FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: FEL02

Product name(s): Cuprofix C/Cuprofix C Disperss

Chemical active substances:

Copper (Bordeaux mixture), 200 g/kg

Cymoxanil, 40 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Art. 33 New authorization)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: March 2023

MS Finalisation date: November 2023; April 2024

Version history

When	What
March 2023	Part B-Section 9 -Core assessment, Version 01 of applicant
November 2023	dRR version by zRMS
April 2024	The final version of the RR after the commenting period

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9 Ecotoxicology (KCP 10)

This dossier is intended for the application for the national authorisation of the product FEL02 according to Article 33 of Regulation (EC) No 1107/2009. The product FEL02 is based on the active substances Copper (as Bordeaux mixture), 200 g/kg, and Cymoxanil, 40 g/kg.

The active substance Copper compounds was first included in Annex I of Directive 91/414/EEC on 1 December 2009 (Commission Directive 2009/37/EC of 23 April 2009). The original rapporteur Member State France provided a Monograph in April 2007 and an Addendum in July 2008. A list of endpoints agreed at the original approval can be found in the Review Report on Copper compounds (SANCO/150/08 final 26 May 2009).

With Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011, the active substance Copper compounds was included in the list of approved active substances according to Regulation (EC) No 1107/2009.

The renewal of approval of Copper compounds (Copper hydroxide, Copper oxychloride, Copper oxide, Bordeaux mixture, tribasic Copper sulphate) according to Regulation (EC) No 1107/2009 was confirmed with Commission Implementing Regulation (EU) 2018/1981 of 13 December 2018, coming into force by 1 January 2019. The rapporteur Member State for the renewal of the EU Review, France, prepared a Renewal Assessment Report in December 2016, with updates in September and November 2017. The conclusion of the Peer Review can be found in EFSA Journal 2018;16(1):5152. The renewal the approval of Copper compounds as candidates for substitution pursuant to Article 24 of Regulation (EC) No 1107/2009 was agreed.

The product (FEL02) was not one of the representative products of the EU Review procedure for renewal of approval of Copper compounds, however, the applicant UPL Europe Ltd. is a member of the European Union Copper Task Force, (EUCuTF) and was one of the notifiers of the renewal procedure. UPL Europe Ltd. has full access to the active substance data package submitted to the rapporteur Member State France.

The active substance Cymoxanil was first included in Annex I of Directive 91/414/EEC on 1 September 2009 (Commission Directive 2008/125/EC of 19 December 2008). The original rapporteur Member State Austria provided a Monograph in June 2007. A list of endpoints agreed at the original approval can be found in the Review Report on Cymoxanil (SANCO/179/08 final 9 July 2010).

With Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011, the active substance Cymoxanil was included in the list of approved active substances according to Regulation (EC) No 1107/2009.

Cymoxanil is in the process of renewal of approval according to Regulation (EC) No 1107/2009. The rapporteur Member State for the renewal of the EU Review, Lithuania, prepared a Renewal Assessment Report in July 2020, and the public consultation was finished in October 2020.

The product (FEL02) is not one of the representative products of the EU Review procedure for renewal of approval of cymoxanil, however, the applicant UPL Europe Ltd. is a member of the Cymoxanil Task Force and was one of the notifiers of the renewal procedure. UPL Europe Ltd. has full access to the active substance data package submitted to the rapporteur Member State Lithuania.

This application follows the data requirements for the plant protection product laid down in Regulation (EC) No 284/2013. Data submitted on the formulated product are owned by the applicant UPL Europe Ltd. A summary of the data is provided in dRR format. Only summaries of studies and risk assessments which have not yet been assessed in any EU Member State are included in dRR Part B.

The technical active substance Copper (Bordeaux mixture) used in FEL02 was evaluated during the EU Review for the renewal of approval of Copper compounds. Thus, an assessment of technical equivalence is not required for the current application.

General observation: Deviation from standard Guidance Documents and EFSA conclusion is necessary and unavoidable for Copper.

The RMS and EFSA are held to assess plant protection products according to the existing methodology described in a series of guidance documents (GDs). Those have been developed for synthetic, organic molecules, and are in most cases not applicable to minerals and Copper. This has led to an EFSA conclusion[1] that indicated a number of critical concerns, or assessments that could not be finalized, which do not reflect any realistic risk, but rather illustrate the inappropriateness of the current GDs for the assessment of Copper. This can easily be seen in a number of endpoints that suggest a high risk exists at concentrations below natural background of this essential micronutrient. The inap-

[1] EFSA Journal 2018;16(1):5152

propriateness of current guidelines for the assessment of Copper compounds has been recognised by the EU Commission, EFSA, the RMS and several MS (see comments from DE and IT in the Peer review Report), and this is now fully justified by the documents made available recently by EFSA[2,3] . Those documents confirm that the approaches and methodology suggested by the EUCuTF already during the EU renewal and also presented by its members for Art. 43 and Art. 33 authorizations can be used for transition metals like copper. In addition, and noticeably, the use of the EUCuTF approach is a prerequisite to enable a meaningful assessment and avoid conservative outcomes for copper products.

The applicant UPL Europe Ltd. presents several statements explaining and justifying the risk assessment approach and deviations from the EU agreed endpoints in the present dossier and in line with the EU dossier submitted for the renewal. The statements are referred to in the dossier where applicable.

The present submission and its evaluation by MS are due before this GD will be available, explaining and justifying the risk assessment approach herein proposed.

The current EFSA conclusion[4] and list of endpoints could at best be considered as a first tier, and applicants as well as MS are required to deviate from the standard procedures described in the GD for the following reasons:

- The current GD do not consider bioavailability; for an essential, ubiquitous micronutrient that is a metal it is indispensable to provide assessment methodologies that consider the bioavailability and the potentially toxic fraction in each real-world exposure scenario. Total concentrations do not result in any meaningful outcome.
- Data normalisation to enable comparison of toxicological lab and field data as well as data obtained with different bioavailable fractions is a pre-requisite to allow a realistic assessment of potential risk. Simplistic worst-case scenarios will always indicate a high risk already at naturally occurring concentrations.
- For a homeostatically tight controlled essential element the application of assessment factors is meaningless. The question whether an excess exposure or deficiency leads to an adverse disruption of the homeostatic control cannot be approached in this way. Further, the exceptional data richness of the Copper dossier and more than 100 years of experience with the use as fungicide make safety factors unnecessary.

These unique features of Copper are already considered in the assessment of Copper under separate legislation (REACH, BPR).

Therefore, applicants as well as zRMS are required to deviate from the LoEP and the standard procedures described in the GD. This can now be fully justified by the documents made available recently by EFSA[5,6]. Those documents confirm that the approaches and methodology suggested by the EUCuTF already during the EU renewal and also presented by its members for Art. 43 and Art. 33 authorizations will find their way into the evaluation system and can be used for transition metals.

[2] Statement of the PPR Panel on a framework for conducting the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products (PPP) | European Food Safety Authority (europa.eu)
[3] Outcome of the Public Consultation on the draft statement of the PPR Panel on a framework for conducting the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products (PPP) - - 2021 - EFSA Supporting Publications - Wiley Online Library
[4] *Ibidem*
[5] EFSA Journal 2021;19(3):6498
[6] EFSA Journal 2021;18(3):EN-6501

9.1 Critical GAP and overall conclusions

Table 9.1-1 Table of critical GAP

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I ****	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks: e.g. g safener/ synergist per ha**	**Conclusion**						
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season**	**kg a.s./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	Central	Potatoes	F	Late blight (*Phytophthora infestans*)	Spraying	(BBCH 21 to 95)	6	7	a) 3.0 b) 18.0	a) 0.120 + 0.600 b) 0.720 + 3.6	100 - 1000	7	Month of application: 04 to 09	C	C	C	C	C	C	C

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

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

Explanation for column 15 - 21 “Conclusion”

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

Remarks table:

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

zRMS comments:

The dRR was prepared by applicant. All comments and conclusions of the zRMS are presented in grey and yellow after commenting. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency. **After Commenting period the updated information were indicated in yellow.**

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)

~~A weight of evidence paper was submitted as part of the renewal of approval and the conclusion is that the long-term risks to birds and mammals and additional information submitted by the applicant were considered and then the acceptable risk can be concluded for application rates of up to 4 kg Cu/ha.~~

Updated 04.2024r.

Birds

The risk assessment to birds was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and considering the EU agreed endpoints. The first-tier assessment of the acute and long-term/reproductive indicated unacceptable risk.

It has been submitted a weight of evidence (WoE) approach. No additional information has been provided to address the data gap from EFSA conclusion-2018 (EFSA Journal 2018; 16(1):5152). Thus, further information for the acute and long-term risk to omnivorous and frugivorous birds would have to be provided.

Zonal-RMS is of the same opinion as RMS in RAR revised and, taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on *more than a realistic worst case scenario*, the WoE approach could be used to conclude acceptable risk at the dose rate requested (maximum annual application rate of 4 kg Cu/ha) until the existence of an accepted guidance document.

Mammals

The risk assessment to mammals was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) and considering the EU agreed endpoints. The first-tier assessment of the acute and long-term/reproductive indicated unacceptable risk.

It has been submitted a weight of evidence (WoE) approach. No additional information has been provided to address the data gap from EFSA conclusion-2018 (EFSA Journal 2018; 16(1):5152). Thus, further information for the acute and long-term risk to frugivorous mammal and the generic focal species large herbivorous “lagomorph” would have to be provided.

Zonal-RMS is of the same opinion as RMS in RAR revised and, taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on *more than a realistic worst case scenario*, the WoE approach could be used to conclude acceptable risk at the dose rate requested until the existence of an accepted guidance document.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

In conclusion, the risk assessment for copper indicated acceptable risk to aquatic organisms from the use of FEL02.

The risk assessment for cymoxanil indicated acceptable risk to aquatic organisms from the use of FEL02.

The risk assessment for the product FEL02 (combined exposure of copper and cymoxanil) based on mixture toxicity indicated that the toxicity of FEL02 is driven by the active substance copper. The risk for the product is therefore adequately covered by the risk assessment of copper alone.

For registration in line with country specific requirements, different mitigation measures may apply.

Based on the lowest endpoint for fish agreed at EU level (EFSA Conclusion 2018) and PECsw calculations agreed at Section 8, the following risk mitigation measures should be applied to surface water bodies:

- 20 meter vegetative buffer strip and 90% drift reduction nozzels for potatoes

For sediment dwelling organism the risk is not finalized in the Core Assessment.
However, there is no approved guideline for calculating PECsed values to determine protective measures for copper compounds. zRMS proposes only for Poland to apply existing default mitigation measures for PECsw for copper for aquatic organism.

The final risk mitigation measures should be decided at MS level.

9.1.1.3 Effects on bees (KCP 10.3.1)

The first-tier risk assessment of the oral acute and chronic risk to bees and bumblebees from the use of FEL02 indicates that there may be unacceptable risks.

The use of **Cuprofix C** to bees based on the proposed GAP is considered acceptable under the following restrictions:
SPe 8: Dangerous to bees.
- To protect bees and other pollinating insects do not apply to crop plants when in flower.
- Do not use where bees are actively foraging.
- Do not apply when flowering weeds are present.
- Remove weeds before flowering.

The risk assessment for bees should be considered by MSs level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk to non-target arthropods from the use of copper, cymoxanil and FEL02 is acceptable.

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

The risks following exposure of Copper to earthworms and other non-target soil macro-organisms are considered to be acceptable at annual doses of up to 4.0 kg Cu/ha.
The risks following exposure of Copper to soil micro-organisms are acceptable at doses of up to 4.0 kg Cu/ha.

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

The risk of FEL02 to non-target plants is acceptable based on the first-tier assessment. Since copper is persistent also an assessment based on literature data was performed. Based on that it can be concluded that the risks following exposure of copper to non-target plants are acceptable at annual doses of up to 6 kg Cu/ha. Since the annual dose resulting from the proposed use of FEL02 is 720 g cu/ha, no adverse effects resulting from the proposed applications are expected.

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

No further testing on, or assessment of risk to, other terrestrial organisms is considered necessary as this is considered to have been addressed in the previous sections.

9.1.2 Grouping of intended uses for risk assessment

Not applicable as only one use is applied for.

9.1.3 Consideration of metabolites

Copper is an element and therefore the formation of metabolites or breakdown products is not possible. Therefore, conducting metabolite-specific risk assessments for copper is not required.

In the EFSA Conclusion on Cymoxanil (EFSA Scientific Report (2008) 167, 1 – 116), no metabolites of ecotoxicological concern were identified. Thus, the risk assessments based on Cymoxanil are considered to cover the toxicity of potential metabolites.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Table 9.2.1-1 Endpoints and effect values for Copper relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Coturnix japonica	Copper oxychloride WP	Oral acute	LD_{50} = 173 mg Cu/kg bw	EFSA Conclusion (2018)[7]
Colinus virginianus	Copper hydroxide	Long-term	NOEL = 5.05 mg Cu/kg bw/d	EFSA Conclusion (2018)

A literature review provides a weight of evidence approach concluding to acceptable risks to birds for doses of 5 kg Cu/ha/year, for granivorous and insectivorous birds.

Table 9.2.1-2 Endpoints and effect values for Cymoxanil relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	Cymoxanil a.s.	Oral acute	LD_{50} > 2000 mg/kg bw/day	EFSA Scientific Report 167 (2008)[8]
Anas platyrhynchos	Cymoxanil a.s.	Short-term	LD_{50} > 260*	EFSA Scientific Report 167 (2008)[4]

[7] EFSA (European Food Safety Authority), 2018. Conclusion on the peer review of the pesticide risk assessment of the active substance Copper compounds Copper(I), Copper(II) variants namely Copper hydroxide, Copper oxychloride, tribasic Copper sulfate, Copper(I) oxide, Bordeaux mixture. EFSA Journal 2018;16(1):5152, 144 pp. https://doi.org/10.2903/j.efsa.2018.5152, hereafter referred to as EFSA Conclusion (2018)

[4] EFSA (European Food Safety Authority), 2008. Conclusion regarding the peer review of the pesticide risk assessment of the active substance cymoxanil. EFSA Scientific Report (2008) 167, 1-116 pp. https://www.efsa.europa.eu/en/efsajournal/pub/rn-167

Species	Substance	Exposure System	Results	Reference
Anas platyrhynchos	Cymoxanil a.s.	Long-term	NOAEL = 14.9 mg/kg bw/day	EFSA Scientific Report 167 (2008)[4]

* 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.

Metabolites
Metabolites are not relevant for copper compounds. In the EFSA Conclusion on Cymoxanil (EFSA Scientific Report (2008) 167, 1 – 116), no metabolites of ecotoxicological concern were identified for birds and mammals. Thus, the risk assessments based on cymoxanil are considered to cover the toxicity of potential metabolites.

9.2.1.1 Justification for new endpoints

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

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)

FEL02 is proposed for 6 applications at a minimum interval of 7 days in potatoes at a dose rate of 3.0 kg formulation per ha (0.6 kg copper and 0.12 kg cymoxanil). The results of the acute and reproductive first-tier risk assessments for use in potatoes are summarised in the following tables.

Copper

Table 9.2.2.1-1 First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of FEL02 in potato - Copper

Intended use	**Potato**				
Active substance/product	Copper				
Application rate [kg/ha]	6 × 0.6				
Acute toxicity [mg/kg bw]	173				
TER criterion	10				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg bw/d]**	**TER_A**
Potatoes BBCH 10-39	Small omnivorous bird “lark”	24.0	1.9	27.36	**6.3**
Potatoes BBCH ≥ 20	Small insectivorous bird “wag-tail”	25.2		28.73	**6.0**
Potatoes BBCH ≥ 40	Small omnivorous bird “lark”	7.2		8.21	21.1
Reprod. toxicity [mg/kg bw/d]	5.05				
TER criterion	5				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Potatoes BBCH 10-39	Small omnivorous bird “lark”	10.9	1.3	8.67	**0.6**
Potatoes BBCH ≥ 20	Small insectivorous bird “wag-tail”	9.7		7.71	**0.7**
Potatoes BBCH ≥ 40	Small omnivorous bird “lark”	3.3		2.62	**1.9**

As shown in the table above the TER for the majority of scenarios are below the trigger of 10 or 5 for acute toxicity and long-term toxicity respectively. Therefore, a higher tier risk assessment is presented under point 9.2.2.2.

Cymoxanil

Table 9.2.2.1-2 First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of FEL02 in potato - Cymoxanil

Intended use	**Potato**				
Active substance	Cymoxanil				
Application rate [kg/ha]	6 × 0.12				
Acute toxicity [mg/kg bw]	>2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg bw/d]**	**TER_A**
Potatoes BBCH 10-39	Small omnivorous bird "lark"	24.0	1.9	5.47	>365
Potatoes BBCH ≥ 20	Small insectivorous bird "wag-tail"	25.2		5.75	>348
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	7.2		1.64	>1218
Reprod. toxicity [mg/kg bw/d]	14.9				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Potatoes BBCH 10-39	Small omnivorous bird "lark"	10.9	2.5 × 0.53	1.73	8.6
Potatoes BBCH ≥ 20	Small insectivorous bird "wag-tail"	9.7		1.54	10
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	3.3		0.52	28

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.

As shown in the table above all TER are higher than the trigger of 10 and 5 for long term and acute toxicity respectively. The risk of cymoxanil as a result of applications with FEL02 according to the proposed GAP are therefore acceptable.

Combined toxicity

For the combined risk assessment of FEL02, a surrogate LD_{50} mix was estimated following the approach proposed in the EFSA GD (2009):

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(\text{a.s.}_i)}{LD_{50}(\text{a.s.}_i)} \right)^{-1}$$

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture; (please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

A comparison between the mixture toxicity and the toxicity of the active substances should be made to test whether there is a change in the predicted risk by using the modelled LC_{50} mix value instead of the measured LD_{50} of the a.s. To achieve a basis for this comparison, a `*tox per fraction*` quotient can be calculated for each active substance and can be compared to the corresponding quotient of the mixture.

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

If the `*tox per fraction a.s*` and the `*tox per fraction mixture*` deviate by < 10%, this indicates, that this active substance will contribute > 90 % to mixture toxicity and the impact of the other component is marginal. Consequently, the risk assessment can be performed for the most toxic component. Otherwise, the LC_{50} mix should be used in the risk assessment. In table 9.2.2.2.1-1 all input parameter needed to estimate the surrogate LD_{50} mix and the tox per fraction comparison are summarized for FEL02.

Table 9.2.2.1-3 Calculation of surrogate LD_{50} for the mixture of active substances

Active substance	Concentration a.s. in mixture [g/kg]	Fraction a.s. in mixture	LD_{50} a.s. [mg/kg bw/d]	Fraction a.s./ LD_{50} a.s.	Surrogate LD_{50} [mg/kg bw/d]	Tox per fraction (a.s.)	Deviation tox per fraction (a.s.) from the tox per fraction (mix) [%]
Copper	200	0.83	173	0.00482	204.1	207.6	1.7%
Cymoxanil	40	0.17	>2000	8.33E-5		12000	5780%

Based on the calculation above, copper contributes over 90% to the acute toxicity of the mixture. Therefore, the acute risk assessment for the active substance copper, covers that of the formulation. Since a higher tier acute assessment is required for the active substance copper, the combined toxicity of both active substances in FEL02 is addressed under point 9.2.2.2. as well.

The long-term combitox should be addressed via the concentration addition (CA) model. When for each substance the trigger values are equal, the combined TER value can be calculated according to:

TERcombi = 1/((1/TERsubstance 1)+(1/TERsubstance 2))

The resulting TERcombi based on the long-term TER values taken from tables 9.2.2.1-2 and -3 are presented in the table below.

Table 9.2.2.1-4 First-tier assessment of the long-term/reproductive risk for birds due to the use of FEL02 in potato

Product	FEL02 (copper + cymoxanil)		
Application rate [g/ha]	6 × 3000 (600 + 120)		
Reprod. toxicity [mg/kg bw/d]	**-***		
TER criterion	5		
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**$TER_{LT\ combi*}$**
Potatoes BBCH 10-39	Small omnivorous bird "lark"	10.9	**0.6**
Potatoes BBCH ≥ 20	Small insectivorous bird "wag-tail"	9.7	**0.7**
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	3.3	**1.8**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
* No reproductive endpoint for FEL02 is available. A TER combi was determined with TER for individual active substances taken from tables 9.2.2.1-1 and -2, using the following formula: TERcombi = 1/((1/TERcopper)+(1/TERcymoxanil)).

As shown in the table above all TERcombi are below the trigger of 5 for long term toxicity. Therefore, a higher tier approach is needed and the combined toxicity of both active substances in FEL02 is addressed under point 9.2.2.2.

9.2.2.2 Higher-tier risk assessment

Copper

The current EFSA/2009/1438 approach to risk assessment for birds, is not applicable to determine the risks for avian dietary exposure to copper, since it makes no allowance for the mechanisms involved in the regulation of copper – a naturally occurring element and essential micronutrient – in the body, or of the compensatory responses of vertebrates to excess copper intake.

A weight of evidence paper was submitted by the EUCuTF members for the renewal of approval of copper which provided evidence that owing to homeostatic control, the acute and long-term risks to all birds is acceptable. The RMS/EFSA partially agreed with this and considered that this weight of evidence approach could be used to conclude acceptable acute and long-term risks for granivorous and insectivorous birds for application doses of up to 8 kg Cu/ha as long as the amount applied during the breeding period did not exceed 5 kg/ha (EFSA, 2018).

The method of determining the short-term/acute risks to birds can be regarded as an unrealistic worst-case estimation. Even after the application of fungicides, a percentage of feed items will remain uncontaminated and will be available within the field margins (Schabaker & Rastall, 2009a,). For those feed items that do contain residues of copper, the level of these residues shows a high level of variability.

