae bee at the Tier 1 is considered to be acceptable.
In the same time an unacceptable chronic risk for adult bees was identified from exposure via flowering at BBCH 10-39. Further-consideration of refinement risk should be considered at MS level.
Risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

Overall, no unacceptable acute effects for bees are expected following application of IN002B1760.

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

Not required.

9.6.4 Effects on bumble bees

No data or information are currently available for bumble bees.

Bumblebee acute oral and contact studies, chronic toxicity to adults and larvae are not required under Regulation (EC) No 1107/2009 therefore currently there is no data requirement for these study types.

9.6.5 Effects on solitary bees

No data or information are currently available for solitary bees.

There are no official validated test guidance documents for solitary bee testing.

For solitary bees there are currently no validated accepted test guidelines or guidance documents available. Ring tests are ongoing for the Osmia acute (contact and oral) study and semi field test design. Studies on solitary bees are not required under Regulation (EC) No 1107/2009, therefore currently there is no data requirement for these study types.

9.6.6 Overall conclusions

The risk to bees is assessed following the provisions of the 'Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC' (Anonymous 2002)[3], considering the EU agreed endpoints of the active ingredient, and the highest application rate of 148.5 g a.s./ha.

The result of the HQ calculation shows values well below the trigger, thus providing a wide safety margin to take into account any possible effect related to the formulation.

[3] Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002, 17th October 2002.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with representative cymoxanil-based formulations in the context of the EU assessment of the active substance. Full details of these studies are provided in the EU DAR and related documents.

Effects on non-target arthropods of IN002B1760 were not evaluated as part of the EU assessment of cymoxanil.

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

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

Species	Substance	Exposure System	Results	Reference
~~Laboratoy studies with Cymoxanil 50% WP~~				
Aphidius rhopalosiphi (adults)	~~Cymoxanil 50 WP~~	~~Laboratory test glass plates (2D), 48 hours~~	~~LR_{50} > 480 g a.s./ha~~ ~~No significant effects on reproduction at 480 g a.s./ha~~	~~EFSA, 2008~~ ~~Geuijen, W.H.C. (1999a)~~ ~~DAR IIA, 8.3.2~~
Typhlodromus pyri (protonymphs)	~~Cymoxanil 50 WP~~	~~Laboratory test glass plates (2D), 7 days~~	~~LR_{50} > 480 g a.s./ha~~ ~~Significant effects on reproduction at 240 g a.s./ha~~	~~EFSA, 2008~~ ~~Geuijen, W.H.C. (2000)~~ ~~DAR IIA, 8.3.2~~
Laboratory studies with Cymoxanil 45 WG (IN002B1760)				
Aphidius rhopalosiphi (adults)	Cymoxanil 45 WG (IN002B1760)	Laboratory test glass plates (2D), 48 hours	**LR_{50} > 960 g a.s./ha** No significant effects on reproduction up to and at 960 g a.s./ha	Moll, M. (2008a) Doc. No. 39701001, KCP 10.3.2.1/01
Typhlodromus pyri (protonymphs)	Cymoxanil 45 WG (IN002B1760)	Laboratory test glass plates (2D), 7 days	**LR_{50} > 480 g a.s./ha** Significant effects on reproduction at 30 g a.s./ha	Moll, M. (2008b) Doc. No. 39702063, KCP 10.3.2.1/02
Extended laboratory studies with Cymoxanil 45 WG				
Poecilus cupreus (adults)	Cymoxanil 45 WG	Extended laboratory test soil (2D), 7 days	No effects on either mortality, feeding activity or behaviour at 480 g a.s./ha	Schmitzer, S. (2008) Doc. No. 39703007, KCP 10.3.2.2/01
Chrysoperla carnea (larvae)	Cymoxanil 45 WG	Extended laboratory test bean leaves (2D), 10 days	No effects on mortality and reproduction performance at 480 g a.s./ha	Moll, M. (2008c) Doc. No. 39704047, KCP 10.3.2.2/02
Field studies ~~with Cymoxanil 45 WG~~				
Typhlodromus	Cymoxanil 45 WG	Field test	No effects on mobile stages or	Rosenkranz, B. and

Species		Substance	Exposure System	Results	Reference
pyri		vineyard (Germany), 41 days	eggs at 4×180 g a.s./ha		Schabio, S. (2009) Doc. No. 39706064, KCP 10.3.2.4/01
Typhlodromus pyri	Cymoxanil 50 WP	Field test vines (France), 29 days	no adverse effects on abundance (mites + eggs) 5×240 g a.s./ha or 4×480 g a.s./ha		EFSA, 2008 Müther, J. (2002) DAR IIA, 8.3.2
Typhlodromus pyri	DPX-KX007 52.5WG (famoxadone 22.5% and cymoxanil 30%, WG)	Field test vineyard (two locations in Germany), 4/5 weeks	Transient reductions in abundance (mites + eggs), no long-term effect 6×120–168 g a.s./ha		EFSA, 2008 Oberwalder, C. (1997) DAR IIIA, 10.5.1

~~The risk assessment will be carried out based on the available endpoints for Cymoxanil 45 WG and the EU representative formulation Cymoxanil 50 WP.~~

zRMS comment:

NTA toxicity endpoints cymoxanil provided in Tables 9.7-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 167, 1-116.

The laboratory studies performed with IN002B1760 (Cymoxanil 45 WG) were evaluated by the zRMS and considered acceptable.
In addition, one field study by Rosenkranz, B. and Schabio, S. (2009) was performed but it was considered by zRMS as additional information only.
For the studies summary and its evaluation by the zRMS, please refer to Appendix 2.

9.7.2 Justification for new endpoints

No new endpoint has been considered.

9.7.3 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.

9.7.3.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of IN002B1760 in potato

Intended use	Potato
Active substance/product	cymoxanil
Application rate (g/ha)	6 × 148.5
MAF	3.2

Test species Tier I	**LR_{50} (lab.)/ER_{50} (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
~~**Cymoxanil 50 WP (EFSA, 2008)**~~			
~~*Typhlodromus pyri*~~	~~> 480~~	~~475.20~~	~~< 0.990~~
~~*Aphidius rhopalosiphi*~~	~~> 480~~		~~< 0.990~~

Laboratory studies Cymoxanil 45 WG (IN002B1760)			
Typhlodromus pyri	> 960	475.20	< 0.495
*Typhlodromus pyri**	30 *		15.84*
Aphidius rhopalosiphi	> 480		< 0.990
Extended labolatory studies Cymoxanil 45 WG (IN002B1760)			
Species	ER_{50} (lab.) (g/ha)	**PERin field (g/ha)**	**In-field exposure > Effects value (trigger 50%)**
Poecilus cupreus (adults)	>480	475.20	No
Chrysoperla carnea (larvae)	>480	475.20	No

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.

*Based on effects on reproduction in laboratory glass plates study (indicative risk only)

9.7.3.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of IN002B1760 in potato

Intended use	Potato				
Active substance/product	cymoxanil / IN002B1760				
Application rate (g/ha)	6 × 148.5				
MAF	3.2				
vdf	10, 5**– (2D)				
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**
~~**Cymoxanil 50 WP (EFSA, 2008)**~~					
~~*Typhlodromus pyri*~~	~~> 480~~	~~1.64 %*~~	~~7.79~~	~~10~~	~~< 0.02~~
~~*Aphidius rhopalosiphi*~~	~~> 480~~				~~< 0.02~~
Cymoxanil 45 WG (IN002B1760)					
Typhlodromus pyri	> 960	1.64 %*	7.79	10	< 0.01 <0.02**
Typhlodromus pyri	30***		7.79**		0.26*** 0.52***
Aphidius rhopalosiphi	> 480		7.79		< 0.02 <0.04**
Extended labolatory studies Cymoxanil 45 WG (IN002B1760)					
Poecilus cupreus (adults), 2D	>480	1.64 %*	7.79	5 ~~10~~	<0.04
Chrysoperla carnea (larvae), 2D	>480				<0.08 ~~< 0.02~~

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.

* Drift value for field crops, 6 applications (70^{th} percentile)

** according to recommendation given in Harmonisation meeting in CZ.

*** Indicative risk assessment with consideration of effects on reproduction from laboratory study for T.pyri with consideration of VDF=10 and with assumption VDF=5, respectively.

zRMS comment:

The in-field exposure to the formulated product is amended in the Table 9.7-3 and Table 9.7-4 by the zRMS.

Based on the hazard quotients calculated based on Tier I (glass plate) LR_{50} values for Aphidius rhopalosiphi and Typhlodromus pyri, Cymoxanil 45 WG poses an acceptable risk to the indicator species following the proposed uses as the values calculated were lower than the trigger value of 2.

It is noted that if the ESCORT 2 guidance is strictly followed (use of just the ER50/LR_{50} values) then the first-tier risks are found acceptable for the proposed uses and in theory no further steps would have been required.

However, in all glass-plate studies with T. pyri effects in reproduction in all tested rates (for the lowest rate; 30 g.a.s/ha - 85.4% reduction in reproduction) were observed which cannot be overlooked.
Based on the an indicative risk assessment performed by zRMS based on effects at 30 g a.s./ha in the Table 9.7-3 the refinement for in-field exposure for T.pyri was needed for this species.

Therefore, field studies were carried out to investigate the effects of cymoxanil to populations of Typhlodromus pyri under field conditions in vineyard. A total of three field studies are available: one with Cymoxanil 50% WP (with application rate 5 x 240 or 4 x 480 g a.s./ha, one with DPX-KX007 52.5WG (famoxadone 22.5% and cymoxanil 30%, WG with application rate 6 x 120-168 g a.s/ha) evaluated at EU level and one with Cymoxanil 45 WG (with application 4 x 180 g a.s/ha) performed and evaluated for this submission.

All of them showed transient or no effect on the abundance of mites and eggs and it could be concluded that under field conditions no long-term impact on reproduction is expected (in line with the conclusion of EFSA, 2008).
In case of higher tier study by Rosenkranz, B (2009) performed under field conditions in vineyard the application did not cover the proposed GAP with 6 x 148.5 g a.s./ha and for this reason can be considered as additional information only.
In addition, extended laboratory studies were also performed with two additional species with different biologies; Poecilus cupreus and Chrysoperls carnea.
An acceptable risk to non-target arthropods can be concluded as the effects from the highest application rate in the additional studies were below 50% .

For off-field exposure as a worst case the VDF of 5 has been considered by zRMS, 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.

In line with Bullet Points: Ecotoxicology (CZSC November 2021) as long as adjustment to the guidance document has not been made, a VDF of 10 should be applied in core risk assessment.

We are aware that VDF of 10 should be used until the update of the guidance document
However, despite these agreements, we constantly receive comments from several Central Zone Member States to present the off-field risk assessment performed with consideration of VDF of 5. Taking this into account, it was decided to present such calculation to avoid these potential comments. Instead, we receive comment that we should not use VDF of 5.
Nevertheless, calculations for both VDF values are presented in Table 9.7-3 and the concerned Member States may decide which calculation is relevant at the national level.

Based on calculations performed with consideration of the Tier I laboratory data an acceptable off-field risk to non-target arthropods from the intended uses of IN002B1760 may be concluded with no need for risk mitigation measures.

Overall, no unacceptable effects for NTA are expected following application of IN002B1760.

9.7.3.3 Additional higher-tier risk assessment

A higher-tier risk assessment is not required.

Several studies on 9 different arthropods species (including the two indicator species, *Aphidius rhopalosiphi* and *Typhlodromus pyri*) and with different exposure systems were carried out with the EU representative formulation Cymoxanil 50% WP, with cymoxanil+famoxadone formulations, with Moximate 505 WP (a cymoxanil+mancozeb formulation) and with formulation Cymoxanil 45 WG. The studies carried out with the cymoxanil+famoxadone formulations were evaluated during the EU approval of the active substance; the studies with Moximate 505 WP, owned by the applicant, have been already submitted and evaluated under the framework of Reg. (EC) 1107/2009 for product authorisation; the studies with Cymoxanil 45 WG were performed and submitted for the Annex II matching dossier owned by the applicant.

The same level of toxicity was derived for the formulation Cymoxanil 50 WP and Cymoxanil 45 WG in studies with the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* and the additional species *Poecilus cupreus* and *Chrysoperla carnea*. For *Aphidius rhopalosiphi*, *Poecilus cupreus*, and *Chrysoperla carnea*, low mortality (<< 50%) and no significant effects on reproduction (for *A. rhopalosiphi* and *C. carnea*) or food consumption (for *P. cupreus*) were observed. For *Typhlodromus pyri*, however, the laboratory studies indicated potential adverse effects on reproduction.

The available studies carried out with cymoxanil+famoxadone and cymoxanil+mancozeb formulations confirm a general low toxicity to non-target arthropods, except in two studies on *Typhlodromus pyri* that showed again an inhibition of reproduction.

Therefore, field studies were carried out to investigate the effects of cymoxanil to populations of *Typhlodromus pyri* under field conditions. A total of three field studies are available: one with Cymoxanil 50% WP, one with DPX-KX007 52.5WG (famoxadone 22.5% and cymoxanil 30%, WG) and one with Cymoxanil 45 WG. All of them showed transient or no effect on the abundance of mites and eggs and it could be concluded that under field conditions no long-term impact on reproduction is expected (in line with the conclusion of EFSA, 2008).

Overall, no further information is deemed to be required and the risk assessment will be carried out based on the available endpoints for Cymoxanil 45 WG and the EU representative formulation Cymoxanil 50 WP.

Species	**Substance**	**Exposure System**	**Results**	**Reference**
Laboratoy studies with Cymoxanil 50% WP				
Aphidius rhopalosiphi (adults)	Cymoxanil 50 WP	Laboratory test glass plates (2D), 48 hours	LR_{50} > 480 g a.s./ha No significant effects on reproduction at 480 g a.s./ha	EFSA, 2008 Geuijen, W.H.C. (1999a) DAR IIA, 8.3.2
Typhlodromus pyri (protonymphs)	Cymoxanil 50 WP	Laboratory test glass plates (2D), 7 days	LR_{50} > 480 g a.s./ha Significant effects on reproduction at 240 g a.s./ha	EFSA, 2008 Geuijen, W.H.C. (2000) DAR IIA, 8.3.2
Poecilus cupreus (adults)	Cymoxanil 50 WP	Laboratory test quartz sand (2D). 14 days	LR_{50} > 480 g a.s./ha No significant effects on food consumption at 480 g a.s./ha	EFSA, 2008 Geuijen, W.H.C. (1999b) DAR IIA, 8.3.2
Chrysoperla carnea (larvae)	Cymoxanil 50 WP	Laboratory test glass plates (2D). 9 weeks	LR_{50} > 480 g a.s./ha No significant effects on reproduction at 480 g a.s./ha	EFSA, 2008 Geuijen, W.H.C. (1999c) DAR IIA, 8.3.2

Species	Substance	Exposure System	Results	Reference
Field studies with Cymoxanil 50% WP				
Typhlodromus pyri	Cymoxanil 50 WP	Field test vines (France), 29 days	no adverse effects on abundance (mites + eggs) @ 5×240 g a.s./ha or @ 4×480 g a.s./ha	EFSA, 2008 Müther, J. (2002) DAR IIA, 8.3.2
Laboratory studies with DPX-KX007				
Typhlodromus pyri (protonymphs)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Laboratory test glass plates (2D), 21 days	Mortality: 98% @ 132 g a.s./ha Reproduction: 100% @ 132 g a.s./ha	EFSA, 2008 Kuehner, C. (1996b) DAR IIIA, 10.5.1
Chrysoperla carnea (larvae)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Laboratory test glass plates (2D), 21+28 days	Mortality: -7.3% @ 132 g a.s./ha Reproduction: 17.3% @ 132 g a.s./ha	EFSA, 2008 Kuehner, C. (1995) DAR IIIA, 10.5.1
Poecilus cupreus (adults)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Laboratory test quartz sand (2D), 14 days	Mortality: 0% @ 132 g a.s./ha	EFSA, 2008 Kuehner, C. (1996a) DAR IIIA, 10.5.1
Extended laboratory studies with DPX-KX007				
Aphidius rhopalosiphi (adults)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Extended laboratory test tomato plants (3D), 48 hours + 10 days	Mortality: 0% @ 1×264 g a.s./ha 33% @ 3×264 g a.s./ha 25% @ 6×264 g a.s./ha Reproduction: 0% @ 1×264 g a.s./ha 34% @ 3×264 g a.s./ha 23% @ 6×264 g a.s./ha	EFSA, 2008 Mead-Briggs, M. (1996) DAR IIIA, 10.5.1
Typhlodromus pyri (protonymphs)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Extended laboratory test vine leaves (2D), 14 days	Mortality: 22.5% @ 1×120 g a.s./ha 40% @ 3×120 g a.s./ha 37.5 % @ 6×120 g a.s./ha 23.9% @ 9×120 g a.s./ha 21.2% @ 12×120 g a.s./ha Beneficial capacity: 24.8% @ 1×120 g a.s./ha 28.6% @ 3×120 g a.s./ha 27.5% @ 6×120 g a.s./ha 23.9% @ 9×120 g a.s./ha 16.5% @ 12×120 g a.s./ha	EFSA, 2008 Grove, A.J. (1996a) DAR IIIA, 10.5.1
Typhlodromus pyri (protonymphs)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Extended laboratory test vine leaves (2D), 14 days	Mortality: 25.3% @ 1×159 g a.s./ha Beneficial capacity: 65% @ 1×159 g a.s./ha 89% @ 3×159 g a.s./ha 82% @ 6×159 g a.s./ha	EFSA, 2008 Grove, A.J. (1996b) DAR IIIA, 10.5.1
Episyrphus balteatus (larvae)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Extended laboratory test bean plants (3D), 3 weeks	Mortality: 1.1% @ 1×289 g a.s./ha -3.4% @ 3×289 g a.s./ha -0.3% @ 6×289 g a.s./ha Reproduction: 33.4% @ 1×289 g a.s./ha 26.3% @ 3×289 g a.s./ha 23.2% @ 6×289 g a.s./ha	EFSA, 2008 Nengel, S. (1996a) DAR IIIA, 10.5.1

Species	Substance	Exposure System	Results	Reference
Poecilus cupreus (adults)	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	Semi-field test silty sand soil with potato plants, 7 days	Mortality: 11.3% @ 1×289 g a.s./ha 18.9% @ 3×289 g a.s./ha 7.5% @ 6×289 g a.s./ha	EFSA, 2008 Nengel, S. (1996b) DAR IIIA, 10.5.1
Laboratory studies with DPX-KP481				
Episyrphus balteatus (larvae)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Laboratory test glass plates (2D), 22 days	Mortality: 1.2% @ 186 g a.s./ha Reproduction: 33.5% @ 186 g a.s./ha	EFSA, 2008 Kuehner, C. (1997a) DAR IIIA, 10.5.1
Poecilus cupreus (adults)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Laboratory test quartz sand (2D), 14 days	Mortality: 0% @ 186 g a.s./ha	EFSA, 2008 Kuehner, C. (1997b) DAR IIIA, 10.5.1
Chrysoperla carnea (larvae)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Laboratory test glass plates (2D), 21+28 days	Mortality: -8.4% @ 186 g a.s./ha Reproduction: 4.1% @ 186 g a.s./ha	EFSA, 2008 Kuehner, C. (1997c) DAR IIIA, 10.5.1
Aleochara bilineata (larvae)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Laboratory test quartz sand (2D), 10 weeks	Mortality: 7.5% @ 186 g a.s./ha Parasitic capacity: 2.6% @ 186 g a.s./ha	EFSA, 2008 Kuehner, C. (1997d) DAR IIIA, 10.5.1
Extended laboratory studies with DPX-KP481				
Poecilus cupreus (adults)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Extended laboratory test soil (2D), 7 days	Mortality: 0% @ 1×264 g a.s./ha 0% @ 3×264 g a.s./ha 0% @ 6×264 g a.s./ha 0% @ 9×264 g a.s./ha	EFSA, 2008 Beech, P., Mead-Briggs, M. (1998) DAR IIIA, 10.5.1
Episyrphus balteatus (larvae)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Extended laboratory test potato leaves (2D), 4 weeks	Mortality: 0% @ 3×186 g a.s./ha 7% @ 6×186 g a.s./ha Reproduction: 69% @ 3×186 g a.s./ha 50% @ 6×186 g a.s./ha	EFSA, 2008 Thompson, B. (1999a) DAR IIIA, 10.5.1
Chrysoperla carnea (larvae)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Extended laboratory test potato leaves (2D), 10 days	Mortality: 23% @ 1×179 g a.s./ha 0% @ 3×179 g a.s./ha 13% @ 6×179 g a.s./ha	EFSA, 2008 Thompson, B. (1999c) DAR IIIA, 10.5.1
Episyrphus balteatus (larvae)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Semi-field test buckwheat plants, 14 days	Mortality: -1.3% @ 181 g a.s./ha 2.8% @ 363 g a.s./ha Reproduction: -58.1% @ 181 g a.s./ha -94.6% @ 363 g a.s./ha	EFSA, 2008 Hermann, P. (2001) DAR IIIA, 10.5.1
Aphidius colemani (adults)	DPX-KP481 (famoxadone 254 g/kg and cymoxanil 266 g/kg, WG)	Semi-field test potato plants, 12 days	Parasitation: -7% @ 1×179 g a.s./ha 25/26% @ 3×179 g a.s./ha 5/52% @ 6×179 g a.s./ha -43% @ 9×179 g a.s./ha	EFSA, 2008 Thompson, B. (1999b) DAR IIIA, 10.5.1
Extended laboratory studies with DPX-KX007 52.5WG				
Typhlodromus pyri	DPX-KX007 52.5WG (famoxadone 22.5%	Extended laboratory test	Mortality: 34.7% @ 1×134 g a.s./ha	EFSA, 2008 Grove, A.J. (1996b)

Species	Substance	Exposure System	Results	Reference
(protonymphs)	and cymoxanil 30%, WG)	grape leaves (2D), 14 days	42.4% @ 1×268 g a.s./ha Beneficial capacity: 85% @ 1×134 g a.s./ha 100% @ 3×134 g a.s./ha 100% @ 6×134 g a.s./ha 100% @ 1×268 g a.s./ha 100% @ 3×268 g a.s./ha 100% @ 6×268 g a.s./ha	DAR IIIA, 10.5.1
Trichogramma cacoeciae (adults)	DPX-KX007 52.5WG (famoxadone 22.5% and cymoxanil 30%, WG)	Extended laboratory test grape leaves (2D), 8 days	Reproduction: -11.7% @ 1×120 g a.s./ha -50.5% @ 3×120 g a.s./ha 13.1% @ 6×120 g a.s./ha 8.1% @ 9×120 g a.s./ha -5.6% @ 12×120 g a.s./ha	EFSA, 2008 Kuehner, C. (1998) DAR IIIA, 10.5.1
Field studies with DPX-KX007 52.5WG				
Typhlodromus pyri	DPX-KX007 52.5WG (famoxadone 22.5% and cymoxanil 30%, WG)	Field test vineyard (two locations in Germany), 4/5 weeks	Transient reductions in abundance (mites + eggs), no long-term effect @ 6×120–168 g a.s./ha	EFSA, 2008 Oberwalder, C. (1997) DAR IIIA, 10.5.1
Extended laboratory studies with Moximate 505 WP				
Typhlodromus pyri (protonymphs)	Moximate 505 WP (mancozeb 465 g/kg and cymoxanil 40 g/kg, WP)	Extended laboratory test bean leaves (2D), 14 days	LR_{50} > 10500 g prod./ha ER_{50} > 10500 g prod./ha	Colli, M. (2009c) Doc. No. 834-003, KCP 10.3.2.2/03
Aphidius rhopalosiphi (adults)	Moximate 505 WP (mancozeb 465 g/kg and cymoxanil 40 g/kg, WP)	Extended laboratory test wheat plants (3D), 48 hours + 10 days	LR_{50} > 10500 g prod./ha ER_{50} > 10500 g prod./ha	Colli, M. (2009b) Doc. No. 834-006, KCP 10.3.2.2/04
Chrysoperla carnea (larvae)	Moximate 505 WP (mancozeb 465 g/kg and cymoxanil 40 g/kg, WP)	Extended laboratory test bean plants (2D), 14 days	LR_{50} > 10500 g prod./ha ER_{50} > 10500 g prod./ha	Colli, M. (2009d) Doc. No. 834-004, KCP 10.3.2.2/05
Coccinella septempunctata (larvae)	Moximate 505 WP (mancozeb 465 g/kg and cymoxanil 40 g/kg, WP)	Extended laboratory test bean plants (2D), 7 days	LR_{50} > 10500 g prod./ha ER_{50} > 10500 g prod./ha	Colli, M. (2010) Doc. No. 834-005, KCP 10.3.2.2/06
Laboratory studies with Cymoxanil 45 WG				
Aphidius rhopalosiphi (adults)	Cymoxanil 45 WG	Laboratory test glass plates (2D), 48 hours	LR_{50} > 960 g a.s./ha No significant effects on reproduction up to and at 960 g a.s./ha	Moll, M. (2008a) Doc. No. 39701001, KCP 10.3.2.1/01
Typhlodromus pyri (protonymphs)	Cymoxanil 45 WG	Laboratory test glass plates (2D), 7 days	LR_{50} > 480 g a.s./ha **Significant effects on reproduction at 30 g a.s./ha**	Moll, M. (2008b) Doc. No. 39702063, KCP 10.3.2.1/02
Extended laboratory studies with Cymoxanil 45 WG				
Poecilus cupreus (adults)	Cymoxanil 45 WG	Extended laboratory test soil (2D), 7 days	No effects on either mortality, feeding activity or behaviour at 480 g a.s./ha	Schmitzer, S. (2008) Doc. No. 39703007, KCP 10.3.2.2/01
Chrysoperla carnea (larvae)	Cymoxanil 45 WG	Extended laboratory test bean leaves (2D),	No effects on mortality and reproduction performance at 480 g a.s./ha	Moll, M. (2008c) Doc. No. 39704047, KCP 10.3.2.2/02

Species	Substance	Exposure System	Results	Reference
		10 days		
Field studies with Cymoxanil 45 WG				
Typhlodromus pyri	Cymoxanil 45 WG	Field test vineyard (Germany), 41 days	No effects on mobile stages or eggs at 4×180 g a.s./ha	Rosenkranz, B. and Schabio, S. (2009) Doc. No. 39706064, KCP 10.3.2.4/01

9.7.3.4 Risk mitigation measures

No risk mitigation needed.

9.7.4 Overall conclusions

The risk assessment is conducted according to the ESCORT 2 Guidance Document (2000) and the Guidance Document on Terrestrial Ecotoxicology (2002).

Several studies on different arthropods species and with different cymoxanil formulations are available. Laboratory and extended laboratory studies show that cymoxanil poses low toxicity to non-target arthropod species. Some concerns were raised in some of the available studies with *Typhlodromus pyri*, however, field data demonstrated that the adverse effect on this species is absent or transient under field conditions.

The quantitative risk assessment has been performed on the basis of the endpoints of Cymoxanil 45 WG which are in line with EU agreed endpoints coming from the studies with the EU representative formulation Cymoxanil 50% WP. No unacceptable risk is expected for population of non-target arthropods living in the in-field and off-field areas, and no mitigation measure is required.

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 cymoxanil and the representative product Cymoxanil 50 WP. 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 IN002B1760 were not evaluated as part of the EU assessment of cymoxanil.

New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance / Product	Exposure System	Results	Reference
Eisenia fetida	cymoxanil	14 d, acute 10 % peat content	LC_{50} > 1000 mg a.s./kg dw $LC_{50,corr}$ > 500 mg a.s./kg dw*	EFSA, 2008 Rodgers, M.H. et al. (1995) DAR
Eisenia fetida	Cymoxanil 50 WP	14 d, acute 10 % peat content	LC_{50} > 505 mg a.s./kg dw $LC_{50,corr}$ > 252.5 mg a.s./kg dw*	EFSA, 2008 Rodgers, M.H. (1995) DAR
Eisenia	DPX-KX007 DF	14 d, acute	LC_{50} = 297 mg a.s./kg dw	EFSA, 2008

Species	Substance / Product	Exposure System	Results	Reference
~~*fetida*~~	~~famoxadone 22.5% and cymoxanil 30%, WP~~	~~10 % peat content~~	~~$LC_{50,corr}$ = 148.5 mg a.s./kg dw*~~	~~Wachter, S. (1997) DAR~~
~~*Eisenia fetida*~~	~~TANOS (DPX-KP481) famoxadone 254 g/kg and cymoxanil 266 g/kg, WG~~	~~56 d, chronic 10 % peat content~~	~~NOEC = 6.6 mg a.s./kg dw~~ ~~$NOEC_{corr}$ = 3.3 mg a.s./kg dw*~~	~~EFSA, 2008 Lührs, U. (2000) DAR~~
Eisenia fetida	IN002B1760	Mixed into substrate 56 d, chronic 10 % peat content	$NOEC_{repro}$ = 95.3 mg prod./kg dw (equivalent to 44.8 mg a.s./kg dw) $NOEC_{repro,corr}$ = 47.7 mg prod./kg dw (equivalent to **22.4** mg a.s./kg dw)*	Dini, R. (2022a) Doc. No. CH–0259/2021 KCP 10.4.1.1/01
Folsomia candida	IN002B1760	Mixed into substrate 21 d, chronic 5 % peat content	~~EC_{10} = **17.5** mg a.s. /kg~~** $NOEC_{repro}$ = 52.9 mg prod./kg dw (equivalent to 24.9 mg a.s./kg dw) , nom NOEC rep =31.584 mg a.s kg soil based on initial measured con.	Dini, R. (2022b) Doc. No. CH–0260/2021 KCP 10.4.2.1/01
Hypoaspis aculeifer	IN002B1760	Mixed into substrate 14 d, chronic 5 % peat content	~~EC_{10} = **69.2** mg a.s. /kg~~ ~~$NOEC_{repro}$ = 296.3 mg prod./kg dw (equivalent to 139.3 mg a.s./kg dw)~~ NOECrepro = 296.3 mg prod./kg dw (equivalent to 109.2 mg a.s./kg dw)	Dini, R. (2022c) Doc. No. CH–0261/2021 KCP 10.4.2.1/02
Field studies				
-				
Litter bag test				
-				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
** Not reliable value.

As stated by the *EFSA Scientific Report of Cymoxanil* (EFSA, 2008), the risk to earthworms for the major soil metabolites of Cymoxanil (IN-U3204, IN-W3595 and IN-JX915) can be considered adequately addressed by the risk assessment of the parent compound, due to the rapid degradation of the active ingredient and because these metabolites are assumed to have been formed in the test system during the studies on *Eisenia foetida* with the a.s. cymoxanil. For this reason, only the risk for the parent compound is hereunder addressed.

zRMS comments:

Soil meso-fauna toxicity endpoints for cymoxanil provided in Tables 9.8-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 167, 1-116. However, due to that LC_{50} values are not required now they were crossed out by zRMS in the Table above.
The studies performed with IN002B1760 (Cymoxanil 45 WG) were evaluated by the zRMS and considered acceptable.
For the study summary and its evaluation by the zRMS, please refer to Appendix 2.

9.8.1.1 Justification for new endpoints

New data requirements, introduced by the Regulation (EU) 284/2013, related to the reproductive toxicity to earthworms and on other soil non-target macro-organisms are covered by the studies carried out with formulation IN002B1760, submitted with this application.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for cymoxanil.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of IN002B1760 in potato

Intended use	Potato		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg a.s./kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
IN002B1760	~~22.4~~ 44.8	0.419	~~53.5~~ 106.92
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC**/~~EC_{10}~~ **(mg a.s./kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
IN002B1760 (*Folsomia candida*)	31.584 ~~17.5~~	0.419	75.38 ~~41.77~~
IN002B1760 (*Hypoaspis aculeifer*)	109.2 ~~69.2~~	0.419	260.2 ~~165.16~~

TER values shown in bold fall below the relevant trigger.

zRMS comments:

Based on calculations in the Table 9.8-2 above, acceptable risk to earthworms can be concluded, based on toxicity endpoints obtained for formulation expressed in a.s. kg/ dws. It should be noted that EC_{10} values for Folsomia candidia and Hypoaspis were considered not reliable taken into account the recommendation given In EFSA 2019 and for this reason NOEC values were considered in the risk assessment.

Overall, no unacceptable effects for earthworm and soil macro-organism are expected following application of IN002B1760.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

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" (2002).

~~TER_A values for acute risk assessment to earthworms are above the trigger of 10, demonstrating that no unacceptable acute risk is expected following the application of IN002B1760 according to the proposed use pattern.~~

New information on the reproductive effects on earthworms and soil macro-organism on the reproductive effects on the standard soil-invertebrate species are available and have been taken into account. Corresponding TER_{LT} values are above the Regulation (EU) 546/2011 trigger of 5, demonstrating that no unacceptable long-term risk is expected following the application of IN002B1760 according to the proposed use pattern.

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

Effects on soil microorganisms of IN002B1760 were not evaluated as part of the EU assessment of cymoxanil. However, the provision of further data on the formulation IN002B1760 is not considered essential since the risk to soil microorganisms following the use of IN002B1760 can be adequately assessed through the risk assessment for its active substance.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	cymoxanil	28 d, aerobic loamy sand soil	Nitrate formation rate: -15.5 % @ 1.6 mg a.s./kg soil dw (equivalent to 1.2 kg a.s./ha)	EFSA, 2008 Kölzer, U. (2003) DAR IIA, 8.5
N-mineralisation	DPX-KX007 (famoxadone 90 g/L and cymoxanil 120 g/L, SC)	28 d, aerobic sandy loam soil	Nitrate formation rate: -0.3 % @ 0.016 mL prod./kg soil dw (equivalent to 1.44 kg a.s./ha)	EFSA, 2008 Carter, J.N. (1995) DAR IIIA, 10.7
~~N-mineralisation~~	~~cymoxanil~~	~~28 d, aerobic loamy sand soil~~	~~Nitrate formation rate: -3.0 % @ 1.6 mg a.s./kg soil dw (equivalent to 1.2 kg a.s./ha)~~	~~Feil, N. (2008) Doc. No. 41382080, KCP 10.5.1/01~~

No studies on effects on soil microbial activity were carried out with IN002B1760. This formulated product contains cymoxanil as the only active ingredient, and formulants (mainly filler and dispersing agents) which are not expected to display any toxicity to non-target organisms. It is therefore reasonable to expect that any adverse effect possibly caused by IN002B1760 to soil microflora is to be ascribed to the a.s. cymoxanil.

zRMS comments:

Micro-organism toxicity endpoints for cymoxanil provided in Table 9.9-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 167, 1-116.

It should be noted that no studies for formulation IN002B1760 are available.

We agree that as the product IN002B1760 contains cymoxanil in amount of 450 g /L as the only active ingredient, and formulants (mainly filler and dispersing agents) are not expected to display toxicity to microorganism.
Therefore, the risk assessment based on the a.s.. studies are considered sufficient in this case.
Overall, no unacceptable effects for soil micro-organism are expected following application of IN002B1760.

9.9.1.1 Justification for new endpoints

An additional test on the effects cymoxanil technical on soil microbial activity carried out with the applicant source of active substance is available. The outcome of this study is in line with the EU agreed endpoint. The study was submitted and evaluated for the Annex II matching dossier owned by the applicant.

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 IN002B1760 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?**
cymoxanil	1.6 (at 28 d)	0.419	yes

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 IN002B1760.

9.9.3 Overall conclusions

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (2002).

The risk to soil micro-organisms was evaluated by comparison of the maximum cymoxanil concentrations with effects $\leq$ 25% derived from laboratory tests, with the highest PEC_{soil}.

The effect levels exceeded the relevant PEC_{soil} values, indicating that no unacceptable risk to soil micro-organisms is expected following the applications of IN002B1760 according to the proposed use pattern.

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

Effects on non-target terrestrial plants of IN002B1760 were not evaluated as part of the EU assessment of cymoxanil. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Allium cepa $_{m}$ [1)] *Avena sativa* $_{m}$ [2)] *Beta vulgaris* $_{d}$ [3)] *Cucumis sativus* $_{d}$ [4)] *Glycine max* $_{d}$ [5)] *Helianthus annus* $_{d}$ [6)]	Cymoxanil 50 WP	21 d Vegetative vigour	Plant height: [1-6)] ER_{50} > 240 g a.s./ha	EFSA, 2008 Balluff, M. (2003) DAR IIA, 8.6
Allium cepa $_{m}$ [1)] *Avena sativa* $_{m}$ [2)] *Lolium perenne* $_{m}$ [3)] *Beta vulgaris* $_{d}$ [4)] *Cucumis sativus* $_{d}$ [5)] *Glycine max* $_{d}$ [6)] *Helianthus annus* $_{d}$ [7)]	Cymoxanil 45 WG (IN002B1760)	21 d Vegetative vigour	Plant height: [1-7)] ER_{50} > 533 g prod./ha (equivalent to 240 g a.s./ha)	Bützler, R. and Meinerling, M. (2008) Doc. No. 39709087 KCP 10.6.2/01
Allium cepa $_{m}$ [1)] *Avena sativa* $_{m}$ [2)] *Beta vulgaris* $_{d}$ [3)] *Cucumis sativus* $_{d}$ [4)] *Glycine max* $_{d}$ [5)] *Helianthus annus* $_{d}$ [6)]	Cymoxanil 45 WG IN002B1760	21 d Seedling emergence	Emergence: [1-6)] ER_{50} > 550 g prod./ha (equivalent to 258.5 g a.s./ha) Plant weight: [1-6)] ER_{50} > 550 g prod./ha (equivalent to 258.5 g a.s./ha) Shoot height: [1-6)] ER_{50} > 550 g prod./ha (equivalent to 258.5 g a.s./ha)	Noè, F. (2022) Doc. No. CH–0262/2021 KCP 10.6.2/02

m: monocotyledonous; d: dicotyledonous

zRMS comments:

NTP toxicity endpoints for cymoxanil provided in Table 9.10-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 167, 1-116.

The studies performed with IN002B1760 (Cymoxanil 45 WG) were evaluated by the zRMS and considered acceptable.

For the study summary and its evaluation by the zRMS, please refer to Appendix 2.

9.10.1.1 Justification for new endpoints

No new endpoint has been considered.

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 IN002B1760 in potato

Intended use	Potato			
Active substance/product	cymoxanil / IN002B1760			
Application rate (g/ha)	148.5			
MAF	- *			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Allium cepa (vegetative vigour)	> 240	2.77 %	4.11	> 58.35
Allium cepa (seedling emergence)	> 258.5	2.77 %	4.11	> 62.84

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

* During the Pesticides Peer Review meeting of September 2015, it was agreed that for the risk assessment of active substances, no MAF values should be used by default, until a guidance document is developed.

zRMS comments:

According to the risk assessment provided with formulation IN002B1760 with most sensitive endpoints ER_{50} of 240 g a.s./ha, an acceptable risk to non-target plants was indicated for the proposed use of IN002B1760.

No risk mitigation measures are required.

Overall, no unacceptable effects on non-target plants are expected following application of IN002B1760.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risk assessment for non-target terrestrial plants in off-field areas is based on the "Guidance Document on Terrestrial Ecotoxicology" (2002).
No unacceptable risk to non-target terrestrial plants in off-crop areas is expected following the use of IN002B1760 according to the proposed use pattern.

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

Potential risks to terrestrial organisms have been assessed considering a broad range of species. Further testing is not deemed to be required.

9.12 Monitoring data (KCP 10.8)

No additional data submitted.

9.13 Classification and Labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to ecotoxicological data is proposed for the preparation:

Table 9.13-1: Justified proposals for classification and labelling for IN002B1760 according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	~~Aquatic Acute 1~~ Aquatic Chronic 1
Hazard pictograms or Code(s) for hazard pictogram(s):	GHS09
Signal word:	Warning
Hazard statement(s):	~~H400: Very toxic to aquatic life~~ H410: Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	~~P273: Avoid release to the environment.~~ P391: Collect spillage P501: Dispose of contents/container to a licensed hazardous-waste disposal contractor or collection site except for empty clean containers which can be disposed of as non-hazardous waste.'
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]

zRMS comment:

Based on data for for formulation IN002B1760 for the most sensitive organism algae with ErC_{50} >1 (being 15.93 mg formulation/L) and based on the chronic data for the a.s. classified as H410 the final classification for formulation IN002B1760 is as following:
Hazard statement: H410, Aquatic Chronic 1
Signal word: Warning
Pictogram: GHS09
Safety phrases: P391, P501

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	[redacted]	2009a	Acute toxicity of Moximate 505 WP to Zebra fish (*Danio rerio*) in a 96-hour study under semi-static exposure. [redacted] GLP Unpublished	Y	Indofil Industries Ltd.
KCP 10.2.1/02	Neri, M.C.	2009b	Acute toxicity of Moximate 505 WP to *Daphnia magna* in a 48-hour immobilisation test under semi-static exposure. Report No. CH-E-004009/2009 (Doc. No. 822-004) Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.2.1/03	Neri, M.C.	2009c	Toxicity of Moximate 505 WP to green algae *Pseudokirchneriella subcapitata* determined in a growth inhibition study. Report No. CH-E-004010/2009 (Doc. No. 823-004) Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.2.1/04	Venkanna, B.	2024	Fresh water Algae Growth Inhibition Test with Cymoxanil 45% WG. Report No. 23/0177 Test Facility: Vivo Bio Tech Limited. Survey # 349/A, Pregnapur Village – 502311, Gajwel Mandal, District - Siddipet, Telangana (India) GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.1.1/01	Schmitzer, S.	2007	Effects of Cymoxanil Technical (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory (Limit Test). Report No. 36571035 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH. Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.1.1/02	Colli, M.	2009a	Effects, acute oral and acute contact toxicity of Moximate 505 WP on the honeybee *Apis mellifera* L. in the laboratory	N	Indofil

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
			~~(dose response test).~~ ~~Report No. BT013/09 (Doc. No. 832-004)~~ ~~Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy~~ ~~GLP~~ ~~Unpublished~~		~~Industries Ltd.~~
KCP 10.3.1.2/01	Ponti, B.	2021	Cymoxanil 45 WG (IN 002B1760): Chronic Oral Toxicity to adult worker honeybees *Apis mellifera* L. (10-day feeding). Report No. CH–0257/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.1.3/01	Noè, F.	2022	Cymoxanil 45 WG (IN 002B1760): Honey bees (*Apis mellifera* L.) Larval Toxicity Test with Repeated Exposure. Report No. CH–0258/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.1.3/01a	Noè, F.	2022	STUDY PLAN AMENDMENT No. 1 to the report "Cymoxanil 45 WG (IN 002B1760): Honey bees (*Apis mellifera* L.) Larval Toxicity Test with Repeated Exposure." Report No. Study Plan Amendment No. 1 CH–0258/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.1/01	Moll, M.	2008a	Effects of Cymoxanil 45 WG on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory - Dose Response Test. Report No. 39701001 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH. Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.1/02	Moll, M.	2008b	Effects of Cymoxanil 45 WG on the Predatory Mite *Typhlodromus pyri* in the Laboratory - Dose Response Test. Report No. 39702063 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.

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 10.3.2.2/01	Schmitzer, S.	2008	Effects of Cymoxanil 45 WG on the Carabid Beetle *Poecilus cupreus* L. - Extended Laboratory Study. Report No. 39703007 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.2/02	Moll, M.	2008c	Effects of Cymoxanil 45 WG on the Lacewing *Chrysoperla carnea* under Extended Laboratory Conditions. Report No. 39704047 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
~~KCP 10.3.2.2/03~~	~~Colli, M.~~	~~2009b~~	~~Effects of Moximate 505 WP on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under extended laboratory conditions (rate response test).~~ ~~Report No. BT035/09 (Doc. No. 834-006)~~ ~~Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Indofil Industries Ltd.~~
~~KCP 10.3.2.2/04~~	~~Colli, M.~~	~~2009c~~	~~Effects of Moximate 505 WP on the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under extended laboratory conditions (rate response test).~~ ~~Report No. BT034/09 (Doc. No. 834-003)~~ ~~Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Indofil Industries Ltd.~~
~~KCP 10.3.2.2/05~~	~~Colli, M.~~	~~2009d~~	~~Effect toxicity evaluation of Moximate 505 WP on the *Chrysoperla carnea* L. (Neuroptera, Chrysopidae) under extended laboratory conditions (rate response test).~~ ~~Report No. BT033/09 (Doc. No. 834-004)~~ ~~Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Indofil Industries Ltd.~~
~~KCP 10.3.2.2/06~~	~~Colli, M.~~	~~2010~~	~~Effect of Moximate 505 WP on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) under extended laboratory conditions (rate response test).~~ ~~Report No. BT032/09 (Doc. No. 834-005)~~ ~~Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy~~	~~N~~	~~Indofil Industries Ltd.~~

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			~~GLP~~ ~~Unpublished~~		
~~KCP 10.3.2.2/07~~	~~Rosenkranz, B. & Wirzinger, G.~~	~~2011~~	~~Statistical re-evaluation of effects of Moximate 505 WP on *Aphidius rhopalosiphi* and *Eisenia fetida*.~~ ~~Report No. not indicated (Doc. No.: 882-002)~~ ~~Test Facility: Scientific Consulting Company, Bad Kreuznach, Germany~~ ~~Not GLP~~ ~~Unpublished~~	~~N~~	~~Indofil Industries Ltd.~~
KCP 10.3.2.4/01	Rosenkranz, B. & Schabio, S.	2009	Effects of Cymbal 45 WG on Predatory Mites (Acari, Phytoseiidae) under Field Conditions in Vine (4 Applications) Report No. 39706064 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Industriestrasse 1, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.4.1.1/01	Dini, R.	2022a	Cymoxanil 45 WG (IN 002B1760): Effects on Reproduction of Earthworm *Eisenia fetida* in an Artificial Soil Study. Report No. CH–0259/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.4.2.1/01	Dini, R.	2022b	Cymoxanil 45 WG (IN 002B1760): Effects on Collembolan Reproduction in an Artificial Soil Study. Report No. CH–0260/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.4.2.1/02	Dini, R.	2022c	Cymoxanil 45 WG (IN 002B1760): Effects on *Hypoaspis (Geolaelaps) aculeifer* Reproduction in an Artificial Soil Study. Report No. CH–0261/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
~~KCP 10.5.1/01~~	~~Feil, N.~~	~~2008~~	~~Effects of Cymoxanil technical on the Activity of the Soil Microflora in the Laboratory.~~ ~~Report No. 41382080~~ ~~Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany~~ ~~GLP~~	~~N~~	~~Indofil Industries Ltd.~~

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			~~Unpublished~~		
KCP 10.6.2/01	Bützler, R. & Meinerling, M.	2008	Effects of Cymoxanil 45 WG on Terrestrial (Non-Target) Plants: Vegetative Vigour Test. Report No. 39709087 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.6.2/02	Noè, F.	2022	Cymoxanil 45 WG (IN 002B1760): Seedling Emergence and Seedling Growth Test of Terrestrial Plants. Report No. CH–0262/2021 Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
IIA, 8.1.1	[redacted]	1996	Cymoxanil Technical acute oral toxicity (LD_{50}) to the bobwhite quail. [redacted] GLP: Yes Published: No	Y	Oxon
IIIA, 10.1.1	[redacted]	2003	Avian toxicity study of Cymoxanil 50 WP oral toxicity in the Japanese quail – limit test according to SETAC guideline document. [redacted] GLP: Yes Published: No	Y	Oxon
IIA, 8.1.2	[redacted]	1999	5-Day dietary toxicity study in mallard duck [redacted]	Y	Oxon

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
			[redacted] GLP: Yes Published: No		
IIA, 8.1.3	[redacted]	1996b	DPX-T3217-113 (cymoxanil): A reproduction study with the mallard (*Anas platyrhynchos*) [redacted] GLP: Yes Published: No	Y	DuPont
IIA, 5.2.1	[redacted]	1992	Acute oral toxicity study with DPX-T3217-113 (Cymoxanil)in male and female rats E.I. du Pont de Nemours and Company [redacted] GLP not published	Y	DuPont
IIIA, 7.1.1	[redacted]	1995a	Cymoxanil 50 % WP: Acute oral toxicity study to the rat [redacted] GLP not published	Y	Oxon
IIIA, 7.1.1	[redacted]	1997a	Acute oral toxicity study with DPX-KP481-25 50WG in male and female rats [redacted] GLP not published	Y	DuPont
IIA, 5.6.2	[redacted]	1993	Developmental toxicity study of DPX-T3217-113 (cymoxanil) in rats [redacted] GLP not published	Y	DuPont
IIA, 8.2.1	[redacted]	1993b	Static, acute, 96-hour LC_{50} of DPX-T3217-113 (cymoxanil) to bluegill sunfish, *Lepomis macrochirus* [redacted] GLP: Yes Published: No	Y	DuPont

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
IIA, 8.2.1	[redacted]	2002b	IN-U3204: Static-renewal, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* [redacted] GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	[redacted]	2002b	IN-W3595: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* [redacted] GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	[redacted]	2002a	IN-KQ960: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* [redacted] GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	[redacted]	2002a	IN-T4226: Static-renewal, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* [redacted] GLP: Yes Published: No	Y	DuPont
IIIA 10.2.1	[redacted]	1999a	96-hour acute toxicity study in rainbow trout with Cymoxanil 50 % WP (semi-static) [redacted] GLP: Yes Published: No	Y	Oxon
IIIA, 10.2.1	[redacted]	1997a	DPX-KP481-25 50WG: Flow-through, acute, 96- hour LC50 to rainbow trout, *Oncorhynchus mykiss* [redacted] GLP: Yes Published: No	Y	DuPont
IIA, 8.2.2.2	[redacted]	1996	DPX-T3217-113 (Cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss* [redacted]	Y	DuPont

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP: Yes Published: No		
IIA, 8.2.4	Baer, K.N.	1993c	Static, acute, 48-hour EC_{50} of DPX-T3217-113 (cymoxanil) to *Daphnia magna* GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Samel, A.	2002c	IN-U3204: Static-renewal, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory DuPont-9557 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002d	IN-W3595: Acute, 48-hour EC_{50} to *Daphnia magna* T. R. Wilbury Laboratories, Inc. DuPont-9383 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Samel, A.	2002d	IN-KQ960: Static, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory DuPont-9559 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002c	IN-T4226: Acute, 48-hour EC_{50} to *Daphnia magna* T. R. Wilbury Laboratories, Inc. DuPont-9385 GLP: Yes Published: No	N	DuPont
IIIA 10.2.1	Migchielsen, M.H.J.	1999	Acute toxicity study in *Daphnia magna* with Cymoxanil 50 % WP (semi-static) NOTOX B.V., The Netherlands Report 262439 GLP: Yes Published: No	N	Oxon
IIIA, 10.2.1	Brown, M.R.	1997b	DPX-KP481-25 50WG: Flow-through, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory HL-1997-00437	N	DuPont

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	Owner
			GLP: Yes Published: No		
IIA, 8.2.5	Baer, K.N.	1993d	Chronic toxicity of DPX-T3217-113 (cymoxanil) to *Daphnia magna*: 24-Hour renewal DuPont Haskell Laboratory HLR 354-93, Revision No. 1 GLP: Yes Published: No	N	DuPont
IIA, 8.2.5	Samel, A.	2003	IN-KQ960: 21-Day chronic toxicity to *Daphnia magna* DuPont Haskell Laboratory DuPont-11971 GLP: Yes Published: No	N	DuPont
IIA, 8.2.6	Hughes, J.S., Williams, T.L., Conder, L.A.	1996a	DPX-T3217-113 (cymoxanil): Influence on growth and reproduction of *Anabaena flos-aquae* Carolina Ecotox, Inc. AMR 4109-96 GLP: Yes Published: No	N	DuPont
IIA, 8.2.6	Sloman, T.L.	2001b	IN-W3595: Influence on growth and growth rate of the blue-green alga *Anabaena flos-aquae* DuPont Haskell Laboratory DuPont-3748 GLP: Yes Published: No	N	DuPont
IIA, 8.2.6	Sloman, T.L.	2001a	IN-T4226: Influence on growth and growth rate of the blue-green alga *Anabaena flos-aquae* DuPont Haskell Laboratory DuPont-3747 GLP: Yes Published: No	N	DuPont
IIIA 10.2.1	Bogers M.	1999b	Fresh water algal growth inhibition test with Cymoxanil 50 % WP NOTOX B.V., The Netherlands, Report 262441 GLP: Yes Published: No	N	Oxon
IIIA, 10.2.1	Leva, S.E., Sloman, T.L.	1997	DPX-KX007: Influence on growth and growth rate of the green alga *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*)	N	DuPont

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**
			DuPont Stine Research Center AMR 4116-96 GLP: Yes Published: No		
IIA, 8.2.8	Leva, S.E., Sloman, T.L.	1996	Cymoxanil: Influence on growth and reproduction of *Lemna gibba* G3 DuPont Stine-Haskell Research Center AMR 3775-96 GLP: Yes Published: No	N	DuPont
IIA, 8.3.1.1	Schur, A.	1999	Assessment of side effects of cymoxanil technical to the honey bee, *Apis mellifera* L. in the laboratory GAB Biotechnologie GmbH and IFU Umweltanalytik GmbH 99063/01-BLEU GLP: Yes Published: No	N	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	1999a	Effects of Cymoxanil 50% WP on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi* in the laboratory NOTOX BV. 257816 GLP: Yes Published: No	N	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	2000	Effects of Cymoxanil 50% WP on survival and reproduction of the phytoseiid mite *Typhlodromus pyri* Scheuten NOTOX BV. 257827 GLP: Yes Published: No	N	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	1999b	Effects of Cymoxanil 50% WP on survival of the carabid beetle *Poecilus cupreus* NOTOX BV. 257838 GLP: Yes Published: No	N	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	1999c	Effects of Cymoxanil 50% WP on survival and reproduction of the green lacewing *Chrysoperla carnea* (laboratorium test) NOTOX BV. 257849 GLP: Yes	N	Oxon

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
			Published: No		
IIA, 8.3.2	Müther, J.	2002	A field study to evaluate the effects of Cymoxanil 50% WP on predatory mites (Acari: phytoseiidae) in vines in France Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Germany 20021069/F1-NFTp GLP: Yes Published: No	N	Oxon
IIIA, 10.5.1	Kuehner, C.	1996b	DPX-KX007, a suspension concentrate (SC) formulation, containing JE874 (90 g/L) and cymoxanil (120 g/L): Acute toxicity to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory Report No. AMR 3530-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Kuehner, C.	1995	DPX-KX007, a suspension concentrate (SC) formulation, containing JE874 (90 g/L) and cymoxanil (120 g/L): Acute toxicity to the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory. Report No. AMR 3529-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Kuehner, C.	1996b	DPX-KX007, a suspension concentrate (SC) formulation, containing JE874 (90 g/L) and cymoxanil (120 g/L): Acute toxicity to the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae) in the laboratory Report No. AMR 3528-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Mead-Briggs, M.	1996	DPX-KX007, a suspension concentrate (SC) formulation, containing JE874 (90 g/L) and cymoxanil (120 g/L): An extended laboratory test to evaluate the effects on the parasitic wasp *Aphidius rhopalosiphi* when applied in up to six serial applications to tomato plants Report No. AMR 3593-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Grove, A.J.	1996a	Effects of DPX-KX007-03 on juvenile survival, reproduction and egg hatch success of *Typhlodromus pyri* (Acari: Phytoseiidae), following multiple applications on grapevine leaf: Extended laboratory test Report No. AMR 3571-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Grove, A.J.	1996b	Extended laboratory test comparing the effects of DPX-KX007 (containing DPX-JE874 and cymoxanil) WG and SC	N	DuPont