It is therefore reasonable to consider that for any individual bird the availability of contaminated and non-contaminated feed items is equally available to them. This is an important factor because there are indications that birds actively avoid contaminated feed items or that they adjust their copper intake through selective feeding (Schabaker & Rastall, 2009a) and the total copper intake is within the range of their natural homeostatic control mechanism. The calculation of TER values also assumes that birds will consume all their daily intake of food within one 'sitting' and within the area of application, i.e. PT = 1, this too is a very conservative assumption.

To provide weight to this premise, there are no descriptions of bird poisoning events that can be clearly traced to copper ingestion noted in the different wildlife monitoring programmes being maintained by various Member States.

The lack of any reports detailing copper-related mortality to bird populations suggests that the treatment of crops with copper poses less acute risks of direct mortality to birds than indicated by the risk assessment according to the EFSA/2009/1438, confirming again that the method in the EFSA/2009/1438 is inadequate for copper.

With regard to the potential long-term risks to birds, again the method of determining these can be regarded as an unrealistic worst-case estimation as discussed for the acute/short-term risks. Additional information relating to the long-term risk to birds was presented in the weight of evidence paper submitted by the EUCuTF members for the renewal of approval of copper. This information included recent observations on bird communities in copper-treated orchards and vineyards in southern Europe (Italy, Spain, France) and central Europe (Germany, Poland) which consistently showed no obvious effects on breeding parameters and bird abundance and diversity. In general, the amounts of copper ingested in the diet are almost never harmful to wildlife because they are relatively low and because birds and mammals have the ability to maintain copper homeostasis by a combination of decreased absorption and enhanced excretion when exposed to higher levels (Schabacker and Rastall, 2009a; Schabacker and Rastall, 2009b).

Field monitoring results indicate that copper uses can result in different copper exposure levels in birds but, as expected from the physiologic background (homoeostasis), no evidence was found that in sites where copper is used as a pesticide adverse effects on wildlife occur.

In summary, the presence of species from different dietary guilds and their active and flourishing populations in copper treated habitats (such as for example copper treated orchards and vineyards) indicate that birds as well as mammals with insectivorous as well as frugivorous and omnivorous diets are able to cope with the copper levels they find in their diets. Copper homeostasis based on physiological and behavioural mechanisms will keep internal copper levels below toxic thresholds over extended environmental concentrations.

After consideration of the above arguments, it is considered that both the short-term and long-term risk to birds (including frugivorous and omnivorous birds) from exposure to copper residues on feed items for application doses of up to 8 kg Cu/ha is acceptable, as long as the amount applied during the breeding period does not exceed 5 kg/ha. Since the maximal total copper application for the defended uses of FEL02 is 3.6 kg Cu/ha, the risk to birds following the application of FEL02 according to the defended uses is acceptable.

Cymoxanil

No higher tier risk assessment required as the risk was determined to be acceptable at the 1st Tier assessment.

FEL02 / combination toxicity

The Tier 1 assessment for combined acute toxicity of copper and cymoxanil indicates that copper contributes >90% to the acute toxicity of the mixture. In line with the approach as described for the surrogate LD_{50} under point 9.2.2.1, the TER based on the reproductive endpoints for individual active substances can be compared with the reproductive TERcombi as presented in the table below.

Based on the calculations in Table **9.3.2.2-2**, copper also contributes over 90% to the reproductive toxicity of the mixture. Therefore, both the acute and long-term risk assessment for the active substance copper, covers that of the formulation. As discussed above, the risk of copper to birds is acceptable for application doses of up to 8 kg Cu/ha as long as the amount applied during the breeding period does not exceed 5 kg/ha. When applied according to the proposed GAP of FEL02, the total amount of copper applied per growing season is 3.6 kg cu/ha. Therefore, the risk of FEL02 to birds is acceptable as well.

Table 9.2.2.2-2 Comparison of the long-term/reproductive risk of FEL02 to birds due to the use in potato, to that of copper and cymoxanil individually.

Copper Reprod. Toxicity 5.05 mg/kg bw/d						
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**	**Difference with TER-combi [%]**
Potatoes BBCH 10-39	Small omnivorous bird "lark"	10.9	2.5 × 0.53	8.67	**0.6**	7
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	9.7		7.71	**0.7**	7
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	3.3		2.62	**1.9**	7
Cymoxanil Reprod. Toxicity 14.9 mg/kg bw/d						
Potatoes BBCH 10-39	Small omnivorous bird "lark"	10.9	2.5 × 0.53	1.73	8.6	1475
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	9.7		1.54	10	1475
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	3.3		0.52	28	1475
Combination of cymoxanil and copper						
Crop scenario Growth stage		**Indicator/generic focal species**			**$TER_{LT\ combi}$***	
Potatoes BBCH 10-39		Small omnivorous bird "lark"			**0.6**	
Potatoes BBCH ≥ 20		Small insectivorous bird "wagtail"			**0.7**	
Potatoes BBCH ≥ 40		Small omnivorous bird "lark"			**1.8**	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio, TER values shown in bold fall below the relevant trigger.
* No reproductive endpoint for FEL02 is available. A TER combi was determined with TER for individual active substances taken from tables 9.3.2.1-1 and -2, using the following formula: TERcombi = 1/((1/TERcopper)+(1/TERcymoxanil)).

zRMS comments:

Copper

For copper toxicity endpoints in line with EFSA Journal 2018;16(1):5152 were considered.
The Tier I acute and long-term risk assessment to birds was indicated as high for all the representative uses without:

Potatoes BBCH ≥ 40	Small omnivorous bird "lark"

Further, as risk refinement, position papers were provided where a Weight of Evidence (WoE) approach was presented to support a homeostatic mechanism in birds and mammals. The WoE was discussed at the Pesticides Peer Review Meeting 169; the experts considered the evidence provided as not satisfactory to exclude the acute risk to birds and mammals. Furthermore, the experts concluded that the data from the wildlife reports which were part of the evidence provided along with information of bird population (e.g. abundance and density), may be indicative of the absence of incidents but not sufficient to address the acute risk identified. The experts concluded that the WoE could be considered acceptable for addressing the long-term risk to birds and mammals for application rate up to 5 kg a.s./ha for granivorous and insectivorus birds; however, further data were considered necessary to draw a conclusion covering all the feeding guild categories, i.e. omnivorous and frugivorous birds and large herbivorous and frugivorous mammals (data gap). By generating further data, the experts considered it useful to focus on, e.g. further investigation of the avoidance and further data on residue in food items. There-fore, based on this conclusion further refinement is required at MSs level for omnivorous and frugivor-ous birds for all proposed uses for copper hydroxide depended on own indicator focal species.

ZRMS-PL is of the same opinion as RMS in RAR revised and, taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on more than a realistic worst case scenario, this WoE approach could be used to conclude acceptable risk at dose requested (maximum annual application rate of 4 kg Cu/ha) until the existence of an accepted guidance document.
The final decision should be considered at MSs level.

Cymoxanil
Acute and long-term risk assessment was accepted by zRMS.
No higher tier risk assessment required as the risk was determined to be acceptable at the 1st Tier assessment.

Combined toxicity
zRMS point out that that copper contributes to more than 90% to the mixture toxicity. Hence, it is considered acceptable to conduct the risk assessment for the active substances alone.

All TER_{combi} are below the trigger of 5 for long term toxicity. Refinement for TER_{combi} for long-term toxicity should be considered by MSs level.

The final decision should be considered at MSs level.

Updated 04.2024

Zonal-RMS comments 9.2	The first-tier assessment of the acute and long-term/reproductive indicated unacceptable risk. The applicant has submitted a weight of evidence (WoE) approach: according to the information on RAR, EFSA conclusion-2018 and Final Renewal report SANTE/10506/2018, the long-term risk could be considered acceptable for insectivorous and granivorous birds. In detail, it is indicated: *"... the weight of evidence could be considered acceptable for addressing the long term risk to birds and mammals for application of up to 5 Kg copper/ha but further data would be neened to draw a conclusion covering all feeding guild categories"*; and:*"a literature review provides a weight of evidence approach concluding to acceptable risks to birds for doses of 5 kg copper/ha/year, for granivorous and insectivorous birds."(SANTE/10506/2018, Final Renewal report copper compounds, page 5).* Taking into account these considerations, zonal-RMS has performed calculations for the risk assessment for **frugivorous and omnivorous birds,** following the GD EFSA Journal 2009; 7(12): 1438, considering the MAF and a TWA of 1 (according to the conclusions of the Pesticides Peer Review Meeting 169) and considering single maximum annual application. The following table shows the calculations for the critical doses (bold results are below the trigger): **Acute risk for birds (LD_{50} = 173 mg/kg bw)**

Intended use	**Potato**
Active substance/product	Copper
Application rate [g/ha]	3600
Acute toxicity [mg/kg bw]	173

TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg b w/d]**	**TER_A**
Potatoes BBCH 10-39	Small omnivorous bird "lark"	24.0	1.0	86.4	**2.0**
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	25.2		90.72	**1.9**
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	7.2		25.92	**6.67**

Long-term/reproductive risk for birds (NOEL = 5.05 mg/kg bw/d)

Intended use	Potato				
Active sub-stance/product	Copper				
Application rate [g/ha]	3600				
Reprod. toxicity [mg/kg bw/d]	5.05				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**$MAF_m \times TWA$**	**DDD_m [mg/kg b w/d]**	**TER_{LT}**
Potatoes BBCH 10-39	Small omnivorous bird "lark"	10.9	1.0	39.24	**0.13**
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	9.7		34.92	**0.14**
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"	3.3		11.88	**0.43**

The results obtained entail important limitations in the application pattern for an use of the product with acceptable risk.

Secondary poisoning.
According the information provided in RAR the risk to birds via consumption of contaminated water or via secondary poisoning is considered to be acceptable.

Conclusion:
According to the Review Report of copper compounds:

The experts concluded that the WoE could be considered acceptable for addressing the long-term risk to birds and mammals for application rate up to 5 kg a.s./ha for granivorous and insectivorus birds; however, further data were considered necessary to draw a con-clusion covering all the feeding guild categories, i.e. omnivorous and frugivorous birds and large herbiv-orous and frugivorous mammals (data gap). By generating further data, the experts considered it useful to focus on, e.g. further investigation of the avoidance and further data on residue in food items. There-fore, based on this conclusion further refinement is required at MSs level for omnivorous and frugivor-ous

	birds for all proposed uses for copper hydroxide depended on own indicator focal species. ZRMS-PL is of the same opinion as RMS in RAR revised and, taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on more than a realistic worst case scenario, this WoE approach could be used to conclude acceptable risk at dose requested (maximum annual application rate of 4 kg Cu/ha) until the existence of an accepted guidance document. According to the information provided and information from RAR, the risk to birds via consumption of contaminated water or via secondary poisoning is considered to be acceptable. ***The final decision should be considered at MSs level.***
Agreed endpoints	Acute toxicity endpoint: 173 (mg/kg bw). Reproduction toxicity endpoint: 5.05 (mg/kg bw/d).

9.2.2.3 Drinking water exposure

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).

A K_{oc} value for copper, as a measure of its absorbance to soil, is not scientifically relevant as it is a metal. Instead, the lowest K_{doc} value will be used, 19509.9 mL/g (EFSA Conclusion, 2018).

The K_{oc} of Cymoxanil is 43.6 mL/g (EFSA conclusion 2008).

Table 9.2.2.3-1 Application rate to endpoint ratios for birds exposed to Copper and Cymoxanil for the intended use of FEL02 in potatoes

Crop	Exposure Scenario	Effective Application Rate [g a.s./ha]*	K_{doc}/ Koc [mL/g]	LD_{50} / NOEL [mg ai/kg bw]	Ratio Application Rate:endpoint	Trigger
Potatoes	**Copper**					
	Acute	1500	19509.9	173	8.7	3000
	Long-term			5.05	297	3000
	Cymoxanil					
	Acute	300	43.6	>2000	<0.15	50
	Long-term			14.9	20.1	50

* Effective application rate = application rate multiplied by mean MAF

Since for copper and cymoxanil the ratio of the effective application rate and the relevant endpoint are well below 3000 and 50 respectively, no specific calculations of exposure and TER are necessary. Since there is a large margin of safety and considering the fast degradation of cymoxanil (DT_{50} of 0.3 days in surface water and 1.3 days in soil (EFSA 2008)), the risk of combined exposure to both actives via drinking water resulting from the proposed use of FEL02 in potatoes is considered acceptable as well and no specific calculations of exposure and TERcombi are necessary.

zRMS comments: Agreed. Since for copper and cymoxanil the ratio of the effective application rate and the relevant endpoint are well below 3000 and 50 respectively, no specific calculations of exposure and TER are necessary.

9.2.2.4 Effects of secondary poisoning

According to the Guidance Document on Risk Assessment for Birds and Mammals (EFSA, 2009), substances with a log $P_{ow} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

A partition coefficient (Log P_{ow}) is not relevant to copper as it is a metal, however, an estimated log P_{ow} value of 2.78 can be determined by the ratio of the water and n-octanol solubilities (EFSA Conclusion, 2018).

The estimated log P_{ow} value of copper is < 3 and the log P_{ow} of cymoxanil is 0.67, the active substances are not considered to accumulate in the food chain and therefore a risk assessment for effects due to secondary poisoning is not required.

No experimentally derived log P_{ow} values are available from the DAR for the metabolites of Cymoxanil. However, the RMS estimated log P_{OW} values with the software KOWWIN (US-EPA) for the metabolites INKQ960, IN-U3204, IN-T4226, IN-JX915, IN-R3273 and IN-KP533 and IN-W3595. For the metabolites IN-KQ960, IN-U3204, IN-T4226, IN-JX915, IN-R3273 and IN-KP533 the modelled log P_{ow} values were below the trigger of 3 and hence no bioaccumulation was expected. For IN W3595 a log P_{ow} of 4.27 was derived with KOWWIN. IN-W3595 is an organic acid, and the software estimates the log P_{ow} for the non-dissociated form of the acid. However, in aquatic environments the substance was partly dissociated, and it was shown to be highly water soluble. Taking into account this information, it was considered unlikely to accumulate in fat tissue. Overall, it can be concluded that the risk of secondary poisoning with metabolites of cymoxanil is acceptable.

zRMS comments: Agreed.

Copper

A partition coefficient (Log Pow) is not relevant to copper as it is a metal, however, an estimated log Pow value of 2.78 can be determined by the ratio of the water and n-octanol solubilities (EFSA Journal

2018;16(1):5152). Therefore, it does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Cymoxanil

Cymoxanil has a log P_{OW} of 0.67. As the log P_{OW} values are below the trigger of 3, it was not necessary to consider the risk from secondary poisoning further. Therefore, based on the low log P_{OW} values the risk from bioaccumulation to fish-eating and worm-eating birds is negligible.

9.2.2.5 Biomagnification in terrestrial food chains

A low potential for copper compounds and cymoxanil in animal tissue was concluded in the EU reviews of these substances (EFSA 2018 and EFSA 2008 respectively).

zRMS comments: Agreed.
According to EFSA conclusion (EFSA Journal 2018;16(1):5152), a literature review provides evidence of lack of bioaccumulation in aquatic food chain.
A low potential for cymoxanil in animal tissue was concluded in the EU reviews (EFSA 2008).

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

Not relevant since FEL02 is not intended to be used as a bait, pellet, granule, prill or for treating seed.

9.2.4 Overall conclusions

The risk to birds from application of FEL02 in potatoes in accordance with the proposed GAP is acceptable.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with copper and cymoxanil. Full details of these studies are provided in the respective EU DAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report (new studies).

Effects on mammals of the formulated product, FEL02, were not evaluated as part of the EU assessment of copper, nor cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report. The oral LD_{50} of FEL02 was determined as > 2000 mg product/kg bw. A comparison of the acute LD_{50} values derived for the formulation and the active substance indicates that the formulation is not more toxic than expected based on its active ingredient content.

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

Table 9.3.1-1 Endpoints and effect values relevant for the risk assessment for mammals – Copper compounds

Species	Substance	Exposure System	Results	Reference
Rat	Tribasic copper sulphate	Acute oral	LD_{50} = 162.6 mg Cu/kg bw	EFSA Conclusion (2018)
Rat	Copper sulphate	Long-term (90 days)	NOAEL = 16 mg Cu/kg bw/d	EFSA Conclusion (2018)

Table 9.3.1-2 Endpoints and effect values relevant for the risk assessment for mammals – Cymoxanil

Species	Substance	Exposure System	Results	Reference
Rat	Cymoxanil a.s.	Acute oral	LD_{50} = 760 mg/kg bw	EFSA Scientific Report 167 (2008)
Rat	Cymoxanil a.s.	2-Generation study	NOAEL = 10.5 mg/kg bw/d (parental/offspring)	EFSA Scientific Report 167 (2008)

Table 9.3.1-3 Endpoints and effect values relevant for the risk assessment for mammals – FEL02

Species	Substance	Exposure System	Results	Reference
Rat	ATO FDH01*	Acute oral	LD_{50} > 2000 mg product/kg bw	[redacted]

* The acute oral toxicity study with the preparation is summarised in dRR, Part B, Section 6, Mammalian Toxicology, KCP 7.1.1/01. The oral LD50 of ATO FDH01 was determined as > 2000 mg product/kg bw. FDH01 is a formulation containing 20.3% Copper, 4.43% Cymoxanil and 2.21% Famoxadone and is considered to be appropriate for the risk assessment for FEL02, please refer to Part B, Section 4 of the dRR.

Metabolites
Metabolites are not relevant for copper compounds and in the EFSA Conclusion on Cymoxanil (EFSA Scientific Report (2008) 167, 1 – 116), no metabolites of ecotoxicological concern were identified for birds and mammals. Thus, the risk assessments based on cymoxanil are considered to cover the toxicity of potential metabolites.

9.3.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Additionally, since formulation data is available to assess acute toxicity, a first-tier risk assessment performed with the acute formulation endpoint has been included to address combination toxicity.

9.3.2 Risk assessment for spray applications

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

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

FEL02 is proposed for 6 applications at a minimum interval of 7 days in potatoes at a dose rate of 3.0 kg formulation per ha (0.6 kg copper and 0.12 kg cymoxanil).

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

Copper

Table 9.3.2.1-1 First-tier assessment of the acute and long-term/reproductive risk of copper to mammals due to the use of FEL02 in potato

Intended use	**Potato**				
Active substance/product	Copper				
Application rate [g/ha]	6 × 0.6				
Acute toxicity [mg/kg bw]	162.6				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg bw/d]**	**TER_A**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	5.40		6.16	26.4
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	40.90		46.63	**3.5**
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	35.10		40.01	**4.1**
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.50	1.9	11.97	13.6
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	17.20		19.61	**8.3**
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”	5.20		5.93	27.4
Reprod. toxicity [mg/kg bw/d]	16				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	1.90		1.51	10.6
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	21.70		17.25	**0.9**
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	14.30	2.5 × 0.53	11.37	**1.4**
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.30		3.42	**4.7**
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	7.80		6.20	**2.6**
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”	2.30		1.83	8.8

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

As shown in the table above, the TERs for most scenarios are below the trigger of 10 or 5 for acute toxicity and long-term toxicity respectively. Therefore, a higher tier risk assessment is presented under point 9.3.2.2.

Cymoxanil

Table 9.3.2.1-2 First-tier assessment of the acute and long-term/reproductive risk of cymoxanil to mammals due to the use of FEL02 in potato

Intended use	**Potato**				
Active substance/product	Cymoxanil				
Application rate [g/ha]	6 × 120				
Acute toxicity [mg/kg bw]	760				
TER criterion	10				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg bw/d]**	**TER_A**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	5.40	1.9	1.23	617
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	40.90		9.33	81
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	35.10		8.00	95
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.50		2.39	317
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	17.20		3.92	194
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”	5.20		1.19	641
Reprod. toxicity [mg/kg bw/d]	10.5				
TER criterion	5				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	1.90	2.5 × 0.53	0.30	34.8
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	21.70		3.45	**3.0**
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	14.30		2.27	**4.6**
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.30		0.68	15.4
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	7.80		1.24	8.5
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”	2.30		0.37	28.7

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

As shown in the table above the acute TER values are all higher than the trigger of 10. The acute risk of cymoxanil as a result of applications with FEL02 according to the proposed GAP are therefore acceptable. In addition, for 4 of the scenarios for long term risk to mammals the TER value was higher than the trigger of 5, indicating an acceptable risk. However, a risk for small herbivorous mammals and for large herbivorous mammals could not be excluded. Therefore, a higher tier risk assessment for these groups is presented under point 9.3.2.2.

Combined toxicity

For the combined risk assessment of FEL02, a surrogate LD_{50} mix was estimated following the approach proposed in the EFSA GD (2009):

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(\text{a.s.}_i)}{LD_{50}(\text{a.s.}_i)} \right)^{-1}$$

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture; (please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

A comparison between the mixture toxicity and the toxicity of the active substances should be made to test whether there is a change in the predicted risk by using the modelled LC_{50} mix value instead of the measured LD_{50} of the a.s. To achieve a basis for this comparison, a `*tox per fraction*` quotient can be calculated for each active substance and can be compared to the corresponding quotient of the mixture.

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

If the `*tox per fraction a.s*` and the `*tox per fraction mixture*` deviate by < 10%, this indicates, that this active substance will contribute > 90 % to mixture toxicity and the impact of the other component is marginal. Consequently, the risk assessment can be performed for the most toxic component. Otherwise, the LC_{50} mix should be used in the risk assessment. In table 9.3.2.1-3 all input parameter needed to estimate the surrogate LD_{50} mix and the tox per fraction comparison are summarized for FEL02.

Table 9.3.2.1-3 Calculation of surrogate LD_{50} for the mixture of active substances

Active substance	Concentration a.s. in mixture [g/kg]	Fraction a.s. in mixture	LD_{50} a.s. [mg/kg bw/d]	Fraction a.s./ LD_{50} a.s.	Surrogate LD_{50} [mg/kg bw/d]	Tox per fraction (a.s.)	Deviation tox per fraction (a.s.) from the tox per fraction (mix) [%]	Experimental formulation endpoint [mg total a.s./kg bw/d]	MDR
Copper	200	0.83	162.6	0.00514	186.7	194.6	4.3%	480	2.6
Cymoxanil	40	0.17	760	0.00022		4560	2342%		

The MDR value (experimental formulation endpoint divided by the calculated surrogate endpoint) shows that the toxicity of the formulations is in agreement with the surrogate value based on concentration addition (value is between 0.2 and 5). In addition, the calculation above shows that copper contributes over 90% to the acute toxicity of the mixture. Therefore, the acute risk assessment for the active substance copper, covers that for the formulation. However, since formulation data is available to assess acute toxicity, a first-tier risk assessment performed with the acute formulation endpoint has been included to address combination toxicity.