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
			formulations on *Typhlodromus pyri*, following multiple applications on grapevine leaves under greenhouse conditions MITOX Stichting Bevordering Duurzame Plaagbestijding AMR 3812-96 GLP: Yes Published: No		
IIIA, 10.5.1	Nengel, S.	1996a	Assessment of side effects of DPX-KX007 20 SC on the hoverfly, *Episyrphus balteatus* DEG. (Diptera, Syrphidae): Extended lab test Report No. AMR 3569-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Nengel, S.	1996b	Assessment of side effects of DPX-KX007 20SC on the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae): Semi-field test Report No. AMR 3570-95 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Kuehner, C.	1997a	DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the hoverfly, *Episyrphus balteatus* Deg. (Diptera, Syrphidae) in the laboratory GAB Biotechnologie GmbH AMR 4232-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Kuehner, C.	1997b	DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae) in the laboratory GAB Biotechnologie GmbH AMR 4233-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Kuehner, C.	1997c	DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory GAB Biotechnologie GmbH AMR 4234-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Kuehner, C.	1997d	DPX-KP481, a water-dispersible granular (WG) formulation containing DPX-JE874 (254 g ai/kg) and cymoxanil (266 g	N	DuPont

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**
			ai/kg): Acute toxicity to the staphylinid beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae) in the laboratory GAB Biotechnologie, GmbH AMR 4235-96 GLP: Yes Published: No		
IIIA, 10.5.1	Beech, P. & Mead-Briggs, M.	1998	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%). An extended laboratory study to evaluate the effects on the ground beetle, *Poecilus cupreus* University of Southampton, Agrochemical Evaluation Unit (AEU) AMR 4539-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Thompson, B.	1999a	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%) an extended laboratory study to evaluate the effects on the hoverfly, *Episyrphus balteatus* University of Southampton, Agrochemical Evaluation Unit (AEU) AMR 4541-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Thompson, B.	1999c	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%). An extended laboratory study to evaluate the effects on the green lacewing, *Chrysoperla carnea* University of Southampton, Agrochemical Evaluation Unit (AEU) Report No.: AMR 4540-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Hermann, P.	2001	DPX-KP481-25: A semi-field study to evaluate the effects on the hoverfly, *Episyrphus balteatus* Deg. (Diptra, Syrphidae) GAB Biotechnologie, GmbH DuPont-3881 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Thompson, B.	1999b	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%): A semi-field study to evaluate the effects on aphid parasitoids, (Hymenoptera, Aphidiinae) in a potato crop University of Southampton, Agrochemical Evaluation Unit (AEU)	N	DuPont

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
			Report No.: AMR 4542-97 GLP: Yes Published: No		
IIIA, 10.5.1	Kuehner, C.	1998	Cymoxanil/famoxadone (DPX-KX007) 52.5% WG (30% : 22.5%): An extended laboratory study (field aged residue) to evaluate the effects on the parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae) Report No.: AMR 4573-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1	Oberwalder, C.	1997	A field study to evaluate the effects of DPX-KX007 WG, a formulation containing DPX-JE874 (225 g ai/kg) and cymoxanil (300 g ai/kg), on the predatory mite *Typhlodromus pyri* in vines (two locations in Germany) Report No.: AMR 3969-96, Revision No.1 GLP: Yes Published: No	N	DuPont
IIA, 8.4.1	Rodgers, M.H., Cameron, D.M., Mansell, P.	1995	Cymoxanil technical acute toxicity (LC_{50}) to the earthworm (*Eisenia foetida*) Huntingdon Life Sciences Ltd. OXN 78A/950675 GLP: Yes Published: No	N	Oxon
IIIA, 10.6.1.1	Rodgers, M.H.	1995	Cymoxanil 50 % WP - Acute toxicity (LC_{50}) to the earthworm (*Eisenia foetida*) Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England OXN 81A/943245 GLP: Yes Published: No	N	Oxon
IIIA, 10.6.1.1	Wachter, S.	1997	Acute toxicity of DPX-KX007-5 on earthworms, *Eisenia foetida* using an artificial soil test GAB Biotechnologie, GmbH AMR 4136-96 GLP: Yes Published: N	N	DuPont
IIA, 8.4.2	Lührs, U.	2000	Cymoxanil/famoxadone (DPX-KP481): A dose response sublethal study to the earthworm, *Eisenia fetida* (Savigny 1826), in artificial soil IBACON DuPont-4611 GLP: Yes Published: No	N	DuPont

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
IIA, 8.5	Kölzer, U.	2003	Assessment of the side effects of cymoxanil technical on the activity of the soil microflora Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. 20031314/01-ABMF GLP: Yes Published: No	N	Oxon
IIIA, 10.7	Carter, J.N.	1995	DPX-KX007 (20 SC) effects on soil non-target micro-organisms Report No.: AMR 3560-95 GLP: Yes Published: No	N	DuPont
IIA, 8.6	Balluff, M.	2003	Vegetative vigour limit test for non target plants following single rate application of Cymoxanil 50 WP Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. 20033057/S1-FGW GLP: Yes Published: No	N	Oxon

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	[redacted]	2009a	Acute toxicity of Moximate 505 WP to Zebra fish (Danio rerio) in a 96-hour study under semi-static exposure. [redacted] GLP Unpublished	Y	Indofil Industries Ltd.
KCP 10.2.1/02	Neri, M.C.	2009b	Acute toxicity of Moximate 505 WP to Daphnia magna in a 48-hour immobilisation test under semi-static exposure. Report No. CH-E–009/2009 (Doc. No. 822-004) Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.2.1/03	Neri, M.C.	2009c	Toxicity of Moximate 505 WP to green algae Pseudokirchneriella subcapitata determined in a growth inhibition study.	N	Indofil Industries Ltd.

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
			Report No. CH-E–00104/2009 (Doc. No. 823-004) Test Facility: ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy GLP Unpublished		
KCP 10.3.1.1/01	Schmitzer, S.	2007	Effects of Cymoxanil Technical (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory (Limit Test). Report No. 36571035 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH. Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.1.1/02	Colli, M.	2009a	Effects, acute oral and acute contact toxicity of Moximate 505 WP on the honeybee Apis mellifera L. in the laboratory (dose response test). Report No. BT013/09 (Doc. No. 832-004) Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.2/03	Colli, M.	2009b	Effects of Moximate 505 WP on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under extended laboratory conditions (rate response test). Report No. BT035/09 (Doc. No. 834-006) Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.2/04	Colli, M.	2009c	Effects of Moximate 505 WP on the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under extended laboratory conditions (rate response test). Report No. BT034/09 (Doc. No. 834-003) Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.2/05	Colli, M.	2009d	Effect toxicity evaluation of Moximate 505 WP on the *Chrysoperla carnea* L. (Neuroptera, Chrysopidae) under extended laboratory conditions (rate response test). Report No. BT033/09 (Doc. No. 834-004) Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy GLP	N	Indofil Industries Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.3.2.2/06	Colli, M.	2010	Effect of Moximate 505 WP on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) under extended laboratory conditions (rate response test). Report No. BT032/09 (Doc. No. 834-005) Test Facility: BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy GLP Unpublished	N	Indofil Industries Ltd.
KCP 10.3.2.2/07	Rosenkranz, B. & Wirzinger, G.	2011	Statistical re-evaluation of effects of Moximate 505 WP on *Aphidius rhopalosiphi* and *Eisenia fetida*. Report No. not indicated (Doc. No.: 882-002) Test Facility: Scientific Consulting Company, Bad Kreuznach, Germany Not GLP Unpublished		
KCP 10.5.1/01	Feil, N.	2008	Effects of Cymoxanil technical on the Activity of the Soil Microflora in the Laboratory. Report No. 41382080 Test Facility: Institut fiir Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished.	N	Indofil Industries Ltd.

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 Acute toxicity of Moximate 505 WP to Zebra fish (*Danio rerio*) in a 96-hour study under semi-static exposure

Comments of zRMS:	The study was not evaluated by zRMS.

~~Reference:~~ ~~KCP 10.2.1/01~~

~~Report~~ [redacted] ~~Acute toxicity of Moximate 505 WP to Zebra fish (*Danio rerio*) in a 96-hour study under semi-static exposure.~~ [redacted]

~~Guideline(s):~~ ~~OECD Guideline for Testing of Chemicals, N. 203 (1992)~~
~~OPPTS 850.1075 guideline (1996)~~

~~Deviations:~~ ~~The temperature values through the aquaria ranged between 17.9 and 24.0°C during the test period, instead of 23.0 ± 2.0°C (OECD recommended range), however, this deviation did not affect the results of the study.~~
~~The dissolved oxygen concentration provided for the semi-static test shall be maintained above the 60 % of the air saturation while as reported in the study plan, for flow-through test, the dissolved oxygen concentration shall be maintained above 75 % of the air saturation.~~

~~GLP:~~ ~~Yes~~

~~Acceptability:~~ ~~Yes~~

~~Duplication~~ ~~No~~
~~(if vertebrate study)~~

~~**Materials and methods**~~

~~The acute toxicity of Moximate 505 WP (batch number: 05, active substances 4 % Cymoxanil and 46.5 %~~

Mancozeb) to zebra fish (*Danio rerio*) was determined in fresh water using a semi static system at nominal concentrations of 10, 17.8, 31.6, 56.2 and 100 mg formulation/L over 96 hours, corresponding to 0.400, 0.71, 1.26, 2.25 and 4.0 mg/L Cymoxanil and to 4.65, 8.28, 14.7, 26.1 and 46.5 mg/L Mancozeb. Replicate groups of 7 fish each were prepared for each experimental group. The test item was tested at five concentrations and in addition to this, one group without the test item was prepared and acted as a negative control. The temperature during the exposure period ranged from 17.9 to 24.0°C. The fish size ranged from 1.3 to 2.6 cm (mean value 1.9 cm) with a weight of 0.10 – 0.26 g (mean value 0.16 g), the loading rate did not exceed 0.8 g fish/L. Records of mortality and sublethal effects of exposure were made at 2, 24, 48, 72 and 96 hours after the start of the exposure. The analytical verification of the actual test concentrations was performed on three test concentrations, namely 17.8, 31.6 and 56.2 mg/L.

Results and discussions

Water quality parameters like dissolved oxygen and pH value were within ranges requested by guideline OECD 203.

The temperature values through the aquaria ranged between 17.9 and 24.0°C during the test period, instead of 23.0 ± 2.0°C (OECD recommended range), however this deviation did not affect the results of the study as these changes occurred for short periods. The dissolved oxygen concentration provided for the semi-static test was maintained above the 60 % of the air saturation while as reported in the study plan, for flow-through test, the dissolved oxygen concentration shall be maintained above 75 % of the air saturation.

Analytical results

Test concentrations were determined from samples taken at the beginning of the test, after 24 hours of exposure, in the freshly prepared solutions to renew the test medium every 24 hours and at the end of the test period. The analytical verification was carried out on three concentrations (17.8, 31.6 and 56.2 mg/L). The test item treatment group of 56.2 mg/L was only analyzed at the beginning of the study and after 24 hours as all the fish were dead at that time.

Due to the instability of both active ingredients in water solutions, the analytical recoveries were lower than the range of 80 – 120 %. The determination of Mancozeb concentrations was carried out based on the amount of Manganese in the water samples.

The average analytical recovery of Cymoxanil in the freshly prepared test solutions at time 0, 24, 48, 72 hours was 62.1 % of the nominal values (range 58.5 – 67.2 %). In the test solutions after 24 hours, the average analytical recovery of quantifiable concentrations was 18.4 % of the nominal concentrations (range 16.2 – 23.8 %). The authors of the study stated that as the light sensitivity of Cymoxanil affected the concentration in water, the biological results were based on the nominal concentrations. The results are summarised in Table A 2.2-1.

Table A 2.2-1: Analytical results for Cymoxanil over a period of 96 h

Nominal concentration [mg/L]		Measured concentrations [mg/L]					Geometric mean [mg/L]
Moximate 505 WP	Cymoxanil	0 h	24 h old / new	48 h old / new	72 h old / new	96 h	
17.8	0.71	0.42	0.05* / 0.60	0.10 / 0.37	0.10 / 0.34	0.13	0.19
31.6	1.26	0.83	0.23 / 0.90	0.20 / 0.49	0.11 / 0.73	0.28	0.37
56.2	2.25	1.51	0.54 / n.a.	n.a.	n.a.	n.a.	0.90

* test concentration below the limit of quantification of 0.10 mg a.s./L , therefore, it was set as half of the limit of quantification

n.a. not analysed as all fish were dead at that time

The average analytical recovery of Mancozeb in the freshly prepared test solutions at time 0, 24, 48, 72 hours was 13.7 % (range 12.7 – 14,3 %) of the nominal values. In the test solutions after 24 hours, the concentration was stable with an average analytical recovery of 13.7 % of the nominal concentrations. The results are summarised in Table A 2.2-2.

Table A 2.2-2: Analytical results for Mancozeb over a period of 96 h

Nominal concentration [mg/L]		Measured concentrations [mg/L]					Geometric mean [mg/L]
Moximate 505 WP	Mancozeb	0 h	24 h old / new	48 h old / new	72 h old / new	96 h	
17.8	8.28	0.87	0.88 / 1.02	1.04 / 1.40	1.44 / 1.33	1.33	1.14
31.6	14.7	1.77	1.81 / 2.03	2.01 / 2.57	2.54 / 2.03	2.07	2.09
56.2	26.1	3.33	3.33 / n.a.	n.a.	n.a.	n.a.	3.33

n.a. not analysed as all fish were dead at that time

As stated by the authors of the study, the biological results refer to the nominal concentrations of the formulation and to the geometric mean of the measured concentrations for Cymoxanil and Mancozeb (calculated from the measured Manganese concentration). Where the concentration was below the limit of quantification (LOQ), it was set as half the LOQ.

Biological test results

The validity criteria were met as no fish died in the control during 96 hours and the oxygen concentration at the end of the test was more than 60 %. The mortalities observed are presented in Table A 2.2-3. No mortality was observed in the control. After 24 hours, all fish in the two highest test concentrations (56.2 and 100 mg/L) had died.

The LC_{50} value for the formulation was based on nominal concentrations and amounted to 30.34 mg/L (95 % confidence interval 24.5 – 37.6 mg/L), corresponding to 0.37 mg/L Cymoxanil (nominal concentration) and 1.94 mg/L Mancozeb (measured concentration), respectively. The no observed effect concentration (NOEC) was determined to be 17.8 mg/L (corresponding to 0.19 mg/L Cymoxanil (nominal concentration) and 1.14 mg/L Mancozeb (measured concentration), respectively), the lowest observed effect concentration (LOEC) was 31.6 mg/L (corresponding to 0.37 mg/L Cymoxanil (nominal concentration) and 2.09 mg/L Mancozeb (measured concentration).

Table A 2.2-3: Observed mortality of zebra fish exposed to Moximate 505 WP for 96 hours in a semi-static acute test

Nominal Concentration [mg formulation/L]	Cumulative Mortality (Number of dead/Number introduced)			
	24 hr	48 hr	72 hr	96 hr
Control	0/7	0/7	0/7	0/7
10	0/7	0/7	0/7	0/7
17.8	0/7	0/7	0/7	0/7
31.6	0/7	2/7*	4/7*	4/7*
56.2	7/7*	7/7*	7/7*	7/7*
100	7/7*	7/7*	7/7*	7/7*

* statistically significant compared to control (Fisher's Exact test, $p < 0.05$)

Conclusion

The NOEC, LOEC and LC_{50} values after 96 hours were calculated to be 17.8, 31.6 and 30.3 mg/L (95 % confidence range: 24.5 – 37.6 mg/L), respectively, based on the nominal concentrations of the product.

A 2.2.1.2 Acute toxicity of Moximate 505 WP to *Daphnia magna* in a 48-hour immobilisation test under semi-static exposure

Comments of zRMS:	The study was not evaluated by zRMS.

Reference:	KCP 10.2.1/02
Report	Neri, M.C. (2009b). Acute toxicity of Moximate 505 WP to *Daphnia magna* in a 48-hour immobilisation test under semi-static exposure. Report No. CHE-004/2009 (Doc. No. 822-004), ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 202 (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The acute toxicity of Moximate 505 WP (batch number: 05, active substances: 4 % Cymoxanil and 46.5 % Mancozeb) to *Daphnia magna* was determined in fresh water at 20.4 – 22.1°C using a semi-static system at nominal concentrations of 10.0, 17.8, 31.6, 56.2 and 100 mg formulation/L over 48 hours, corresponding to 0.40, 0.71, 1.26, 2.25 and 4.0 mg/L Cymoxanil and to 4.65, 8.28, 14.7, 26.1 and 46.5 mg/L Mancozeb. Four groups of five daphnids each with an age of 6 – 24 hours were used for each treatment group. The loading rate was at least 2 mL test solution per daphnid. Records of immobility were made at 24 and 48 hours after the start of the exposure. Those animals unable to swim within 15 seconds after gentle agitation of test beaker were considered to be immobile. For determination of the test item concentrations, samples were taken from each test group at the start of the test, after 24 hours and at the end of the test. Furthermore, samples of the freshly prepared solutions were also analysed. The samples were analysed by HPLC/UV for Cymoxanil and from quantification of manganese by atomic absorption for Mancozeb.

Results and discussions

Water quality parameters like temperature, dissolved oxygen and pH value were within ranges requested by guideline OECD 202.

Analytical results

The analytical recovery of Cymoxanil for all samples was in the range of 7.3 and 11.0 % of the nominal values. This was probably due to the low concentration of Cymoxanil tested and to the high light sensitivity of Cymoxanil. The results of the analytical measurements are summarised in Table A 2.2.4.

Table A 2.2-4: Analytical results for Cymoxanil over a period of 48 h

Nominal concentration [mg/L]		Measured concentrations [mg/L] [1]				Geometric mean [mg/L]
Moximate 505 WP	Cymoxanil	0 h	24 h (old)	24 h (new)	48 h	
10.0	0.40	0.05*	0.05*	0.05*	0.05*	0.05
17.8	0.71	0.05**	0.05*	0.05**	0.05*	0.05
31.6	1.26	0.05**	0.05*	0.10 (8.0)	0.05*	0.06
56.2	2.25	0.16 (7.3)	0.05*	0.22 (9.8)	0.05*	0.10
100	4.00	0.34 (8.6)	0.05*	0.44 (11.0)	0.05*	0.14

[1] values in parentheses represent percentage ratio measured to nominal concentration
* test concentration below the limit of detection of 0.05 mg a.s./L , therefore, it was set as the limit of detection itself
** test concentration below the limit of quantification of 0.1 mg a.s./L, therefore, it was set as half of the limit of quantification

In the freshly prepared solutions (time 0 and 24 hours), the average analytical recovery of Mancozeb (based on the Manganese content in the samples) was 18.2 % of the nominal concentrations. During the test period, the Mancozeb concentrations were stable with an average analytical recovery of 17.5 %. The results of the analytical measurements are summarised in Table A 2.2-5.

Therefore, the biological results refer to both the nominal concentrations of the formulation and to the geometric mean of the measured concentrations of Mancozeb (calculated from the measured Manganese concentration) and Cymoxanil. Where the concentration was below the limit of quantification (LOQ), it was set as half the LOQ, while where the concentration was below the limit of detection (LOD), it was set as the LOD.

Table A 2.2-5: Analytical results for Mancozeb over a period of 48 h

Nominal concentration [mg/L]		Measured concentrations [mg/L] 1				Geometric mean [mg/L]
Moximate 505 WP	Mancozeb	0 h	24 h (old)	24 h (new)	48 h	
10.0	4.65	0.92 (19.7)	0.67 (14.4)	0.78 (16.8)	0.30 *	0.61
17.8	8.28	1.56 (18.9)	1.53 (18.5)	1.32 (16.0)	1.26 (15.2)	1.41
31.6	14.7	2.82 (19.2)	2.84 (19.3)	2.47 (16.8)	2.43 (16.6)	2.63
56.2	26.1	5.09 (19.5)	5.15 (19.7)	4.51 (17.2)	4.47 (17.1)	4.80
100	46.5	9.39 (20.2)	9.18 (19.7)	8.26 (17.8)	8.32 (17.9)	8.77

1 values in parentheses represent percentage ratio measured to nominal concentration
* test concentration below the limit of quantification of 0.59 mg a.s./L, therefore, it was set as half of the limit of quantification

Biological test results

The results of the most recently performed test with a reference substance were reported. The 24 h IC_{50} of potassium dichromate for *Daphnia magna* was 1.22 mg/L (95 % confidence interval: 0.75 – 1.77 mg/L).

The validity criteria were met as the mortality in the control was not more than 10 % after 48 hours (actual mortality: 0 %), the oxygen concentration at the end of the test was equal to or more than 3 mg/L (actual concentration: > 6.0 mg/L) and no control daphnids were trapped at the surface of the test water.

The results for immobility are presented in Table A 2.2-6. In the control and in the lowest concentration of 10.0 mg/L no immobile daphnids were observed. Immobility increased with increasing test concentrations up to 100 % immobile daphnids at 100 mg/L.

Table A 2.2-6: Observed immobility of *Daphnia magna* exposed to Moximate 505 WP for 48 hours in a semi-static acute test

Nominal concentration [mg formulation/L]	Immobility			
	24 hr		48 hr	
	No immobile	% immobile	No immobile	% immobile
Control	0	0	0	0

10.0	0	0	0	0
17.8	1	5	2	10
31.6	0	0	1	5
56.2	2	10	10	50
100	15	75	20	100

20 animals were introduced per concentration

The EC_{50} value (48 h) for the formulation was based on nominal concentrations and amounted to 56.2 mg/L (95 % confidence interval: 49.1 – 63.2 mg/L). For the active substances, the EC_{50} values corresponded to 0.10 mg Cymoxanil/L (measured concentration) and 4.80 mg Mancozeb/L (measured concentration). The no observed effect concentration (NOEC) was determined to be 31.6 mg/L (corresponding to 0.06 mg/L Cymoxanil (measured concentration) and 2.63 mg/L Mancozeb (measured concentration), respectively), the lowest observed effect concentration (LOEC) was 56.2 mg/L (corresponding to 0.10 mg/L Cymoxanil (measured concentration) and 4.80 mg/L Mancozeb (measured concentration), respectively).

Conclusion

The EC_{50} value (48 h) for the formulation was based on nominal concentrations and amounted to 56.2 mg/L (95 % confidence interval: 49.1 – 63.2 mg/L). For the active substances, the EC_{50} values corresponded to 0.10 mg Cymoxanil/L (measured concentration) and 4.80 mg Mancozeb/L (measured concentration).

A 2.2.1.3 Toxicity of Moximate 505 WP to green algae *Pseudokirchneriella subcapitata* determined in a growth inhibition study

Comments of zRMS:	The study was not evaluated by zRMS.

Reference:	KCP 10.2.1/03
Report	Neri, M.C. (2009c). Toxicity of Moximate 505 WP to green algae *Pseudokirchneriella subcapitata* determined in a growth inhibition study. Report No. CH-E 004/2009 (Doc. No. 823 004), ChemService S.r.l. Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 201 (2006)
Deviations:	According to OECD guidance, the recommended temperature range is 24.0 ± 2.0°C. During the test period the temperature is reported as being in the range of 24.5 – 26.3°C. The deviation of 0.3°C occurred for one hour during the test period. Nevertheless, this deviation did not affect the final results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The toxicity of Moximate 505 WP (batch: 05, active substances: 4 % Cymoxanil and 46.5 % Mancozeb) to *Pseudokirchneriella subcapitata* was determined in a static system at nominal concentrations of 0, 1.0, 3.16, 10.0, 31.6 and 100 mg formulation/L, corresponding to 0.04, 0.13, 0.40, 1.26 and 4.0 mg/L Cymoxanil and to 0.47, 1.47, 4.65, 14.7 and 46.5 mg/L Mancozeb over 72 hours at 24.5 – 26.3°C and continuous lighting. Test media were inoculated with 10^4 cells/mL. Six replicates for the treatments and con-

trol were established. Algal cell density (biomass) was measured at 24, 48 and 72 hours after test initiation. At the same time points, the algal growth rate and yield were also determined.

Cell density was measured every 24 hours by fluorescent reading with a spectrofluorophotometer in few millilitre samples taken from each test concentration replicate and from controls. The observed values were used to calculate the percentage inhibition of cell growth as yield and as growth rate in comparison to the control. A reference test with 3.5 dichlorophenol was conducted under similar conditions. Concentrations were analysed by HPLC-UV/VIS.

Results and discussions

Water quality parameters like temperature, dissolved oxygen and pH value were within ranges requested by guideline OECD 202.

Analytical results

Cymoxanil and Mancozeb measured concentrations deviated to more than 20 % of the nominal concentrations, therefore, the biological results are referred both to the nominal concentrations of the formulations and to the geometric means of the measured concentrations of the active substances. Results of the analytical determinations are presented in Table A 2.2-7.

Table A 2.2-7: Analytical results for Cymoxanil and Mancozeb over a period of 72 h

Nominal concentration [mg/L]			Measured concentrations [mg/L]					
			Cymoxanil			Mancozeb		
Maximate 505 WP	Cymoxanil	Mancozeb	0 h	72 h	Geometric mean	0 h	72 h	Geometric mean
0	0	0	0	0	0	0	0	0
1.0	0.04	0.47	0.05*	0.05*	0.05	0.08	0.25**	0.14
3.16	0.13	1.47	0.05*	0.05*	0.05	0.28	0.25**	0.26
10.0	0.40	4.65	0.13	0.05*	0.08	0.86	0.96	0.91
31.6	1.26	14.7	0.52	0.05*	0.16	2.86	3.36	3.10
100.0	4.00	46.5	1.26	0.05*	0.25	9.17	9.84	9.50

* concentration was below the limit of detection (LOD) of 0.05 mg/L for Cymoxanil, therefore the value was set to the LOD
** concentration was below the limit of detection (LOD) of 0.25 mg/L for Mancozeb, therefore the value was set to the LOD

Biological test results

The validity criteria were met as the biomass in the control increased by a factor of at least 16 (actual increase: 272), the shift of the pH value in the control was not more than 1.5 units (actual shift: 1.44), the coefficient of variation for specific growth rate was not more than 35 % (actual coefficient of variation: 33.4 %) and the coefficient of variation of average growth in the control replicates was not more than 7 % (actual coefficient of variation: 3.8 %). The E_rC_{50} value for the reference item 3.5 dichlorophenol was determined to be 4.08 mg/L (95 % confidence intervals: 3.04 – 5.18 mg/L). The mean cell densities after 24, 48 and 72 hours are summarised in Table A 2.2-8.

Table A 2.2-8: Mean cell concentrations of *Pseudokirchneriella subcapitata* after exposure to Maximate 505 WP

Nominal Concentration [mg formulation/L]	Mean Cell Concentration [x 10^4 cells/mL] after		
	24 h	48 h	72 h
Control	7.18	82.0	271.6
1.0	6.79	81.6	295.0
3.16	5.40	75.4	308.3
10.0	1.79	18.3	178.4
31.6	1.06	1.19	1.07
100.0	1.01	1.04	0.961

Initial cell density was estimated to be 10⁴/mL

The average specific growth rates are summarised in Table 10.2.2.3-3. The mean coefficient of variation (CoV) for the average specific growth rate of the controls was 33.4 % during the 72-hour exposure period. In the test solutions, the algal growth rate was inhibited by 7.5 % in the 10 mg/L treatment group and to 100.7 % in the 100 mg/L treatment group. The two lowest concentrations did not show any significant effect compared to the control. The NOEC for the growth rate after 72 hours was 3.16 mg/L and the LOEC was 10 mg/L.

Table A 2.2-9: Average specific growth rate of *Pseudokirchneriella subcapitata* after exposure to Moximate 505 WP

Nominal Concentration [mg formulation/L]	Average specific growth rate (x 10^{-3}) * after		
	24 h	48 h	72 h
Control	82.1 (-)	91.8 (-)	77.8 (-)
1.0	79.8 (2.9)	91.7 (0.1)	79.0 (-1.5)
3.16	70.3 (14.5)	90.1 (1.9)	79.6 (-2.3)
10.0	24.2 (70.5) **	60.6 (34.0) **	72.0 (7.5) **
31.6	2.5 (96.7) **	3.6 (96.1) **	0.9 (98.9) **
100.0	0.5 (99.4) **	0.8 (99.1) **	-0.6 (100.7) **

* values in parentheses represent mean percentage of inhibition compared to the control
**significantly different compared to control (Dunnett's Multiple Comparison, $p \leq 0.05$)

After 72 hours of exposure, the yield (y), i.e. the difference of algal density between the end and the beginning of the test, decreased by a minimum value of 34.4 % for the nominal concentration of 10 mg/L, to a maximum value of 100 % for the highest concentration (100 mg/L). The two lowest concentrations did not show significant effects compared to the control. The percentages of growth inhibition in terms of biomass (yield) are summarised in Table A 2.2-9.

Table A 2.2-10: Algal yield inhibition of Moximate 505 WP after 72 hours of exposure

Nominal Concentration [mg formulation/L]	Mean biomass after 72 hours [cells/ml]	Mean growth inhibition [%]
Control	2715754	-
1.00	2950358	-8.7
3.16	3082792	-13.6
10.0	1784130	34.4 *
31.6	106467	99.8 *
100.0	9614	100 *

*significantly different compared to control (Dunnett's Multiple Comparison, $p \leq 0.05$)

The 72-h E_rC_{50} and E_rC_{10} were calculated to be 16.8 and 10.9 mg formulation/L, respectively based on nominal concentrations. The E_yC_{50} and E_yC_{10} were determined to be 18.3 and 5.38 mg formulation/L, respectively, based on nominal concentrations.

~~The two lowest nominal product concentrations (1.00 and 3.16 mg/L) did not show any significant effect compared with the negative control.~~

~~**Table A 2.2.11: EC_{50} values for growth rate and yield of *Pseudokirchneriella subcapitata* after 72 hours of exposure to Moximate 505 WP in a static test system**~~

~~Growth Function~~	~~EC_{10} [mg/L]~~	~~EC_{50} [mg/L]~~	~~LOEC [mg/L]~~	~~NOEC [mg/L]~~
~~**Moximate 505 WP ***~~				
~~Average specific growth rate~~	~~10.9 (9.14 – 12.0)~~	~~16.8 (12.9 – 20.8)~~	~~10.0~~	~~3.16~~
~~Yield~~	~~5.38 (3.73 – 6.86)~~	~~18.3 (17.3 – 19.4)~~	~~10.0~~	~~3.16~~
~~**Cymoxanil ****~~				
~~Average specific growth rate~~	~~0.081 (0.081 – 0.082)~~	~~0.117 (0.116 – 0.118)~~	~~0.4~~	~~0.13~~
~~Yield~~	~~0.058 (0.057 – 0.058)~~	~~0.096 (0.093 – 0.098)~~	~~0.4~~	~~0.13~~
~~**Mancozeb ****~~				
~~Average specific growth rate~~	~~0.992 (0.819 – 1.11)~~	~~1.57 (0.995 – 2.17)~~	~~4.65~~	~~1.47~~
~~Yield~~	~~0.523 (0.368 – 0.663)~~	~~1.74 (1.65 – 1.85)~~	~~4.65~~	~~1.47~~

~~* based on nominal concentrations~~
~~** based on mean measured concentrations~~

~~**Conclusion**~~

~~The E_yC_{50} is 18.3 mg/L, corresponding to 0.096 mg Cymoxanil/L and to 1.74 mg Mancozeb/L. The E_rC_{50} is 16.8 mg/L, corresponding to 0.117 mg Cymoxanil/L and to 1.57 mg Mancozeb/L.~~

A 2.2.1.4 Fresh water Algae Growth Inhibition Test with Cymoxanil 45% WG

Comments of zRMS:	The study was conducted in line with OECD 201. The test concentrations of the active substances were verified at the beginning and at the end of the exposure for only three tested concentration levels. 1.00, 6.76, 45.70 mg test item/L The measured concentration of were in the range 97% to 111.04% of the nominal values at the beginning (day 0) and from 96.60% to 110.01 % of the nominal values at the test end (72h). Performed chemical analyses were sufficient to confirm stability of the test item in the test solution over the study period. The endpoints are based on nominal test item concentration. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment. 72 h E_rC_{50}=15.93 mg test item /L (corresponding to 7.22 mg Cymoxanil/L) based on nominal concentrations. 72-h E_yC_{50}= 4.02 mg test item/L (corresponding to 1.82 mg Cymoxanil/L) based on nominal concentrations. NOE_rC = 1.0 mg test item/L (0.45 mg Cymoxanil/L) based on nominal concentrations. The zRMS additionally evaluated the reliability of the derived EC10 according to the criteria specified in Appendix E of EFSA (2019). For this purpose, the normalised width (NW) of the 95 %-Confidence Interval (CI) was calculated as follow: 72 h EyC_{10} = 0.84 mg product /L (CI: 0.00014-1.81) (nom); NW = 2.15 According to Appendix E of EFSA (2019), NW value are rated as bad (>2) indicating no reliability of the estimated 72 h EyC_{10}. 72 h ErC_{10} = 0.84 mg product /L (CI: NA) (nom); NW = no 95% CI is given. No reliability can be confirmed. The NOEC value was used in the risk assessment.

Reference:	KCP 10.2.1/04
Report	Venkanna, B. (2024). Fresh water Algae Growth Inhibition Test with Cymoxanil 45% WG. Report No. 23/0177. Vivo Bio Tech Limited. Survey # 349/A, Pregnapur Village – 502311, Gajwel Mandal, District - Siddipet, Telangana (India)
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 201 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

The objective of this study was to determine the toxicity of Cymoxanil 45% WG on algal growth by exposing fresh water green alga, *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) to an aqueous medium containing test item at five different nominal test concentrations for 72 hours. The study was performed according to OECD Guidelines for the Testing of Chemicals, Section 2. Number 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test", adopted on 23rd March 2006. Annex 5 corrected: 28th July 2011.

Materials and methods

Based on the results of a range finding experiment, the main test was conducted with control (six replicates) and test item concentrations of 1.00, 2.60, 6.76, 17.58 and 45.70 mg test item/L corresponding to 0.45, 1.18, 3.07, 7.97 and 20.72 mg active ingredient/L, respectively. During the conduct of the main experiment, approximately 100 mL of sample was collected from the control, 1.00, 6.76 and 45.70 mg/L concentrations on the 0 day of exposure and on completion at 72 hours (Pooled samples of the replicate) and sent to Analytical Chemistry for concentration verification. The concentration of samples was analysed using a fully validated HPLC-DAD method under GLP (Study No. 23/0181).

Test system

Species (Test system)	:	*Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) (Unicellular green alga)
Source	:	Department of Botany, Osmania University.
Sub culture	:	In-house culture, Vivo Biotech Ltd.
Algae Inoculated	:	8575 cells/mL
Test Method	:	Static Test

Test Conditions

Temperature (Main experiment)	:	Control : 22.3 - 22.8 °C Treatment : 22.2 - 22.8 °C
Test Vessel	:	250 mL conical flask
No. of Replicate	:	Control - 6 replicates, treatment - 3 replicates for each test concentration
Light (Main experiment)	:	Continuous Light Intensity of 5657-5705 Lux
pH range	:	Start of the test on day (0) in bulk of medium (main experiment) Control : 8.10 Treatment : 7.80-7.91 End of the test (72 hr) Control : 8.05 - 8.10 Treatment : 7.74 - 7.93
Medium	:	OECD growth medium
Pre-culture	:	3 days
Test Duration	:	72 Hours

Results and discussions

Water quality parameters like temperature, dissolved oxygen and pH value were within ranges requested by guideline OECD 201.

Analytical results

Cymoxanil measured concentrations deviated less than 20 % of the nominal concentrations, therefore, the biological results are referred as the nominal concentrations of the formulations Results of the analytical determinations are presented in Table A 2.2-12.

Table A 2.2-12: Analytical results over a period of 72 h

Nominal test item concentration	Measured test item concentrations at 0 h		Measured test item concentrations at 72 h	
[mg/L]	[mg/L]	% Recovery	[mg/L]	% Recovery
1.00	0.970	97.00	0.966	96.60
6.76	7.506	111.04	7.437	110.01
45.70	45.719	100.04	44.730	97.88

Biological test results

The validity criteria were met as the biomass in the control increased by a factor of at least 16 (actual increase: 117), the coefficient of variation for section-by-section specific growth rate was not more than 35 % (actual coefficient of variation: 2.5 %) and the coefficient of variation of average specific growth rates in the control replicates was not more than 7 % (actual coefficient of variation: 1.3 %).

The Growth Inhibition after 72 hours is summarised in Table A 2.2-13.

Table A 2.2-13: Percent Growth Inhibition

Nominal Concentration [mg test item/L]	0-24 h		0-48 h		0-72 h	
	Mean growth rate	Growth inhibition (%)	Mean growth rate	Growth inhibition (%)	Mean growth rate	Growth inhibition (%)
Control (0)	0.10	0.00	0.17	0.00	0.20	0.00
1.00	0.10	-0.38	0.17	-0.04	0.20	-0.05
2.60	0.09	9.07	0.16	3.65	0.18	7.71*
6.76	0.08	17.74	0.14	18.90	0.15	26.61*
17.58	0.06	35.27	0.10	42.61	0.10	51.11*
45.70	0.06	37.01	0.06	62.75	0.04	81.50*

Key: * indicates statistically significant from control (Dunnett's Multiple Comparison, $p \leq 0.05$).

The Yeld Inhibition after 72 hours is summarised in Table A 2.2-14.

Table A 2.2-14: Percent Yield Inhibition

Nominal Concentration [mg test item/L]	0 h	24 h			48 h			72 h		
	Cell count	Cell count	Yield (0-24 h)	Inhibition [%]	Cell count	Yield (0-48 h)	Inhibition [%]	Cell count	Yield (0-72 h)	Inhibition [%]
Control (0)	8575	94167	85592	0.00	472500	463925	0.00	1005000	996425	0.00
1.00	8575	95000	86425	-0.97	473333	464758	-0.18	1008333	999758	-0.33
2.60	8575	75833	67258	21.42	408333	399758	13.83	694167	685592	31.19*
6.76	8575	61667	53092	37.97	221667	213092	54.07	283333	274758	72.43*
17.58	8575	40833	32258	62.31	85833	77258	83.35	88333	79758	92.00*
45.70	8575	39167	30592	64.26	38333	29758	93.59	20833	12258	98.77*

Key: * indicates statistically significant from control (Dunnett's Multiple Comparison, $p \leq 0.05$).

Results allowed the endpoints derivation. $EC_{10,20,50}$, NOEC and LOEC values are reported in the following tables.

Table A 2.2-15: Inhibition of Yield and Growth rate at 72 hours of exposure assessed on the basis of nominal test item concentrations

Endpoint	**0 - 72 h EC_{10}** (mg test item/L)	**0 - 72 h EC_{20}** (mg test item/L)	**0 - 72 h EC_{50}** (mg test item/L)	**0 - 72 h NOEC** (mg test item/L)	**0 - 72 h LOEC** (mg test item/L)
Growth rate	2.10 (NA - NA)	5.41 (NA - NA)	15.93 (14.05 - 18.09)	1.0	2.60
Yield	0.84 (0.00014 - 1.81)	1.40 (0.00086 - 2.51)	4.02 (3.44 - 4.71)	1.0	2.60

Key: Values in the parentheses are 95% confidence limits.

Table A 2.2-16: Inhibition of Yield and Growth rate at 72 hours of exposure assessed on the basis of nominal active ingredient Cymoxanil concentrations

Endpoint	**0 - 72 h EC_{10}** (mg a.i./L)	**0 - 72 h EC_{20}** (mg a.i./L)	**0 - 72 h EC_{50}** (mg a.i./L)	**0 - 72 h NOEC** (mg a.i./L)	**0 - 72 h LOEC** (mg a.i./L)
Growth rate	0.95 (NA - NA)	2.45 (NA - NA)	7.22 (1.84 - 8.20)	0.45	1.18
Yield	0.38 (0.000063 – 0.82)	0.63 (0.00039 – 1.14)	1.82 (1.56 - 2.14)	0.45	1.18

Key: Values in the parentheses are 95% confidence limits.

Conclusion

The effects of the test item Cymoxanil 45% WG on the fresh water green algae, *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) were evaluated in a 72-hour growth inhibition test under static conditions.

The NOEC for the inhibition of growth rate after 72 hours was 1.0 mg test item/L, corresponding to 0.45 mg Cymoxanil/L. The 72-h E_rC_{50} and E_rC_{10} were calculated to be 15.93 and 2.10 mg test item/L, respectively (corresponding to 7.22 and 0.95 mg Cymoxanil/L) based on nominal concentrations.

The NOEC for the inhibition of yield after 72 hours was 1.0 mg test item/L, corresponding to 0.45 mg Cymoxanil/L. The 72-h E_yC_{50} and E_yC_{10} were calculated to be 4.02 and 0.84 mg test item/L, respectively (corresponding to 1.82 and 0.38 mg Cymoxanil/L) based on nominal concentrations.

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 Effects of Cymoxanil Technical (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory (Limit Test)

Comments of zRMS:	The a.s. new data are not evaluated by zRMS during authorisation of the product at zonal level.

~~Reference: KCP 10.3.1.1/01~~

Report	Schmitzer, S. (2007). Effects of Cymoxanil Technical (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory (Limit Test). Report No. 36571035, Institut fiir Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 213 and 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test Item:	Cymoxanil Technical, Batch No.: PP#26, content:
	Cymoxanil Technical: 98.48 % (w/w) (analytical); 98.0 % (w/w) (nominal).
Test Species:	Honey bee *(Apis mellifera* L.); female worker bees; obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.
Test Design:	Limit test; acute oral and contact toxicity test; duration 48 h; 5 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hours; Reference Item: Dimethoate 400 g/L (nominal).
Test Concentrations:	Contact test: 100 µg of the a.i. of Cymoxanil Technical/bee,
	Oral test (nominal): 100 µg of the a.i. of Cymoxanil Technical/bee
	Oral test (measured): 106.6 µg of the a.i. of Cymoxanil Technical/bee
	* in the following, *e.g.* 100.0 µg of the a.i. of Cymoxanil Technical/bee, will be referred as 100.0 [µg a.i./bee. The dose levels applied were adjusted to reflect the percentage a.i. Cymoxanil (analytical).
Test Conditions:	Temperature: 25 °C; relative humidity: 47 % - 74 %; photoperiod: 24 h darkness.

Results and discussions

At the end of the contact toxicity test (48 hours after application), there was no mortality at the single test concentration of 100.0 µg a.i./bee. No mortality occurred in the solvent control (acetone) and in the negative control (water + 0.5 % Adhasit). No behavioural abnormalities occurred at any time in the contact test.

In the oral test, the single nominal dose level (100 µg a.i./bee) resulted in an actual dose of 106.6 µg a.i./bee. This dose level resulted in no mortality at the end of the test (48 hours after application). No mortality occurred in both the negative control (50 % sugar solution) and the solvent control (acetone + water + sugar solution (50 % sugar and 5 % Acetone; the use of 5 % acetone instead of 1 % as stated in the guideline is due to the high test concentration (100 µg a.i./bee); internal IBACON experiences has shown that this higher acetone concentration does not influence the outcome of the study, as also shown by the valid control mortality). No behavioural abnormalities occurred at any time in the oral test.

Table A 2.3-1: Toxicity of Cymoxanil Technical to honey bees (*Apis mellifera* L.) in contact and oral toxicity (limit test)

	Contact Test [48 h]	**Oral test [48 h]**
LD_{50} [µg a.i./bee]	> 100.0	> 106.6

The contact and oral LD_{50} (24 h) values for the reference item (dimethoate) were calculated to be 0.16 and 0.10 µg a.i./bee, respectively.

Conclusion

The toxicity of Cymoxanil Technical was tested in both acute contact and oral toxicity test on honey bees. The LD_{50} (48 h) was > 100.0 µg a.i./bee in the contact toxicity test. The LD_{50} (48 h) was > 106.6 µg a.i./bee in the oral toxicity test.

A 2.3.1.1.2 Effects, acute oral and acute contact toxicity of Moximate 505 WP on the honeybee *Apis mellifera* L. in the laboratory (dose response test)

Comments of zRMS:	The study was not evaluated by zRMS.

Reference:	KCP 10.3.1.1/02
Report	Colli, M. (2009a). Effects, acute oral and acute contact toxicity of Moximate 505 WP on the honeybee *Apis mellifera* L. in the laboratory (dose response test). Report No. BT013/09 (Doc. No. 832-004), BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 213 and 214 (1998)
Deviations:	Only one concentration of the reference item was tested instead of 3 concentrations as recommended in the guidelines and no LD_{50} was determined. However, the mortality of bees exposed to the reference item were in the range of the LD_{50} stated in the guidelines, indicating that the test system was sensitive.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The 48 hour acute contact and oral toxicity of Moximate 505 WP (batch 05, active substances 4.4 % Cymoxanil and 48.3 % Mancozeb) to young adult worker bees was determined by topical application (test item dispersed in deionised water) and offering test item treated sucrose solution under laboratory conditions at 24.3 – 24.7°C. The different doses for the oral toxicity study were prepared by mixing a definite amount of stock solution with a definite amount of a 50 % (w/v) aqueous sucrose solution to give the test concentrations of 37.5, 75.0, 150.0, 300.0 and 600.0 µg product/bee. The study included 5 test item treatment groups, a control and 1 reference item group with 3 replicates each. A single replicate contained a group of 10 bees. Before the test items administration, the bees were starved for approximately two hours. Quantities of 200 µl (20 µL/bee) of treated solutions were offered to each cage of 10 bees for four hours.

In the acute contact toxicity study, the bees were anaesthetized and then were individually treated by topical application with a micro applicator. 1 µl of test solution was applied to the dorsal side of the thorax of each bee. After application, the bees were returned to the cages and fed a 50 % aqueous sucrose solution *ad libitum*. Five test concentrations were examined, 18.8, 37.5, 75.0, 150.0 and 300.0 µg product/bee. The study included 5 test item treatment groups, a control and 1 reference item group with 3 replicates each. A single replicate contained a group of 10 bees.

Dimethoate was used as reference item at a dose of 0.5 µg/bee (corresponding to 0.20 µg a.s./bee). Assessments of mortality and behavioural abnormalities were performed at 4, 24 and 48 hours after treatment for both acute oral and acute contact toxicity tests.

Results and discussions

In order to determine the sensitivity of the test, the oral and contact toxicity of the reference item were investigated. The acute oral and contact toxicity of Perfekthion (active substance Dimethoate) after 48 hours at a dose rate of 0.5 µg/bee was 96.4 and 100 % mortality, respectively. These values correspond to the specified range for the contact and oral 24 h LD_{50} of 0.1 – 0.3 µg/bee and 0.1 – 0.35 µg/bee, respectively, as stated in the OECD Guidelines 213/214 and showed that the test system was sensitive. Moreover, the mortality caused by the reference item is within the range of the historical data generated in the testing facility (i.e. > 70 % at the dose of 0.20 µg a.s./bee). Therefore, this deviation is considered to have no impacts on the results of the study.

Oral toxicity test

Mortality in the control was 6.67 % after 48 h (validity criteria 10 %). The statistical analysis showed a statistically significant difference between the effects of the test item at the maximum dose and the effects obtained in the untreated control group, p-value < 0.05. The oral LD_{50} (48 h) was determined to be > 600 µg/bee, corresponding to > 26.4 µg Cymoxanil/bee and > 289.6 µg Mancozeb/bee. The results are summarised in the table below. During the first 4 hours, the bees in the treated groups consumed all the provided solution. Thus, Moximate 505 WP did not appear to be repellent. Furthermore, no behavioural abnormalities were noticed after oral exposure.

Table A 2.3 2: Mortality of honeybees exposed to Moximate 505 WP for 48 hours in an oral toxicity test

Nominal dose [µg formulation/bee]	Mean dose consumed [µg/bee] [1]	Acute oral mortality [%] after	
		24 h [2]	48 h [2]
Moximate 505 WP			
Control (sucrose solution)	0	6.67	6.67
37.5	37.5	0	0
75.0	75.0	0	0
150	150	0	3.57
300	300	7.14	10.7
600	600	17.9	17.9*
Reference Item (Dimethoate)			
0.5	0.5	96.4	96.4

[1] during the first 4 hours, honey bees have consumed all the treated solution offered to them.
[2] mortality of the treated groups are corrected according to Abbott (1925)
* statistically significant compared to the control ($p \leq 0.05$)

Contact toxicity test

Mortality in the control was 6.67 % after 48 h. The statistical analysis showed a statistically significant difference between the effects of the test item at the maximum dose and the effects obtained in the untreated control group, p-value < 0.05. Corrected mortality in the treatment groups of 18.8, 37.5, 75, 150 and 300 µg formulation/bee after 48 hours was 0, 7.14, 3.57, 10.7 and 17.9 %, respectively. Therefore, the calculated contact LD_{50} (48 h) was determined to be > 300 µg formulation/bee. The LD_{50} values corresponded to > 13.2 µg Cymoxanil/bee and > 144.8 µg Mancozeb/bee. The results are summarised in Table A 2.3 2.

During the contact and oral toxicity tests, no abnormal behavioural effects (e.g. uncoordinated movement, increased rate of grooming or constant grooming, lethargy, lack of feeding or diarrhoea) was noticed in the treated honey bees. Furthermore, no repellent effects of the formulation were observed.

Table A 2.3.3: Mortality of honeybees exposed to Moximate 505 WP for 48 hours in a contact toxicity test

Nominal dose [µg/bee]	Acute contact mortality [%] [1] after	
	24 h	48 h
Moximate 505 WP		
Control	3.33	6.67
18.8	0	0
37.5	10.3	7.14
75.0	6.9	3.57
150	10.3	10.7
300	17.2	17.9 *
Reference Item (Dimethoate)		
0.50	93.1	100

[1] mortality of the treated groups are corrected according to Abbott (1925)
* statistically significant compared to the control ($p \leq 0.05$)

Conclusion
Under the conditions of this study, it can be concluded that Moximate 505 WP does not cause adverse effects on *Apis mellifera* L. after oral and contact exposure. Therefore, the LD_{50} for both endpoints was set above the highest tested dose. The oral LD_{50} (48 h) was determined to be > 600 µg/bee, corresponding to > 26.4 µg Cymoxanil/bee and > 289.6 µg Mancozeb/bee. The contact LD_{50} (48 h) was determined to be > 300 µg formulation/bee, corresponding to > 13.2 µg Cymoxanil/bee and > 144.8 µg Mancozeb/bee.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Cymoxanil 45 WG (IN 002B1760): Chronic Oral Toxicity to adult worker honeybees *Apis mellifera* L. (10-day feeding)

Comments of zRMS:	The study was conducted in line with OECD 245 with no deviations. All validity criteria were met. • Controls mortality in the negative control and Xanthan: 6.7 and 10% respectively. • In positive control: 100% mortality. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LC_{50} (10 days) = 343.1 mg product/kg feeding solution (equivalent to 161 mg a.s/kg feeding solution) LDD_{50} = (10 days) = 10.7 µg product/bee/day (equivalent to 5.1 µg a.s product/bee/day). NOEC (10 days) = 209 mg product/kg feeding solution (equivalent to 98.2 mg a.s kg feeding solution) NOEC (10 days) = 95 mg product/kg feeding solution (equivalent to 44.63 mg a.s kg feeding solution) NOEDD = 7.3 µg product bee/day (equivalent to 3.4 µg a.s. bee/day)

	NOEDD =3.32 µg product bee/day (equivalent to 1.56 µg a.s. bee/day)

Reference:	KCP 10.3.1.2/01
Report	Ponti, B. (2021). Cymoxanil 45 WG (IN 002B1760): Chronic Oral Toxicity to adult worker honeybees *Apis mellifera* L. (10-day feeding). Report No. CH–0257/2021, ChemService S.r.l. Controlli e Ricerche, Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 245 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The purpose of this study was to assess the chronic oral effects of the test item Cymoxanil 45 WG (IN 002B1760) to adult worker honey bees of *Apis mellifera* L. For this purpose, young adult worker honey bees (max. 2 days old at test start) were exposed to five doses of the test item dispersed in a 50 % w/v aqueous sucrose solution by continuous and *ad libitum* feeding over a period of 10 days. Mortality and behavioural abnormalities were recorded daily during the 10-day test period. The test was carried out according to OECD guideline 245 (2017) and in compliance with the Principles of Good Laboratory Practice (GLP).

Test Item:	Cymoxanil 45 WG (IN 002B1760), Batch No.: IND_F040_0421_1 Active substance: cymoxanil, 45 % (w/w) (nominal); 47 % (w/w) (analytical).
Test Species:	Honey bee *(Apis mellifera* L.); young adult worker honey bees (max. 2 days old at test start).
Test Design:	Dose-response with five doses; chronic oral toxicity test; duration 10 d; 3 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality every day; Reference Item: Dimethoate.
Test Concentrations:	95.0, 209.0, 459.0, 1010.0, 2222.0 mg test item/kg feeding solution corresponding to 114.0, 250.8, 550.8, 1212.0 and 2666.4 mg test item/L (according to the aqueous sucrose solution density of 1.20 g/mL). Since the test item was not water soluble/miscible and the use of solvent is not recommended to solubilize a formulation, a thickener (Xanthan gum) was used with the aim to increase the suspensibility of the test item. Additionally, a negative control (sucrose solution without test item), a thickener control (sucrose solution with 0.1 % w/v of Xanthan) and positive control (dimethoate) were tested.
Test Conditions:	Temperature: 31.4°C to 33.1 °C (mean value 33.0°C); Relative humidity: 37.2 % to 67.6 % (mean value 59.9%); Photoperiod: constant darkness (except during assessments)..

Results and discussions

The bees were observed for mortality each 24 hours during the 10-day test period. Results for the treated groups were corrected for the xanthan control mortality, while the reference item dimethoate was corrected for the negative control. The amount of feeding solutions consumed were determined daily by initially weighting the feeders before and after feeding. Results were adjusted for evaporation of test solutions from the feeders.