The long-term combitox should be addressed via the concentration addition (CA) model. When for each substance the trigger values are equal, the combined TER value can be calculated according to:

TERcombi = 1/((1/TERsubstance 1)+(1/TERsubstance 2))

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

Table 9.3.2.1-4 First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of FEL02 in potato

Intended use	Potato (use group potato)				
Active substance/product	FEL02 (copper + cymoxanil)				
Application rate [g/ha]	6 × 3000 (600 + 120)				
Acute toxicity [mg/kg bw]	>2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg bw/d]**	**TER_A**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	5.40	1.9	30.78	>65
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	40.90		233.13	>9
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	35.10		200.07	>10
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.50		59.85	>33
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	17.20		98.04	>20
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”	5.20		29.64	>67
Reprod. toxicity [mg/kg bw/d]	No data available*				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**			**$TER_{LT\ combi}$***
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	1.90			8.1
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	21.70			**0.7**
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	14.30			**1.1**
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.30			**3.6**
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	7.80			**2.0**
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”	2.30			6.7

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

* No reproductive endpoint for FEL02 is available. A ter combi was determined with TER for individual active substances taken from tables 9.3.2.1-1 and -2, using the following formula: TERcombi = 1/((1/TERcopper)+(1/TERcymoxanil)).

As shown in the table above the acute TER values are all higher than the trigger of 10. The acute risk of FEL02 to mammals is therefore acceptable when the product is applied as proposed. In addition, for 2 of the scenarios for long term risk to mammals the TER value was higher than the trigger of 5, indicating an acceptable risk. However, a risk for small herbivorous mammals, large herbivorous mammals and small omnivorous mammals could not be excluded. Therefore, a higher tier risk assessment for these groups is presented under point 9.3.2.2.

9.3.2.2 Higher-tier risk assessment

Copper

A weight of evidence paper was submitted by the EUCuTF members for the renewal of approval of copper which provided evidence that owing to homeostatic control, the acute and long-term risks to all mammals is acceptable. The RMS/EFSA disagreed with this and considered that there was still a concern regarding the long-term risk to a

single generic focal species, the large herbivorous "lagomorph" (EFSA, 2018).

The agreed long-term endpoint for the mammalian risk assessment is a NOAEL of 16 mg Cu/kg bw/d (EFSA, 2018). Based on this agreed value and a TER trigger value of 5, the lowest Daily Dietary Dose (DDD) that would result in acceptable risks was determined to be 3.2 mg Cu/kg bw/d.

$$DDD = \frac{Toxicity}{TER} = \frac{16}{5} = 3.2$$

Utilizing an ETE approach for a rabbit with a FIR/bw = 0.50 (EFSA, 2009) and assuming a worst-case PT of 1, the required concentration of copper in diet that would result in acceptable risks (C) was calculated to be 6.4 mg/kg fresh diet:

$$DDD = ETE = \frac{FIR}{bw} * C * PT$$

$$\text{Therefore } C = \frac{ETE}{FIR/_{bw}} * \frac{1}{PT} = \frac{3.2}{0.50} * \frac{1}{1} = 6.4$$

In a study to assess the effect of dietary copper addition on lipid metabolism in rabbits (Lei et al., 2017) the authors showed that basal control diet containing 8.19 mg Cu/kg had no adverse effect on rabbits over a 30-Day study period, and supplementation of this basal diet with concentrations up to 45 mg Cu/kg had no significant effect on food intake or resulted in any treatment related mortalities. Copper et al. (1996) reports a typical copper supplementation of 100 ppm copper in the diet of rabbits held in a commercial fryer ranch and one would assume that for the purposes of breeding, this diet must be suitable. EFSA (2016)[9] also documents the Maximum Tolerable Level (MTL) for rabbits to be 500 mg Cu/kg diet based on two studies in which rabbits "received 500 mg Cu/kg diet for up to 32 days without adverse effects on growth performance".

It should also be noted that copper residues after treatment in carrots (STMR 0.87 mg/kg), lettuce (STMR 22.75 mg/kg), leek (STMR 10.98 mg/kg) or fresh beans (STMR 2.52 mg/kg) are all well below the values fed in these studies.

This data indicates that the model for assessing the risks of dietary intake of copper are not appropriate since unacceptable risks are concluded at concentrations that are significantly lower than those that have been assessed in the literature, and at concentrations that are also significantly lower than the EFSA maximum authorised content of copper in rabbit feed.

Therefore, it is considered that the risks to the lagomorph are acceptable and when EFSA publishes its guidance documents on the assessment of risk to mammals from exposure to metals, this assessment will be updated.

Cymoxanil

A risk for small herbivorous mammals and for large herbivorous mammals could not be excluded in the Tier I assessment. Therefore, a higher tier risk assessment for these groups is performed below based on available data on the dissipation of cymoxanil in vegetation. For cymoxanil, the DAR contains information about residue decline in lettuce and potatoes. The data show that a DT_{50} of 2 days can be considered a worst-case assumption for the residue calculation in vegetation. A MAFxTWA of 0.412 was calculated using a moving time frame approach[10].

[9] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the revision of the currently authorised maximum copper content in complete feed. EFSA Journal 2016;14(8):4563, 100 pp. doi:10.2903/j.efsa.2016.4563

[10] Using 'Calculation tool: Moving time window determination (Belgium, v.2)' downloaded from https://fytoweb.be/nl/handleiding/gewasbescherming

Table 9.3.2.2-1 Refined assessment of the long-term/reproductive risk of cymoxanil to mammals due to the use of FEL02 in potato

Intended use	**Potato**				
Active substance/product	Cymoxanil				
Application rate [g/ha]	6 × 120				
Reprod. toxicity [mg/kg bw/d]	10.5				
TER criterion	5				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"	21.70	0.412*	1.07	9.8
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph"	14.30		0.71	14.9

* determined using a moving time frame approach; 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.

As shown in the table above the TER value are higher than the trigger of 5, indicating that the long-term risk to herbivorous mammals is acceptable when the rapid dissipation of cymoxanil in vegetation is taken into account.

FEL02 / combination toxicity

As part of the Tier 1 assessment for combined acute toxicity of copper and cymoxanil it was shown that copper contributes >90% to the acute toxicity of the mixture. In line with the approach as described for the surrogate LD_{50} under point 9.2.2.1, the TER based on the reproductive endpoints for individual active substances can be compared with the reproductive TERcombi.

In Table 9.3.2.2-2 below the Tier 1 TERcombi and the difference with the Tier 1 TER for the individual active substances is presented. Based on that, copper also contributes to the majority of the reproductive TERcombi of the mixture but not >90%. Therefore, the comparison was refined using the refined DT_{50} of 2 days available for cymoxanil in plant material as described on page 24 above. For herbivores mammals a DT_{50} of 2 for cymoxanil was considered for the whole diet, while a DT_{50} of 10 was considered for copper for the whole diet. According to EFSA 2009, the diet of the omnivorous mouse consists of 25% weeds, 50% weed seeds and 25% ground arthropods. For cymoxanil, a DT_{50} of 2 days in weeds and weed seeds, and a DT_{50} of 10 days in arthropods is assumed and for copper a DT_{50} of 10 days in the whole diet is considered. In Tables 9.3.2.2-3 and -4 the refined TER combi and the difference with the TER for the individual active substances is presented for herbivorous mammals and omnivorous mammals respectively.

Table 9.3.2.2-2 Comparison of the long-term/reproductive risk of FEL02 to mammals due to the use in potato, to that of copper and cymoxanil individually.

Copper Reprod. toxicity 16 mg/kg bw/d						
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**	**Difference with TERcombi [%]**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	1.90	2.5 × 0.53	1.51	10.6	31
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	21.70		17.25	**0.9**	29
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	14.30		11.37	**1.4**	27
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.30		3.42	**4.7**	30
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	7.80		6.20	**2.6**	30
Cymoxanil Reprod. toxicity 10.5 mg/kg bw/d						
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	1.90	2.5 × 0.53	0.30	34.8	330
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	21.70		3.45	**3.0**	329
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	14.30		2.27	**4.6**	318
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	4.30		0.68	15.4	328
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	7.80		1.24	8.5	325

Combination of cymoxanil and copper		
Crop scenario Growth stage	**Indicator/generic focal species**	**$TER_{LT\ combi*}$**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	8.1
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	**0.7**
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	**1.1**
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	**3.6**
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse”	**2.0**

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.

* No reproductive endpoint for FEL02 is available. A ter combi was determined with TER for individual active substances taken from tables 9.3.2.1-1 and -2, using the following formula: TERcombi = 1/((1/TERcopper)+(1/TERcymoxanil)).

Table 9.3.2.2-3 Refined comparison of the long-term/reproductive risk of FEL02 to herbivorous mammals due to the use in potato, to that of copper and cymoxanil individually.

Copper Reprod. toxicity 16 mg/kg bw/d						
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**$MAF_m \times TWA$**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**	**Difference with TERcombi [%]**
			1.864*			
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"	21.70		24.3	**0.66**	6.7
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph"	14.30		16.0	**1.0**	
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"	4.30		4.81	**3.3**	
Cymoxanil Reprod. toxicity 10.5 mg/kg bw/d						
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"	21.70	0.412**	1.07	**9.8**	1484
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph"	14.30		0.707	**15**	
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"	4.30		0.212	**49**	

Combination of cymoxanil and copper		
Crop scenario Growth stage	**Indicator/generic focal species**	**$TER_{LT\ combi\#}$**
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"	**0.62**
Potatoes BBCH 10 - 40	Large herbivorous mammal "lagomorph"	**0.93**
Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"	**3.1**

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
* Determined with moving time frame, Dt50 = 10 days
** Determined with moving time frame, DT50 = 2 days
# No reproductive endpoint for FEL02 is available. A ter combi was determined using the following formula: TERcombi = 1/((1/TERcopper)+(1/TERcymoxanil)).

Table 9.3.2.2-4 Refined comparison of the long-term/reproductive risk of FEL02 to <u>omnivorous mammals</u> due to the use in potato, to that of copper and cymoxanil individually.

Copper Reprod. toxicity 16 mg/kg bw/d									
Crop scenario Growth stage	**Indicator/generic focal species**	Diet	**FIR/BW**	**Mean RUD**	**Deposition**	**MAF_m × TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**	**Difference with TERcombi [%]**
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse"	25% weeds 50% weed seeds 25% ground arthropods	0.27	29.2	1	1.864*	8.8	**1.8**	12
Cymoxanil Reprod. toxicity 10.5 mg/kg bw/d									
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse"	25% weeds 50% weed seeds 50% weed seeds	0.27	29.2	1	0.412** 0.412** 1.864*	0.73	14.3	789
Combination of cymoxanil and copper									
Crop scenario Growth stage		**Indicator/generic focal species**						**$TER_{LT\ combi\#}$**	
Potatoes BBCH 10 - 39		Small omnivorous mammal "mouse"						**1.6**	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio, TER values shown in bold fall below the relevant trigger
* Determined with moving time frame, DT50 = 10 days
** Determined with moving time frame, DT50 = 2 days
# No reproductive endpoint for FEL02 is available. A TER combi was determined using the following formula: TERcombi = 1/((1/TERcopper)+(1/TERcymoxanil)).

Based on the comparison of the TERcombi and the TER of copper and cymoxanil separately in Table 9.3.2.2-3 above, copper contributes over 90% to the reproductive risk of the mixture to mammals, when based on the diet of herbivorous mammals and taking into account a DT_{50} of 2 days for cymoxanil in vegetation. Therefore, the long-term risk assessment for the individual active substances cymoxanil and copper, cover that of the formulation. As the risk for the active substances has been shown to be acceptable, the risk of the combination of the active substances is acceptable as well.

The tier 1 and higher tier risk assessment for cymoxanil indicate an acceptable risk to small omnivorous mammals for cymoxanil. As shown above in Table 9.3.2.2 4 copper contributes about 88% to the long-term TERcombi of FEL02 to small omnivorous mammals. This is assuming a DT_{50} of 10 days in arthropods, even though this value is likely lower based on the DT_{50} values in vegetation (2 days), water (hydrolysis 1.1 days at pH 7 at 20 °C; 0.3 days in water sediment system at 20 °C) and soil (0.2-7.3 days). Although copper contributes to slightly less than 90% of the toxicity to the omnivorous mouse based on its diet in the above calculation, it is clear that copper is the driver of the toxicity of FEL02. According to the EFSA review the risk of copper to mammals other than herbivorous and frugivorous mammals is safe when no more than 5 kg cu/ha is applied. The proposed GAP will lead to a maximum of 3.6 kg cu/ha per season, and only a limited number of applications is possible in the period between BBCH 20-40 in potatoes when the risk to the omnivorous mouse is highest due to low crop cover. Taking into account the margin of safety for copper due to the relatively low seasonal application rate and the fact that copper drives the long-term toxicity also for omnivorous mouse, the risk of the combined exposure of omnivorous mammals to both active substances as a result of applications with FEL02 according to the proposed GAP is considered acceptable as well.

zRMS comments:

Copper

For copper, the applicant based his argumentation only upon the EFSA conclusion for copper (2018) and the RAR for copper. The acute and long-term TER values for copper are below the relevant trigger values at screening step and at Tier 1 for most of the scenarios, according to the use pattern of the product **Cuprofix C.** The Applicant submitted a weight of evidence (WoE) approach: according to the information on RAR, EFSA conclusion-2018 and Final Renewal report SANTE/10506/2018. During the renewal of copper hydroxide the RMS-France concluded the following: "*A weight-of-evidence based approach to refine the mammals risk assessments is submitted. Together with the studies of Schabacker, J. and Rastall, A. 2009 a & b the effects of copper exposure on wild life is studied. The RMS considers that the literature review provided by the notifier (EUCuTF) gives evidence of homeostatic mechanisms for mammals. Theoretical acute and long-term dietary exposure of shrew and vole observed in the papers performed by Hunter et al. (1987a, b; 1989) is much higher than the one calculated for a standard application rate of copper in vineyard and tomato crops. Thus, the acute and the long-term risk to mammals due to copper exposure can be considered acceptable for the small herbivorous mammal "vole" and the small insectivorous mammal "shrew".* Further, according to EFSA Conclusion 2018, literature review provides evidence of homeostatic mechanisms, and allows concluding to acceptable long-term risks based on weight of evidence except for large herbivorous. Therefore, based on this conclusion further refinement is required at MSs level for all proposed uses for large herbivorous mammals. ZRMS-PL is of the same opinion as RMS in RAR revised, and taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on *more than a realistic worst case scenario*, the WoE approach could be used to conclude acceptable risk at the dose rate requested until the existence of an accepted guidance document. **The final decision should be considered at MSs level.**

Cymoxanil

The acute TER values are all higher than the trigger of 10. The acute risk of cymoxanil as a result of applications with CUPROFIX C according to the proposed GAP are therefore acceptable. In addition, for 4 of the scenarios for long term risk to mammals the TER value was higher than the trigger of 5, indicating an acceptable risk. However, a risk for small herbivorous mammals and for large herbivorous mammals could not be excluded. Therefore, a higher tier risk assessment for these groups is necessary. The long-term risk assessment was accepted by zRMS based on the DT_{50} of 2 days. The TER value are higher than the trigger of 5, indicating that the long-term risk to herbivorous mammals is acceptable when the rapid dissipation of cymoxanil in vegetation is taken into account.

Combined toxicity
zRMS point out that that copper contributes to more than 90 % to the mixture toxicity. Hence, it is considered acceptable to conduct the risk assessment for the active substances alone.

Updated 04.2024r.
According to the AT comment the presented approach by the applicant to use a combined risk assessment like it is presented in the birds and mammals GD cannot be accepted. However, in this case copper clearly driving the risk assessment due to its high toxicity compared to cymoxanil. In this context, a risk assessment for copper is relevant and cover risk assessment for formulation. In our opinion combined risk assessment is not required in this case. The final decision should be considered by MSs level.

~~All TER_{combi} are below the trigger of 5 for long-term toxicity for mammals. The refinement for TER_{combi} for long-term toxicity should be considered by MSs level.~~

The final decision should be considered at MSs level.

Zonal-RMS comments
9.2

Updated 04.2024

According to the information provided and information from RAR, zonal-RMS opinion is as well the risk could be considered acceptable for the shrew and the vole (acute and long-term) and for the mouse (long-term). However, no more data has been made available for the generic focal species large herbivorous “lagomorph” and frugivorous. Taking into account these considerations, zonal-RMS has performed calculations for the risk assessment for **large herbivorous mammal**, following the GD EFSA Journal 2009; 7(12): 1438, considering the MAF and a TWA of 1 (according to the conclusions of the Pesticides Peer Review Meeting 169) and considering single maximum annual application. The following table shows the calculations for the critical doses (bold results are below the trigger):

Copper

First-tier assessment of the acute and long-term/reproductive risk of copper to mammals due to the use of FEL02 in potato

Intended use	**Potato**
Active substance/product	Copper
Application rate [g/ha]	3600
Acute toxicity [mg/kg bw]	162.6
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg/kg bw/d]**	**TER_A**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”	5.40	1	19.44	**8.36**
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole”	40.90		147.24	**1.1**
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph”	35.10		126.36	**1.3**
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”	10.50		37.8	**4.3**

Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse"	17.20		61.92	**2.63**
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse"	5.20		18.72	**8.69**

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.

Long-term/reproductive risk for mammals (NOAEL = 16 mg/kg bw/d)

Intended use	**Potato**
Active sub-stance/product	Copper
Application rate [g/ha]	3600
Reprod. toxicity [mg/kg bw/d]	16
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**$MAF_m \times TWA$**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew"	1.90	1	6.84	**2.3**
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"	21.70		78.12	**0.2**
Potatoes BBCH 10 - 40	Large herbivorous mammal "lag-omorph"	14.30		51.48	**0.3**
Potatoes BBCH ≥ 40	Large herbivorous mammal "lag-omorph"	4.30		15.48	**1.03**
Potatoes BBCH 10 - 39	Small omnivorous mammal "mouse"	7.80		28.08	**0.57**
Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse"	2.30		8.28	**1.93**

The acute and long-term TER values for copper are below the relevant trigger values at Tier 1 for most of the scenarios, according to the use pattern of the product **Cuprofix C** considering the MAF and a TWA of 1 (according to the conclusions of the Pesticides Peer Review Meeting 169) and considering single maximum annual application.

Conclusion:
According to the Review Report of copper compounds:

"The conclusions are based on assessment at first tier where worst case conditions were applied and possibilities for refinement have not yet been investigated. Hence, considering also that copper is a micronutrient, this final conclusion might be overly negative. Indeed, experts considerations which are also therein reported as follows indicate a weight of evidence approach could be applied: "concluded that the weight of evidence could be considered acceptable for addressing the long term risk to birds and mammals for application of up to 5 Kg copper/ha but further data would be needed to draw a conclusion covering all feeding guild categories". To note also that from available studies there is indication of avoidance of copper contaminated food items, avoidance which has not been applied in refinement of risk. Indeed, as

	indicated in the EFSA list of endpoints: "a literature review provides a weight of evidence approach concluding to acceptable risks to birds for doses of 5 kg copper/ha/year, for granivorous and insectivorous birds." On mammals, "a literature review provides evidence of homeostatic mechanisms, and allows concluding to acceptable long-term risks based on weight of evidence except for large herbivorous." No additional information has been provided to address the data gap from EFSA conclusion-2018 (EFSA Journal 2018; 16(1):5152). Thus, further information for the acute and long-term risk to large herbivorous mammal would have been provided. Zonal-RMS is of the same opinion as RMS in RAR revised, and taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on more than a realistic worst case scenario, the WoE approach could be used to conclude acceptable risk at the dose rate requested until the existence of an accepted guidance document. According to the information provided and information from RAR, the risk to mammals via consumption of contaminated water or via secondary poisoning is considered to be acceptable. *The experts concluded that the WoE could be considered acceptable for addressing the long-term risk to birds and mammals for application rate up to 5 kg a.s./ha for granivorous and insectivorus birds; however, further data were considered necessary to draw a conclusion covering all the feeding guild categories, i.e. omnivorous and frugivorous birds and large herbivorous and frugivorous mammals (data gap). By generating further data, the experts considered it useful to focus on, e.g. further investigation of the avoidance and further data on residue in food items. Therefore, based on this conclusion further refinement is required at MSs level for omnivorous and frugivorous birds for all proposed uses for copper hydroxide depended on own indicator focal species. ZRMS-PL is of the same opinion as RMS in RAR revised and, taking into account all the available data and due to the absence of an adapted guide to evaluate elements such as copper and that the conclusions were based on more than a realistic worst case scenario, this WoE approach could be used to conclude acceptable risk at dose requested (maximum annual application rate of 4 kg Cu/ha) until the existence of an accepted guidance document.* **According to the zRMS comments, the first-tier risk assessment indicates to high acute and chronic risk for mammals for all representative uses of FEL02. Thus, a higher-tier risk assessment was required for both, birds and mammals. However, the data submitted for the higher-tier risk assessment (here: weight-of-evidence approach) did not provide substantial additional evidence to those already provided in the EU reports. Thus, in our opinion, the conclusions of those EU reports should be retained and Member states should pay particular attention to their national conditions for the national assessment considering the the relevance of the generic focal species large herbivorous "lagomorph" (acute and long-term risk assessment) and the relevance of the omnivorous birds (the acute and long-term risk assessment) would have to be provided.** ***The final decision should be considered at MSs level.***
Agreed endpoints	Acute toxicity endpoint: 162.6 (mg/kg bw). Reproduction toxicity endpoint: 16 (mg/kg bw/d).