Table A 2.3-4: Summarized mortality data at the end of the test

Treatment group	Concentration (g test item/kg food)	Food uptake (mg feeding solution/bee/day) (1)	Daily dosage		Mortality after 10 days	
			Test item (µg test item/bee/day)	Cymoxanil (µg a.s./ bee/day)	absolute (%)	corrected (%) (2)
Negative control	0.0	40.36	-	-	6.7	-
Xanthan control	0.0	39.15	-	-	10.0	-
Test item	95.0	34.97	3.32	1.56	13.3	3.7
	209.0	34.79	7.27	3.42	36.7	29.6
	459.0	30.03	13.78	6.48	66.7*	63.0
	1010.0	25.58	23.93	11.25	90.0*	88.9
	2222.0	19.26	38.63	18.16	100.0*	100.0
Positive control	0.8 (Dimethoate)	27.49	0.020 (Dimethoate)		100.0	100.0

(1) adjusted for evaporation from the feeders.
(2) corrected according to Abbot's formula. Negative values are to be considered equal to 0.
* statistically significant difference in pairwise comparison between treatment and untreated control (Fisher Exact/Bonferroni Adj Test).

The concentration of active ingredient Cymoxanil in the test medium was measured with a fully validated analytical method. Analytical recovery of Cymoxanil residues were in the range 85.8 % – 99.2 % of the nominal concentrations.
The $LC_{10,20,50}$/$LDD_{10,20,50}$ with 95 % confidence limits and the NOEC/NOEDD values at the end of the exposure (10 days), expressed as nominal Cymoxanil concentration / mean daily Cymoxanil uptake were assessed and are reported hereafter.
All the validity criteria of the test were met.

Conclusion

The effects of the test item Cymoxanil 45 WG (IN 002B1760) on adult worker honey bees (*Apis mellifera* L.) were assessed in a chronic oral toxicity test (10-days feeding).
The $LC_{10,20,50}$/$LDD_{10,20,50}$ with 95 % confidence limits and the NOEC/NOEDD values at the end of the exposure (10 days), expressed as nominal test item concentration / mean daily test item uptake were assessed as follows.

Table A 2.3-5: Statistical Outcome of the Chronic Toxicity Test on the basis of nominal test item concentrations/mean daily uptake doses

Critical concentration (mg test item/kg feeding solution)	**LC_{10}**	**LC_{20}**	**LC_{50}**	**NOEC**
10 days	122.0 (51.0 – 190.9) (*)	174.0 (87.1 – 252.8) (*)	343.1 (230.9 – 454.8) (*)	209
Critical dose (µg test item/bee/day)	**LDD_{10}**	**LDD_{20}**	**LDD_{50}**	**NOEDD**
10 days	5.0 (2.4 – 7.0) (*)	6.5 (3.6 – 8.7) (*)	10.7 (7.7 – 13.3) (*)	7.3

(*) 95% confidence limits

The $LC_{10,20,50}$/$LDD_{10,20,50}$ with 95 % confidence limits and the NOEC/NOEDD values at the end of the exposure (10 days), expressed as nominal Cymoxanil concentration / mean daily Cymoxanil uptake were assessed as follows.

Table A 2.3-6: Statistical Outcome of the Chronic Toxicity Test on the basis of nominal cymoxanil concentrations/mean daily uptake doses

Critical concentration (mg a.s./kg feeding solution)	**LC_{10}**	**LC_{20}**	**LC_{50}**	**NOEC**
10 days	57.3 (24.0 – 89.7) (*)	81.8 (40.9 – 118.8) (*)	161.3 (108.5 – 213.8) (*)	98.2
Critical dose (µg a.s./bee/day)	**LDD_{10}**	**LDD_{20}**	**LDD_{50}**	**NOEDD**
10 days	2.31 (1.1 – 3.3) (*)	3.0 (1.7 – 4.1) (*)	5.1 (3.6 – 6.3) (*)	3.4

(*) 95% confidence limits

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

A 2.3.1.3.1 Cymoxanil 45 WG (IN 002B1760): Honey bees (*Apis mellifera* L.) Larval Toxicity Test with Repeated Exposure

Comments of zRMS:	The study was conducted in line with OECD 239 with slight deviations. -During the test period, some punctual measurements of humidity were outside the acceptable range. All validity criteria were met. • The analysis of the stock solutions used to treat the diet administered to the larvae showed that the test item content was in the range of ± 20 % of nominal concentration (mean recovery rates of 82.7- 106.3%). • Cumulative mortality of the larvae from D3 to D8 from the negative control: 2.8% • Emergency rate of adults at D22 for the negative control: 77.8% • Cumulative mortality in the reference substance treated group (48.0 mg a.s./kg diet) at D8. Therefore, the endpoints were calculated on the basis of the nominal doses of test item. Since the validity criteria for the control were met the deviations are not impact on the study results. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 22 d NOED adult emergence rate =3.80 µg prod./larva (1.79 µg a.s./larva) 22 d ED_{10} =2.21 µg a.s./larva (1.53-2.41) 22 d NOEC adult emergence rate = 24.7 mg prod./kg diet (11.6 mg a.s./kg diet)

	The zRMS additionally evaluated the reliability of the derived EC10 according to the criteria specified in Appendix E of EFSA (2019). For this purpose, the normalised width (NW) of the 95 %-Confidence Interval (CI) was calculated as follow: 22 d ED_{10} =2.21 µg a.s./larva (1.53-2.41), NW=0.39 According to Appendix E of EFSA (2019), all NW values are rated as 'fair' (<1) or 'good' (0.2-0.5) indicating reliability of the estimated EC_{10}. However, NOEC provide a lower endpoint and will be considered for risk assessment.

Reference:	KCP 10.3.1.3/01 (and KCP 10.3.1.3/01a – STUDY PLAN AMENDMENT No. 1)
Report	Noè, F. (2022). Cymoxanil 45 WG (IN 002B1760): Honey bees (*Apis mellifera* L.) Larval Toxicity Test with Repeated Exposure. Report No. CH–0258/2021, ChemService S.r.l. Controlli e Ricerche, Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD Guideline for Testing of Chemicals, N. 239 (2021)
Deviations:	During the test period, some punctual measurements of humidity were outside the acceptable range. Reason of change: Handling the animals required an opening of the climatic chamber. Impact on the study: None, since the validity criteria for the control were met.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The purpose of this study was to assess the effects of the test item Cymoxanil 45 WG (IN 002B1760) to honey bee larvae *Apis mellifera* L. in a 22-day toxicity test (i.e. to adult emergence) with repeated exposure. The test method and the test species are as recommended by international test guidelines (OECD guidance document No. 239) and in compliance with the principles of Good Laboratory Practice (GLP).

Test Item:	Cymoxanil 45 WG (IN 002B1760), Batch No.: IND_F040_0421_1 Active substance: cymoxanil, 45 % (w/w) (nominal); 47 % (w/w) (analytical).
Test Species:	Honey bee *(Apis mellifera* L.); first instar synchronised larvae (larvae of the same age).
Test Design:	Dose-response with five doses; repeated exposure toxicity test; duration 22 day; 3 replicates, each consisting of 12 larvae in one plate per test concentration; mortality and other observations/abnormal effects were recorded daily from D3 to D8 (for larvae), from D8 to D15 (for pupae mortality) and emergence rate was recorded at day 22; Reference Item: Dimethoate.
Test Concentrations:	negative control (larvae not exposed to test item) and 2.7, 8.2, 24.7, 74.1 and 222.2 mg test item/kg diet, corresponding to 0.42, 1.27, 3.80, 11.41 and 34.22 µg test item/larvae and to 0.20, 0.60, 1.79, 5.36 and 16.08 µg Cymoxanil/larvae.
Test Conditions:	Temperature: 34.26 °C as mean value.; Relative humidity: 97.3 % as mean value (D1 - D8); 82.7 % as mean value (D8 – D15); 71.4 % as mean value (D15 – D22); Photoperiod: constant darkness (except during assessments).

Results and discussions

Mortality

Table A 2.3-7: Mortality assessment

Nominal concentration (mg test item/kg larval diet)	Nominal dose (µg test item/larva)	8-Day Larval Mortality (1) (%)	15-Day Pupal Mortality (2) (%)	22-Day Pupal Mortality (3) (%)	22-Day Larval + Pupal Mortality (4) (%)
Negative control	0	2.8	14.3	20.0	22.2
2.7	0.42	0.0	13.9	25.0	25.0
8.2	1.27	5.6	5.9	14.7	19.4
24.7	3.80	2.8	11.4	11.4	13.9
74.1	11.41	8.3	51.5*	72.7*	75.0*
222.2	34.22	0.0	100.0*	100.0*	100.0*

(1) Based on observations from days 3 to 8
(2) Based on observations from days 8 to 15
(3) Based on observations from days 8 to 22
(4) Based on observations from days 3 to 22
* statistically significant difference in pairwise comparison between treatment and untreated control (*Dunnett Multiple Comparison Test*)

Day-22 Adult emergence

Emerged adults that showed a normal development, alive adult bees and dead adults which had left their cell, were recorded at D22. Data on emergence of bee at day 22 are reported in the following table.

Table A 2.3-8: Adult emergence rate

Nominal concentration (mg a.s./kg larval diet)	Nominal dose (µg test item/larva)	Emergence/Replicate			Total emerged adults	Emergence rate [a]	Sign. [b] (+/-)
		A	B	C			
Negative control	0	10	9	9	28	77.8	n.a.
2.7	0.42	9	6	12	27	75.0	-
8.2	1.27	9	10	10	29	80.6	-
24.7	3.80	10	11	10	31	86.1	-
74.1	11.41	6	1	2	9	25.0	+
222.2	34.22	0	0	0	0	0.0	+

[a] based on 36 larvae at initiation
[b] statistically significant difference in pairwise comparison between treatment and untreated control (*Dunnett Multiple Comparison Test*) + significant; - non-significant
n.a. not applicable

The $EC_{10/20/50}$ and the $ED_{10/20/50}$ with 95 % confidence limits and the NOEC/NOED values for emerged adult bees on D22 were assessed on the basis of nominal test item concentrations/doses and are reported below.

A concentration analytical check of cymoxanil residues were, performed at the time D3, D4, D5 and D6 in the lowest and in the highest test solutions used to make up diets. The results of the analytical phase showed that recoveries ranged between 82.7% and 106.3%.

Conclusion

The effects of test item Cymoxanil 45 WG (IN 002B1760) on honey bee larvae (*Apis mellifera* L), were assessed in a 22-day toxicity test (i.e. to adult emergence) with repeated exposure.

The EC_{50} and the ED_{50} and the NOEC/NOED values at the end of the test (22 days) were assessed on adult emergence.

Statistical endpoints assessed on the basis of nominal test item concentrations/doses:

Table A 2.3-9: Statistical endpoints (test item) based on adult emergence rate at D22

End points (mg test item/kg diet)	**EC_{10}**	**EC_{20}**	**EC_{50}**	**NOEC**	**LOEC**
22 days	29.0 (21.6 – 33.0) (*)	34.1 (26.2 – 43.9) (*)	55.1 (35.6 – 99.7) (*)	24.7	74.1
End points (µg test item/larvae)	**ED_{10}**	**ED_{20}**	**ED_{50}**	**NOED**	**LOED**
22 days	4.51 (3.26 – 5.13) (*)	5.33 (4.14 – 7.94) (*)	8.58 (5.57 – 19.92) (*)	3.80	11.41

(*) 95% confidence limits

Statistical endpoints assessed on the basis of nominal Cymoxanil concentrations/doses:

Table A 2.3-10: Statistical endpoints (a.s.) based on adult emergence rate at D22

End points (mg a.s./kg diet)	**EC_{10}**	**EC_{20}**	**EC_{50}**	**NOEC**	**LOEC**
22 days	13.6 (10.2 – 15.5) (*)	16.0 (12.3 – 20.6) (*)	25.9 (16.7 – 46.9) (*)	11.6	34.8
End points (µg a.s./larvae)	**ED_{10}**	**ED_{20}**	**ED_{50}**	**NOED**	**LOED**
22 days	2.21 (1.53 – 2.41) (*)	2.51 (1.95 – 3.73) (*)	4.03 (2.62 – 9.36) (*)	1.79	5.36

(*) 95% confidence limits

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

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

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

A 2.3.2.1.1 Effects of Cymoxanil 45 WG on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory - Dose Response Test

Comments of zRMS:	The study was conducted in line with Mead-Briggs et al. (2000), method with no deviations. All validity criteria were met: • No mortality in the control group (0%). • 100% corrected mortality in reference item. • 26.3 mummies per female (mean value). • There was no parasitoid producing zero values. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} 48 h > 960 g a.s./ha

Reference:	KCP 10.3.2.1/01
Report	Moll, M. (2008a). Effects of Cymoxanil 45 WG on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory - Dose Response Test. Report No. 39701001, Institut far Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	Mead-Briggs et al. (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test Item:	Cymoxanil 45 WG; batch no. 070724; content of a.s.: Cymoxanil: 45 % w/w (nominal), 449.2 g/kg corresponding to 44.9 % w/w (analysed).
Test Species:	Parasitoid *(Aphidius rhopalosiphi),* adults less than 48 hours old; source: Katz Biotech AG, Baruth, Germany.
Test Design:	This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 4 replicates each containing 10 adult parasitoids. The parasitoids were exposed to dried residues on treated glass plates. Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups where > 50 % of parasitoids survived they were removed and their reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi.* The females were removed after 24 hours and the aphid-infested plants left for further 11 - 12 days before the numbers of aphid mummies that had developed were assessed.
Endpoints:	Mortality of exposed parasitoids; LR_{50}: lethal rate producing 50 % mortality after 48 h of exposure. Additionally reproductive capacity for female survivors.
Reference Item:	Perfekthion (Dimethoate, 395.9 g/L (nominal: 400 g/L)).
Test Rates:	Control, 60.0, 120, 240, 480 and 960 g a.i./ha and reference item. The reference item was applied at an application rate of 0.3 mL Perfekthion/ha. All treatments were applied in 200 L water/ha. The spraying dilutions were sprayed onto glass plates via laboratory spraying equipment, and air dried.
Test Conditions:	Temperature: 18 °C - 22 °C; relative humidity: 67 - 90 % (acclimatisation and exposure period), 65 - 73 % (post-exposure period, within the test units); photo-

period: 16 h light: 8 h dark; light intensity: 780 - 1620 lux (acclimatisation, exposure and parasitisation period), 5600 - 9400 lux (postparasitisation period).
Statistics: Mortality: Fisher Exact Test, reproduction: Dunnett-Test

Results and discussions
All study validity criteria were met. No mortality was observed in the control groups. In the different Cymoxanil 45 WG treatment groups mortalities ranged between 0.0 % and 2.5 %. At all dose rates up to and including 960 g a.i./ha mortality was not statistically significantly different compared to the control (Fisher Exact Test, a = 0.05). The LR_{50} was estimated to be greater than 960 g a.i./ha.
Reproduction was tested at 60.0, 120, 240, 480 and 960 g a.i./ha. There was no statistically significant effect on reproduction up to and including 960 g a.i./ha compared to the control (Dunnett-Test, a = 0.05).

Table A 2.3-11: Effects of Cymoxanil 45 WG on mortality and reproduction of the parasitoid, *Aphidius rhopalosiphi*, exposed to fresh dried residue in the laboratory

	Rate [1] [g a.i./ha]	Mortality [2] [%]	Mortality Mortality [%]	Reproduction [4] [mummies/female]	Effect on reproduction [5] [%]
Control	0	0.0	--	26.3	--
Cymoxanil 45 WG	60.0	2.5 n.s.	2.5	35.0 n.s.	-33.2
Cymoxanil 45 WG	120	0.0 n.s.	0.0	38.2 n.s.	-45.5
Cymoxanil 45 WG	240	0.0 n.s.	0.0	43.3 n.s.	-64.8
Cymoxanil 45 WG	480	0.0 n.s.	0.0	37.1 n.s.	-41.3
Cymoxanil 45 WG	960	2.5 n.s.	2.5	35.7 n.s.	-36.0
Endpoint					
LR_{50}: > 960 g a.i./ha					

1) Application rate in 200 L water/ha
2) Mortality: after 48 hours of exposure to spray residues on glass plates (Fisher Exact Test, a = 0.05; n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of parasitised aphids/female, (Dunnett-Test, a = 0.05; n.s. = not significant)
5) calculated on the exact raw data; negative value means increased reproduction compared to the control

The reference item applied at a rate of 0.3 mL Perfekthion/ha produced a statistically significant mortality of 100.0 % after 48 hours.

Conclusion

Under worst case laboratory conditions, the LR_{50} of Cymoxanil 45 WG is estimated to be greater than 960 g a.i./ha in 200 L water/ha.
Reproduction of *Aphidius rhopalosiphi* was not statistically significantly affected up to and including 960 g a.i./ha compared to the control.

A 2.3.2.1.2 Effects of Cymoxanil 45 WG on the Predatory Mite *Typhlodromus pyri* in the Laboratory - Dose Response Test

Comments of zRMS:	The study was conducted in line with Blümel et al. (2000) method with slight deviations: - The age of protonymphs at test start was: not older than 24 hours and 10 minutes, instead of 24 hours. Test conditions were not recorded for four days during reproduction due to a breakdown of the computer. In the same time the air conditioner was running correctly as checked. - At 240 and 480 g a.i./ha in some replicates, the sex ratio was not balanced at day 7. The sex of the mites could not be definitely determined on day 7, 10 or 11 because the mites were under developed. There is no impact on the study results due to reproduction

	was affected at both dose rates in all replicates and this was not caused by the absence of males. All validity criteria were met: • Mortality in the control group 6.7 %. • 92.9 % corrected mortality in reference item. • 10 eggs per female mummies per female. Since the validity criteria for the control were met the deviations are not impact on the study results. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: LR_{50} 48 h > 480 g a.s./ha Statistically significant effect on reproduction at 30 g a.s./ha (85.7% reduction reproduction)

Reference:	KCP 10.3.2.1/02
Report	Moll, M. (2008b). Effects of Cymoxanil 45 WG on the Predatory Mite *Typhlodromus pyri* in the Laboratory - Dose Response Test. Report No. 39702063, Institut far Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	Blümel *et al.* (2000)
Deviations:	The age of protonymphs at test start was: not older than 24 hours and 10 minutes, instead of 24 hours. Impact on the study: None, only slight deviation. Test conditions were not recorded for four days during reproduction due to a breakdown of the computer. Impact on the study: None, the air conditioner was running correctly as checked by IBACON personnel. At 240 and 480 g a.i./ha in some replicates, the sex ratio was not balanced at day 7. The sex of the mites could not be definitely determined on day 7, 10 or 11 because the mites were underdeveloped. Impact on the study: None, reproduction was affected at both dose rates in all replicates and this was not caused by the absence of males.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test Item:	Cymoxanil 45 WG; batch no. 070724; content of a.s.: Cymoxanil: 45 % w/w (nominal), 449.2 g/kg corresponding to 44.9 % w/w (analysed).
Test Species:	Predatory Mite (*Typhlodromus pyri*), protonymphs not older than 24 hours and 10 minutes; source: Katz Biotech AG, Baruth, Germany.
Test Design:	This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 3 replicates each containing 20 mites. The mites were exposed to dried residues on treated glass plates. Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups displaying less than 50 % corrected mortality were sexed

	and the number of eggs per females was recorded at 3 assessment days within one week.
Endpoints:	Mortality after 7 days of exposure; LR50: lethal rate producing 50 % mortality after exposure over 7 days, additionally reproduction capacity for all variants with less than 50 % corrected mortality.
Reference Item:	Perfekthion (Dimethoate, 395.9 g/L (nominal: 400 g/L)).
Test Rates:	Control, 30.0, 60.0, 120, 240 and 480 g a.i./ha and reference item. The reference item was applied at an application rate of 8.0 mL Perfekthion/ha. All treatments were applied in 200 L water/ha. The spraying dilutions were sprayed onto glass plates via laboratory spraying equipment, which were then air dried.
Test Conditions:	Temperature: 25 °C - 26 °C; relative humidity: 60 % - 82 %; photoperiod: 16 h light: 8 h dark; light intensity: 390 lux - 630 lux.
Statistics:	Mortality: Fisher Exact Test, reproduction: Welch-t-Test with Bonferroni Adjustment.

Results and discussions

All study validity criteria were met.
In the different Cymoxanil 45 WG treatment groups mortalities between 20.0 % and 56.7 % were observed. This results in corrected mortalities between 14.3 % and 53.6 %. At 120 g a.i./ha mortality was not statistically significantly different and at 30.0, 60.0, 240 and 480 g a.i./ha mortality was statistically significantly different compared to the control where 6.7 % mortality was observed (Fisher Exact Test, a = 0.05).
The LR_{50} was estimated to be greater than 480 g a.i./ha.
Reproduction was tested at all dose rates. There was a statistically significant effect on reproduction up to and including 480 g a.i./ha compared to the control (Welch-t-Test, a = 0.05). No eggs were observed at dose rates of 60 g a.i./ha and above.

The results are summarized in Table A 2.3-12.

Table A 2.3-12: Effects of Cymoxanil 45 WG on mortality and reproduction of the predatory mite, *Typhiodromus pyri*, exposed to fresh dried residue in the laboratory

	Rate [1] [g a.i./ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [4] [eggs/female]	Effect on reproduction [5] [%]
Control	0	6.7	--	10.0	--
Cymoxanil 45 WG	30.0	28.3 *	23.2	1.4 *	85.7
Cymoxanil 45 WG	60.0	56.7 *	53.6	0.0 *	100.0
Cymoxanil 45 WG	120	20.0 n.s.	14.3	0.0 *	100.0
Cymoxanil 45 WG	240	33.3 *	28.6	0.0 *	100.0
Cymoxanil 45 WG	480	33.3 *	28.6	0.0 *	100.0
Endpoint					
LR_{50}: > 480 g a.i./ha					

1) Application rate in 200 L water/ha
2) Mortality: after 7 days of exposure to spray residues on glass plates (Fisher Exact Test, α = 0.05: n.s. = not significant, * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of eggs/female (Welch-t-Test with Bonferroni Adjustment, α = 0.05: * = significant)
5) calculated on the exact raw data

The reference item applied at a rate of 8.0 mL Perfekthion/ha produced a statistically significant mortality of 93.3 % (corrected mortality 92.9 %) after 7 days.

Conclusion

Under worst case laboratory conditions, the LR_{50} of Cymoxanil 45 WG is estimated to be greater than 480 g a.i./ha. The highest mortality observed at 60 g a.i./ha is considered an outlier since affected by one replicate and since corrected mortalities never exceeded 30 % at the higher dose levels.
The reproduction of *T. pyri* was statistically significantly reduced at all dose rates. No eggs were observed at treatment rates of 60 g a.i./ha and above.

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

A 2.3.2.2.1 Effects of Cymoxanil 45 WG on the Carabid Beetle *Poecilus cupreus* L. - Extended Laboratory Study

Comments of zRMS:	The study was conducted in line with method with BBA guideline part VI, 23-2.1.8 (1991) and Heimbach et al. (2000) with no deviations. All validity criteria were met: • Mortality in the control group 0%. • 100 % corrected mortality in reference item. Since the validity criteria for the control were met the deviations are not impact on the study results. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: NOER=480 g a.s./ha ER_{50} > 480 g a.s./ha

Reference:	KCP 10.3.2.2/01
Report	Schmitzer, S. (2008). Effects of Cymoxanil 45 WG on the Carabid Beetle *Poecilus cupreus* L. - Extended Laboratory Study. Report No. 39703007, Institut far Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	BBA guideline part VI, 23-2.1.8 (1991) and Heimbach *et al.* (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test Item:	Cymoxanil 45 WG, Batch No.: 070724, content of al.: Cymoxanil: 45 % w/w (nominal), 449.2 g/kg corresponding to 44.9 % w/w (analysed).
Test Species:	Carabid beetle (*Poecilus cupreus* L.), age: about 6 weeks old; source: Bio-Test Labor GmbH, Sagerheide, Germany.
Test Design:	The Beetles were exposed to direct application on natural soil LUFA 2.1. Three treatments (test item, water treated control, reference item), each treatment consists of 5 test units each containing 6 individuals (3 males and 3 females)/test unit. As-

	sessment of mortality and behaviour 2 hours, 1, 2, 4, 7, 10 and 14 days after application. Food consumption was assessed on day 2, 4, 7, 10 and 14 after application.
Reference item:	Perfekthion EC (Dimethoate, 400 g/L).
Endpoints:	Mortality, behaviour and feeding rate of exposed beetles.
Test Rates:	Control, Cymoxanil 45 WG at a rate of 480 g a.i./ha (taking into consideration the nominal concentration of the product of 450 g/kg Cymoxanil). The reference item (Perfekthion EC containing 400 g/L Dimethoate) was applied at a dose equivalent to 1200 mL/ha. The substances were applied as a spray application in 400 L water/ha upon the substrate and the beetles via laboratory spray applicator.
Test Conditions:	Temperature: 19 °C - 22 °C; relative humidity: 68 % - 89 %; photoperiod: 16 h light: 8 h dark; light intensity: 570 - 1530 lux; food: fly pupae (*Calliphora spec.*).

Results and discussions

After 14 days no mortality occurred in the Cymoxanil 45 WG treatment group and in the water treated control. The mean food consumption (mean number of eaten pupae per beetle over the entire observation period) was 1.9 in the test item group and 1.7 in the control, respectively. This results in 112 % food consumption in the test item variant relative to the control (see Table A 2.3-13).
No test item induced behavioural impairments were found at any time during the entire time of the experiment.

Table A 2.3-13: Effects on ground beetles (*Poecilus cupreus*) exposed to Cymoxanil 45 WG in an extended laboratory trial

	Rate [1)] [a.i./ha]	Mortality [2)] [%]	corrected Mortality [3)] [%]	Pupae consumed per beetle [4)]	Food consumption [5)] [%]
Control	--	0.0	--	1.7	--
Cymoxanil 45 WG	480	0.0	0.0	1.9	112 %
Perfekthion EC	480	100.0	100.0	--	--

1) Application rate in 400 L water/ha
2) Mortality: mean of 5 replicates with 6 beetles/replicates, 30 beetles in total
3) corrected mortality according to Abbott, 1925
4) rounded values
5) compared to the control (control = 100 %)

Statistics mortality and food consumption: since in the test item treatment group no mortality occurred and the food consumption was higher compared to that in the control group, no statistics were performed.

The reference item resulted in a mortality of 100.0 % within 2 days of exposure.

Conclusion

No effects of Cymoxanil 45 WG on either mortality, feeding activity or behaviour of the ground dwelling predator *Poecilus cupreus* were observed after exposure up to an application rate of 480 g a.i. in 400 L water/ha on natural soil.