9.3.2.3 Drinking water exposure

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).

A K_{oc} value for copper, as a measure of its absorbance to soil, is not scientifically relevant as it is a metal. Instead, the lowest K_{doc} value will be used, 19509.9 mL/g (EFSA Conclusion, 2018).

The K_{oc} of Cymoxanil is 43.6 mL/g (EFSA conclusion 2008).

Table 9.3.2.3-1 Application rate to endpoint ratios for birds exposed to Copper and Cymoxanil for the intended use of FEL02 in potatoes

Crop	Exposure Scenario	Effective Application Rate [g a.s./ha]*	K_{doc}/ Koc [mL/g]	LD_{50} / NOEL [mg ai/kg bw]	Ratio Application Rate:endpoint	Trigger
Potatoes	**Copper**					
	Acute	1500	19509.9	162.6	9.2	3000
	Long-term			516	2.9	3000
	Cymoxanil					
	Acute	300	43.6	760	0.39	50
	Long-term			10.5	29	50

* Effective application rate = application rate multiplied by mean MAF

Since for copper and cymoxanil the ratio of the effective application rate and the relevant endpoint are well below 3000 and 50 respectively, no specific calculations of exposure and TER are necessary. Since there is a large margin of safety and taking into account the fast degradation of cymoxanil (DT_{50} of 0.3 days in surface water and 1.3 days in soil (EFSA 2008)), the risk of combined exposure to both actives via drinking water resulting from the proposed use of FEL02 in potatoes is considered acceptable as well and no specific calculations of exposure and TERcombi are necessary.

zRMS comments: Agreed. Since for copper and cymoxanil the ratio of the effective application rate and the relevant endpoint are well below 3000 and 50 respectively, no specific calculations of exposure and TER are necessary.

9.3.2.4 Effects of secondary poisoning

According to the Guidance Document on Risk Assessment for Birds and Mammals (EFSA, 2009), substances with a log $P_{ow} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

A partition coefficient (Log P_{ow}) is not relevant to copper as it is a metal, however, an estimated log P_{ow} value of 2.78 can be determined by the ratio of the water and n-octanol solubilities (EFSA Conclusion, 2018).

The estimated log P_{ow} value of copper is < 3 and the log P_{ow} of cymoxanil is 0.67, the active substances are not considered to accumulate in the food chain and therefore a risk assessment for effects due to secondary poisoning is not required.

No experimentally derived log P_{ow} values are available from the DAR for the metabolites of Cymoxanil. However, the RMS estimated log P_{ow} values with the software KOWWIN (US-EPA) for the metabolites INKQ960, IN-U3204, IN-T4226, IN-JX915, IN-R3273 and IN-KP533 and IN-W3595. For the metabolites IN-KQ960, IN-U3204, IN-T4226, IN-JX915, IN-R3273 and IN-KP533 the modelled log P_{ow} values were below the trigger of 3 and hence no bioaccumulation was expected. For IN W3595 a log P_{ow} of 4.27 was derived with KOWWIN. IN-W3595 is an organic acid, and the software estimates the log P_{ow} for the non-dissociated form of the acid. However, in aquatic environments the substance was partly dissociated, and it was shown to be highly water soluble. Taking into account this information, it was considered unlikely to accumulate in fat tissue. Overall, it can be concluded that the risk of secondary poisoning with metabolites of cymoxanil is acceptable.

zRMS comments: Agreed.

Copper

A partition coefficient (Log Pow) is not relevant to copper as it is a metal, however, an estimated log Pow value of 2.78 can be determined by the ratio of the water and n-octanol solubilities (EFSA Journal 2018;16(1):5152). Therefore, it does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Cymoxanil

Cymoxanil has a log P_{OW} of 0.67. As the log P_{OW} values are below the trigger of 3, it was not necessary to consider the risk from secondary poisoning further. Therefore, based on the low log P_{OW} values the risk from bioaccumulation to fish-eating and worm-eating birds is negligible.

9.3.2.5 Biomagnification in terrestrial food chains

A literature review that was submitted for the renewal of approval of copper compounds provided evidence of a lack of bioaccumulation in the food chain (EFSA Conclusion, 2018).

zRMS comments: Agreed.
According to EFSA conclusion (EFSA Journal 2018;16(1):5152), a literature review provides evidence of lack of bioaccumulation in aquatic food chain.
A low potential for cymoxanil in animal tissue was concluded in the EU reviews (EFSA 2008).

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

Not relevant since FEL02 is not intended to be used as a bait, pellet, granule, prill or for treating seed.

9.3.4 Overall conclusions

The risks to mammals form the use of copper is acceptable.

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

A literature review that was submitted for the renewal of approval of copper compounds (EFSA Conclusion, 2018) provided evidence of a range of median lethal or effective concentrations for amphibians from 19.5 to 180 µg Cu/L while the lowest value that caused significant effects to an amphibian (toad) was 4.25 µg/L (measured concentrations). A NOAL value of 283.3 mg/kg soil (mean measured concentrations) was identified. Given the extremely high NOAL value, it is considered that the literature review data provides sufficient evidence of a lack of risk to other terrestrial wildlife (reptiles and amphibians).

No data on reptiles and terrestrial amphibians are available for cymoxanil.

zRMS comments: Agreed.

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 for a broad range of Copper compounds. 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).

Studies on the toxicity of Cymoxanil and four aquatic metabolites (IN-T4226, IN-KQ960, IN-U3204, IN-W3595) were carried out for the approval of the active substance and are provided in the EU DAR. Agreed endpoints were taken from the EFSA Conclusion (2008).

Effects on aquatic organisms of FEL02 were not evaluated as part of the EU assessment of copper or cymoxanil. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2. Additional toxicity data for FEL02 have been generated for fish, daphnia and algae. FEL02 is not more toxic than the active substances based on available acute toxicity data for FEL02 and the active substances.

Table 9.5.1-1 Endpoints and effect values relevant for the risk assessment for aquatic organisms – copper compounds

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Copper oxide	96 h, f	Mortality, LC_{50} = 0.207 mg a.s./L (total Cu) $_{mm}$ LC_{50} = 0.0344 mg a.s./L (dissolved Cu) $_{mm}$	EFSA Conclusion (2018)
Acipenser transmontanus	Copper sulphate	14 d; 28 d; 53 d, f	53-d EC_{10} (growth) = 0.00112 mg a.s./L (dissolved Cu)	EFSA Conclusion (2018)
Daphnia magna	Copper hydroxide	48 h, s	LC_{50} (mortality) = 0.0266 mg a.s./L (dissolved Cu) $_{mm}$	EFSA Conclusion (2018)
Daphnia magna	Copper oxychloride	21 d, ss	NOEC (reproduction) = 0.0076 mg a.s./L, $_{geometric\ mm}$	EFSA Conclusion (2018)
Chironomus riparius	Tribasic copper sulphate	28 d, s, spiked sediment	NOEC = 0.50 mg a.s./L (total Cu) $_{nom}$	EFSA Conclusion (2018)
Tubifex tubifex	Copper chloride	28 d, ss, spiked sediment	NOEC (reproduction, growth) = 16.17 mg a.s./kg dry weight normalized to 2.5% OC	EFSA Conclusion (2018)
S. capricornutum	Copper hydroxide WP	72 h, s	Growth rate: E_rC_{50} = 0.02229 mg a.s./L (total Cu) $_{nom}$	EFSA Conclusion (2018)
Higher-tier studies (micro- or mesocosm studies)				
Indoor microcosm study	Copper hydroxide WP	6 applications at 10-d intervals followed by 250 days of monitoring	NOEC = 0.0048 mg a.s./L (dissolved Cu) $_{mm}$ (AF = 2 applied)	EFSA Conclusion (2018)

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 9.5.1-2 Endpoints and effect values relevant for the risk assessment for aquatic organisms – Cymoxanil

Species	Substance	Exposure System	Results	Reference
Lepomis macrochirus	Cymoxanil	96 h, s	LC_{50} = 29 mg a.s./L $_{mm}$ NOEC = 17 mg a.s./L $_{mm}$	EFSA Conclusion (2008)
Oncorhynchus mykiss	Cymoxanil	90 d, f	NOAEC = 0.044 mg a.s./L $_{mm}$ [a]	EFSA Conclusion (2008)
Daphnia magna	Cymoxanil	48 h, s	EC_{50} (immobility) = 27 mg a.s./L $_{mm}$ NOEC = 15 mg a.s./L $_{mm}$	EFSA Conclusion (2008)
Daphnia magna	Cymoxanil	21 d, ss	NOEC (reproduction) = 0.067 mg a.s./L $_{mm}$	EFSA Conclusion (2008)
Anabaena flos-aquae	Cymoxanil	96 h, s	E_rC_{50} = 0.254 mg a.s./L $_{im}$ NOE_rC = 0.0652 mg a.s./L $_{im}$ E_bC_{50} = 0.122 mg a.s./L $_{im}$ NOE_bC = 0.034 mg a.s./L $_{im}$	EFSA Conclusion (2008)
Lemna gibba	Cymoxanil	14 d, s	E_rC_{50} = >0.7 mg a.s./L $_{im}$ NOE_rC = 0.7 mg a.s./L $_{im}$ E_bC_{50} = >0.7 mg a.s./L $_{im}$ NOE_bC = 0.7 mg a.s./L $_{im}$	EFSA Conclusion (2008)
Higher-tier studies (micro- or mesocosm studies)				
None				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations
a: c This value is a NOAEC. The NOAEC was the lowest relevant endpoint from three studies. No NOEC could be derived as effects were seen at the lowest concentration. However, these effects were not considered relevant.

Table 9.5.1-3 Endpoints and effect values relevant for the risk assessment for aquatic organisms – Cymoxanil metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	IN-T4226	96 h, ss limit test	LC_{50} > 111 mg/L $_{mm}$ NOEC = 111 mg/L $_{mm}$	EFSA Conclusion (2008)
Oncorhynchus mykiss	IN-KQ960	96 h, s limit test	LC_{50} > 120 mg/L $_{nom}$ NOEC = 120 mg/L $_{nom}$	EFSA Conclusion (2008)
Oncorhynchus mykiss	IN-U3204	96 h, ss limit test	LC_{50} > 97 mg/L $_{mm}$ NOEC = 97 mg/L $_{mm}$	EFSA Conclusion (2008)
Oncorhynchus mykiss	IN-W3595	96 h, s limit test	LC_{50} > 130 mg/L $_{mm}$ NOEC = 130 mg/L $_{mm}$	EFSA Conclusion (2008)
Daphnia magna	IN-T4226	48 h, ss	EC_{50} (immobility) > 116 mg/L	EFSA Conclusion

Species	Substance	Exposure System	Results	Reference
			$_{mm}$ NOEC = 116 mg/L $_{mm}$	(2008)
Daphnia magna	IN-KQ960	48 h, s	EC_{50} (immobility) = 0.8 mg/L $_{mm}$ NOEC = n.d.	EFSA Conclusion (2008)
Daphnia magna	IN-KQ960	21 d, ss	NOEC (reproduction) = 0.302 mg/L $_{mm}$	EFSA Conclusion (2008)
Daphnia magna	IN-U3204	48 h, ss	EC_{50} (immobility) = 100 mg/L $_{mm}$ NOEC = 53 mg/L $_{mm}$	EFSA Conclusion (2008)
Daphnia magna	IN-W3595	48 h, s	EC_{50} (immobility) > 126 mg/L $_{mm}$ NOEC = 126 mg/L $_{mm}$	EFSA Conclusion (2008)
Anabaena flos-aquae	IN-T4226	96 h, s	E_rC_{50} = 35.9 mg/L $_{nom}$ NOE_rC = 20 mg/L $_{nom}$ E_bC_{50} = 25.8 mg/L $_{nom}$ NOE_bC = 20 mg/L $_{nom}$	EFSA Conclusion (2008)
Anabaena flos-aquae	IN-W3595	96 h, s	E_rC_{50} = 19.9 mg/L $_{nom}$ NOE_rC = 5 mg/L $_{nom}$ E_bC_{50} = 12.7 mg/L $_{nom}$ NOE_bC = 5 mg/L $_{nom}$	EFSA Conclusion (2008)
Higher-tier studies (micro- or mesocosm studies)				
None				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations; n.d.: not derived

Table 9.5.1-4 Endpoints and effect values relevant for the risk assessment for aquatic organisms –FEL02

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Cuprofix C Disperss (FEL02)	96 h, s	LC_{50} = 47 mg prod./L $_{nom}$ LC_{50} = 6.7 mg Cu/L $_{mm}$ LC_{50} = 1.5 mg Cym/L $_{im}$	[redacted]
Daphnia magna	Cuprofix C Disperss (FEL02)	48 h, s	EC_{50} = 0.35 mg prod./L $_{nom}$ EC_{50} = 0.062 mg Cu/L $_{mm}$ EC_{50} = 8.7 µg Cym/L $_{im}$	Hutchinson, Sharpe, 2012b KCP 10.2.1/02
Pseudokirchneriella subcapitata	Cuprofix C Disperss (FEL02)	72 h, s	E_rC_{50} = 0.22 mg prod./L $_{nom}$ E_rC_{50} = 0.023 mg Cu/L $_{mm}$ E_rC_{50} = 3.7 µg Cym/L $_{im}$ NOE_rC = 0.16 mg prod./L $_{nom}$ E_yC_{50} = 0.049 mg prod./L $_{nom}$ E_yC_{50} = 0.008 mg Cu/L $_{mm}$ E_yC_{50} = 1.1 µg Cym/L $_{im}$ NOE_yC = 0.025 mg prod./L $_{nom}$	Hutchinson, Sharpe, 2012c KCP 10.2.1/03
Higher-tier studies (micro- or mesocosm studies)				
None				

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

9.5.1.1 Justification for new endpoints

9.5.1.1.1 Use of Biotic Ligand Model - copper

Both EFSA and the RMS rejected all novel methods proposed by the notifier for assessing the exposure and risks from the use of copper to aquatic organisms, stating that the approval review process is not the correct forum for such an assessment. All the details requested regarding the Biotic Ligand Model have been provided and this method has been successfully applied for REACH and BPR dossiers. In addition, further data was provided in two dossier updates in April 2016, where the SSD derivation was explained and a link to the Cu-VRAR (2008) was provided (see http://echa.europa.eu/nl/copper-voluntary-risk-assessment-reports) regarding the Biotic Ligand Model; and in July 2017, with a detailed explanation on how the toxicity data was normalised for bioavailability using the Biotic Ligand Model, from which realistic endpoints were derived. The applicant insists that without such normalisation to take into account the bioavailability of copper in different water bodies, the resulting endpoint would be meaningless. Indeed, neglecting the bioavailability could also result in under-protective effect thresholds for highly vulnerable media (with high Cu bioavailability) when the media used for toxicity testing do not adequately cover such scenarios.

Following EFSA comments, a position paper has been developed (Van Sprang, 2019) which provides additional detail on the update of bioavailability models for copper and provides realistic endpoints for copper. This position paper is summarized below and based on the conclusions of this position paper, while awaiting the copper GD, the EUCuTF members will continue to use the BLM approach unless different methodology appropriate for data normalisation is provided by MS.

Reference:	**KCP 10.2/01, Van Sprang, P., 2019**
Title:	Response to EFSA comments on the aquatic effects assessment for Cu - extension
Report No.:	Not applicable
Guidelines:	Not applicable
Deviations:	Not applicable
GLP:	No
Published	No
Comment:	-

Executive Summary

During the past years, biotic ligand models (BLM) have increasingly been used to account for the influence of water chemistry variables (e.g., pH, water hardness and dissolved organic carbon, DOC) in the evaluation of ecological risks of copper in surface waters. For instance, copper BLMs have been implemented to derive predicted no effect concentrations (PNEC) in the risk assessments performed in the European Union (EU) (ECI 2008). However, recently optimizations of the Cu bioavailability models have been proposed through the use of generalized bioavailability models (gBAMs) (Van Regenmortel et al. 2015, De Schamphelaere 2018). gBAMs are an alternative to the existing BLMs to predict chronic effect concentrations for copper towards freshwater organisms. The main difference between both models is that in a gBAM the effect of pH on metal toxicity is incorporated as a log-linear relation between pH and free Me^{2+} toxicity, while in a traditional BLM the effect of pH is modelled via a linear relation between pH and free Me^{2+} toxicity (parametrised via the biotic ligand stability constant; $K_{H\text{-}BL}$). Hence, gBAMs may account for other factors that determine the effect of pH on Me^{2+} toxicity besides the competitive effect of H^+ at the biotic ligand site. At the moment, chronic Cu gBAMs are available for four taxonomic groups: algae (De Schamphelaere & Janssen 2006), the crustacean *Daphnia magna* (Van Regenmortel et al. 2015), fish (De Schamphelaere 2018) and the higher plant *Lemna minor*.

Currently, the PNEC derivation for Cu includes traditional BLMs, except for algae, for which a gBAM is used. All current bioavailability models are used in combination with WHAM V as speciation program. However, WHAM V is not always practical in use and is not available online anymore. WHAM VII is the most recent version of the Windermere Humic Aqueous Model and is more user-friendly compared to WHAM V. WHAM VII incorporates the improved Humic-Ion binding model VII (Tipping et al. 2011). It was shown that free metal ion activity in natural waters could be calculated rather accurately using WHAM VII (Lofts & Tipping 2011). Hence, WHAM VII can be considered as the most appropriate speciation software to model metal speciation. While the chronic gBAMs for fish (De Schamphelaere 2018) and *L. minor* were directly developed in combination with WHAM VII, the chronic Cu gBAMs for *D. magna* (Van Regenmortel et al. 2015) and algae (De Schamphelaere & Janssen 2006) have been originally developed in combination with WHAM V. However, Van Regenmortel (2017) recently evaluated the predictive performance of the *D. magna* and algae gBAMs in combination with WHAM VII.

The present report summarizes all available information underlying the update of the Cu bioavailability normalization procedure to the $gBAM_{WHAMVII}$-approach.

Overall, the chronic Cu gBAMs for *D. magna* and algae performed relatively well when the models were calibrated on metal speciation calculated with WHAM VII. A bioavailability model is generally accepted to be sufficiently accurate if the majority of ECx_{Mediss} of an independent dataset is predicted within 2-fold error (Di Toro et al. 2001; De Schamphelaere & Janssen 2006; Van Regenmortel et al. 2015), this was the case for both gBAMs. Additionally, the prediction performance in WHAM VII approached those in the original publications reported for the original gBAMs calibrated with WHAM V (De Schamphelaere & Janssen 2006; Van Regenmortel et al. 2015).

The Fish $gBAM_{WHAMVII}$ developed by De Schamphelaere (2018) based on juvenile rainbow trout has also been successfully extrapolated to early life stages of fathead minnow and rainbow trout. The available evidence suggests that at least the pH effect on Cu toxicity of the Fish $gBAM_{WHAMVII}$ can be extrapolated to early life stage toxicity data for rainbow trout and fathead minnow. However, because of the limited available bioavailability data for fish it is difficult to evaluate the cross-species and cross-lifestage applicability of the protective effects of other competing ions on early life stage Cu toxicity.

The *L. minor* $gBAM_{WHAMVII}$ predicts chronic Cu toxicity to *L. minor* for three endpoints relatively accurately, but a validation with an independent dataset has not yet been performed.

Overall, these combined conclusions indicate that the chronic Cu $gBAM_{WHAMVII}$ can be used for predicting chronic Cu toxicity in risk assessment applications, such as deriving site-specific bioavailable PNECs.

(Van Sprang, 2019)

9.5.1.1.2 Relevance of Standard Assessment Factors for Risk Assessment of Copper

In the July 2017 dossier update, the applicant provided a position paper that thoroughly investigated the relevance of standard assessment factors for risk assessment of the essential element Copper. This position paper has since been updated with additional information and is summarised below (Oorts and Verdonck, 2019). It was concluded that an assessment factor (AF), which is typically used to compensate for levels of uncertainties, is not justified since most sources of uncertainty (e.g. inter-species variation) are largely covered by the amount of available data on chronic toxicity of Cu to aquatic organisms. Hence, **the use of an assessment factor in this case could lead to wrong decision making process when based on RAC values within background levels of copper**.

Reference:	**KCP 10.2/02, Oorts, K., Verdonck, F., 2019**
Title:	Relevance of Standard Assessment Factors for Risk Assessment of the Essential Element Copper
Report No.:	CuPPP20170705
Guidelines:	Not applicable
Deviations:	Not applicable
GLP:	No
Published	No
Comment:	-

Executive Summary

Defining regulatory accepted concentrations for copper is a complex process since there are adverse effects from both copper deficiency and copper excess (U shape curve). Moreover, the bioavailability of copper depends on the physicochemical properties of the receiving environment.

For all environmental compartments (water, sediment and soil), reliable chronic toxicity data for Cu overlap with the range in Cu background concentrations in the European environment. Worst-case approaches based on the lowest toxicity thresholds, as typically used in a standard risk assessment framework, without consideration of bioavailability and application of additional assessment factors results in concentrations within the natural background ranges for Cu in European water, sediment and soil. This will lead to over-conservative conclusions and risk identification at natural background concentrations and even may result in maximum thresholds in deficiency conditions in environments with low bioavailability of Cu. When ignoring bioavailability, the selection of a regulatory acceptable concentration strongly depends upon the combinations of sensitive species and sensitive environmental media (water, sediment or soil) that were tested, without considering their relevance for other environments. As such, neglecting bioavailability may also result in under-protective effect thresholds for highly vulnerable media (with high Cu bioavailability) when the media used for toxicity testing do not adequately cover such scenarios.

The application of assessment factors is built in risk characterisation to ensure decision-makers they don't make wrong decisions in case of uncertainty. However, an overestimation of uncertainty in case of data-rich dossiers (like for essential metals as copper) can also lead to making wrong decisions. A sound risk assessment for Cu should therefore consider the uncertainty on the bioavailability of Cu by the use of proper correction and normalization models and worst-case assumptions instead of the application of standard assessment factors that were derived for organic (anthropogenic) chemicals. Because of the data richness of chronic toxicity data for the effect of Cu to aquatic and terrestrial organisms, most sources of uncertainty (e.g. inter-species variation) are also largely covered by the available data. Therefore, the use of a low assessment factor (even 1) for ecological risk assessment of Cu fungicides is justified and will avoid making wrong decisions such as RAC values within background or deficiency ranges for some soils.

9.5.1.1.3 Aquatic dwelling organisms - Copper

While awaiting the copper GD, the EUCuTF members will continue to use the SSD and BLM approach and no AF unless different methodology appropriate for data normalisation is provided by MS. Art.43 submissions will provide an update of the approach already used in the EU dossier.

Acute and chronic fish endpoints

It is incongruous that the critical aquatic endpoint for fish is less than the 95th percentile concentration of copper in European surface waters. The applicant would like to point out that the RAC derived by EFSA for Plant Protection Products is also much lower than the endpoint derived for REACH and BPR dossier (0.37 µg/L for PPP vs. 7.8 µg/L for REACH/BPD), highlighting large inconsistencies in the methodologies used and leading to an unrealistic refined endpoint.

All relevant PEC_{sw} values were higher than the acute and chronic first-tier RAC_{sw} values and hence a refined $HC_{5\text{-}50}$ value was calculated from a species sensitivity distribution (SSD) based on reliable quality-screened data found in the open literature regarding chronic toxicity of copper to fish. These data before being used in the SSD were normalised for bioavailability towards specific European eco-regions using the Chronic Biotic Ligand Model (BLM) and geometric mean values for the most sensitive endpoints have been calculated for 11 different fish species (see document Van Sprang, 2015). As discussed above, no assessment factor should be applied and hence the BLM-normalised SSD-$RAC_{sw,ch}$ was determined to be 7.9 µg/L.

Since effects of chronic exposure normally occur at lower concentrations than those of acute exposure, $RAC_{sw,ch}$ are expected to be lower than and therefore protective for the $RAC_{sw;ac}$.

Aquatic invertebrate and algae endpoint

All PEC_{sw} values were higher than the relevant acute and chronic first-tier RAC_{sw} values for algae and aquatic invertebrates.

In a microcosm study (Schäfers, 2000), a NOEC of 4.8 µg/L (dissolved copper) was determined for the most sensitive species *Chydorus sphaericus*. This study was performed with a mean pH of 9.4; mean DOC of 9.4 mg/L; and a total study duration of 385 days (i.e., the treatment period was 56 days and the post-treatment period (recovery) was 329 days). A very similar microcosm study (mean pH of 9.0; mean DOC of 4.4 mg/L) with a total study duration of 111 days with 2 × weekly addition of equilibrated Cu-salt in order to achieve constant copper concentrations was also performed (Schäfers, 2001). Given that DOC, known to mitigate copper toxicity, was much lower in the second study one would expect a lower NOEC in the second study. This was not the case as the NOEC for *Chydorus sphaericus* was found to be much higher, i.e. between 33 and 64 µg/L dissolved Copper. This suggests that the NOEC of 4.8 µg/L found in the initial study was a very conservative endpoint.

Given the exceptionally data richness and the particularity of a homeostatically tight controlled essential element, no further AF should be applied to the endpoint derived from the mesocosm and hence the ETO- $RAC_{sw;ch}$ will be 4.8 µg/L.

For the acute risks to invertebrates, since effects of chronic exposure normally occur at lower concentrations than those of acute exposure, The $RAC_{sw,ch}$ is expected to be lower than and therefore protective for the $RAC_{sw;ac}$.

Overall endpoint

The BLM-normalised SSD-$RAC_{sw,ch}$ value of 7.9 µg/L for fish is significantly higher than the aquatic invertebrate and algae ETO-$RAC_{sw;ch}$ of 4.8 µg/L thereby confirming that fish are not the most sensitive species. **The ETO-$RAC_{sw;ch}$ of 4.8 µg/L is therefore considered by the applicants as sufficiently protective of all aquatic organisms and hence is used as the critical endpoint for the aquatic risk assessment for all aquatic organisms**. Looking on the monitoring data and natural copper contents in surface water, this seems to be a sufficiently conservative value, still significantly lower as those derived under REACH and BPR.

A position paper relating to the use of the updated BLM model (Van Sprang, 2019) provided Cu PNEC values for PPP-zones. According to the PPP, a zonal system of authorisation operates in the EU to enable a harmonised and efficient system to operate. The EU is divided into 3 zones; North (Zone A), Central (Zone B) and South (Zone C). Therefore, Cu HC5 values which are representative for these 3 zones were calculated based on the HC_5 values for the individual countries, i.e. Denmark, Estonia, Latvia, Lithuania, Finland and Sweden for Zone A; Austria, Belgium, Czech Republic, Germany, Hungary, Ireland, The Netherlands, Poland, Slovakia, Slovenia and United Kingdom for Zone B; Spain, France, Greece, Italy and Portugal for Zone C. An overview of the Cu HC_5 cumulative distributions for the different zones, based on the physico-chemical parameters (DOC, pH) from Foregs, is provided in the **Figure 9.5.1.1.3-1** below:

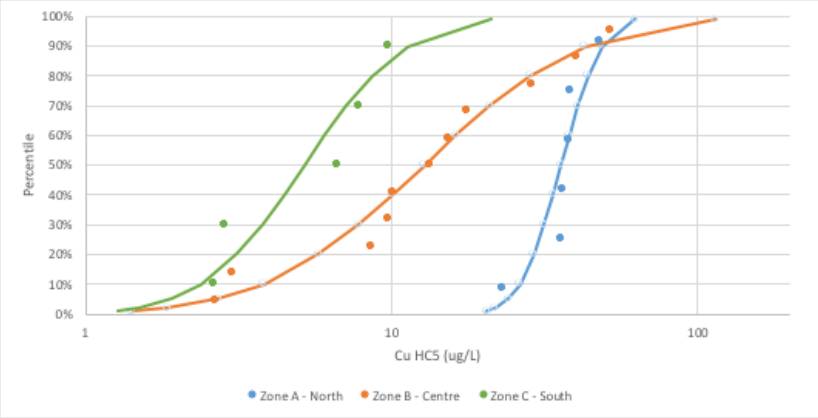

Figure 9.5.1.1.3-1 Overview of the Cu HC5 cumulative distributions for the different PPP zones

From **Figure 9.5.1.1.3-1** both median (50th %) and realistic worst case (10th %) HC5 for Cu could be calculated as shown in **Table 9.5.1.1.3-1**. Increasing sensitivity towards copper is observed when moving from North to South Europe, with a median HC5 value of 35.75 µg/L for Zone A (North EU), 12.81µg/L for Zone B (Central EU) and 5.2 µg/L for Zone C (South EU). As the DOC is the main driver in the mitigation of Cu toxicity, it is no surprise that the highest DOC are noted in North Europe (median DOC of 12.1 mg/L), an intermediate DOC in Central Europe (median DOC of 4.4 mg/L) and a lowest DOC in South Europe (median DOC of 2.9 mg/L).

Table 9.5.1.1.3-1 Overview of the Cu HC5 values for the different PPP zones

Percentile	HC5 – Zone A	HC5 – Zone B	HC5 – Zone C
1%	20.44	1.43	1.28
2%	21.82	1.85	1.50
5%	24.07	2.72	1.92
10%	26.27	3.83	2.40
20%	29.20	5.80	3.12
30%	31.51	7.81	3.78
40%	33.63	10.09	4.45
50%	35.75	12.81	5.19
60%	37.99	16.26	6.05
70%	40.55	20.99	7.12
80%	43.76	28.30	8.62
90%	48.64	42.83	11.24
99%	62.52	114.60	21.12

The results of this modelling of copper HC5 values supports the use of the ETO-RAC$_{sw;ch}$ of 4.8 µg/L as being sufficiently protective of all aquatic organisms in the majority of areas where agricultural use of copper occurs, however the MS should pay particular attention to areas where low DOC may occur as this could have a significant effect on the sensitivity of aquatic organisms to dissolved copper.

(Oorts and Verdonck, 2019)

A study to model the effects of copper exposure on trout populations was undertaken using experimental data derived from an early-life stage toxicity test with rainbow trout to predict effects of trout populations in realistic conditions. The results of this modelling are summarized below:

Reference:	[redacted]
Title:	Modelling of the Funguran-OH Effects on *Onchorhynchus mykiss* Populations
Report No.:	Not applicable
Guidelines:	Not applicable
Deviations:	Not applicable
GLP:	No
Published	No
Comment:	-

Executive Summary

An earlier study on the toxicity of Funguran-OH on early life stage of the rainbow trout was performed by the Fraunhofer Institute in 2000 (URA-001/4-18). Recently, issues have arisen on the applicability of this study under the plant protection products regulation. Arche Consulting was asked to interpret these lab results in a more ecologically realistic context. It is important to understand how the effects of a toxicant on individual-level endpoints (i.e. survival, reproduction) translate to effects on populations. Therefore, in this study the effect of Funguran-OH on the population density of a trout population due to mortality of early life stages was modelled.

To this end, the Fraunhofer Institute data was used to parameterize a toxicity model for survival. This model was combined with a population model for trout species and used to predict effects on trout populations in realistic exposure conditions for different application scenarios. A constant exposure to a fixed dissolved copper concentration was used to mimic the conditions of the Fraunhofer test. However, this exposure pattern is not realistic as Funguran-OH is typically applied multiple times per year and will not remain constant in the water. A typical exposure pattern will consist of pulses of copper: peak water concentrations after each application which decrease over time. Therefore, the second scenario "Pulse exposure" includes a worst-case realistic use of Funguran-OH with a maximum number of applications during the sensitive life stage possible according to the application guidelines of Funguran-OH.

In a population context, effects of Funguran-OH were observed at higher concentrations compared to the toxicity test (EC_{10} = 1.7 µg Cu/L and EC_{50} = 4.4 µg Cu/L): the EC_{10} for population density (3.51 µg Cu/L) was a factor two higher for the continuous exposure scenario and more than a factor 4 higher for the pulse exposure scenario (7.99 µg/L). Although roughly the same for the continuous exposure scenario, the EC_{50} value for the pulse exposure scenario (9.57 µg/L) was a factor 2 higher compared to the toxicity experiment.

(Janssen, et al., 2019)

The EC_{10} of 7.99 µg/L from the pulse exposure scenario further supports the use of the ETO-$RAC_{sw;ch}$ of 4.8 µg/L as being sufficiently protective of all aquatic organisms.

9.5.1.1.4 Sediment dwellers - Copper

It is incongruous that the critical endpoint for sediment dwellers of 3.23 mg/kg is significantly less than the average natural concentration of copper in European sediments (17 mg/kg). The applicants would like to point out that the RAC derived by EFSA for Plant Protection Products is also much lower than the endpoint derived for REACH and BPR dossier, highlighting large inconsistencies in the methodologies used and leading to unrealistic refined endpoint (3.23 mg/kg for PPP versus 87 mg/kg for REACH/BPR). The applicant insists that neglecting the bioavailability also leads to meaningless endpoints.

The EUCuTF has submitted a position paper on a revised PNEC sediment for copper for sediment effects which is summarised below (Vangheluwe, 2019). While awaiting the copper GD, the EUCuTF members will continue to use the bioavailability approach (e.g. AVS) and no AF unless different methodology appropriate for data normalisation is provided by MS. Art.43 submissions provides an update of the approach already used in the EU dossier, but **accept the normalization should be done to sediments containing 2.5% organic carbon, which will lower the RAC to 40.4 mg/kg dry wt.**

Reference:	**KCP 10.2/04, Vangheluwe, M., 2019**
Title:	Revised PNEC sediment copper for the sediment effects assessment for Cu: extending the database with additional species
Report No.:	Not applicable
Guidelines:	Not applicable
Deviations:	Not applicable
GLP:	No
Published	No
Comment:	-

Executive Summary

Currently, a quality-screened database on the toxicity of Cu towards freshwater sediment-dwelling organisms representing a variety of feeding strategies and taxonomic groups has been compiled (Vangheluwe et al, 2016) combining the data from the VRAR (2008) and the results from newly retrieved literature (search 2007-2015). The following species are covered in the database: amphipods (*Hyalella azteca*, *Gammarus pulex*), mayfly (*Hexagenia sp*), oligochaetes (*Tubifex tubifex*, *Lumbriculus variegatus*), Gastropod (*Bellamya aeruginosa*) and the midge (*Chironumus riparius*). The chronic toxicity tests covered different endpoints such as abundance, survival, growth/biomass, reproduction and fecundity. Geometric mean values for the most sensitive endpoints were calculated for 7 different sediment species (representing 65 NOEC values) and were used to populate a species sensitivity distribution curve (SSD) and to derive a realistic worst-case Predicted No Effect Concentration (RWC-PNEC) for copper. In order to capture the variability introduced by the presence of toxicity values generated at different organic carbon concentrations each NOEC value was normalised for organic carbon. The safe threshold for freshwater sediment organisms towards Cu was then calculated from the 5th percentile (HC5) of the SSD based on chronic toxicity and yielded a value of 1,360 µg Cu/g OC. This can be translated to a HC5 value of 68 mg/kg dry weight (for sediments with 5% O.C.) or a HC5 value of 34 mg/kg dry weight (for sediments with 2.5% O.C.) as suggested by the EFSA guidance.

Recently, this database and approach to derive the HC5 value was discussed at EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, France, and co-rapporteur Member State, Germany, for the pesticide active substance copper compounds (EFSA Journal 2018;16(1):5152). The endpoints to be used in the risk assessment for aquatic organisms (including sediment dwellers) were further discussed at the Pesticide Peer Review meeting 133-169. It was concluded that

1. the data set as such is based on different ecologically relevant chronic endpoints for risk assessment purposes (NOEC) derived for observations made for reproduction, survival, growth, emergence, fecundity and biomass. However, it was pointed out that these endpoints should not be used altogether to derive a HC5. HC5 calculated for survival, reproduction, biomass, etc. independently, shall be calculated if enough data are available". Concerning the dataset in the present study, enough data are available for chronic endpoints based on survival and growth to derive a SSD and calculate a HC5 (according to EFSA 6 data are available in both cases). The endpoint growth is the most conservative for all tested species.
2. according to EFSA aquatic guidance toxicity data for at least eight different benthic species should be used as a required minimum to derive a SSD.
3. use of geomean with chronic data is not recommended by the opinion of EFSA regarding sediment organisms (2015, EFSA Journal 13(7): 4176).

The present study aimed to re-evaluate and extend the current database in order to set a safe threshold of copper for the freshwater compartment taking into account the recently made comments of EFSA. Different scenarios were presented including the use of the geomean and the use of the lowest NOECs.

The copper database has been extended with two additional species (the macrophyte *V. spiralis* and the gastropod *P. antipodarum*) resulting in a database with 9 species and 5 taxonomic groups representing different feeding strategies and living habits. If the geometric mean is used **a HC5 of 40.4 mg/kg dry wt for a sediment containing 2.5% OC is obtained**. The use of a geometric mean for the chronic sediment data as has been proposed here by the EUCuTF in the copper case is deemed to be justified since all sediment test concentrations have been normalized for the organic carbon content allowing to compare the different tests at similar test conditions.

(Vangheluwe, 2019)

9.5.1.1.5 Cymoxanil

No new endpoints were used.

zRMS comments:

In response to decision from EFSA conclusion 2018 the applicant has submitted some summaries of new reports intending to justify the new endpoints used in this risk assessment in the current RAR. However, it should be noted, that the studies in which the new documents are based on, were included in the update of the RAR and were considered in the EFSA's Peer Review and, at zonal level, the endpoints would not be refined with documentation previously submitted in the renewal of approval of the active substance.

The short summary of these reports are summarised below.

1.Van Sprang, P, 2019, Response to EFSA comments on the aquatic effects assessment for Cu – extension.

According to the this report, the applicant indicates the results from Biotic Ligan Models (BLM) may be more realistic and could be useful for refining.
This model used by the applicant for the refinement of the endpoints was not admitted by the experts: the peer review did not consider acceptable the use of the BLM as proposed parameters were not duly validated. –The RMS indicated that new tools such as model and therefore the BLM should be validated and discussed at the European level first before being used in monographs in order to refine and predict toxicity values for various aquatic taxa across Europe (Copper_RAR_11_Volume_3CA_B-9_2018-05).-In the current report by Van Sprang-2019, different models are summarized to predict the bioavailability of copper to adjust its toxicity in different organisms.-On one hand the increased use of the traditional Biotic Ligand Models (BLM) approach is exposed (it has been observed that the bioavailability of metals has a strong influence by the chemistry factors such as pH, water hardness and dissolved organic carbon (DOC)). This models have also been optimized with the new generalized bioavailability models (gBAMs) as an alternative to predict chronic effects concentrations for copper, incorporating a log-linear relation between pH and free metal ion toxicity (instead of using a stability constant as a biotic ligand).–On the other hand, the models have also been used together with the speciation programs WHAM V and WHAM VII (Windermere Humic Aqueous Model) which aims to predict the competitive reactions of the metal with natural organic matter.

The report offers a study of the development and validation of these models, comparing observed dissolved Cu toxicity to the predicted dissolved Cu toxicity obtained in the models, using the dataset on which the model is developed in one way and using an independent dataset in other way (preferably toxicity data in natural waters). The next groups of aquatic organism data were considered: *Daphnia magna* chronic toxicity, algae chronic toxicity (*Pseudokirchneriella subcapitata*, *Chlorella vulgaris*), *Lemma minor* chronic toxicity and fish chronic (*Pimephales promelas*, *Oncorhynchus mykiss*; early life and juvenile stages). The report summarizes the current available models to account for the bioavailability of metals in the aquatic environment. This compilation of the methods (development and validation) is described in order to show that this bioavailability should be considered when evaluating the risk of copper compounds, and that nowadays they are the only way to estimate possible PNECs of bioavailability for chronic copper toxicity. The modeling indicated has enough data for *Daphnia* and algae species. Nevertheless, due to the limited available chronic bioavailability data for fish it was considered difficult to evaluate the protective effects of other ions on early life stage copper toxicity (at least, the pH effect on copper predicted toxicity can be extrapolated to early life stage toxicity data for the two species). However it should be noted that the dissolved organic carbon is an important factor in this model, but the extrapolation to region with lower concentration of dissolved organic carbon can be difficult.
The extrapolation of this model to the different agro-environmental characteristic in Europe should be ensured.

2. Oorts, Kand Verdonck, F, 2019:
Relevance of Standard Assessment Factors for Risk Assessment of the Essential Element Copper.
The applicant is in the opinion that assessment factors should not be applied to endpoints for chronic toxicity of copper to aquatic organisms, as the bioavailability of copper has not been considered and standard factors would not be applied to counteract uncertainty in organic compounds.
It is exposed that for elements such as Cu, a particularized and standardized correction would be necessary instead of the application of standardized AF for organic compounds.
The text in Executive Summarize and Conclusion is exactly the same as the previous 2017 document included in the Revised RAR (Copper_RAR_11_Volume_3CA_B-9_2017-09).
In this report, an uncertainty assessment has been added to the original document (point 4), which refers to the Guidance on Uncertainty Analysis in Scientific Assessments-2018 (EFSA Journal 2018; 16 (1): 5123).
An uncertainty analysis is carried out in which it is considered that the use of AF can be too conservative and that it is possible to take uncertainty into account through other approaches, distinguishing between quantifiable and non-quantifiable uncertainty, considering that the latter can only be described qualitatively.
It is proposed to cover uncertainties, that are normally covered with the AF, with other types of factors such as: assumption of worst case, inter-species variability reduced by sufficient number of tests available (to SSD - HC_5), the use of high quality data on SSD, the normalization of data for SSD for a worse case of bioavailability, taking into account bioavailability model, (facing laboratory-to-field uncertainty), the historical burden of expression of the RAC as total Cu.

In opinion of the applicant a more coherent approach would be guaranteed with the allocation of AF, distinguishing between those cases in which the availability of data is greater and those in which the number of data is less.

It is indicated that in those cases where it is not possible to express the effects in quantitative terms (which is recommended by the EFSA 2018 guide), it would be necessary to carry out a qualitative analysis. This occurs in elements such as Cu, in which a high AF has been derived considering a very worst case.
It was concluded that if the effects of bioavailability are considered, which is in turn strongly depends on the physical-chemical properties of the environment, the AF should be reduced.

3. [redacted] of the Funguran-OH Effects on Onchorhynchus mykiss Populations.

In this report it is indicated that the effects can be extrapolated/calculated to population based on a new modelling, with original data from a study in the year 2000th with Funguran-OH ([redacted]), in which the exposure (only for *Onchorhynchus mykiss* populations) is assumed to occur in more realistic conditions.
Specifically, it is a matter of measuring the effects of the product on the density of trout population considering mortality in the early stages of life, establishing a toxicity model for survival.
The reference data was taken from the [redacted] study, and those data for dissolved copper concentration were used and only related to larval survival in yolk-sac stage, since only significant effects were found in that stage. It should be indicated that not enough information was available on the water chemistry to perform a bioavailability correction and for this reason the dissolved copper concentrations were used for the toxicity model
The model used was the General Unified Threshold for Survival (GUTS) in its "reduced" form (combining the toxicokinetic model with the damage model: the external concentration is directly translated to the damage level) and using the mean concentration of Cu dissolved in the yolk-sac stage as input of the toxicide.
The worst case resulting from the possible death mechanisms of the model (stochastic, individual limit or both combined) is chosen and implemented in the population model InSTREAM-Gen (Ayllón et al. 2016): individual model for trout in stream environments, in which the entire life cycle is modeled

following daily routine. Adaptations were made for rainbow trout and a mortality function for copper toxicity was added. The model was implemented by adding two redd variables, the level of damage and limit levels of the eggs/larvae in the redd.
To consider the exposure, several scenarios were simulated and only two of them have been included in the report: one for continuous exposure of the toxicide (it has to be noted that a NOEC of 2 µg dissolved Cu/L was observed on the population) and another one for exposure by pulses (worst realistic case in which 1.2 kg Funguran-OH/ha was applied, which would be a PECini of 4.8 microg Cu dissolved/L , was applied 5 times with an interval of 7 days, DT_{50} Funguran-OH = 1 day).
In the continuous exposure scenario, clear effects would be found on the mean population density at 4 µg/L (EC_{10} = 3.51; EC_{50} = 3.97).
On the other hand, in the pulse exposure scenario, no effects on population density were predicted at the concentration of 4.8 µg/L. The mean values of the effects obtained per year were EC_{10} = 7.99 µg/L and EC_{50} = 9.57 µg/L.