A 2.3.2.2.2 Effects of Cymoxanil 45 WG on the Lacewing Chrysoperla carnea under Extended Laboratory Conditions

Comments of zRMS:	The study was conducted in line with Vogt et al. (2000) method with slight deviations: - The determination of unhatched (infertile) eggs was done on the day when the last larvae had hatched or 1 day after the last larvae had hatched due to a mistake. - There is no impact on the study: According to IBACON experience, the hatching of the

	larvae from eggs is always finished within two days after the first larvae had hatched. All validity criteria were met: • 17.5% control mortality. • 100% corrected mortality for reference item. • Fecundity in the control group: 25.4 eggs per female per day (mean number). • Fertility in the control group: 79.2% larval hatching rate (mean value). Since the validity criteria for the control were met the deviations are not impact on the study results. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: ER_{50} > 480 g a.s./ha

Reference:	KCP 10.3.2.2/02
Report	Moll, M. (2008c). Effects of Cymoxanil 45 WG on the Lacewing *Chrysoperla carnea* under Extended Laboratory Conditions. Report No. 39704047, Institut far Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	Vogt *et al.* (2000)
Deviations:	The determination of unhatched (infertile) eggs was done on the day when the last larvae had hatched or 1 day after the last larvae had hatched due to a mistake. Impact on the study: None, like in this study and according to IBACON experience, the hatching of the larvae from eggs is always finished within two days after the first larvae had hatched.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test Item:	Cymoxanil 45 WG; batch no. 07024; content of a.s.: Cymoxanil: 45 % w/w (nominal), 449.2 g/kg corresponding to 44.9 % w/w (analysed).
Test Species:	Lacewing (*Chrysoperla carnea*), larvae (3 days old); source: Katz Biotech AG, Baruth, Germany.
Test Design:	This study encompassed 3 treatment groups (1 dose rate of the test item, control, reference item) with 40 replicates each containing 1 larva. The larvae were exposed to dried residues on treated leaf surfaces (bean leaves). Exposure time lasted as long as pupae were transferred to the reproduction units for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. In addition, he reproduction performance, i.e. egg deposition and larval hatching rate, was determined (2 checks/week, 24 hours period each check).
Endpoints:	Larval and pupal mortality, additionally reproductive capacity for female survivors.
Reference Item:	Perfekthion (Dimethoate, 395.9 g/L (nominal: 400 g/L)).
Test Rates:	Control, 480 g a.i./ha and reference item. The reference item was applied at an application rate of 100 mL Perfekthion/ha. All treatments were applied in 200 L water/ha. The spraying dilutions were sprayed onto bean leaves from bean

	plants that were 16 days old via laboratory spraying equipment, which were then air dried.
Test Conditions:	Temperature: 23 °C - 26 °C; relative humidity: 62 % 89 %; photoperiod: 16 h light: 8 h dark; light intensity: 1500 lux - 5140 lux.
Statistics:	Mortality: Fisher Exact Test

Results and discussions

All study validity criteria were met.
In the control 17.5 % and in the Cymoxanil 45 WG treatment group 7.5 % mortality was observed. This resulted in a corrected mortality of -12.1 %. At the only dose rate of 480 g a.i./ha mortality was not statistically significantly different compared to the control (Fisher Exact Test, a 0.05). The toxic control caused 100% mortality. Therefore, it can be concluded that the test item had no effect on mortality at the limit rate of 480 g a.i./ha.
Reproduction was > 15 eggs per female per day and the mean hatching rate was 79.2 and 84.9 % in the control and at the only dose rate of 480 g a.i./ha, respectively. This indicates that there is no negative effect of the test item on reproductive performance of *Chrysoperla carnea* at the limit rate of 480 g a.i./ha. Due to the high mortality to larvae, no reproductive performance test was possible with the toxic reference substance.

The results are summarized in Table A 2.3-14.

Table A 2.3-14: Effects of Cymoxanil 45 WG on pre-imaginal mortality and reproduction of *Chrysoperla carnea*, exposed to fresh dried residue in the laboratory

	Rate [g a.i./ha] 1)	Mortality [%] 2)	Mortality corr. [%] 3)	Reproduction [eggs/female/day]	Hatching rate [%]
Control	--	17.5	--	25.4	79.2
Cymoxanil 45 WG	480	7.5 n.s.	-12.1	27.3	84.9
Reference Item (Perfekthion)	100 milha	100.0 *	100.0	-	–

1) Application rate in 200 L deionised water/ha
2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces (Fisher Exact Test, $\alpha = 0.05$: n.s. = not significant, * = significant)
3) Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli; negative value means lower morality compared to the control

Conclusion

At the dose rate of 480 g a.i./ha Cymoxanil 45 WG showed no adverse effect on mortality and reproduction performance of the lacewing *Chrysoperla carnea*.

A 2.3.2.2.3 Effects of Moximate 505 WP on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under extended laboratory conditions (rate response test)

Comments of zRMS:	The study was not evaluated by zRMS.

~~Reference:~~ ~~KCP 10.3.2.2/03~~

~~Report~~ ~~Colli, M. (2009b). Effects of Moximate 505 WP on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under extended laboratory conditions (rate response test). Report No. BT039/09 (Doc. No. 834-006),~~

	BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy
Guideline(s):	ESCORT I Guidance Document (Barrett et al., 1994) ESCORT II Guidance Document (Candolfi et al., 2001) IOBC (Blümel et al., 2000).
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The effects of Moximate 505 WP (batch 05, active substances 4.4 % Cymoxanil and 48.26 % Mancozeb) on the survival and reproduction of *Typhlodromus pyri* exposed to residues of the test item were determined under extended laboratory conditions at 24.3 – 26.0°C during 14 days. The aim of the study was to establish the rate producing 50 % mortality (LR_{50} at 7 days) and the rate producing 50 % reduction in reproduction (ER_{50} at 14 days) where possible. The test product was applied to leaf discs cut from bean plants at five concentrations, the application rates of 656.3, 1312.5, 2625, 5250 and 10500 g product/ha. Other plants were sprayed with deionised water for the untreated control and with Perfekthion (active substance Dimethoate, application rate 10 g/ha) for the reference item. Once residues had dried 1- to 2-day old protonymphs of *T. pyri* (no older than 24 hours after moulting) were confined to the surface of the leaf discs and fed with pollen and *Tetranichus urticae* and were provided with water *ad libitum*. Three replicates were prepared for each treatment, each containing 20 mites. The mortality of the mites was assessed 3 and 7 days after treatment. On day 7, the sex ratio was determined. Reproduction per female was recorded from day 7 to day 14. Reproduction in each replicate was determined based on the number of eggs. The number of eggs per female was determined by counting the number of females and eggs on 4 assessments at day 7, 9, 11 and 14.

Results and discussions

In the untreated control group the mortality was 6.67 %. There was 100 % mortality in the reference item group. In the Moximate 505 WP treatment groups, the corrected mortality was < 50 % in each group tested. The LR_{50} of Moximate 505 WP calculated 7 days after treatment is therefore higher than 10500 g/ha, corresponding to 462 g Cymoxanil/ha and 5067 g Mancozeb/ha.

Table A 2.3-15: Effects of Moximate 505 WP on mortality of *Typhlodromus pyri* after 7 days of exposure

Treatment [g formulation/ha]	Mortality after 7 days [%]	Corrected mortality [%] [1]	Mean number of eggs/female	Reduction in fecundity [%] [2]
Control	6.67	-	8.73 ± 2.62	-
656.3	10.0	3.57	8.33 ± 0.70	4.58
1312.5	11.7	5.36	8.98 ± 1.26	-2.86
2625	40.0	35.7	8.12 ± 1.71	6.99
5250	40.0	35.7	6.23 ± 1.09	28.6
10500	48.3	44.6	5.03 ± 1.31*	42.4
Perfekthion: 10	100	100	n/a	n/a

1 mortality for treatment and reference substance croup corrected according to Abbott (1925)
2 negative values indicate an increase of fecundity in comparison to the control, positive values indicate a reduction in fecundity compared to the control
* statistically significant compared to the control ($p < 0.05$)
n/a not applicable, reproduction was not assessed due to high mortality (> 50 %)

The mean egg production was 8.73 eggs per female in the untreated control group. The maximum reduction in reproduction of mites was 42.4 % at the highest test item treatment group. The reduction in all test

item treatment groups was less than 50 %. Therefore, the ER_{50} is > 10500 g/ha (corresponding to > 462 g Cymoxanil/ha and > 5067 g Mancozeb/ha).

Conclusion

The results obtained in the trial demonstrate that the test item Moximate 505 WP caused a statistically significant mortality of the test organism *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) when applied to bean leaves. However even at the highest dose rate (10500 g product/ha) the corrected mortality was < 50%. The LR_{50} of Moximate 505 WP calculated at 7 days after treatment is greater than 10500 g product/ha equivalent to > 462 g Cymoxanil/ha and > 5067.3 g Mancozeb/ha. The ER_{50} is > 10500 g product/ha.

A 2.3.2.2.4 Effects of Moximate 505 WP on the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under extended laboratory conditions (rate response test)

Comments of zRMS:	The study was not evaluated by zRMS.

Reference:	KCP 10.3.2.2/04 (KCP 10.3.2.2-07 statistical re-evaluation)
Report	Colli, M. (2009c). Effects of Moximate 505 WP on the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under extended laboratory conditions (rate response test). Report No. BT034/09 (Doc. No. 834-003), BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy Statistical re-evaluation: Rosenkranz, B. & Wirzinger, G. (2011). Statistical re-evaluation of effects of Moximate 505 WP on *Aphidius rhopalosiphi* and *Eisenia fetida*. Report No. not indicated (Doc. No.: 882-002), Scientific Consulting Company, Bad Kreuznach, Germany
Guideline(s):	ESCORT I Guidance Document (Barrett et al., 1994) ESCORT II Guidance Document (Candolfi et al., 2001) IOBC (Mead-Briggs et al., 2000).
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The effects of Moximate 505 WP (batch 05, active substances 4.4 % Cymoxanil and 48.26 % Mancozeb) on the survival and reproduction of *Aphidius rhopalosiphi* exposed to residues of the test item were determined under Tier II extended laboratory conditions at 20 ± 2°C during 48 hours. All treatments were applied at an application rate of 400 L/ha to wheat plants. Nominal treatment rates were 656.25, 1312.5, 2625, 5250 and 10500 g Moximate 505 WP/ha, corresponding to 28.9, 57.8, 115.5, 231 and 462 g Cymoxanil/ha and 316.7, 633.4, 1266.8, 2533.7 and 5067.3 g Mancozeb/ha. Once residues had dried 5 adult female wasps of *A. rhopalosiphi* (age less than 48 hours) were confined in each plastic cylinder containing the plants. In the bioassay, all treatment groups were replicated six times. Perfekthion (active substance Dimethoate, application rate 10 g/ha) was used as a reference item. The mortality of the wasps was assessed 2, 24 and 48 hours after treatment. To assess the sub-lethal effects of treatment on the fecundity of the wasps, the surviving female wasps from the Moximate 505 WP treatment and from the untreated

control were individually confined in fecundity cages and given 24 hours to parasitize aphids. The number of mummies (parasitized aphids) that developed was assessed 12 days later.

Results and discussions

The test met all validity criteria as control mortality during the first 48 hours did not exceed 13 % (actual mortality of 6.67 %) and mortality in the reference treatment was 78.6 %. Wasps in the control treatment produced a minimum of 5 mummies per female over a 24-hour period with no more than 2 wasps producing zero values (actual values of 5.33 mummies per female, and no wasps producing a zero value).

The affinity of the wasps to the treated plants observed in the beginning of the test was similar to that of the untreated control for each concentration. This indicates no repellent effect of the test item to the wasps. Moximate 505 WP had no effect on the behaviour of the treated wasps. The treated wasps showed no signs of reduced coordination and no difference in activity with respect to the wasps of the control group.

Table A 2.3-16: Effects of Moximate 505 WP on mortality and reproduction of *Aphidius rhopalosiphi*

Treatment [g formulation/ha]	Mortality after 48 h [%] [1]	Mean percentage of wasps staying on plants [%]	Mean number of mummies/wasp	Reduction in fecundity [%] [2]
Control	6.67	24.0	5.33 ± 4.5	-
656.25	0	27.3	5.50 ± 3.29	-3.19
1312.5	17.9	22.0	4.58 ± 3.18	14.1
2625.0	10.7	24.0	5.50 ± 4.10	-3.19
5250.0	21.4	25.3	5.42 ± 3.55	-1.69
10500.0	21.4	25.3	4.75 ± 2.83	10.9
Perfekthion: 10	78.6	4.67	n/a	n/a

1 mortality for treatment and reference substance croup corrected according to Abbott (1925)
2 negative values indicate an increase of fecundity in comparison to the control, positive values indicate a reduced fecundity compared to the control
n/a not assessed, mortality after 48 h too high to assess fecundity

After 48 hours exposure, the mortality in the control was 6.67 %. In the two highest test item treatment groups the mortality was 21.4 %. The mortality of the reference item treatment group was 78.6 %. In the fecundity test, the mean numbers of mummies produced was 4.58 – 5.5 mummies per female in the test item treatment groups and 5.33 mummies per female in the control treatment. There is no statistically significant difference between the untreated control and each treatment rate tested (t-test, $p < 0.0001$). The p value for significance as used for the statistical analysis of the effects on reproduction is very low. Normally, a p value of 0.05 is used, therefore, the statistical analysis was conducted with a p value of 0.05 with ToxRat Professional Version 2.10 (Rosenkranz, B. & Wirzinger, G. (2011), Doc. No. 882-002, KCP 10.3.2.2-07). Based on the statistical re-evaluation, the effects on reproduction were not statistically significantly different (Dunnett's Test, $p < 0.05$).

Conclusion

Moximate 505 WP did not cause a statistically significant mortality of the test organism *Aphidius rhopalosiphi* when applied to wheat plants. Even at the highest dose rate (10500 g product/ha) the corrected mortality was < 30 %. In the fecundity test, the mean numbers of mummies produced in the test item treatment groups was 4.25 – 5.50 mummies per female and 5.33 mummies per female in the control treatment. The LR_{50} and ER_{50} values were determined to be greater than 10500 g product/ha, corresponding to 462 g Cymoxanil/ha and 5067 g Mancozeb/ha.

A 2.3.2.2.5 Effect toxicity evaluation of Moximate 505 WP on the *Chrysoperla carnea* L. (Neuroptera, Chrysopidae) under extended laboratory conditions (rate response test)

Comments of zRMS:	The study was not evaluated by zRMS.

Reference:	KCP 10.3.2.2/05
Report	Colli, M. (2009d). Effect toxicity evaluation of Moximate 505 WP on the *Chrysoperla carnea* L. (Neuroptera, Chrysopidae) under extended laboratory conditions (rate response test). Report No. BT033/09 (Doc. No. 834-004), BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy
Guideline(s):	ESCORT I Guidance Document (Barrett et al., 1994) ESCORT II Guidance Document (Candolfi et al., 2001) IOBC (Vogt, H. et al., 2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The effects of Moximate 505 WP (batch 05, active ingredients 4.4 % Cymoxanil and 48.26 % Mancozeb) on the survival of *Chrysoperla carnea* (first instar larvae, 3 days old) exposed to residues of the test item under Tier II extended laboratory conditions at 25 ± 2°C during 7 days. All treatments were applied to bean plants (*Phaseolus vulgaris*). Nominal treatment rates were 656.25, 1312.5, 2625, 5250 and 10500 g Moximate 505 WP/ha, corresponding to 28.9, 57.8, 115.5, 231 and 462 g Cymoxanil/ha and 316.7, 633.4, 1266.8, 2533.7 and 5067.3 g Mancozeb/ha. Perfekthion (active substance Dimethoate, application rate 250 g/ha) was used as a reference group. Once residues had dried one single lacewing larva was placed on each of the leaves. In the bioassay, all treatment groups were replicated 30 times. The larvae were fed a mix of pollen and *Aphis* spp. *ad libitum*. Survival of the larvae was checked daily. Pupation and the adults hatching were recorded. Cocoons were collected before adults hatching, but not earlier than 5 days after formation in order to avoid damaging of young pupae. For the reproduction phase, all adults from a treatment group were pooled together. They were kept in plastic containers covered with cotton paper. The reproduction test was started one week after the first egg laying had been observed. The lacewings were sexed before starting the test. The females deposited their eggs on the cotton paper. Four egg samples, covered an egg laying period of 24 hours, were taken within two weeks. The eggs of each sample, attached to the filter paper, were incubated for hatching in containers to determine their viability. In the first week the eggs viability was < 70 %, as a result the test was prolonged for another week and two further assessments over 24 hours were carried out.

Results and discussions

The test met all validity criteria as control mortality during the first 7 days did not exceed 20 % (actual mortality 16.7 %) and mortality in the reference treatment was 80 %.

The mortality in the control was 16.7 %. In all test item treatment groups, no tested rate caused a corrected mortality > 50 %. The maximum mortality was 44.0 % in the second highest test item treatment group. In the control the mortality was 16.7 %. The LR_{50} was determined to be > 10500 g formulation/ha, corresponding to > 462 g Cymoxanil/ha and to > 5067 g Mancozeb/ha.

In the fecundity test, the mean numbers of fertile eggs per female in the test item treatment groups was 18.5 in the lowest treatment group and 10.7 in the highest treatment group. In the control, the mean number of fertile eggs/female was 15.3. The reproduction test results showed no significant reduction in reproduction of the treated females in all treatment groups. The ER_{50} was determined to be > 10500 g/ha, corresponding to > 462 g Cymoxanil/ha and to > 5067 g Mancozeb/ha.

Table A 2.3-17: Effects of Moximate 505 WP on mortality and fecundity of *Chrysoperla carnea*

Treatment [g formulation/ha]	Mortality after 7 days [%] [1]	Mean number of eggs/female	Hatching rate [%] [2]	Mean number of fertile eggs/female	Reduction in fecundity [%] [3]
Control	16.7	19.8	77.5	15.3	-
656.25	12.0	20.3	90.8	18.5	-20.7
1312.5	7.99	16.7	88.4	14.7	4.13
2625.0	24.0	20.8	83.3	17.3	-13.0
5250.0	44.0	18.3	82.7	15.3	0.0
10500	32.0	12.2	87.7	10.7	30.4
Perfekthion: 250	80.0	n/a	n/a	n/a	n/a

1 mortality for treatment and reference substance croup corrected according to Abbott (1925)
2 2nd week of reproductive assessment
3 negative values indicate an increase of fecundity in comparison to the control, positive values indicate a reduced fecundity compared to the control
n/a not applicable, reproduction was not assessed due to high mortality (> 50 %)

Conclusion

Up to and including a rate of 10500 g formulation/ha, there were no significant effects on the mortality and reproduction of the larvae of *Chrysoperla carnea*. The LR_{50} and ER_{50} were determined to be > 10500 g formulation/ha, corresponding to > 462 g Cymoxanil/ha and to > 5067 g Mancozeb/ha.

A 2.3.2.2.6 Effect of Moximate 505 WP on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) under extended laboratory conditions (rate response test)

Comments of zRMS:	The study was not evaluated by zRMS.

Reference:	KCP 10.3.2.2/06
Report	Colli, M. (2010). Effect of Moximate 505 WP on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) under extended laboratory conditions (rate response test). Report No. BT032/09 (Doc. No. 834-005), BIOTECNOLOGIE BT S.r.l., Fraz. Pantalla, 06050 Todi (PG), Italy
Guideline(s):	ESCORT I Guidance Document (Barrett et al., 1994) ESCORT II Guidance Document (Candolfi et al., 2001) IOBC (Vogt, H. et al., 2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The effects of Moximate 505 WP (batch 05, active substances 4.4 % Cymoxanil and 46.5 % Mancozeb) on the survival of *Coccinella septempunctata* (first instar larvae, 5 days old) exposed to residues of the test item under Tier II extended laboratory conditions at 25 ± 2°C during 28 days. All treatments were applied to bean plants (*Phaseolus vulgaris*). Nominal treatment rates were 656.25, 1312.5, 2625, 5250 and 10500 g Moximate 505 WP/ha, corresponding to 28.9, 57.8, 115.5, 231 and 462 g Cymoxanil/ha and 316.7, 633.4, 1266.8, 2533.7 and 5067.3 g Mancozeb/ha. Perfekthion (active substance Dimethoate, ap-

plication rate 250 g/ha) was used as a reference group. Once residues had dried one single ladybird larva was placed on each of the leaves. In the bioassay, all treatment groups were replicated 30 times. The larvae were fed fresh aphids *ad libitum*. Survival and development of the larvae was checked daily. The number of eggs per female was assessed daily, in addition to the viability of the eggs. For the reproduction test, assessment of reproductive performance was started one week after the appearance of the first batch of eggs in the untreated control. At the beginning of the egg-laying period, the beetles were sexed. Eggs laid from the beginning to day 7 inclusive were not included and were removed from the test unit. The reproduction performance was only assessed in those groups where the corrected mortality was ≤ 50 %. For the fertility test, the number of eggs per female of the surviving beetles was assessed, where the viability of the eggs was also evaluated.

Results and discussions

The test met all validity criteria as control mortality during the first 7 days did not exceed 30 % (actual mortality 26.7 %), mortality in the reference treatment was higher than 40 % (actual mortality 81.8 %) and the fertility (mean number of fertile eggs per viable female/day) in the control was higher than 2 (exact value 18.9 fertile eggs/female/day).

In the control the mortality was 26.7 %. In the test item treatment groups, no tested rate caused a corrected mortality > 50 %. At the lowest test item rate of 656.3 g/ha, the corrected mortality was 0 % and at the highest application rate of 10500 g/ha the corrected mortality was 9.08 %. The mortality in the highest test item treatment group was not statistically significantly different to the control. The LR_{50} was determined to be > 10500 g/ha, corresponding to > 462 g Cymoxanil/ha and to > 5067 g Mancozeb/ha.

Table A 2.3-18: Effects of Moximate 505 WP on the mortality and fecundity of *Coccinella septempunctata*

Treatment [g formulation/ha]	Mortality [%] [1]	Mean number of eggs/female	Hatching rate [%]	Mean number of fertile eggs/female	Reduction in fecundity [%] [2]
Control	26.7	23.7	81.2	18.9	-
656.3	23.3 (0)	24.6	79.7	17.3	8.56
1312.5	20.0 (0)	25.1	79.2	17.3	8.47
2625.0	23.3 (0)	21.2	74.3	13.4	28.9
5250.0	30.0 (4.54)	23.0	77.6	15.5	17.9
10500.0	33.3 (9.08)	21.5	76.8	14.7	22.4
Perfekthion: 250	86.7 (81.8)	n/a	n/a	n/a	n/a

1 mortality for treatment and reference substance croup corrected according to Abbott (1925)
2 negative values indicate an increase of fecundity in comparison to the control, positive values indicate a reduced fecundity compared to the control
n/a not applicable, reproduction was not assessed due to high mortality (> 50 %)

In the control, the mean number of fertile eggs/female was 18.9. In the fecundity test, the mean numbers of fertile eggs per female in the test item treatment groups ranged from 14.7 in the 10500 g/ha test item treatment group to 17.3 in the two lowest test item treatment groups. The reproduction test results showed no significant reduction in reproduction of the treated females. The ER_{50} is determined to be > 10500 g/ha (corresponding to > 462 g Cymoxanil/ha and to > 5067 g Mancozeb/ha).

Conclusion

Up to and including a rate of 10500 g formulation/ha, there were no significant effects on the mortality or fecundity of the larvae. The LR_{50} and ER_{50} values were determined to be > 10500 g formulation/ha, corresponding to > 462 g Cymoxanil/ha and to > 5067 g Mancozeb/ha.

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

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.2.4.1 Effects of Cymbal 45 WG on Predatory Mites (Acari, Phytoseiidae) under

Field Conditions in Vine (4 Applications)

Comments of zRMS:	The study was evaluated by RMS in ongoing process of renewal of the a.s-cymoxanil in RAR Volume 3CP Cymoxanil 45 WG, Section B.9 dated on 2020-03-09. The purpose of this study was to investigate short and long-term effects on the population development of leaf-dwelling predatory mites (e.g. Typhlodromus pyri) after applying the fungicide Cymbal 45 WG according to practical use conditions in a vineyard. Natural occurring populations of predatory mites were treated with 4 applications of Cymbal 45 WG in a vineyard. The study comprised 3 treatment groups (test item, water treated control and a reference item) with 5 replicates (plots). The applications were carried out from the end of May till the middle of June. The toxic reference item (propineb) was applied 3 times. The population development of predatory mites was assessed determining the total number of mites and eggs on leaf samples. Based on the results of this study and according to the corresponding guideline (Blümel et al., 2000), no unacceptable effects on predatory mite populations (Acari: Phytoseiidae) mobile stages or the eggs were observed if Cymbal 45 WG is applied 4 times at an application rate of 400 g/ha in grapevine (equivalent to 180 g of Cymoxanil/ha). The effects of the toxic reference substance exceeded 50% and demonstrated the sensitivity of the test system. RMS's evaluation from the RAR 2020 is presented below: The study was conducted according to the Blümel et al., 2000 and was well reported. Determination of the Phytoseiidae species (predatory mites) spectrum was done. In addition, the number of spider mites (Tetranychidae) was estimated. No other group of arthropods were identified. The initial population density and the number of eggs per leaf were assessed 3 days before the application. The mean number of predatory mites in the control was more than 30. The effects of the toxic reference substance exceeded more 50% (was 64.5%). In the study 5 plots per treatment group were used with a number of 15 vines per plot, of which 8 vines were evaluated. The plots were distributed and plots size was chosen in appropriate way. In the field study, field history was provided for the year of application only. The used application rates did not fully cover the intended use in vines. In the study were 4 applications with use rates of 180 g a.s./ha (BBCH 19-65). According to the GAP the intended uses in the grapes are (5x120 g a.s./ha, BBCH 11-89). The RMS is of the opinion that the field study despite small deficiencies is acceptable to address the risk to the predatory mite T.Pyri. zRMS agrees that study is considered acceptable. However, in the other side it can be also noted that the used application rates did not fully cover the intended use in potatoes in the current GAP. In the field study were 4 applications with use rates of 180 g a.s./ha (BBCH 19-65). According to the GAP the intended uses in the potatoes are (6 x 148.5 ga.s./ha, BBCH 11-95).Therefore, the max application rate is higher in the GAP being 891 g a.s./ha in comparison to the application rate used in the field study (600 g a.s./ha). For this reason, the study is considered only as additional information

Reference:	KCP 10.3.2.4/01
Report	Rosenkranz, B. & Schabio, S. (2009). Effects of Cymbal 45 WG on Predatory Mites (Acari, Phytoseiidae) under Field Conditions in Vine (4 Applications). Report No. 39706064, Institut far Biologische Analytik und Consult-

	ing IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	Blümel et al. (2000)
Deviations:	The 4th application of the Test Item, the Control and the Reference Item was done even if the wind speed was above 3 m/s (3.4 m/s at maximum). Impact on the study: None, due to the arrangement of the plots, drift is negligible. At the 3rd application, the reference item was not sprayed due to a mistake. Impact on the study: None, the reference item caused an effect > 50% on the population and number of eggs per leaf already after the first and second application, respectively. At the 2nd application the deviation to the target application in the Control plot X exceeded 10% (10.6%). Impact on the study: None, the control was evenly treated with tap water.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Test Item:	Cymbal 45 WG; batch no.: 070724; content of a.s.: Cymoxanil 450g/kg (nominal).
Test Species:	Predatory mites.
Test Design:	Natural occurring populations of predatory mites were treated with 4 applications of Cymbal 45 WG in a vineyard. The study comprised 3 treatment groups (test item, water treated control and a reference item) with 5 replicates (plots). The applications were carried out from the end of May till the middle of June. The toxic reference item (propineb) was applied 3 times. The population development of predatory mites was assessed determining the total number of mites and eggs on leaf samples.
Endpoints:	Predatory mite population development compared to the control.
Test Site:	The test site was located in GroB-Umstadt (low mountain area Odenwald), federal state Hessia, Germany.
Test Conditions:	Temperature: 23 °C - 26 °C; relative humidity: 62 % 89 %; photoperiod: 16 h light: 8 h dark; light intensity: 1500 lux - 5140 lux.
Reference Item:	Antracol WG 70 (Probineb, 70%)
Test Item Rates:	

	Cymbal 45 WG	equivalent to Cymoxanil [1]
1st application	400	180
2nd application	400	180
3rd application	400	180
4th application	400	180

[1] based on nominal content

Control:	The control group was treated with tap water at each application date..
Reference Item Rates:	The reference item (Antracol WG 70) was applied 3 times (at the first two and the last application of the test item) as a 0.2% product concentration in a water volume adapted to the growth stage of vine plants, resulting in application rates of 800 g/ha (1st application), 1200 g/ha (2nd application) 1600 g/ha (4th application).
Growth Stages of the Vine Plants at Application:	

	BBCH
1st application	19
2nd application	53-55
3rd application	57
4th application	65

Water Amount:	All treatments were performed in a water volume depending on the growth stage of the vine plants. Therefore, the following water amounts were used: 1st application 400 L tap water/ha 2nd application 600 L tap water/ha 3rd application 600 L tap water/ha 4th application 800 L/ha
Application Technique:	Application was conducted with a back pack mist blower (SOLO 432, Schachtner Geratetechnik).
Test Conditions:	Natural field conditions. Climatic conditions (temperature and rain) were recorded throughout the experimental period at a local weather station.
Test Parameter:	Number of predatory mites and eggs of predatory mites was assessed using the washing method after Boller (1984).
Statistics:	Kolmogorroff-Smirnov (check for normal distribution), Cochran's test (check for homogeneity of variance), Student-t test (pair-wise); (software: TOX Rat® Pro, version 2.09-Spirit Solutions)

Results and discussions

In the pre-sampling a mean of 2.81 mites/leaf were found in the control treatment. This number was comparable with the number of mites found in the test item and the reference item treatment with 2.99 and 2,90 mites/leaf, respectively. The mean number of the eggs in the control was 1.22 eggs/leaf, in the reference item (propineb) 1.19 eggs/leaf and in the test item Cymbal 45 WG 1.07 eggs/leaf. The population and the number of eggs were homogeneous (no statistically significant difference; Student t-test one-sided smaller, a = 0.05) and sufficiently high to be used as test population. After distribution of the plots no statistically significant differences between the treatments did occur.
In the assessments after the 1st application the mean number of mites per leaf was between 1.90 and 11.44 in the control treatment, between 1.31 and 8.55 mites/leaf in the test item treatment and ranged from 1.07 to 7.62 mites/ leaf in the reference item treatment. The number of eggs in the control treatment was between 0.26 and 2.04 eggs/leaf in the test item treatment between 0.22 and 1.22 eggs/leaf, and in the reference item treatment between 0.24 and 1.23 eggs/leaf.
The effects of the test item Cymbal 45 WG on the predatory mite populations ranged between 7.7 % and 46.9 %. At the 1st assessment date (6 DAA 1) an effect of 46.9 % was observed. This effect was statistically significantly different compared to the control but remain below the 50% limit for unacceptable effect. No statistically significant difference compared to the control was observed at any of the following sampling dates (Student t-test, one-sided smaller, a = 0.05).
For the toxic reference item (propineb) a 64.5 % reduction of the number of mites per leaf could be observed at the 1st sampling date 6 DAA 1 (6 days after the first application) which confirmed the sensitivity of the test conditions. The reduction of the predatory mite population in the plots treated with the reference item was statistically significant in the 1st assessment 6 DAA 1 and the 2nd assessment 6 days after the 2nd application (6 DAA 2) (Student t-test, one-sided smaller, $\alpha = 0.05$).
The effects of the test item Cymbal 45 WG on the eggs ranged between 12.5 % and 47.7 %. At the 1st assessment date (6 DAA 1) an effect of 40.0 % was observed (statistically significant different compared to the control). A further statistically significant difference in the numbers of the eggs (effect of 41.8%) was found at the 5th sampling date (27 DAA 4; Student t-test, one-sided smaller, $\alpha = 0{,}05$). The results of the 6th sampling showed no statistically significant effect compared to the control. It has to be considered that the effects on eggs were always below the trigger value of 50 % throughout the experimental time. Therefore, no unacceptable effects on predatory mites were observed at any sampling date.
For the reference item (propineb) a maximum effect of 60,6 % could be observed at the 2nd sampling 6 DAA 2, thus confirming the sensitivity of the test conditions for this parameter as well. The reduction of the eggs from the predatory mite population in the plots treated with the reference item was statistically significant to the control at the 1st assessment 6 DAA 1 (Student t-test, one-sided smaller, $\alpha = 0.05$).