4. Vangheluwe, M, 2019, Revised PNEC sediment copper for the sediment effects assessment for Cu: extending the database with additional species.

In this report, the new calculations of endpoint for sediment dwelling organism were provide as an extension to the study CA 8.2.5.4/01 "Environmental hazard assessment of copper: sediment-dwelling organisms" (Vangheluve, M., 2015, in RAR Revised 2017).

This calculation by Vangheluve-2015 was not accepted at the expert meeting of October 2017, due to not following the aquatic guidance document indications, which establishes a minimum of 8 species to do the SSD approach and since the use of the geometric mean is not recommended for chronic values. The selected endpoints were based on different types of chronic endpoints, with which the RMS did not agree.
The RMS proposed in the RAR Revised 2017 to obtain an HC_5 through a selection of endpoints of the same type (*growth*) but for only the original 6 different organisms, considering a geometric mean from the most conservative chronic values.
In Vangheluve-2019, two new species-endpoints are assumed to be provided. However, it has to be noted that the study with *V. spiralis* was not used in RAR as results were reported for macrophytes only, and the study with *P. antipodarum* was not considered reliable as there was "a potential bias through copper exposure via the overlying water".
The geometric mean NOEC and the worst case NOEC obtained from these studies have been incorporated to the data base by Vangheluve-2019, to reach 9 intended species data points and new HC_5 have been recalculated (featuring a total of 9 species of 5 different taxonomic groups).
In this report several SSD-HC_5 calculations have been proposed: SSD based on calculations with 6 species (the original ones of the calculation specified in the RAR) or 9 species (the original ones plus *L. Variegatus*, plus the two species mentioned above; the studies for these species were included in the RAR but were not taken into account). The endpoints have been selected only for "growth" and both the average and lowest values have been considered.

- Using 9 species (all available data according to the applicant), geometric mean NOEC and normalizing to 2.5% OC, the HC_5 would be **40.4 mg Cu/kg dw- preferred Applicant's proposal**
- Using the lowest NOEC value per available species data according to the applicant, and normalizing to 2.5% CO content, the HC_5 would be 19.4 mg Cu/kg dw.
- Using the lowest NOEC value per available species data and normalizing to 2.5% CO content, the HC_5 for the 6 original species would be 13.4 mg Cu / kg dw.

The applicant emphasized that it is incongruous that the critical endpoint according to the EFSA conclusion for sediment dwellers was significantly less than the average natural concentration of copper in European sediments.

However, the experts of EFSA as the RMS concluded that "from the results of the updated literature

data on the levels of copper in sediments of water bodies in winegrowing areas, the exposure and the risk in these sites could be higher than the assessment done during the european peer review of the renewal dossier for copper compounds" (EFSA Supporting publication 2018:EN-1486, Outcome of the consultation, August 2018, No. 4(14), page 14), and "no further information was provided in order to investigate if natural background levels of copper increased and consequently to assess the relevance of the median concentrations of copper in European stream sediment".
No further information has been provided in order to assess this formulated product either.

In the overall position for the evaluation of the copper effects in aquatic organisms, it is indicated that the applicant will continue to use the bioavailability approach and will not apply any AF: for sediment dwellers the standardization of the endpoint to an organic content of 2.5% has been applied, which makes it a proposal of RACsed dwellers of 40.4 mg/kg (including the concerning species added to the SSD data, and taking into account the use of a geometric mean for the chronic data -not recommended- and for the rest of aquatic organisms an ETO-RAC of 4.8 µg/L has been considered by the applicant as protective value of all acute and chronic risk.

Overall conclusion

It has to be noted that there is no specific guide to evaluate metal compounds and that the methodology according to EFSA conclusion is based on a conservative worst case and in which it has not been established to take into account the bioavailability of copper, both from the concentration predicted as a result of the current application of the product, and from the accumulated concentration over the years, although it must be said that many of the available data on chronic toxicity to aquatic organisms were obtained under laboratory conditions, where the accumulated copper does not influence the results of the studies. EFSA experts in their 2018 conclusion accepted a PEC_{sw} calculation methodology with which the zRMS agrees. The zonal-RMS-PL is in agreement with the calculated maximum mitigations that could be allowed according to the EFSA conclusion.2018. Therefore, the risk assessment based on EFSA Conclusion 2018 for aquatic organism is considered. With regard to sediment dwellers organisms (concerning the solid medium), it is important to take into account the effect of the accumulation over time of the metal in the sediment medium. The average amount according to the latest monitored studies for conservative calculation is 17 mg/kg. But it would be necessary to follow a more zonally specific monitoring and also consider the non-total mobilisation of copper and therefore the relative availability of it by aquatic organisms.

Therefore, further consideration of the risk assessment for sediment dwelling organism should be decided at MSs level.

9.5.2 Risk assessment - Copper

During the review of the renewal of approval of copper the EUCuTF made the claim that the standard models used to predict the PEC of copper in surface water are not relevant to metals such as copper. The Commission has agreed with this premise and in their Renewal Report (SANTE/10506/2018) called for more relevant models to be developed.

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

The relevant global maximum FOCUS Step 1 and 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. The applicant would like to reiterate that FOCUS modelling is not designed or validated to predict the behaviour of metals in the environment, and thus is not suitable for copper predictions and was only carried out for completeness. The applicant would like to request that more suitable assessment protocols are used for minerals such as copper.

As discussed above, to achieve a concise risk assessment for aquatic dwelling organisms, an ETO-$RAC_{sw;\ ch}$ value of 4.8 µg/L was used as this value was protective of all acute and chronic risks to all relevant aquatic species.

9.5.2.1 Risk assessment for aquatic dwelling organisms - Copper

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{sw}) and regulatory acceptable concentrations (RAC) for aquatic dwelling organisms are given for each FOCUS scenario. As discussed above, to achieve a concise risk assessment for aquatic dwelling organisms, an ETO-$RAC_{sw;\ ch}$ value of 4.8 µg/L was used as this value was protective of all acute and chronic risks to all relevant aquatic species.

Predicted concentrations in surface water have been calculated for copper as follows:

The applicants would like to point out that on page 15 of the EFSA conclusion (2018) they are pleased to see that EFSA recognises that due to the very rapid dissipation of copper (Cu^{2+} ions) from surface waters to sediment, **it was considered that the single application scenario represents the worst-case for the exposure assessment**. As a result of this statement the notifier would like the PEC surface water modelling results for multiple applications from Appendix A (LoEP) to be considered as irrelevant, as they ignore any dissipation from the water phase.

Standard FOCUS Step 1 and 2 PEC_{sw} values as described below were calculated for the use on potatoes:

PEC_{sw} without spray drift mitigation:

FOCUS Step 1 and 2 PEC_{sw} values (FOCUS Steps 1 and 2, version 3.2) were calculated considering all entry routes to water bodies with an interception of 0% (no cover crop) selected as a worst-case scenario.

Table 9.5.2.1-1 Aquatic organisms: acceptability of risk (PEC/RAC < 1) based on standard FOCUS Step 1, 2 maximum PECsw values for the use of FEL02 following a single application to potatoes (all entry routes to water bodies considered) – Total copper

Group	Aquatic dwelling organisms						
Endpoint [NOEC, µg/L]	4.8						
AF	1						
ETO RAC_{sw} [µg/L]	4.8						
Uses	**Application pattern**	**Season of application**	**Region**	**Step 1**		**Step 2**	
				PECsw [µg/L]	**PEC/RAC**	**PECsw [µg/L]**	**PEC/RAC**
Potatoes	1 × 600 g a.s./ha	Mar-May	N	9.85	**2.05**	5.52	**1.15**
			S	9.85	**2.05**	5.52	**1.15**
		Jun-Sep	N	9.85	**2.05**	5.52	**1.15**
			S	9.85	**2.05**	5.52	**1.15**

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

The Step 1 and Step 2 PEC_{sw} values are higher than the ETO $RAC_{sw;ch}$ value for aquatic organisms thus indicating a concern regarding the acute and chronic risk to aquatic organisms from the proposed use of FEL02 in potatoes at the proposed application rates. It is therefore considered that a refined acute risk assessment for aquatic organisms exposed to copper from the proposed uses of FEL02 is required.

Under the spray drift scenario, the particulate, barely water-soluble copper compound that hits the surface water will start dissolving while complexation to Dissolved Organic Carbon and sedimentation remove copper from the dissolved fraction. The results from the Blust and Joosen 2016 study (please refer to B8) have demonstrated that in a realistic water/sediment scenario the total copper declines very rapidly in the water phase while dissolved copper was at least a factor of 10 lower. This study describes best the speciation and kinetic behaviour of copper in an aquatic environment following a spray drift event. Despite this, the EUCuTF has proposed a more conservative

total/dissolved value of 3 for use in the risk assessment, based on the measurements in the mesocosm study.

The EFSA evaluation used a total/dissolved ratio of 1, which suggests that all copper is dissolved. This is against all observations in the monitoring studies and studies from the dossier cited above. This Art. 33 evaluation should apply a total to dissolved copper ratio of at least 3.

The following table summarises the risk assessment for aquatic dwelling organisms based on the FOCUS Step 1 and Step 2 maximum PEC_{sw} values following a single application to potatoes. These PEC_{sw} values are based on the highest acceptable mitigation for all entry routes to water bodies and are converted to dissolved copper concentration using a total to dissolved copper ratio of 3. For details of the calculation of the respective PECsw values please refer to B8.

Table 9.5.2.1-2 Aquatic organisms: acceptability of risk (PEC/RAC < 1) based on standard FOCUS Step 1, 2 maximum PEC_{SW} for the use of FEL02 following a single application to potato (all entry routes to water bodies considered) - Dissolved copper

Group	Aquatic dwelling organisms						
Endpoint [NOEC, µg/L]	4.8						
AF	1						
ETO RAC_{SW} [µg/L]	4.8						
Uses	**Application pattern**	**Season of application**	**Region**	**Step 1**		**Step 2**	
				PEC_{SW} [µg/L]	**PEC/RAC**	**PEC_{SW} [µg/L]**	**PEC/RAC**
Potatoes	1 × 600 g/ha	Mar-May	N	3.28	0.68	1.84	0.38
			S		0.68	1.84	0.38
		Jun-Sep	N	3.28	0.68	1.84	0.38
			S		0.68	1.84	0.38

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

For the intended use in potato, calculated PEC/RAC ratios indicate an acceptable risk for all group of aquatic organisms (risk as characterised by ETO-$RAC_{SW;ch}$ of 4.8 µg/L) in all FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

9.5.2.2 Risk assessment for sediment dwelling organisms - Copper

In the following tables, the ratios between predicted environmental concentrations in sediment (PEC_{sed}) and the regulatory acceptable concentration (RAC) for sediment dwelling organisms are given for the intended use on potatoes for each FOCUS scenario.

To calculate the PEC sediment accumulation over seven years, the FOCUS step 2 sediment via run-off /drainage and spray drift values are multiplied by seven to account for seven years of application (the period of authorization) and then a median **background level of copper in European sediments of 17 mg/kg** is added. For details of the calculation, please refer to the respective part in B8.

For illustrative purposes, mitigation measures were also included in the assessment of risk to sediment dwelling organisms.

Table 9.5.2.2-1 Sediment dwelling organisms: acceptability of risk (PEC/RAC < 1) based on FOCUS Step 2 maximum $PEC_{sed\ accumulation}$ values for the use of FEL02 following seven years application to potatoes (runoff/drainage with risk mitigation) - Copper

Group	Sediment dwelling organisms									
Endpoint [HC_5, mg/kg]	40.4									
AF	1									
ETO RAC_{sed} [mg/kg]	40.4									
Uses	**Application pattern**	**Season of application**	**Region**	**PEC_{sed} [mg/kg]**	**$PEC_{sed,\ accumulation}$ Total copper (7 years accumulation) + background [mg/kg]**					
				Step 2	**Mitigation**					
				1 year	**None**	**PEC/RAC**	**60%**	**PEC/RAC**	**80%**	**PEC/RAC**
Potatoes	1 × 3600 g a.s./ha	Mar-May	N	1.67	28.69	0.7101	21.68	0.5366	19.34	0.4787
			S	3.34	40.38	0.9995	26.35	0.6522	21.68	0.5366
		Jun-Sep	N	1.67	28.69	0.7101	21.68	0.5369	19.34	0.4787
			S	2.5	34.50	0.8540	24.00	0.5941	20.50	0.5074

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 9.5.2.2-2 Sediment dwelling organisms: acceptability of risk (PEC/RAC < 1) based on FOCUS Step 2 maximum $PEC_{Sed\ accumulation}$ values for the use of FEL02 following seven years application to potatoes (spray drift with risk mitigation) - Copper

Group	Sediment dwelling organisms							
Endpoint [HC_5, mg/kg]	40.4							
AF	1							
ETO RAC_{Sed} [mg/kg]	40.4							
Uses	**Application pattern**	**PEC_{sed} [mg/kg]**	**$PEC_{sed,\ accumulation}$ Total copper (7 years accumulation) + background + no spray buffer[mg/kg]**					
		Step 2	**Mitigation**					
		1 year	**Standard distance**	**PEC/RAC**	**5 m**	**PEC/RAC**	**10 m**	**PEC/RAC**
Potatoes	1 × 3600 g a.s./ha	0.717	22.02	0.545	18.64	0.461	17.87	0.442

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

The PEC/RAC values for sediment dwelling organisms were all lower than 1 thus indicating no concerns regarding the acute or chronic risks to sediment dwelling organisms from the proposed use of FEL02 in potatoes.

9.5.3 Risk assessment – Cymoxanil

The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of 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). The risk assessment was quantitatively performed for the active substance itself and for the metabolites for which experimental data was available. In case experimental data were lacking, as a worst-case approach a 10 x higher toxicity than the parent compound was assumed.

For sediment-dwelling organisms, no risk assessment was performed. Cymoxanil is not an insect growth regulator, and the parent and its metabolites are not likely to partition into sediments. In water-sediment studies, partitioning of cymoxanil to the sediment was insignificant. Very rapid degradation from the whole system was observed, with DT_{50} values in the range of 0.1 – 1.6 days (see section B.8). As neither cymoxanil nor any of its metabolites partitioned significantly to sediment, no toxicity test with the sediment dwelling midge *Chironomus* spp. was deemed necessary for cymoxanil or its metabolites, and no risk is assumed.

9.5.3.1 Risk assessment for the active substance cymoxanil

In **Table 9.5.3.1-1**, the ratios between predicted environmental concentrations of cymoxanil in surface water bodies (PEC_{SW}, as provided by the Fate section) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for FOCUS Step 1 and Step 2 scenarios and each organism group for the application on potatoes.

Table 9.5.3.1-1: Risk assessment for aquatic organisms for the use of cymoxanil on potatoes (6 x 120 g a.s./ha)

Group	**Fish acute**	**Fish prolonged**	**Invertebrates acute**	**Invertebrates prolonged**	**Algae**	**Aquatic plants**
Test species	***Lepomis macrochirus***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint	LC_{50}	NOAEC	EC_{50}	NOEC	E_bC_{50}	NOE_rC
(µg/L)	29000	44	27000	67	122	700
AF	100	10	100	10	10	10
RAC (µg a.s./L)	290	4.4	270	6.7	12.2	70
FOCUS Step 1 PEC_{sw} (µg a.s./L)	38.9					
PEC/RAC	0.13	**8.84**	0.14	**5.81**	**3.19**	0.56
FOCUS Step 2 PEC_{sw} (µg a.s./L)	1.1					
PEC/RAC	-	0.25	-	0.16	0.09	-

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

As can be seen in the table above, no unacceptable risks were identified for any of the aquatic dwelling organism groups at FOCUS Step 2 for the use of cymoxanil on potatoes.

9.5.3.2 Risk assessment for the relevant metabolites

The following metabolites were identified to be relevant in water: IN-U3204, IN-W3595, IN-KQ960, IN-T4226, IN-JX915, IN-R3273, IN-KP533 and Metabolite fraction M5. For the first four of these, experimental data was available for (some) of the organism groups, see **Table 9.5.1-3**. The risk assessment of these four metabolites is presented below.

IN-U3204

The risk assessment for metabolite IN-U3204 indicated no unacceptable risk for acute exposure of fish and invertebrates, see **Table 9.5.3.2-1**. This metabolite was not tested in algae. Assuming a 10 x higher toxicity of the metabolite compared to the parent (worst-case assumption), there is no unacceptable risk for algae using the FOCUS Step 2

PECsw value, see **Table 9.5.3.2-1**. No chronic studies are considered required for this metabolite, since the metabolite was not acutely more toxic than the active substance.

Table 9.5.3.2-1: Risk assessment for aquatic organisms for metabolite IN-U3204

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	97000	100000	12.2
AF	100	100	10
RAC (µg/L)	970	1000	1.22
FOCUS Step 1 PEC_{sw} (µg/L)	19.3		
PEC/RAC	0.02	0.02	**15.8**
FOCUS Step 2 PEC_{sw} (µg/L)	0.273		
PEC/RAC	-		0.22

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

IN-W3595

The risk assessment for metabolite IN-W3595 indicated no unacceptable risk for acute exposure of fish, invertebrates and algae, see **Table 9.5.3.2-2**. No chronic studies are considered required for this metabolite, since the metabolite was not acutely more toxic than the active substance.

Table 9.5.3.2-2: Risk assessment for aquatic organisms for metabolite IN-W3595

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	>130000	126000	12700
AF	100	100	10
RAC (µg/L)	>1300	1260	1270
FOCUS Step 1 PEC_{sw} (µg/L)	58.8		
PEC/RAC	<0.05	0.05	0.05

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

IN-KQ960

The risk assessment for metabolite IN-KQ960 indicated no unacceptable risk for acute exposure of fish and invertebrates using FOCUS Step 1 or 2 PECsw value, see **Table 9.5.3.2-3**. A chronic study in invertebrates was performed because this metabolite was more acutely toxic to invertebrates than the active substance. No unacceptable chronic risk was indicated. This metabolite was not tested in algae. Assuming a 10x higher toxicity of the metabolite compared to the parent (worst-case assumption), there is an unacceptable risk for algae using the FOCUS Step 2 PECsw value, see **Table 9.5.3.2-3**. In line with the RR for the product FDJ03 (2013, zRMS AT), the risk for algae can be considered low. The following reasoning was given in the RR, and is applicable to this dossier as well: “All studies with algae were performed under static 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 behaviour information on Cymoxanil it is reasonable to assume that these metabolites 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 considered to be low as it was covered by the assessment of the parent substance”. Therefore, there are no unacceptable risks for the metabolite IN-KQ960 for the use of cymoxanil on potatoes.

Table 9.5.3.2-3: Risk assessment for aquatic organisms for metabolite IN-KQ960

Group	**Fish acute**	**Invertebrates acute**	**Invertebrates chronic**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	NOEC	E_bC_{50}
(µg/L)	>120000	800	302	12.2
AF	100	100	10	10
RAC (µg/L)	>1200	8	30.2	1.22
FOCUS Step 1 PEC_{sw} (µg/L)	54.6			
PEC/RAC	<0.05	**6.83**	**1.81**	**44.75**
FOCUS Step 2 PEC_{sw} (µg/L)	1.7			
	-	0.21	0.06	**1.39**

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

IN-T4226

The risk assessment for metabolite IN-T4226 indicated no unacceptable risk for acute exposure of fish, invertebrates and algae, see **Table 9.5.3.2-4**. No chronic studies are considered required for this metabolite, since the metabolite was not acutely more toxic than the active substance.

Table 9.5.3.2-4: Risk assessment for aquatic organisms for metabolite IN-T4226

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	>111000	>116000	25800
AF	100	100	10
RAC (µg/L)	>1110	1160	2580
FOCUS Step 1 PEC_{sw} (µg/L)	23.6		
PEC/RAC	<0.02	0.02	0.01

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

For the metabolites IN-R3273, IN-KP533, IN-JX915 and Metabolite fraction M5, no experimental data was available. Assuming a 10 x higher toxicity of the metabolite compared to the parent (worst-case assumption), there was no unacceptable risk using the FOCUS Step 2 PECsw value for Metabolite fraction M5 and IN-JX915, see **Table 9.5.3.2-5** and **Table 9.5.3.2-6**, respectively.

Table 9.5.3.2-5: Risk assessment for aquatic organisms for Metabolite fraction M5

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	2900	2700	12.2
AF	100	100	10
RAC (µg/L)	29	27	1.22
FOCUS Step 1 PEC_{sw} (µg/L)	9.3		
PEC/RAC	0.32	0.34	**7.62**
FOCUS Step 2 PEC_{sw} (µg/L)	0.253		
	-	-	0.21

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 9.5.3.2-6: Risk assessment for aquatic organisms for metabolite IN-JX915

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	2900	2700	12.2
AF	100	100	10
RAC (µg/L)	29	27	1.22
FOCUS Step 1 PEC_{sw} (µg/L)	25.4		
PEC/RAC	0.88	0.94	**20.82**
FOCUS Step 2 PEC_{sw} (µg/L)	0.581		
	-	-	0.48

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

For metabolites IN-R3273 and IN-KP533, unacceptable risks to algae were indicated using FOCUS Step 2 PECsw values, see **Table 9.5.3.2-7** and **Table 9.5.3.2-8**, respectively, with PEC/RAC ratios slightly above 1 for both metabolites. However, similar to metabolite IN-KQ960 and in line with the RR for the product FDJ03 (2013, zRMS AT), the risk for algae can be considered low. The following reasoning was given in the RR, and is applicable to this dossier as well: "All studies with algae were performed under static 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 behaviour information on Cymoxanil it is reasonable to assume that these metabolites 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 considered to be low as it was covered by the assessment of the parent substance". Therefore, there are no unacceptable risks for the metabolites IN-R3273 and IN-KP533 for the use of cymoxanil on potatoes.

Table 9.5.3.2-7: Risk assessment for aquatic organisms for metabolite IN-R3273

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	2900	2700	12.2
AF	100	100	10
RAC (µg/L)	29	27	1.22
FOCUS Step 1 PEC_{sw} (µg/L)	76.2		
PEC/RAC	**2.63**	**2.18**	**48.20**
FOCUS Step 2 PEC_{sw} (µg/L)	1.39		
	0.05	0.05	**1.14**

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 9.5.3.2-8: Risk assessment for aquatic organisms for metabolite IN-KP533

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg/L)	2900	2700	12.2
AF	100	100	10
RAC (µg/L)	29	27	**1.22**

Group	Fish acute	Invertebrates acute	Algae
FOCUS Step 1 PEC_{sw} (µg/L)	56.1		
PEC/RAC	**1.93**	**2.08**	**45.98**
FOCUS Step 2 PEC_{sw} (µg/L)	1.24		
	0.04	0.05	**1.02**

AF: Assessment factor; PEC: Predicted environmental

Updated 04.2024

According to the AT comment the WoE to get rid of the risk indicated for metabolites IN-R3273 and IN-KP533 is not supported. It is argued that "these metabolites was considered to be low as it was covered by the assessment of the parent substance" which is not in line with the aquatic GD nor the EFSA conclusion. The risk assessment for metabolites IN-R3273 and IN-KP533 should be considered by MSs level. Further risk refinement may be required based on the step 3/4.

9.5.4 Risk assessment – Mixture toxicity

The combined exposure of copper and cymoxanil was assessed based on the concentration addition model as described in the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface water (EFSA Journal 2013;11(7):3290), taking into account the decision scheme of section 10.3.11. The measured acute endpoint of the formulation is compared to the acute calculated mixture toxicity to determine whether there is any synergism or antagonism between the active substances. The deviation between calculated and measured toxicity (Model Deviation Ratio, MDR) indicates whether the observed and calculated toxicity are in agreement (MDR between 0.2 and 5).

Equation 13 of the EFSA Aquatic Guidance Document (page 148) is used for the calculated mixture toxicity by concentration addition:

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

where:
n = number of mixture components
i = index from 1…n mixture components
p_i = the i^{th} component as a relative fraction of the mixture composition (note $\Sigma\ p_i$ must be 1)
ECx_i = concentration of component i provoking x% effect (pragmatically, $NOEC_i$ may be inserted, too)

The MDR is then calculated using equation 15 of the EFSA Aquatic Guidance (page 149):

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

An MDR between 0.2 and 5 indicates that the mixture toxicity conforms to assumptions of concentration-addition; MDR >5 indicates synergy; MDR <0.2 indicates antagonism. The same test species should be used across this comparison where possible, regardless of whether or not they provide the lowest endpoint for the individual active ingredients, to account for differences in species sensitivity. Formulation studies and studies with copper have been performed with *Oncorhynchus mykiss*, *Daphnia magna* and *Pseudokirchneriella subcapitata*. Studies with cymoxanil have been performed with *Lepomis macrochirus*, *Daphnia magna* and *Anabaena flos-aquae*.

Table 9.5.4-1: Mixture toxicity assessment, Concentration Addition approach

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x,PPP}$) [mg a.s./L]	Toxicity of the a.s. copper ($EC_{x,Cu}$) [mg a.s./L] *	Toxicity of the a.s. cymoxanil ($EC_{x,Cym}$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product, $EC_{x,mix-CA}$) [mg a.s./L] [a]	Model deviation ratio (MDR) [b]	Calculated mixture toxicity at PECmix ($EC_{xmix-CA}$, a.s. in PEC_{mix}) [mg a.s./L] [a]	$EC_{xmix-CA}$ (a.s. in PPP)/$EC_{xmix-CA}$ (a.s. in PECmix)
LC_{50} fish	47	11.28	0.207	29	0.25	0.022	0.996	0.25
EC_{50} daphnids	0.35	0.084	0.0308	27	0.37	4.4	1.46	0.25
ErC_{50} algae	0.22	0.0528	0.02229	0.122	0.026	0.49	0.064	0.40

*: Copper as total copper; [a]: as calculated using Equation 13; [b]: as calculated using Equation 15;

As can be seen in **Table 9.5.4-1**, the calculated MDR values were between 0.2 and 5 for daphnids and algae, indicating that the concentration addition concept holds and the formulation does not cause an (unexpected) increased or decreased toxicity compared to the active substances for these organisms. No synergism or additional toxicity occurs due to the co-formulants. The apparent antagonism for fish (toxicity of the mixture is less than expected based on concentration addition) could be explained by the fact that the studies with fish were not performed with the same species for the formulation and the two active substances. Therefore, the measured mixture toxicity is still plausible and the mixture toxicity assessment continues with step 3 of the decision scheme in 10.3.11. This involved checking whether the mixture composition in the formulation study giving the measured mixture toxicity (ECx_{PPP}) in terms of the relative proportions of the individual a.s. was similar to the mixture composition at the PEC_{mix} (sum of all separate component PEC values). As a direct comparison on the basis of the relative proportions of the a.s. at the ECx_{PPP} with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. The $EC_{x\ mix-CA}$ for the mixture composition of the a.s. at the PEC_{mix} was calculated using Equation 13 and taking into account Equation 19:

$$p_i = \frac{PEC_i}{PEC_{mix}}$$

where:

PEC_i = PEC of the i^{th} component

PEC_{mix} = sum of all PEC_i

If the ECxmix-CA (a.s. in PPP)/ECxmix-CA (a.s. in PECmix) is between 0.8 and 1.2, the mixture can be considered similar. If the ratio is lower than 0.8 or higher than 1.2, the mixture is not similar and it should be considered whether one mixture component clearly drives the toxicity of the mixture (step 5 of the decision scheme). As can be seen in **Table 9.5.4-1**, the ratio is lower than 0.8 for all organism groups, indicating that the mixture is not similar.

The toxic unit (TU) approach is applied to identify if one active substance is driving the risk. Where a single active substance provides > 90 % of the sum of the toxic units for the mixture, that active substance is clearly driving the risk and can be considered alone. Equation 14 of the EFSA Aquatic Guidance shows how to calculate the sum of the toxic units for each component of a mixture:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

where:

c_i = the concentration of a mixture component

ECx_i = the toxicological acute (e.g. EC_{50}) or chronic (e.g. long-term NOEC) endpoint

Table 9.5.4-2 shows the individual toxic units and the percentage contribution of the individual TU to the mixture for each a.s. and each organism group. It is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product, please refer to the copper risk assessment in Section 9.5.2.

Table9.5.4-2: Mixture toxicity assessment, toxic unit approach

Endpoint	TU copper *	TU cymoxanil	Sum TU	%TU copper *	% TU cymoxanil
LC_{50} fish	0.027	0.00004	0.02704	99.9	0.1
EC_{50} daphnids	0.018	0.0004	0.01804	99.8	0.2
ErC_{50} algae	0.248	0.009	0.257	96.5	3.5

*: Copper as total copper

9.5.5 Risk assessment – Product

For the product FEL02, experimental values were available for fish, algae and *Daphnia*. No chronic studies were required, since the formulated product was not more acutely toxic than the active substance. No separate risk assessment for the product is considered necessary, as the risk is adequately assessed by the risk assessment of the active substance copper (please refer to Section 9.5.2 and 9.5.5). However, for illustrative purposes, this risk assessment is provided below. The endpoints from the formulation studies (please refer to KCP 10.2) expressed as total a.s. are shown in **Table 9.5.5-1**. The PEC values relevant for use in the product risk assessment are shown in **Table 9.5.5-2**. As described in Section 9.5.2.1 (risk assessment for copper, aquatic organisms), the dissolved copper is relevant and is thus shown here.

Table 9.5.5-1 Endpoints and effect values relevant for the risk assessment for aquatic organisms –FEL02

Study		**Endpoint (mg prod./L)**	**Endpoint (mg Cu/L)[a]**	**Endpoint (mg cymoxanil/L)[b]**	**Endpoint for use in risk assessment (mg total a.s./L)**
Oncorhynchus mykiss	96 h LC_{50}	47	9.73	1.88	11.61
Daphnia magna	48 h EC_{50}	0.35	0.072	0.014	0.086
Pseudokirchneriella subcapitata	72 h E_rC_{50}	0.22	0.046	0.009	0.054

[a] based on an active substance (copper) content of 20.7% in the formulated product
[b] based on an active substance (cymoxanil) content of 4% in the formulated product

Table 9.5.5-2 PEC_{sw} values for the risk assessment for aquatic organisms – FEL02

	$PEC_{dissolved\ copper}$ (µg/L)	**$PEC_{cymoxanil}$ (µg/L)**	**$PEC_{sw,total\ a.s.}$ (µg/L)**
FOCUS Step 1	3.28	38.9	42.18
FOCUS Step 2	1.84	1.1	2.94

The risk assessment is provided in **Table 9.5.5-3**. As can be seen, using this approach safe use can be demonstrated for fish and algae while safe use cannot be demonstrated for invertebrates. However, as mentioned above, the separate risk assessment for the formulated product is not considered necessary. The mixture toxicity is driven by copper and the risk assessment for copper is considered sufficient to cover also the risk assessment of the formulated product. Please refer to the copper risk assessment in Section 9.5.2.

Table 9.5.5-3: Risk assessment for aquatic organisms for FEL02

Group	**Fish acute**	**Invertebrates acute**	**Algae**
Test species	***Oncorhynchus mykiss***	***Daphnia magna***	***Anabaena flos-aquae***
Endpoint	LC_{50}	EC_{50}	E_bC_{50}
(µg total a.s./L)	11609	86.45	54.34
AF	100	100	10
RAC (µg/L)	116.09	0.8645	5.434

Group	Fish acute	Invertebrates acute	Algae
FOCUS Step 1 $PEC_{sw,total\ a.s.}$ (µg/L)	42.18		
PEC/RAC	0.36	**48.79**	**7.76**
FOCUS Step 2 $PEC_{sw,total\ a.s.}$ (µg/L)	2.94		
	0.03	**3.40**	0.54

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

9.5.6 Overall conclusions

In conclusion, the risk assessment for copper indicated acceptable risk to aquatic organisms from the use of FEL02.

The risk assessment for cymoxanil indicated acceptable risk to aquatic organisms from the use of FEL02.

The risk assessment for the product FEL02 (combined exposure of copper and cymoxanil) based on mixture toxicity indicated that the toxicity of FEL02 is driven by the active substance copper. The risk for the product is therefore adequately covered by the risk assessment of copper alone, and safe use is demonstrated.

For registration in line with country specific requirements, different mitigation measures may apply.

zRMS comments: The risk assessment for aquatic organisms was corrected by zRMS.

According to Part B8 for FEL02 product:

Cymoxanil

PECsw/sed are accepted for risk assessment.

Copper
The endpoints used for surface water exposure assessment are consistent with list of EFSA journal (2018). The application rate used in the calculations was determined assuming the GAP. Based on the results of the study of Blust R and Joosen S (2016; 9d.2.3-1), the correction factor of 3 cannot be agreed by the RMS." (RAR-copper compounds- Volume 3 – B.8 (PPP). The final report of monitoring study was submitted but no used by zRMS. The copper content in stream sediments was examined in field trials including copper treated plots with different application rate 4.0 to 40.0 kg Cu/ha. The sampling points were located close to the field site and represented areas potentially exposed to copper from the treatments on the adjacent field trial. The meteorological data were collected. The Cu content in stream sediments was examined. Sediment was analyzed for residues of copper using the analytical methods reported in the GLP compliant studies. The report was used only for additional information.

PL:

The PEC_{sw} have been calculated by applicant taking into account the protection zones (WBZ and NSZ and the use of appropriate anti-drift techniques. For the entry via drift into water bodies, zRMS is of the opinion that according to the EFSA journal (2018), the single application scenario represents the worst-case for the exposure assessment due to the very rapid dissipation of copper from surface waters. Single application scenario considered as a realistic worst-case for the calculation of PEC_{sw} values.

The PECsw have been calculated by zRMS taking into account the protection zones (WBZ and NSZ and the use of appropriate anti-drift techniques. For the entry via drift into water bodies, zRMS is of the opinion that according to the EFSA journal (2018), the single application scenario represents the worst-case for the exposure assessment due to the very rapid dissipation of copper from surface waters. Single application scenario considered as a realistic worst-case for the calculation of PEC_{sw} values.

Predicted environmental concentrations in surface water bodies (PECsw and PECsed) were calculated to simulate applications of copper to potatoes for exposure via spray drift and run-off. The PECsw and PECsed concentrations of copper were determined using the following assumptions:

PECsw values for active substance copper following a single application to proposed crop in GAP

Crop	Calculations with drift and run off mitigation and 90% mitigation nozzle reduction	
	Exposure by runoff and drain-	Exposure by drift

	age				
	PECsw (runoff and drainage) STEP2 unmitigated µg/L)	PECsw with 90% reduction runoff (20 m VBZ) µg/L)	PECsw (drift) STEP2 unmitigated µg/L)	10m NSZ µg/L)	20m NSZ µg /L)
Potatoes 1x 600g Cu /ha	0.552	0.0552	0.552	0.055	0.0232

Sum PECsw (drift and runoff) values for active substance copper following a single application to proposed crop in GAP after risk mitigation measure

Crop	Sum of concentrations µg/L of copper and 90% mitigation nozzle reduction	
	20 m VBZ 10 m NSZ	20 m VBZ 20 m NSZ
Potatoes 1x 600g Cu /ha	**0.023**	---

Sum PECsed (drift and runoff) values for active substance copper following a single application annual dose for proposed crop in GAP after risk mitigation measure

Crop	**Application (g/ha)**	**PECsed (mg/kg)**				
		1 year	**7 years accumulation**	**Total after 10 years**	**Total after 20 years**	**7 years accumulation + background concentration 17 mg/kg**

Potatoes **90% runoff reduction 20m**	3600 g Cu /ha	0.28	2.02	2.8	5.6	19.02

The PEC_{sw} and PEC_{sed} values may be used in the aquatic risk assessment.

Aquatic organisms: acceptability of risk (PECsw/RAC < 1) for copper for each organism group based on FOCUS Steps 2 calculations for the use of FAP13.

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sediment dwelling**		**Sediment dwelling**
Test species		***Oncorhynchus mykiss***	***Acipenser transmontanus***	***Daphnia magna***	***Daphnia magna***	***Selenasrtum capricornutum***	***Chironomus ripatius***		***Tubifex tubifex***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC		NOEC
(µg/L)		8	1.12	26.6	7.6	22.29	500		16.17 mg/kg
AF		100	10	100	10	10	10		5*
RAC (µg/L)		0.008	0.12	0.266	0.76	2.229	50		3.23
	PECsw-max (µg/L)	**PEC/RAC ratios**						**PEC sed max (mg/kg)**	**PEC/RAC ratios**
Step 2									
	0.552	**69**	**4.6**	**2.075**	0.73	0.25	0.011	0.28 [1] 2.08 [2] 2.8 [3] 5.6 [4] 19.02 [5]	0.0867 0.64 0.87 **1.73** **5.89**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;
PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*according to the EFSA Journal 2018;16(1):5152

[1] 1 year
[2] 7 years accumulation
[3] Total after 10 years
[4] Total after 20 year
[5] 7 years accumulation + background accumulation 17 mg Cu/kg

Based on the results performed in the Tables above, the PECsw/RAC ratio is above trigger of 1 for fish, aquatic invertebrates indicating needs for further refinement. In case of sediment dwelling organism for species Chironmus riparius (spiked in water) an acceptable risk is identified.
However, the risk for sediment dwelling organim (spiked in sediment) needs further consideration at each MSs level.
Refined endpoints based on species sensitivity distribution (SSD) were available for both the acute and chronic risk assessment for fish and were discussed and agreed on in the Pesticide Peer Review meeting. The respective endpoints are reported in the EFSA conclusion (EFSA Journal 2018;16(1):5152) and considered for the higher tier risk assessment below. It was agreed that total or dissolved copper might be considered as equivalent; and that the SSD could be built using data expressed both as total and dissolved copper, depending on how the studies had been designed and reported.
With respect to algae and aquatic invertebrates, a microcosm study was available. The experts at the Pesticide Peer Review meeting agreed to use the end point derived from this study (ETO-RAC) together with an assessment factor of 2.

Aquatic organisms: acceptability of risk (PECsw/RAC < 1) for copper compounds for each organism group based on maximum PEC_{sw} of copper (PEC_{sw}) considering different mitigation options for the use of CUPROFIX C

Group		**Fish acute (higher tier)**	**Fish pro-longed (higher tier)**	**Inverteb. Acute (higher tier)**	**Inverteb. prolonged (higher tier)**	**Algae (higher tier)**
Test species		**7 fish species**		**Indoor microcosm study**		
Endpoint (µg/L)		**SSD-HC_5 3.73**	**SSD-HC_5 1.11**	**ETO-RAC = 4.8**		
AF		3	3	2		
RAC (µg/L)		1.24	0.37	2.4		
Total copper	**Max. PEC_{sw} (µg/L)**					
STEP 2 (all entry routes)						

20 meter vegetative buffer strip and 90% drift reduction nozzles	**0.023**	0.019	0.062	0.0096

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

For the risk assessment with very conservative exposure approach proposed by EFSA, it can be concluded that even with mitigation measures a high risk could be identified for fish and aquatic invertebrates.
Further calculations of PEC_{sw} /RAC ratio was provided by the zRMS with PECsw with buffer zones values agreed be e-fate expert in Section 8, to conclude acceptable risk assessment to aquatic organism.
To protect aquatic organism the following risk mitigation measures should be applied to surface water bodies:

- 20 meter vegetative buffer zone and 90% drift reduction nozzles

The risk mitigation measures should be decided at MSs level.

Sediment dwelling organism:

According to the calculations of PECsed/RAC ratio the risk from the use of active substance for potatoes is not acceptable for sediment dwelling organisms considering the active substance-copper. Further refinement is required.
However, there is no approved guideline for calculating $PECsed_{acc}$ values for non-organic substance agreed at EU level to determine protective measures, similar to PEC_{sw} value approach.
Therefore, a high risk to sediment dwellers (exposure via sediment) was still concluded for proposed use according to EFSA 2018 endpoint.
The MSs should apply their own mitigation measures at national level.
In addition, we would like to emphasize clearly that if the other MSs are different opinion referred to the risk assessment proposed by the zRMS they are considered it further at National level with consideration all data presented in this dossier.

Conclusion - Copper:

In opinion zRMS further calculations provided by the Applicant should be considered at the level of the Member States due to the need to apply risk mitigation measures to aquatic organisms.

To refine the exposure the PECsw have been calculated by zRMS taking into account the protection zones WBZ and NSZ and the use of appropriate anti-drift techniques. In opinion zRMS further calculations provided by the zRMS should be considered at the level of the Member States due to the need to apply risk mitigation measures to aquatic organisms.

Based on the lowest value RAC of 0.37 microgram/L for fish agreed at EU level the PECsw/RAC ratio is below 1, when following risk mitigation measures are applied:

Potatoes
20 m vegetative strip with 90% nozzles reduction.
Justification: PECsw/RAC: 0.0552/0.37 is below 1 (0.149)

The acceptability of the PECsw calculation and final risk mitigation measures should be decided at MSs level.
There is no approved guideline for calculating PECsed values to determine protective measures, similar to PECsw value approach. Therefore, the MSs should apply their own mitigation measure at national level.
In opinion zRMS further calculations provided by the Applicant should be considered at the level of the Member States due to the need to apply risk mitigation measures and specific national information. to aquatic organisms. The additional risk mitigation is presented for illustration only and has to be assessed during the national assessment.
In the CA B9 a safe use on the basis of the agreed EU ERA methodology cannot be concluded. Considering PECsw calculation and the agreed endpoints for aquatic organisms, a safe use was only established after applying additional risk mitigation only available at national level in Poland (20m vegetative strip with 90% nozzles reduction).

zRMS would like to emphasize clearly that if the other MSs are different opinion referred to the risk assessment proposed by the zRMS they are considered it further at National level with consideration all data presented in this dossier. The risk for aquatic organisms remains open for finalization of the assessment by cMS.

Sediment dwelling organism

Based on PEC_{sed} calculation and the agreed endpoints for sediment dwelling organisms a safe use was not established. The risk assessment for sediment dwelling organisms also should be considered by MSs level. Further refinement is required. However, there is no approved guideline for calculating PECsed values to determine protective measures, similar to PECsw value approach. Therefore, a high risk to sediment dwellers (exposure via sediment) was still concluded for proposed use according to EFSA 2018 endpoint.
The MSs should apply their own mitigation measures at national level.

zRMS point out that a safe use was not established for aquatic and sediment dwelling organisms according to the EU agreed assessment in the CA. Any kind of additional risk mitigation going beyond the EU agreed maximum mitigation level does not qualify to conclude on a safe use and should be placed in the national addendum or amended to the CA for illustration purposes only. The high risk to sediment dwellers (exposure via sediment) for copper was still concluded for proposed use according to EFSA 2018 endpoint. The MSs should apply their own mitigation measure at national level.

Cymoxanil

The risk assessment for cymoxanil indicated acceptable risk to aquatic organisms from the use of product Cuprofix C.

Combined risk assessment
It is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product.

Risk assessment for product CUPROFIX C
For the product Cuprofix C, experimental values were available for fish, algae and *Daphnia*. No chronic studies were required, since the formulated product was not more acutely toxic than the active substance. No separate risk assessment for the product is considered necessary, as the risk is adequately assessed by the risk assessment of the active substance copper.