Table A 2.3-19: Summary of results, Effect of Cymbal 45 WG and the reference item on predatory mites according to ABBOTT (1925)

Mean Number of predatory mites per leaf										
time	Control		Cymbal 45 WG				Reference Item			
	Mean [1]	SD [2]	Mean [1]	SD [2]	E [%] [3]		Mean [1]	SD [2]	E [%] [3]	
Pre-application	2,81	± 0.52	2.99	± 1.22	-6.2	n.s.	2.90	± 0.69	-3.1	n.s.
6 DAA [4] 1	10.60	± 4.00	5.63	± 1.71	46.9	*	3.77	± 1.43	64.5	*
6 DAA 2	11.44	± 4.82	8.55	± 1.24	25.2	n.s.	7.50	± 179	34.4	*
5 DAA 3	7.88	± 6.05	6.23	± 3.70	20.9	n.s.	7.62	± 2.01	3.4	n.s.
5 DAA 4	6.91	± 3.55	5.19	± 1.63	24.9	n.s.	6.25	± 1,27	9.6	n.s.
27 DAA 4	3.02	± 0.68	2.79	± 0.93	7.7	n.s.	2,18	± 1.01	27.8	n.s.
41 DAA 4	1.90	± 0.96	1.31	± 0.48	31.1	n.s.	1.07	± 0.84	43.7	n.s.

[1] mean from 5 replicates
[2] standard deviation from 5 replicates
[3] Effect after Abbott
[4] DAA = days after application
n.s. = not statistically significant to the control; * statistically significant to the control
Statistic: Student t test, pair-wise, two-sided, α = 0.05 before application
Student t-test, pair-wise, one-sided smaller, α = 0.05 after application

Table A 2.3-20: Summary of results, Effect of Cymbal 45 WG and the reference item on eggs of predatory mites according to ABBOTT (1925)

Mean Number of predatory mites per leaf										
time	Control		Cymbal 45 WG				Reference Item			
	Mean [1]	SD [2]	Mean [1]	SD [2]	E [%] [3]		Mean [1]	SD [2]	E [%] [3]	
Pre-application	1.22	± 0.35	1.07	± 0.21	12.3	n.s.	1.19	± 0.75	2.1	n.s.
6 DAA [4] 1	2.04	± 0.45	1.22	± 0.56	40.0	*	1.23	± 0.70	39.6	*
6 DAA 2	1.06	± 0.99	0.55	± 0.38	47.7	n.s.	0.42	± 0.28	60.6	n.s.
5 DAA 3	0.78	± 0.65	0.54	± 0.55	30.9	n.s.	0.55	± 0.32	28.9	n.s.
5 DAA 4	0.49	± 0.28	0.34	± 0.22	30.8	n.s.	0.48	± 0.42	1.3	n.s.
27 DAA 4	0.44	± 0.14	0.26	± 0.10	41.8	*	0.38	± 0.18	14.5	n.s.
41 DAA 4	0.26	± 0.14	0.22	± 0.13	12.5	n.s.	0.24	± 0.16	6.3	n.s.

[1] mean from 5 replicates
[2] standard deviation from 5 replicates
[3] Effect after Abbott
[4] DAA = days after application
n.s. = not statistically significant to the control; * statistically significant to the control
Statistic: Student t test, pair-wise, two-sided, α = 0.05 before application
Student t-test, pair-wise, one-sided smaller, α = 0.05 after application

Conclusion

Based on the results of this study and according to the corresponding guideline (BLUMEL *et al.*, 2000), no unacceptable effects on predatory mite populations (Acari: Phytoseiidae) mobile stages or the eggs were observed if Cymbal 45 WG is applied 4 times at an application rate of 400 g/ha in grapevine. The effects of the toxic reference substance exceeded 50% and demonstrated the sensitivity of the test system.

A 2.3.2.1 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Cymoxanil 45 WG (IN 002B1760): Effects on Reproduction of Earthworm *Eisenia fetida* in an Artificial Soil Study

Comments of zRMS:	The study was conducted in line with OECD 222 with slight deviations. - The recorded of the test temperatures was started on 9th September, instead of 8th September (start of the study). All validity criteria are met. • Mean value of juveniles in the control group: 273.9. • % coefficient of variation of reproduction in the controls group : 5.7%. • 0 % mortality in the control group. Since the validity criteria for the control were met the deviation has no impact on the study results. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: $NOEC_{reproduction}$=95.3 mg product/kg dws, correspond to 44.8 mg a.s./kg dws based on nominal concentration $EC_{10\ reproduction}$=113.7 mg product/kg dws, correspond to 53.4 mg a.s./kg dws (44.4 – 57.1), NW= 0.24 based on nominal concentration. According to Appendix E of EFSA (2019), a NW value is rated as 'fair' (<1) or 'good' (0.2-0.5), indicating reliability of the estimated EC_{10}. However, NOEC provides a lower endpoint and will be considered for risk assessment.

Reference:	KCP 10.4.1.1/01
Report	Dini, R. (2022a). Cymoxanil 45 WG (IN 002B1760): Effects on Reproduction of Earthworm *Eisenia fetida* in an Artificial Soil Study. Report No. CH–0259/2021, ChemService S.r.l. Controlli e Ricerche, Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD 222 (2016)
Deviations:	The recorded of the test temperatures was started on 9th September, instead of 8th September (start of the study). Impact on the study: None.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The purpose of this study was to determine the effects of the test item on the survival and reproductive output of the earthworm *Eisenia fetida* determined in an eight weeks exposure test according to the guideline OECD 222 (2016) and in compliance with the Principles of Good Laboratory Practice (GLP).

A. Materials

Test Item

Name	Cymoxanil 45 WG (IN 002B1760)
Batch no.	IND_F040_0421_1
Active ingredient(s)	Cymoxanil
Content of a.s.	45 % (w/w)
(analysed)	47 % (w/w)

Test System

Species	*Eisenia fetida*
Age	Adults
Source	Commercial supplier (Bias Labs Ltd)
Acclimatization	24 hours (at the same environmental conditions of the test)
Diet	Horse manure. Food was first provided one day after the beginning of the test. Thereafter it was provided once a week during the first 4-week period and only once in the second 4-week period, just after removing adults from the soil (on the 28th day).
Water	Deionized water

Experimental Conditions

Artificial soil	10 % sphagnum peat; 20 % kaolin clay; 70 % industrial sand
Temperature	19.9 – 20.4°C
Photoperiod	16 hours light and 8 hours dark per day.
Light Intensity	564 - 771 Lux.

B. Study design and method

Experimental period
8th September – 22nd December 2021

Experimental treatment
The test item solution was prepared and thoroughly mixed into the artificial soil. The treated soil was then wetted up to reach the 40% of WHC (water holding capacity) and introduced in the test containers.
After 4 weeks of exposure to the treated soil, the surviving earthworms were removed from the exposure cages, and weighed. 8 weeks after the treatments the reproduction performance was evaluated by the assessment of the number of juveniles produced.

Application concentrations
The test was performed at eight different concentrations in a geometric series, namely 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg/kg d.w. These test item concentrations correspond to 7.7, 13.8, 24.9, 44.8, 80.6, 145.1, 261.1 and 470.0 mg Cymoxanil/kg d.w.
A negative control (artificial soil without test item) was tested to check the health of the worms.
Four replicates per each test item concentration were prepared, and eight replicates were prepared for the negative control. For each test item concentration as well as for the negative control, 10 worms per replicate were used.

Observations
The mortality rate was assessed after 4 weeks. The adult worms were inspected by emptying the test containers onto a tray, sorting organisms from the soil, and observing their reaction to a mechanical stimulus at the anterior end. Worms were classified as dead if they did not respond to the stimulus or if they were not found in the soil.
The effect on reproduction was assessed after 8 weeks, by counting juveniles appearing on the surface after placing the glass containers in a water bath initially at 40°C and rising to 60°C (as described in OECD No. 222, Annex 5, point a). Moreover, the soil was checked, and the number of cocoons produced by the adult worms was recorded.

Statistics

The CETIS v.1.8.7.7 software was used to carry out the statistical analysis. The 28 d- LC_{10}, LC_{20}, LC_{50} was determined by Linear Interpolation (ICPIN) analysis. The NOEC value was determined by Bonferroni adj t Test after testing the normality of distribution of the data and the homogeneity of variance by Shapiro-Wilk W normality test (normal distribution) and Bartlett equality of variance test (equal variances), respectively. $EC_{10,}$ EC_{20} and EC_{50} values were determined by Linear Interpolation (ICPIN) analysis.

Results and discussions

Table A 2.4-1: Survival and biomass of earthworms after 28 days

Treatment Group	Test item concentration (mg test item/kg soil d.w.)	Cymoxanil concentration (mg test item/kg soil d.w.)	Mortality after 4 weeks	Body weight change after 4 weeks			
			Percentage of dead earthworms (%)	Mean (%)	Mean (g)	SD	cv%
Negative control	0.0	0.0	0.0	0.2	0.42	0.027	6.5
Test item	16.3	7.7	0.0	-1.2	0.43	0.032	7.5
	29.4	13.8	0.0	1.1	0.41	0.036	8.8
	52.9	24.9	0.0	-1.1	0.40	0.028	7.1
	95.3	44.8	0.0	7.6	0.45	0.042	9.4
	171.5	80.6	2.5	4.6	0.45	0.054	12.1
	308.6	145.0	2.5	-1.4	0.42	0.021	5.0
	555.6	261.1	7.5	0.5	0.44	0.062	14.1
	1000.0	470.0	25.0*	-38.2	0.24	0.042	17.4

* statistically significantly different compared to the control (*Bonferroni Adj t Test* α = 0.05)

Table A 2.4-2: Effects on earthworm reproduction after 8 weeks

Treatment Group	Test item concentration (mg test item/kg soil d.w.)	Cymoxanil concentration (mg test item/kg soil d.w.)	Number of unhatched cocoons	Number of juveniles			Inhibition %
			Mean	Mean	SD	CV%	
Negative control	0.0	0.0	2.4	273.9	15.6	5.7	-
Test item	16.3	7.7	2.5	271.3	21.9	8.1	1.0
	29.4	13.8	2.0	270.0	16.8	6.2	1.4
	52.9	24.9	2.3	266.5	12.1	4.5	2.7
	95.3	44.8	1.5	270.5	13.0	4.8	1.2
	171.5	80.6	2.0	195.3*	11.6	5.9	28.7
	308.6	145.0	0.5	93.8*	9.6	10.2	65.8
	555.6	261.1	0.3	11.0*	1.4	12.9	96.0
	1000.0	470.0	0.0	0.0*	0.0	0.0	100.0

* statistically significantly different compared to the control (*Bonferroni Adj t Test* α = 0.05)

The actual test concentrations of the cymoxanil residues were analytically measured at test start (time 0), after 28 days and at the end of the test after 56 days of exposure. Measured cymoxanil concentrations at T0 were in the range of ± 20% (91.0 % - 117.5 %).
All the validity criteria of the OECD guidance No. 222 (2016) were met.

Conclusion

The lethal and sublethal effects of Cymoxanil 45 WG (IN 002B1760) to earthworms *Eisenia fetida* were investigated. Resulting NOEC and L/EC_x are summarised below:

Table A 2.4-3: Statistical endpoints

Endpoint	[mg test item/kg d.w.] based on nominal test item concentration	[mg a.s./kg d.w.] based on nominal cymoxanil concentration
Survival		
NOEC	555.6	261.1
LC_{50} (LCL – UCL)	> 1000.0 (NA – NA)	> 470.0 (NA – NA)
Reproduction		
NOEC	95.3	44.8
EC_{10} (LCL – UCL)	113.7 (94.5 – 121.5)	53.4 (44.4 – 57.1)
EC_{20} (LCL – UCL)	141.7 (120.7– 156.3)	66.6 (56.7 – 73.5)
EC_{50} (LCL – UCL)	240.4 (220.9– 255.4)	113.0 (103.8 – 120.0)

N/A = not applicable
LCL, Lower Confidence Level and UCL, Upper Confidence Level

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Cymoxanil 45 WG (IN 002B1760): Effects on Collembolan Reproduction in an Artificial Soil Study

Comments of zRMS:	The study was conducted in line with OECD 232 with minor no deviations. All validity criteria are met. • Mean value of juveniles in the control group: 434.4. • % coefficient of variation of reproduction in the controls group :14.4%. • 5 % mortality in the control group. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: $NOEC_{reproduction}$ = 52.9 mg prod./kg soil dw (equivalent to 31.584 mg a.s./kg soil dw) EC_{10} = 52.4 mg prod./kg soil dw (equivalent to 17.5 mg a.s./kg soil dw) The NOEC based on nominal concentration is 52.9 mg test item/kg soil d.w. equivalent to 24.9 mg test item/kg soil d.w. (based on the actual active substance content of 47%). The NOEC based on initial measured concentration is 31.584 a.s./kg soil d.w. EC_{10} reproduction = 17.5 mg a.s /kg dws, (CI: 7.7 – 27.2) based on initial measures concentration. NW= 1.11 based on initial concentration, uncertainties according EFSA GD 2019. NOEC should be preferred. The NOEC based on initial measured concentration is 31.584 mg a.s /kg soil d.w and

	this value is used used in the risk assessment. ~~The EC_{10} based on nominal concentration is 52.4 mg test item/kg soil d.w equivalent to 24.6 mg test item/kg soil d.w. (based on the actual active substance content of 47%).~~ ~~The EC_{10} based on initial measured concentration was used in the risk assessment.~~ ~~The conversion of nominal value from product to a.s.is correct and does not need to be recalculated.~~

Reference:	KCP 10.4.2.1/01
Report	Dini, R. (2022b). Cymoxanil 45 WG (IN 002B1760): Effects on Collembolan Reproduction in an Artificial Soil Study. Report No. CH–0260/2021, ChemService S.r.l. Controlli e Ricerche, Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD 232 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The purpose of this study was to determine the effects of the test item Cymoxanil 45 WG (IN 002B1760) on the survival and reproduction of the Collembola (springtail) species *Folsomia candida* in a four-week artificial soil study. The study was carried according to the OECD guideline N. 232 (2016) and in compliance with the Principles of Good Laboratory Practice (GLP).

A. MATERIALS

1. Test Item

Name	Cymoxanil 45 WG (IN 002B1760)
Batch No.	IND_F040_0421_1
Active ingredient(s)	Cymoxanil
Content of a.s.	45 % (w/w)
(analysed)	47 % (w/w)

2. Reference Item

Name	Boric acid analytical standard
Batch No.	AM1193865
Purity	100.1 %

3. Test system

Species	*Folsomia candida* (synchronized females were used)
Age	Juveniles (from 9 to 12 days)
Source	Commercial supplier (Bias Labs Ltd)
Diet	granule yeast. Food was first provided at the beginning of the test, thereafter it was provided after 14 days of exposure.

4. Experimental conditions

Artificial soil	5 % sphagnum peat; 20 % kaolin clay; 75 % industrial sand, based on dry weights, dried to a constant weight at 105°C. The pH of pre-moistened soil was 5.9 since no adjustment with calcium carbonate was necessary.
Temperature	19.3 – 20.2 °C.
Photoperiod	16 hours light and 8 hours dark per day.
Light Intensity	593 - 627 Lux.

B. STUDY DESIGN AND METHODS

1. Experimental period

15th September 2021 – 27th October 2021

2. Experimental treatment

The test item was diluted in deionised water in order to obtain the stock solutions. These stock solutions were added to the pre-moistened soil previously divided into different aliquots of 300 or 500 g soil dry weight for concentrations and for the control, then water was added to reach the final moisture. The soil hydrated was accurately mixed.

3. Application concentrations

The test was performed at eight different concentrations in a geometric series, namely 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000.0 mg/kg soil d.w., chosen according to the literature data and in agreement with the Sponsor. These test item concentrations correspond to 7.7, 13.8, 24.9, 44.8, 80.6, 145.1, 261.1 and 470.0 mg Cymoxanil/kg soil d.w.
Four replicates per each test item concentration were prepared while, for the control, 8 replicates were used. One more replicate for each test concentration and for the control was prepared and used to evaluate the pH and the moisture content of the artificial soil at the end of the test. In addition, one Becker per each concentration and for the control was prepared to perform the analytical check.

4. Observations

At the end of the exposure period the total number of juveniles produced by parent animals and the survival of parent animals were assessed after extraction from the soil.

5. Statistics

The CETIS v.1.8.7.7 software was used to carry out all the statistical analyses. The 28 d-LC_{50} was determined by Linear Interpolation method. The NOEC and LOEC values were determined from the raw data by using the Williams Multiple Comparison test ($p < 0.05$). The 28 d-EC_{50}, EC_{20} and EC_{10} were determined by Nonlinear Regression method.

Results and discussions

Table A 2.4-4: Survival and Reproduction of collembolans after 8 weeks

Nominal concentration of test item (mg test item/kg d.w.)	Nominal a.s. concentration (mg a.s./kg soil d.w.)	Initial measured a.s. concentration (mg a.s./kg soil d.w.)	Mortality	Number of juveniles per replicate			Reduction in reproduction %
			% of dead organisms	Mean	S.D.	CV %	
0.0 (neg. control)	0.0	0.0	5.0	434.4	60.9	14.4	-
16.3	7.661	7.900	20.0	391.8	109.0	27.8	9.8
29.4	13.818	12.261	7.5	391.3	62.1	15.9	9.9
52.9	24.863	21.834	10.0	413.5	99.7	24.1	4.8
95.3	44.791	31.584	5.0	340.8*	125.6	36.9	21.6
171.5	80.605	57.622	12.5	259.0*	67.9	26.2	40.4
308.6	145.042	102.058	22.5	219.3*	81.9	37.4	49.5

Nominal concentration of test item (mg test item/kg d.w.)	Nominal a.s. concentration (mg a.s./kg soil d.w.)	Initial measured a.s. concentration (mg a.s./kg soil d.w.)	Mortality	Number of juveniles per replicate			Reduction in reproduction %
			% of dead organisms	Mean	S.D.	CV %	
555.6	261.132	192.495	7.5	171.5*	73.2	42.7	60.5
1000.0	470.000	334.213	20.0	50.8*	9.0	17.7	88.3

* statistically different from the control group (Williams Multiple Comparison test, $p < 0.05$)

The actual test concentrations of the cymoxanil residues were analytically measured in soil samples at test start (time 0), after 13 days and at the end of the test after 28 days of exposure. Measured cymoxanil concentrations at T0 range between 70.4% - 103.1%. Statistical endpoints were calculated using both nominal concentration and measured concentration at T0.
All the validity criteria of the OECD guidance No. 232 (2016) were met.

Conclusion

The lethal and sublethal effects of Cymoxanil 45 WG (IN 002B1760) on survival and reproduction of the Collembola species *Folsomia candida* were investigated. The data on mortality and reproductive output were statistically analyzed and the following results obtained. The test item caused no statistically significant mortality of the adult Collembola up to and including the maximum tested soil concentration, equal to 1000 mg test item/kg soil d.w.. No statistically significant adverse effect on reproduction was observed up to and including 52.9 mg test item/kg soil d.w. Resulting NOEC and L/ECx are summarised below:

Table A 2.4-5: Statistical endpoints

Endpoint	Test Item concentration (mg test item/kg soil d.w.)	Nominal cymoxanil concentration (mg test item/kg soil d.w.)	Initial measured cymoxanil concentration mg a.s./kg soil d.w.) ~~(mg test item/kg soil d.w.)~~
Mortality			
NOEC	1000.0	470.0	334.213
LC_{50} (LCL – UCL)	> 1000.0 (NA – NA)	> 470.0 (NA – NA)	> 334.2 (NA – NA)
Reproduction			
NOEC	52.9	24.9	31.584
EC_{10} (LCL – UCL)	52.4 (24.2 – 80.6)	24.6 (11.4 – 37.9)	17.5 (7.7 – 27.2)
EC_{20} (LCL – UCL)	101.1 (65.8 – 139.8)	47.5 (30.9 – 65.7)	33.7 (21.8 – 46.7)
EC_{50} (LCL – UCL)	310.8 (247.1 – 390.9)	146.1 (116.1 – 183.7)	103.4 (81.0 – 132.0)

N/A = not applicable
LCL, Lower Confidence Level and UCL, Upper Confidence Level

A 2.4.2.1.2 Cymoxanil 45 WG (IN 002B1760): Effects on *Hypoaspis (Geolaelaps) aculeifer* Reproduction in an Artificial Soil Study

Comments of zRMS:	The study was conducted in line with OECD 226 with no deviations. All validity criteria are met. • Mean value of juveniles per vessel in the control group: 78.9. • % coefficient of variation of reproduction in the controls group :15.3%. • % mortality in the control group: 12.5%. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: $NOEC_{reproduction}$ = 296 mg prod./kg soil dw (equivalent to 109.2 mg a.s./kg soil dw) $EC_{10\ reproduction}$ = 361.0 mg prod./kg soil dw (CI: 242.7 – 485.3), (equivalent to 69.2 mg a.s./kg soil dw), NW value is 0.67. According to Appendix E of EFSA (2019), a NW value is rated as 'fair' (<1) or 'good' (0.2-0.5), indicating reliability of the estimated EC_{10}. It should be noted that EC10 expressed in a.s.unit are not considered reliable as no 95%CI lower limit was not given in the study report. It was not possible to calculate NW value according EFSA 2019. For this reason, NOEC=109.2 mg a.s./kg dws based on initial concentration for cymoxanil was used in the risk assessment.
Reference:	KCP 10.4.2.1/02
Report	Dini, R. (2022c). Cymoxanil 45 WG (IN 002B1760): Effects on *Hypoaspis (Geolaelaps) aculeifer* Reproduction in an Artificial Soil Study. Report No. CH–0261/2021, ChemService S.r.l. Controlli e Ricerche, Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The purpose of this study was to determine the effects of Cymoxanil 45 WG (IN 002B1760) on the survival and reproduction of the soil mite species *Hypoaspis (Geolaelaps) aculeifer* in a two-week artificial soil study according to the guideline OECD 226 (2016) and in compliance with the Principles of Good Laboratory Practice (GLP).

A. MATERIALS

1. Test Item

Name	Cymoxanil 45 WG (IN 002B1760)
Batch No.	IND_F040_0421_1
Active ingredient(s)	Cymoxanil
Content of a.s.	45 % (w/w)
(analysed)	47 % (w/w)

2. Test system

Species	*Hypoaspis aculeifer*

Age	Females with an age between 28 and 35 days after the first spawning
Source	Commercial supplier (Bias Labs Ltd)
Diet	Juvenile organism of *Folsomia candida* (*ad libitum*), were added to each container at test start and 2 or 3 times per week.
Water	Deionised water

3. Experimental conditions

Artificial soil	5% sphagnum peat; 20% kaolin clay; 75% industrial sand, based on dry weights, dried to a constant weight at 105°C. The pH of pre-moistened soil was 5.9 since no adjustment with calcium carbonate was necessary.
Temperature	19.3 – 20.2°C.
Photoperiod	16 hours light and 8 hours dark per day.
Light Intensity	593 - 615 Lux.

B. STUDY DESIGN AND METHODS

1. Experimental period

14th September 2021 – 2nd December 2021

2. Experimental treatment

The test item was added to the artificial soil by mixing the soil substrate with eight different stock solutions of the test item. The stock solutions were prepared by directly weighing the test item into deionised water. Then, the stock solutions were added to the pre-moistened soil previously divided into different aliquots of 300 or 500 g dry weight for concentrations and for the control. Finally, water was added to reach the final moisture and the hydrated soil was accurately mixed.

3. Application concentrations

The test was performed at eight different test item concentrations in soil, in a geometric series, namely 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7 and 1000.0 mg test item/kg d.w. These test item concentrations correspond to nominal Cymoxanil concentrations equal to 27.5, 41.3, 61.9, 92.8, 139.3, 208.9, 313.3 and 470.0 mg a.s./kg d.w. A negative control (artificial soil without test item) was tested to check the health of mites.

Four replicates per each test item concentration were prepared while, for the control, 8 replicates were used. One more replicate for each test concentration and for the control was prepared and used to evaluate the pH and the moisture content of the artificial soil at the end of the test. Additionally, one further aliquot of untreated and treated soil for the concentrations (without test organisms) was prepared, in order to extract the soil and perform the analytical measurements of the exposure concentrations.

4. Observations

At the end of the exposure period the total number of living juveniles produced during the test and the total number of surviving females was counted after extraction from the soil.

5. Statistics

The CETIS v.1.8.7.7 software was used to carry out all the statistical analysis. The 14 d-LC_x was determined by Linear Interpolation (ICPIN) method. The 14 d-EC_x on the reproductive output was determined by Non-linear regression. The NOEC and LOEC values were determined from the raw data by using the Bonferroni Adj t test.

Results and discussions

Table A 2.4-6: Mortality of mites after 14 days

Test item concentration (mg test item/kg soil d.w.)	Nominal Cymoxanil concentration (mg a.s./kg soil d.w.)	Cymoxanil concentration measured at time 0 (mg a.s./kg soil d.w.)	Mortality after 2 weeks	
			Percentage of dead mites (%)	Significance [a] (+/-)
0.0 (negative control)	0.0	0.0	12.5	n.a.

Test item concentration (mg test item/kg soil d.w.)	Nominal Cymoxanil concentration (mg a.s./kg soil d.w.)	Cymoxanil concentration measured at time 0 (mg a.s./kg soil d.w.)	Mortality after 2 weeks	
			Percentage of dead mites (%)	Significance [a] (+/-)
0.0 (negative control)	0.0	0.0	12.5	n.a.
58.5	27.495	21.056	7.5	-
87.8	41.266	35.256	12.5	-
131.7	61.899	65.479	5.0	-
197.5	92.825	71.878	5.0	-
296.3	139.261	109.216	15.0	-
444.4	208.868	155.810	12.5	-
666.7	313.349	224.490	7.5	-
1000.0	470.000	377.224	22.5	-

[a] statistically significant difference in pairwise comparison between treatment and untreated control (*Bonferroni Adj Test* α = 0.05)
n.a. not applicable
+ significant; - non-significant

Table A 2.4-7: Effects on mite reproduction after 14 days

Test item concentration (mg test item/kg soil d.w.)	Cymoxanil concentration (mg active substance/kg soil d.w.)	Cymoxanil concentration measured at time 0 (mg a.s./kg soil d.w.)	Number of juveniles			Inhibition %
			Mean	SD	CV%	
0.0 (negative control)	0.0	0.0	78.9	12.1	15.31	-
58.5	27.495	21.056	111.0	9.7	8.74	-40.7
87.8	41.266	35.256	98.3	14.5	14.78	-24.6
131.7	61.899	65.479	98.3	18.9	19.25	-24.6
197.5	92.825	71.878	95.5	3.7	3.87	-21.1
296.3	139.261	109.216	74.5	6.0	8.02	5.5
444.4	208.868	155.810	49.8*	18.0	36.19	36.9
666.7	313.349	224.490	41.8*	13.5	32.39	47.1
1000.0	470.000	377.224	26.5*	6.6	24.75	66.4

* statistically significantly different compared to the control (*Bonferroni Adj t Test* α = 0.05)

The actual test concentrations of the cymoxanil residues were analytically measured in soil samples at test start (time 0), after 7 days and at the end of the test after 14 days of exposure. Measured cymoxanil concentrations at T0 range between 71.6% - 105.8%. Statistical endpoints were calculated using both nominal concentration and measured concentration at T0.
All the validity criteria of the OECD guidance No. 226 (2016) were met.

Conclusion

The effects of the test item Cymoxanil 45 WG (IN 002B1760) on survival and reproduction of the mite species *Hypoaspis (Geolaelaps) aculeifer* were investigatedin a GLP laboratory study according to OECD test guideline No. 226. The test item caused no statistically significant mortality of the adult mites up to and including the maximum tested soil concentration, equal to 1000 mg test item/kg soil d.w. No statistically significant adverse effect on reproduction was observed up to and including 296.3 mg test item/kg soil d.w. Resulting NOEC and L/EC_x are summarised below:

Table A 2.4-8: Statistical endpoints

Endpoint	**[mg test item/kg d.w.]** based on nominal test item concentration	**[mg a.s./kg d.w.]** based on nominal cymoxanil concentration	**[mg a.s./kg d.w.]** based on measured cymoxanil concentration at time 0
Survival			
NOEC	1000.0	470.0	377.2
LC_{50} (LCL – UCL)	> 1000. (NA – NA)	> 470.0 (NA – NA)	> 377.2 (NA – NA)
Reproduction			
NOEC	296.3	139.3	109.2
EC_{10} (LCL – UCL)	361.0 (242.7 – 485.3)	169.7 (114.1 – 228.1)	69.2 (N/A – 103.4)
EC_{20} (LCL – UCL)	446.0 (331.1 – 581.3)	209.6 (155.6 – 273.6)	100.5 (55.6 – 138.8)
EC_{50} (LCL – UCL)	814.7 (585.6 – 1301.0)	382.9 (275.2 – 611.5)	190.1 (145.0 – 249.1)

N/A = not applicable
LCL, Lower Confidence Level and UCL, Upper Confidence Level

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Effects of Cymoxanil technical on the Activity of the Soil Microflora in the Laboratory

Comments of zRMS:	The study is not considered by zRMS in evaluation of the product at zonal level.

~~Reference: KCP 10.5.1/01~~

~~Report Feil, N. (2008). Effects of Cymoxanil technical on the Activity of the Soil Microflora in the Laboratory. Report No. 41382080, Institut far Biologische Analytik und Consulting IBACON GmbH, Ar heilger Weg 17, 64380 Rossdorf, Germany~~

~~Guideline(s): OECD 216 (2000) and OECD 217 (2000)~~

~~Deviations: 2 g/kg glucose was used instead of 3 g/kg which was determined to be the optimal glucose concentration, giving the highest respiration rates, due to a transcription error (human mistake). Impact on the study: None, the optimum amount of glucose should be in the range of 2-4 g/kg, therefore the smaller amount of 2 g/kg is still sufficient for the respiration activity of the soil microflora.~~

~~GLP: Yes~~

~~Acceptability: Yes~~

Duplication -
(if vertebrate study)

Materials and methods

Test Item: Cymoxanil technical; Batch No. 52; Purity: 99.1% w/w

Test Species: Biologically active agricultural soil: loamy sand.

Test Design: Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. A BSB-Sensomat System® was used to determine the CO2 production over a period of up to 24 hours at different sampling intervals.
Determination of nitrogen transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. NH_4^+, NO_2^- and NO_3^- nitrogen formed from the nitrification process were determined by means of a Dionex ion chromatography system (DX-120 IC, AS-50 autosampler, ECD and UVD 340S UV photometer).

Test Rates: Control;
Lower test concentration: 0.32 mg/kg soil dry weight (corresponding to a field application rate of 240 g/ha and assuming a homogeneous distribution in the top 5 cm of soil and a soil density of 1.5 g/cm3);
Higher test concentration: 1.60 mg/kg soil dry weight (corresponding to 5 times the field application rate).

Endpoints: Effects on CO_2 consumption after 28 days of exposure.
Effects on NO_3 nitrogen production after 28 days exposure.

Reference Item: Sodium chloride was applied at a rate of 16 g/kg dry soil in a separate study (study code: 30692080) within one year of the start of the experimental phase of this study.

Test Conditions: Soil moisture: 47.0 to 49.7 % of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in plastic boxes covered by perforated lids.

Statistics: Calculation of mean values per treatment, standard deviation and coefficient of variation. Normality and homogeneity of variances were tested using the R/S-Test Test ($\alpha = 0.05$) and Cochran's-Test ($\alpha = 0.05$), respectively and pairwise comparisons of treated and control values according to Student t-test + Welch t-test ($\alpha = 0.05$) were performed.

Results and discussions

Soil Respiration Rates: The soil respiration rates were within the trigger value of ±25% set by OECD guideline 217 throughout the experiment. On day 28 the values differed by 2.1% and 11.3% from the control for the low and high dose rate, respectively. There were no statistically significant differences between control and the lower test item treated group within the experiment (Student t-test + Welch t-test, $\alpha = 0.05$). A summary of the results is shown in Table A 2.5-1.

Soil Nitrate Content: The soil nitrate content deviated less than ±25% from the control within the experiment. On day 28 the deviations were -9.1% and -3.0% at the dose rates of 0.3 and 1.6 mg/kg soil dry weight respectively. The statistical evaluation resulted in significant differences for the lower treatment group for all sampling dates (Student t-test + Welch-test, $\alpha = 0.05$). A summary of the results is shown in Table A 2.5-2.

Nitrate Formation Rate: The soil nitrate formation rates were calculated on an incremental basis (i.e. between successive sampling dates). The difference in the soil nitrate for-

mation rate between the control and the lower test concentration was only higher than 25% in the first interval between days 0 and 7. In the last interval between days 14 and 28, the deviations from control were 2.2% and 9.8% for the lower and higher test concentration of Cymoxanil technical. Thus, the difference in the soil nitrate formation rates between the control and both test item treatments was clearly below the OECD guideline 216 trigger value of 25 % at the 14 to 28 day interval. These deviations were statistically not significant different (Student t-test, a = 0.05). A summary of the results is shown in Table A 2.5-2.

Soil Mineral Nitrogen Content: The mineral nitrogen content (as required by the EPPO and SETAC guidelines) at both concentrations of Cymoxanil technical was continuously within the ±25% difference from control. At day 28 the values were below the trigger value (-9.1% and -2.8% for the lower and higher concentration).

Validity Criteria: The variation between the replicate control samples for both parameters was within the validity criterion of 15% for both the carbon and nitrogen transformation tests (OECD test guidelines 216/217) throughout the study. The validity of the test system was further confirmed by the sensitivity established in separate positive control experiments using sodium chloride at a concentration of 16 g/kg soil dry weight.

Table A 2.5-1: Effects of Cymoxanil technical on Soil Respiration in a Loamy Sand Soil

Soil Respiration (mg CO_2 / kg soil dry weight / hr) Mean Values						
Sampling	Control		Cymoxanil technical			
			0.32 mg/kg soil dw		1.60 mg/kg soil dw	
	Respiration Rate	Replicate Variation[1]	Respiration Rate	Deviation[2]	Respiration Rate	Deviation[2]
Day 0	13.872	1.91	13.309	-4.06	13.632	-1.73
Day 7	13.734	4.09	14.051	2.31	15.385	12.02
Day 14	11.695	0.15	12.158	3.96	12.818*	9.60
Day 28	12.712	1.60	12.973	2.05	14.144*	11.26

1 = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value * 100)
2 = % deviation to control

Table A 2.5-2: Effects of Cymoxanil technical on Soil Nitrogen Transformation in a Loamy Sand Soil

NO_3 – Nitrogen (mg / kg soil dry weight) Mean Values						
Sampling	Control		Cymoxanil technical			
			0.32 mg/kg soil dw		1.60 mg/kg soil dw	
	Nitrate-N Content	Replicate Variation[1]	Nitrate-N Content	Deviation[2]	Nitrate-N Content	Deviation[2]
Day 0	23.113	1.12	22.611*	-2.17	22.781*	-1.44
Day 7	15.942	5.30	13.574*	-14.85	15.098	-5.29
Day 14	22.436	2.35	18.987*	-15.37	20.251*	-9.74
Day 28	35.389	0.94	32.153*	-9.14	34.327	-3.00
NO_3 – Nitrogen Formation Rate (mg / kg soil dry weight per day)[3]						

Interval[3]	Control	Cymoxanil technical			
		0.32 mg/kg soil dw		1.60 mg/kg soil dw	
	Nitrate-N Formation	Nitrate-N Formation	Deviation[2]	Nitrate-N Formation	Deviation[2]
Day 0 – 7	-1.02	-1.29*	26.5	-1.10	7.8
Day 7 – 14	0.93	0.77	-17.2	0.73	-21.5
Day 14 – 28	0.92	0.94	2.2	1.01	9.8

NO_3 – Nitrogen Formation Rate (mg / kg soil dry weight per day)[5]

Interval[3]	Control	Cymoxanil technical			
		0.32 mg/kg soil dw		1.60 mg/kg soil dw	
	Nitrate-N Formation	Nitrate-N Formation	Deviation[2]	Nitrate-N Formation	Deviation[2]
Day 0 – 7	-1.02	-1.29*	26.5	-1.10	7.8
Day 0 – 14	-0.05	-0.26*	420.0	-0.18*	260.0
Day 0 – 28	0.44	0.34*	-22.7	0.41	-6.8

Mineral Nitrogen[4] (mg / kg soil dry weight) Mean Values

Sampling	Control		Cymoxanil technical			
			0.32 mg/kg soil dw		1.60 mg/kg soil dw	
	Mineral-N Content	Replicate Variation[1]	Mineral-N Content	Deviation[2]	Mineral-N Content	Deviation[2]
Day 0	29.816	1.83	30.364	1.83	30.498	2.28
Day 7	16.903	5.10	14.614*	-13.54	16.521	-2.26
Day 14	24.024	1.66	20.460*	-14.84	21.756*	-9.44
Day 28	36.374	1.01	33.080*	-9.06	35.354	-2.80

1 – % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value * 100)
2 – % deviation to control
3 – related to successive intervals between samplings
4 – mineral nitrogen = sum of nitrite- nitrate- and ammonium-nitrogen
5 – related to sampling at test start
+ – stimulating effect
– – inhibitory effect
dw - dry weight
* – statistically significant different from control (Student t test + Welch t test; a = 0.05)

Conclusion
Based on the results of this study, Cymoxanil technical had no adverse effect on soil microflora (carbon mineralisation and nitrogen transformation) when applied at concentrations up to 1.60 mg/kg soil dry weight. It can be concluded that Cymoxanil technical will not have any long-term influence on soil microflora.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Effects of Cymoxanil 45 WG on Terrestrial (Non-Target) Plants: Vegetative Vigour Test

Comments of zRMS:	The study was conducted in line with OECD 227 with minor deviations. -The day temperature temporarily exceeded 28 °C (maximum 33°C) on six days for maximal 11 hours; the night temperature temporarily exceeded 23 °C (maximum 27°C) on one day for 7

	hours. Impact on the study: None, the temperature was not higher than the temperature of 22°C ± 10°C recommended by the OECD guideline 227 (with two exceptions of one to two hours at 33°C), the development of seedlings was not affected. -In the 1st experiment, the samples taken from the stock solution for the analytical determination of the a.i. had not been immediately frozen due to a human error. Impact on the study: Insufficient analytical results for the first experiment. An additional experiment was performed with the highest application rate. -With respect to the analytical method defined in SOP-05/TES, the HPLC conditions were changed during the measurements of the first and second experiment due to an error. Impact on the study: None, since at both experiments fortified samples were analysed and showed acceptable recovery All validity criteria are met: • Mean survival of control plants: 100%. • Growth and morphology of the control plants: The control plants exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species; validity criterion was met. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: <u>Plant height:</u> ER_{50} > 533 g prod./ha (equivalent to 240 g a.s./ha) The very small signs of phytotoxicity in Glycine max were not considered to affect the further development and growth of the plants.

Reference:	KCP 10.6.2/01
Report	Bützler, R. & Meinerling, M. (2008). Effects of Cymoxanil 45 WG on Terrestrial (Non-Target) Plants: Vegetative Vigour Test. Report No. 39709087, Institut far Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Guideline(s):	OECD 227 (2006)
Deviations:	The day temperature temporarily exceeded 28 °C (maximum 33°C) on six days for maximal 11 hours; the night temperature temporarily exceeded 23 °C (maximum 27°C) on one day for 7 hours. Impact on the study: None, the temperature was not higher than the temperature of 22°C ± 10°C recommended by the OECD guideline 227 (with two exceptions of one to two hours at 33°C), the development of seedlings was not affected (see results of the controls). In the 1st experiment, the samples taken from the stock solution for the analytical determination of the a.i. had not been immediately frozen due to a human error. Impact on the study: Insufficient analytical results for the first experiment. An additional experiment was performed with the highest application rate. With respect to the analytical method defined in SOP-05/TES, the HPLC conditions were changed during the measurements of the first and second experiment due to an error. Impact on the study: None, since at both experiments fortified samples were analysed and showed acceptable recovery.
GLP:	Yes
Acceptability:	Yes
Duplication	-

(if vertebrate study)

Materials and methods

Test Item:	Cymoxanil 45 WG;
Batch:	070724; active ingredient: 45 % w/w Cymoxanil (nominal) 44.9 % w/w (analysed)
Test Species and Rates:	Seven plant species from six different plant families were tested: *Beta vulgaris, Glycine max, Helianthus annuus, Cucumis sativus, Lolium perenne, Avena sativa* and *Allium cepa* with following rates: 1st experiment: 240, 120, 60.0, 30.0 and 15.0 g a.i./ha 2nd experiment: 240 g a.i./ha
Test Design:	Plants were grown until they had reached the 2 to 4 true leaf stage prior to dosing. Test rates were calculated for a water amount of 500 L/ha and were administered onto the plants using a laboratory spray equipment. A minimum of 20 plants was tested per rate and species. The concentration of the active ingredient in the stock solution was verified analytically. The exposure time was 21 days.
Endpoints:	NOER based on fresh weight; Observation of mortality and phytotoxicity.
Dates of Work:	1st experiment: Pre-application period: May 21, 2008 – June 19, 2008 (non GLP) Application: June 19, 2008 2nd experiment: Pre-application period: August 21, 2008 – September 19, 2008 (non GLP) Application: September 19, 2008 Exposure period: 21 days after application
Test Conditions:	The study was performed in a growth chamber. Exposure conditions were as follows: 1st experiment: Day temperature was 24 °C to 33 °C, night temperature was 19 °C to 29 °C. Humidity was 46% to 100%. Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 6792 Lux (5010 to 10690 Lux). 2nd experiment: Day temperature was 23 °C to 28 °C, night temperature was 18 °C to 21 °C. Humidity was 48% to 86%. Photoperiod: 16 hours light / 8 hours dark. Mean light intensity during the day was 6913 Lux (5010 to 9990 Lux).

Results and discussions

The analytical recovery rate of the active ingredient cymoxanil in the stock solution was 77 % of the nominal value in the 1st experiment. The recovery was poor because of the instability of cymoxanil in

aqueous solution. Therefore a 2nd experiment was performed. In the 2nd experiment the analytical recovery rate of the active ingredient cymoxanil in the stock solution was 98 % of the nominal value in a sample of stock solution which was diluted immediately after sampling.
The fresh weight was not statistically significantly reduced for any of the plant species tested (*Beta vulgaris, Glycine max, Helianthus annuus, Cucumis sativus, Lolium perenne, Avena sativa* and *Allium cepa)* up to a test concentrations of 240 g a.i./ha (the highest rate tested) in both experiments.
No mortality was observed for any species tested.
Some small signs of phytotoxicity were observed on some *Glycine max* plants (chlorosis, necrosis, growth reduction and abnormal growth of the leaves and the stems) and were limited to 1 – 2 % (max. 5 %) of the surface area. One pot with *Beta vulgaris* seedlings showed a slight chlorosis in the 2nd experiment only.

Table A 2.6-1: Summary of effect rates for both experiments (based on fresh weight)

	NOER [g a.i./ha]	**LOER [g a.i./ha]**	**Statistical Analysis**
Beta vulgaris	240	> 240	n.s. [1,2]
Glycine max	240	> 240	n.s. [1,2]
Helianthus annuus	240	> 240	n.s. [1,2]
Cucumis sativus	240	> 240	n.s. [1,2]
Lolium perenne	240	> 240	n.s. [1,2]
Allium cepa	240	> 240	n.s. [1,3]
Avena sativa	240	> 240	n.s. [1,2]

n.s. not statistically significant differences to control
1 multiple comparison Dunnett-test, $\alpha = 0.05$
2 pairwise comparison Student-t-test, $\alpha = 0.05$
3 pairwise comparison Welch-t-test, $\alpha = 0.05$

Conclusion

Based on the results of the study the no adverse effect rate was 240 g ai/ha, the highest dose tested. The very small signs of phytotoxicity in *Glycine max* were not considered to affect the further development and growth of the plants.

A 2.6.2.2 Cymoxanil 45 WG (IN 002B1760): Seedling Emergence and Seedling Growth Test of Terrestrial Plants

Comments of zRMS:	The study was conducted in line with OECD 208 with no deviations. All validity criteria are met. • The seedling emergence was above 90 % for all the species; the seedlings did not exhibit visible phytotoxic effects (modification in colour, necrosis, leaf and stem deformations were 0% in all tested species) and the plants showed only normal variation in growth and morphology for that particular species. • The mean survival of emerged control seedlings was 100% at the end of the study for all the tested species. • The environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix from the same source. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:

	Emergence: ER_{50} > 550 g prod./ha (equivalent to 258.5 g a.s./ha) Plant weight: ER_{50} > 550 g prod./ha (equivalent to 258.5 g a.s./ha) Shoot height: ER_{50} > 550 g prod./ha (equivalent to 258.5 g a.s./ha) At the end of the test no phytotoxic effects were shown in all tested species.

Reference:	KCP 10.6.2/02
Report	Noè, F. (2022). Cymoxanil 45 WG (IN 002B1760): Seedling Emergence and Seedling Growth Test of Terrestrial Plants. Report No. CH–0262/2021, ChemService S.r.l. Controlli e Ricerche, Via Fratelli Beltrami, 15, 20026 Novate Milanese (MI), Italy
Guideline(s):	OECD 208 (2006) EPPO bulletin 1/135 (4), Phytotoxicity assessment, 2014
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

The purpose of the study was the assessment of the potential phytotoxic effects of the test item on seedling emergence and early growth of terrestrial plants following the application of test item to a standard soil. Plants of six different species (onion, oats, cucumber, soybean, sugar beet, sunflower) were treated at one single application rate, which was chosen according to the Sponsor. The test duration was 21 days after the 50% emerged seeds in negative control. The test method and the test species are recommended by the international test guidelines

A. MATERIALS

1. Test item

Name:	Cymoxanil 45 WG (IN 002B1760)
Batch:	IND_F040_0421_1
Active substance and CAS No.:	Cymoxanil [57966-95-7]
Active substance content	45% w/w (nominal) 47% w/w (measured)

2. Test system

Species:	*Dicotyledonae: Cucumis sativus, Glycine max (G. soia), Beta vulgaris, Helianthus annus* *Monocotyledonae: Allium cepa, Avena sativa*
Stage:	Seeds
Soil:	loamy sand standard soil (soil type 5M) with an organic carbon percentage of 0.88% (± 0.18), sieved with a mesh of 2 mm. The pH of soil

at the test start was equal to 7.31 and its moisture content was 6.1%.

3. Experimental conditions

Temperature:	Day: 27.10 – 28.80°C (mean value 27.88°C, S.D. 0.35°C), Night: 20.40 – 21.90°C (mean value 21.30°C, S.D. 0.32°C).
Relative Humidity:	61.11 % - 82.31 % (mean value 75.55 %, S.D. 4.50 %)
Photoperiod:	16 hours light and 8 hours darkness, with an average wavelength of 400 to 700 nm.
Light intensity:	in the range 10190 - 11720 lux.

B. STUDY DESIGN AND METHODS

1. Experimental period:

16th September – 14th October 2021

2. Experimental treatment

A test item stock solution was prepared by directly adding the test item into deionized water. Test item solutions were sprayed using a sprayer specifically designed to simulate spray application in field (producer: G. Revello, Stefano Belbo Italy). It is provided with a 12 L-tank, it has a spray boom 130 cm long with 3 orange flat fan nozzles 45 cm apart (ISO 11001), a pressure gauge to reach pressure of 2 Bar. Application volume was equal to 200 L/ha and spray hight was 35 cm above the plants. The nozzles were adjusted on the boom so that the fan pattern, viewed from above, was 5-10 degrees out of alignment with respect to the boom in order to avoid the overlap area.
A concentration analytical check on all stock solutions used to spray was performed

3. Experimental design

One test item application rate was chosen in agreement with the Sponsor. For all species test item application rate was 550 g test item/ha, corresponding to 258.5 g cymoxanil/ha. A negative control (test soil without test item) was also tested.

4. Observations

Plants emergence in the negative control group was checked and recorded every 2 working days after the test start until 50% of plants have emerged. Once the 50% of emergence was reached, the number of emerged plants, visual rating of plant conditions and the number of plants surviving was recorded on days 7, 14 and 21. In addition, plants shoot height and biomass (as fresh weight) of surviving plants for all species was recorded at the end of the test (21 days).
Percentage of inhibition compared with negative control was calculated.

5. Statistics

The CETIS v.1.8.7.7 software was used to carry out the statistical analysis.

Results and discussions

Table A 2.6-1: Summary of the biological results

Application rate (g test item/ha)	Mortality		Biomass (as fresh weight)		Shoot height	
	Number of dead plants	Mortality (%)	Average fresh weight (g)	Inhibition of fresh weight (%)	Average shoot height (cm)	Inhibition of shoot height (%)
Oats *(Avena sativa)*						
0 (neg. control)	0	0.0	1.55	-	40.5	-

Application rate (g test item/ha)	Mortality		Biomass (as fresh weight)		Shoot height	
	Number of dead plants	Mortality (%)	Average fresh weight (g)	Inhibition of fresh weight (%)	Average shoot height (cm)	Inhibition of shoot height (%)
550	0	0.0	1.52	2.01	40.0	1.2
Onion (Allium cepa)						
0 (neg. control)	0	0.0	0.28	-	18.5	-
550	0	0.0	0.23	19.19	15.8	14.7*
Cucumber (Cucumis sativus)						
0 (neg. control)	0	0.0	8.37	-	6.4	-
550	0	0.0	8.25	1.39	6.4	0.4
Soybean (Glycine max (G. soia))						
0 (neg. control)	0	0.0	6.35	-	61.3	-
550	0	0.0	6.07	4.34	59.1	3.6
Sugar beet (*Beta vulgaris*)						
0 (neg. control)	0	0.0	4.03	-	19.0	-
550	0	0.0	3.98	1.10	18.9	1.0
Sunflower (*Helianthus annus*)						
0 (neg. control)	0	0.0	7.32	-	25.1	-
550	0	0.0	7.13	2.62	25.0	0.5

* Significantly different from the control according to the Equal Variance t Two-Sample rate ($\alpha = 0.05$) method

At the end of the test no phytotoxic effect was shown in tested species.
All the validity criteria of the study were met.
The analytical recovery of the cymoxanil concentration measured in the stock solution was equal to 100.7% of the nominal value.

Conclusion

Regarding mortality and biomass (fresh weight), no significant effects were observed in any of the tested species with respect to the untreated control. Therefore, the ER_{50} was evaluated to be higher than the application rate used in this study (i.e., 550 g test item/ha). The NOER for mortality and biomass (fresh weight) corresponded to 550 g test item/ha for all the tested species.
For shoot eight, a significant reduction was measured for the plant species *Allium cepa* (onion), while for the other species no significant effects were observed. For onion, the inhibition of shoot height was lower than 50%, thus, the ER_{50} was evaluated to be higher than 550 g test item/ha for all the tested species. The

NOER for shoot height was evaluated to be lower than the tested application rate for onion. For the other species, the NOER for shoot height corresponded to 550 g test item/ha.

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

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

A 2.8 KCP 10.8 Monitoring data