However, the chemical analysis for the product Cuprofix C and fish, algae and Daphnia revealed, that the concentration of cymoxanil was below LOQ at the end of the test concentrations. Hence, it is concluded the toxicity endpoints from this studies may be questionable due to cannot be determined as exposure of the test compound was not maintained throughout the study. On the other hand, the combined risk assessment confirmed that it is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product and the copper concentration were properly maintained throughout the studies for fish, algae and Daphnia. ~~*The reliable endpoint for these studies and risk assessment for plant product protection CUPROFIX C should be considered at MSs level.*~~
Updated 04.2024 r.
The chemical analysis for the product Cuprofix C and fish, Daphnia and algae revealed, that the concentration of cymoxanil was below LOQ at the end of the test concentrations. Please noticed that in this case the calculate the endpoint with the geomean concentration for cymoxanil is probably impossible in mathemathical reason. In opur opinion no separate risk assessment for the product is considered necessary, as the risk is adequately assessed by the risk assessment of the active substance copper. The combined risk assessment confirmed that it is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The validity criteria for these studies were met. Apart from minor deviations from the methodology (such as the temperature range), the tests were carried out in accordance with the methodology recommendations. in this specific situation, RMS proposes due to the limitations of testing on vertebrates (test for fish), the validity criteria was met and the fact that the toxicity comes from 90% of the copper in the formulation, not to establish a data gap. However, this approach and the assessment of the studies for aquatic organisms for the product Cuprofix C and fish, algae and Daphnia should be considered at MSs level. In our opinion – the final conclusion for these studies in this specific situation should be considered by MSs level.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Toxicity data for Copper, Cymoxanil and the product FEL02

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

Toxicity data on cymoxanil were obtained from the EFSA Scientific Report for Cymoxanil (2008).

Toxicity data on FEL02 is also presented and were considered in the risk assessment.

Table 9.6.1-1 Endpoints and effect values – Copper compounds

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Copper hydroxide technical	Acute	Contact toxicity LD_{50} = 44.46 µg/bee	EFSA Conclusion (2018)
Apis mellifera	Copper hydroxide WP	Acute	Oral toxicity LD_{50} = 49.0 µg/bee Contact toxicity LD_{50} > 57 µg/bee	EFSA Conclusion (2018)
Apis mellifera	Copper oxychloride	Acute	Oral toxicity LD_{50} = 12.1 µg/bee Contact toxicity LD_{50} = 44.3 µg/bee	EFSA Conclusion (2018)
Apis mellifera	Bordeaux Mixture WP	Acute	Oral toxicity **LD_{50} = 23.3 µg/bee** Contact toxicity **LD_{50} > 25.2 µg/bee**	EFSA Conclusion (2018)
Apis mellifera	Tribasic copper sulfate SC	Acute	Oral toxicity LD_{50} = 40 µg/bee Contact toxicity LD_{50} > 23.5 µg/bee	EFSA Conclusion (2018)
Apis mellifera	Copper oxide technical	Acute	Contact toxicity LD_{50} > 22.0 µg/bee	EFSA Conclusion (2018)
Apis mellifera	Copper oxide WG	Acute	Oral toxicity LD_{50} > 116.0 µg/bee Contact toxicity LD_{50} = 82.5 µg/bee	EFSA Conclusion (2018)
Apis mellifera	Copper oxychloride 50% WP	Adult, chronic oral	**LDD_{50} = 0.466 µg copper/bee/day**	Colli, 2018a KCP 10.3.1.2/02
Apis mellifera	Copper oxychloride 50% WP	Larval mortality Adult emergence	**NOED 14.17 µg copper/lava/day** NOED 14.17 µg copper/larva/day	Colli, 2018b KCP 10.3.1.3/03
Higher-tier studies (tunnel test, field studies)				
Field or semi-field tests (EFSA Conclusion, 2018) Two outdoor cages were performed with copper oxychloride WP and Bordeaux Mixture. No significant effects at rates up to 1.25 kg/ha. Tunnel test performed with copper oxychloride WP on *Phacelia* – single application of 2.5 kg a.s/ha. Statistically significant reduction is observed on flight intensity at a rate of 2.5 kg a.s/ha.				

Table 9.6.1-2 Endpoints and effect values – Cymoxanil

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Cymoxanil	Acute	Oral toxicity: **LD_{50} > 85.29 µg a.s./bee** Contact toxicity: **LD_{50} > 100 µg a.s./bee**	Schur A., 1999 (In EFSA Conclusion (2008)
Higher-tier studies (tunnel test, field studies)				
None				

Values in **bold** are used in the risk assessment

Table 9.6.1-3 Endpoints and effect values relevant for the risk assessment for bees – FEL02

Species	Substance	Exposure System	Results	Reference
Apis mellifera	ATOFEL02	Oral acute	**LD_{50} = 51.6 µg prod./bee**	Vinall, 2011 KCP 10.3.1.1.1/01
		Contact acute	**LD_{50} > 419 µg prod./bee**	
Bombus terrestris	FEL02	Oral acute	**LD50 = 107 µg prod./bbee**	McVean, 2022 KCP 10.3.1.1.1/02
Bombus terrestris	FEL02	Contact acute	**LD50> 500 µg prod./bee**	McVean, 2022 KCP 10.3.1.1.2/01
Apis mellifera	Copper 20% + Cymoxanil 4% WG	Oral chronic	**LDD_{50} = 5.51 µg prod./bee/d**	Ruhland, 2018 KCP 10.3.1.2/01
Apis mellifera	Copper 20% + Cymoxanil 4% WG	Chronic larvae, 8-day, repeated exposure	NOED = 20.1 µg prod./larvae/dev.period	Scheller, 2018a KCP 10.3.1.3/01
Apis mellifera	Copper 20% + Cymoxanil 4% WG	Chronic larvae, 22-day, repeated exposure	**NOED = 45.2 µg prod./larvae/dev.period**	Scheller, 2018b KCP 10.3.1.3/02
Higher-tier studies (tunnel test, field studies)				
None				

Values in **bold** are used in the risk assessment

9.6.1.1 Justification for new endpoints

Additional studies addressing the acute oral and contact, adult chronic and larvae toxicity of the formulation FEL02 on honeybees and bumblebees are available and are used in the present risk assessment.

9.6.2 Risk assessment

Honeybees may be exposed to the active substances copper and cymoxanil, present in the formulation FEL02, by direct spraying of the plant protection product while honeybees are foraging on flowers and weeds present in or adjacent to the crop treated. They may also be exposed through contact with fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew.

The risk assessment for acute effects on bees is conducted in accordance with the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). Following the data requirement according to Regulation (EU) No. 284/2013, data on the chronic risk to adult honeybees and honeybee larvae are available. Further, data on the acute risk to bumblebees have been submitted. However, in the currently notified SANCO Guidance Document, these data are not considered in the risk assessment scheme. A new guidance document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp*. and solitary bees) has been published in 2013 by EFSA, in which risk assessment schemes for the chronic risk to adult honeybees and honeybee larvae, and for the risk to bumblebees are described. Although this Guidance Document is not yet noted by the Standing Committee on Plants, Animals, Food and Feed, a risk assessment for the chronic risk to honeybees and for the acute risk bumblebees according to the new EFSA Guidance Document is included below. That way, all available data on bees is taken into account in a risk assessment.

9.6.2.1 Risk quotients for bees

Copper

The in-field risk of copper to honeybees was calculated as Application Rate/LD_{50}. The result of the risk assessment is presented in **Błąd! Nie można odnaleźć źródła odwołania.**9.6.2.1-1. Hazard Quotient (HQ) values are below the

trigger value of 50, with a large margin of safety. Therefore, the risk of the active substances copper and cymoxanil for bees is considered to be acceptable, according to SANCO/10329/2002 framework. Since the in-field risk is acceptable, the off-field risk is concluded to be acceptable as well since this risk will by definition be lower than the in-field risk.

Table 9.6.2.1-1 First-tier assessment of the risk for bees for the active substances copper for adult bees (SANCO/10329/2002)

Type	Route	Application rate (g a.s./ha)	Exposure (g a.s./ha)	Endpoint (µg a.s./bee)	HQ	Trigger
Copper						
In-field	Oral	600	600	23.3	25.8	50
	Contact			25.2	23.8	50

EFSA risk assessment scheme

An illustrative honeybee risk assessment has been conducted according to the EFSA risk assessment scheme, including the results of the adult chronic and larval studies, although the scheme is not yet Noted and is currently under review. The assessment was conducted using the EFSA spreadsheet "EFSA Bee Tool v3". Considering the use in potatoes in the GAP, the downward spraying category was used as well as the application rate of the commercial product (3.0 kg product/ha). For the contact assessment, hazard quotients (HQ) were calculated, and for the oral assessment exposure toxicity ratios (ETR) were calculated.

The results of the screening risk assessment are presented in **Błąd! Nie można odnaleźć źródła odwołania.** for the active substance copper.

Table 9.6.2.1-2 Screening assessment of the risk of the active substance copper to adult and larvae bees based on EFSA Guidance on risk assessment for honeybees (2013)

Species	Test design	Endpoint	Calculation factor	HQ	Trigger
Honeybee	Contact, acute	LD_{50} = 25.2	1	23.8	42
Species	**Test design**	**Endpoint**	**SV**	**ETR**	**Trigger**
Honeybee	Oral, acute	LD_{50} = 23.3	7.6	0.20	0.20
Honeybee	Oral, chronic	LDD_{50} = 0.466	7.6	**9.78**	0.03
Honeybee	Larvae	NOED = 14.17	4.4	0.19	0.20

HQ/ETR values in **bold** breach the relevant trigger

The screening assessment indicates that there may be unacceptable risks to honeybees: HQ values are below the relevant trigger values whilst ETR values for oral chronic risk to adults exceeded the triggers. Therefore, a first-Tier assessment was performed, taking into consideration the application timing in potatoes (BBCH 21-95). The resulting ETR are presented in the table below:

Table 9.6.2.1-3 Adult honeybee – First-Tier risk assessment for copper based on EFSA Guidance on risk assessment for bees (2013)

category	scenario	BBCH	Honeybee	
			ETR	trigger
chronic	treated crop	10 - 39	**0.85**	0.03

chronic	treated crop	40 - 69	**0.85**	0.03
chronic	treated crop	≥ 70	0.00	0.03
chronic	weeds	10 - 39	**2.69**	0.03
chronic	weeds	40 - 69	**0.81**	0.03
chronic	weeds	≥ 70	**0.81**	0.03
chronic	field margin	10 - 39	0.02	0.03
chronic	field margin	40 - 69	0.02	0.03
chronic	field margin	≥ 70	0.02	0.03
chronic	adjacent crop	10 - 39	0.02	0.03
chronic	adjacent crop	40 - 69	0.02	0.03
chronic	adjacent crop	≥ 70	0.02	0.03
chronic	next crop	10 - 39	**0.50**	0.03
chronic	next crop	40 - 69	**0.50**	0.03
chronic	next crop	≥ 70	**0.50**	0.03

Values in **bold** indicate unacceptable risks
*no pollen present at BBCH ≥ 70

The first-tier risk assessment of the oral acute and chronic risk to bees from the use of FEL02 indicates that there may be unacceptable risks, please see also point 9.6.2.2 for further consideration.

Cymoxanil

The in-field risk of cymoxanil to honeybees was calculated as Application Rate/LD_{50}. The result of the risk assessment is presented in **Błąd! Nie można odnaleźć źródła odwołania.**9.6.2.1-4.

Table 9.6.2.1-4 First-tier assessment of the risk of cymoxanil to adult bees (SANCO/10329/2002)

Type	Route	Application rate (g a.s./ha)	Exposure (g a.s./ha)	Endpoint (µg a.s./bee)	HQ	Trigger
Cymoxanil						
In-field	Oral	120	120	85.29	1.4	50
	Contact			100	1.2	50

Hazard Quotient (HQ) values are below the trigger value of 50, with a large margin of safety. Therefore, the risk of the active substances copper and cymoxanil for bees is considered to be acceptable, according to SANCO/10329/2002 framework. Since the in-field risk is acceptable, the off-field risk is concluded to be acceptable as well since this risk will by definition be lower than the in-field risks.

EFSA risk assessment scheme

An illustrative honeybee risk assessment has been conducted according to the EFSA risk assessment scheme, although the scheme is not yet Noted and is currently under review. The assessment was conducted using the EFSA spreadsheet “EFSA Bee Tool v3”. Considering the use in potatoes in the GAP, the downward spraying category was used as well as the application rate of the commercial product (3.0 kg product/ha). For the contact assessment, hazard quotients (HQ) were calculated, and for the oral assessment exposure toxicity ratios (ETR) were calculated.

Table 9.6.2.1-5 Screening assessment of the risk of cymoxanil to adult bees based on EFSA Guidance on risk assessment for honeybees (2013)

Species	Test design	Endpoint	Calculation factor	HQ	Trigger

Honeybee	Contact, acute	LD_{50} > 100	1	1.2	42
Species	**Test design**	**Endpoint**	**SV**	**ETR**	**Trigger**
Honeybee	Oral, acute	LD_{50} = 85.29	7.6	0.01	0.20

HQ/ETR values in **bold** breach the relevant trigger

The screening assessment indicates that there are no unacceptable acute risks to bees expected for cymoxanil as the HQ and ETR values are below the relevant trigger values. Chronic risk of cymoxanil is addressed as part of the risk assessment for FEL02, containing both copper and cymoxanil, as no EU agreed chronic endpoints are available for cymoxanil.

FEL02/ combination toxicity
The in-field risk of FEL02 for honeybees was calculated as Application Rate/LD_{50}. The result of the risk assessment is presented in **Błąd! Nie można odnaleźć źródła odwołania.**9.6.2.1-6.

Table 9.6.2.1-6 First-tier assessment of the risk for bees for the product FEL02 for adult bees (SANCO/10329/2002)

Type	Route	Application rate (g product/ha)	Exposure (g product/ha)	Endpoint (µg product/bee)	HQ	Trigger
FEL02, honeybees						
In-field	Oral	3000	3000	51.6	**58**	50
	Contact			>419	<7.2	50

Hazard Quotient (HQ) values are below the trigger value of 50 for honeybees in respect to the contact toxicity. The first-tier risk assessment of the oral acute risk to bees from the use of FEL02 indicates that there may be unacceptable risks, therefore please see also point 9.6.2.2 for further consideration.

EFSA risk assessment scheme

An illustrative honeybee risk assessment has been conducted according to the EFSA risk assessment scheme, including the results of the adult chronic and larval studies, although the scheme is not yet Noted and is currently under review. The assessment was conducted using the EFSA spreadsheet "EFSA Bee Tool v3". Considering the use in potatoes in the GAP, the downward spraying category was used as well as the application rate of the commercial product (3.0 kg product/ha). For the contact assessment, hazard quotients (HQ) were calculated, and for the oral assessment exposure toxicity ratios (ETR) were calculated.

Table 9.6.2.1-4 Screening assessment of the risk of FEL02 to adult bees and larvae based on EFSA Guidance on risk assessment for honeybees (2013)

Species	Test design	Endpoint	Calculation factor	HQ	Trigger
Honeybee	Contact, acute	LD_{50} >100 µg prod./bee	1	<7.2	42
Species	**Test design**	**Endpoint**	**SV**	**ETR**	**Trigger**
Honeybee	Oral, acute	LD_{50} = 51.6 µg prod./bee	7.6	**0.44**	0.20
Honeybee	Oral, chronic	LDD_{50} = 5.5 µg prod./bee 1	7.6	**4.14**	0.03
Honeybee	Larvae	NOED = 45.2 µg	4.4	**0.29**	0.20

		prod./bee			

HQ/ETR values in **bold** breach the relevant trigger

The screening assessment for the use of FEL02 in potatoes indicates that there may be unacceptable risks to honeybees. Although the HQ value for acute contact risk is below the relevant trigger value, the ETR values for oral acute risk to adult honeybees and bumblebees and oral chronic risk to adult honeybees and larvae of honeybees exceed the triggers. Therefore, a First Tier Assessment was performed, taking into consideration the application timing in potatoes (BBCH 21-95), and are presented in the table below:

Table 9.6.2.1-5 Adult and larvae honeybee – First-Tier risk assessment based on EFSA Guidance on risk assessment for bees (2013)

category	scenario	BBCH	Honeybee	
			ETR	trigger
acute	treated crop	10 - 39	0.05	0.2
acute	treated crop	40 - 69	0.05	0.2
acute	treated crop	≥ 70	0.00	0.2
acute	weeds	10 - 39	**0.22**	0.2
acute	weeds	40 - 69	0.06	0.2
acute	weeds	≥ 70	0.06	0.2
acute	field margin	10 - 39	0.00	0.2
acute	field margin	40 - 69	0.00	0.2
acute	field margin	≥ 70	0.00	0.2
acute	adjacent crop	10 - 39	0.00	0.2
acute	adjacent crop	40 - 69	0.00	0.2
acute	adjacent crop	≥ 70	0.00	0.2
acute	next crop	10 - 39	0.04	0.2
acute	next crop	40 - 69	0.04	0.2
acute	next crop	≥ 70	0.04	0.2
chronic	treated crop	10 - 39	**0.36**	0.03
chronic	treated crop	40 - 69	**0.36**	0.03
chronic	treated crop	≥ 70	0.00	0.03
chronic	weeds	10 - 39	**1.14**	0.03
chronic	weeds	40 - 69	**0.34**	0.03
chronic	weeds	≥ 70	**0.34**	0.03
chronic	field margin	10 - 39	0.01	0.03
chronic	field margin	40 - 69	0.01	0.03
chronic	field margin	≥ 70	0.01	0.03
chronic	adjacent crop	10 - 39	0.01	0.03
chronic	adjacent crop	40 - 69	0.01	0.03
chronic	adjacent crop	≥ 70	0.01	0.03
chronic	next crop	10 - 39	**0.21**	0.03
chronic	next crop	40 - 69	**0.21**	0.03
chronic	next crop	≥ 70	**0.21**	0.03
larva	treated crop	10 - 39	0.01	0.2
larva	treated crop	40 - 69	0.01	0.2
larva	treated crop	≥ 70	0.00	0.2
larva	weeds	10 - 39	0.12	0.2
larva	weeds	40 - 69	0.04	0.2
larva	weeds	≥ 70	0.04	0.2
larva	field margin	10 - 39	0.00	0.2

category	scenario	BBCH	Honeybee	
			ETR	trigger
larva	field margin	40 - 69	0.00	0.2
larva	field margin	≥ 70	0.00	0.2
larva	adjacent crop	10 - 39	0.00	0.2
larva	adjacent crop	40 - 69	0.00	0.2
larva	adjacent crop	≥ 70	0.00	0.2
larva	next crop	10 - 39	0.02	0.2
larva	next crop	40 - 69	0.02	0.2
larva	next crop	≥ 70	0.02	0.2

Values in **bold** indicate unacceptable risks

The first-tier risk assessment of the oral acute and chronic risk to bees from the use of FEL02 indicates that there may be unacceptable risks, please see also point 9.6.2.2 for further consideration.

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

Copper

As presented in the (EFSA Conclusion, 2018), two outdoor cage tests were performed with copper oxychloride WP and Bordeaux Mixture. No significant effects at rates up to 1.25 kg/ha.

A semi-field study was conducted with copper oxychloride WP on *Phacelia* with a single application at 2.5 kg a.s/ha. Statistically significant reduction (30%) is observed on flight intensity at a rate of 2.5 kg a.s/ha and only on the day of application, total recovery was noted from Day 2, suggesting a transient effect. (France, 2017)

In addition, literature data was submitted for the renewal of approval of copper compounds which provided evidence that chronic exposure of copper via feeding of copper solutions as an anti-varroa treatment in hives did not show adverse effects on bees at doses of 1-2 g copper/L. The RMS (France, 2018) concluded that "*…the articles submitted by the applicant show that chronic exposure of bees to copper does not induce adverse effects at individual colony level. Therefore, this literature review and the tunnel tests submitted in the frame of the re-approval of copper bring evidence that no chronic adverse effects are expected for bees and colonies when exposed to copper following the application of copper-based formulations".*

Cymoxanil

The screening assessment indicates that no unacceptable acute risks to bees are expected for cymoxanil as the HQ and ETR values are below the relevant trigger values. Chronic risk of cymoxanil is addressed as part of the risk assessment for FEL02, containing both copper and cymoxanil, as no EU agreed chronic endpoints are available for cymoxanil.

FEL02/Combined toxicity

The First-tier assessment for the product FEL02 for adult honeybees according to SANCO/10329/2002 indicated that honeybees may be acutely at risk based on the oral toxicity test in honeybees. Additionally, the first-tier risk assessment of the oral acute and chronic risk to bees from the use of FEL02 in accordance with the EFSA risk assessment scheme indicates that there may be unacceptable risks for bees foraging in the treated crop, the following crop and in the weeds. A higher tier assessment considering a more realistic exposure scenario would then be the necessary. While higher tier data is available for copper, no higher tier data for the product FEL02 is available. Therefore, the contribution of each of the active substances to the toxicity of the formulation is assessed below, to show that a higher tier risk assessment can be based on active substance data.

For the combined risk assessment of FEL02, a surrogate LD50 mix was estimated following the approach proposed in the EFSA GD for birds and mammals (2009):

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(\text{a.s.}_i)}{LD_{50}(\text{a.s.}_i)} \right)^{-1}$$

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

A comparison between the mixture toxicity and the toxicity of the active substances should be made to test whether there is a change in the predicted risk by using the modelled LD50 mix value instead of the measured LD50 of the a.s. To achieve a basis for this comparison, a `*tox per fraction*` quotient can be calculated for each active substance and can be compared to the corresponding quotient of the mixture.

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

If the `*tox per fraction a.s*` and the `*tox per fraction mixture*` deviate by < 10%, this indicates, that this active substance will contribute > 90 % to mixture toxicity and the impact of the other component is marginal. Consequently, the risk assessment can be performed for the most toxic component. In table 9.6.6.2-1 all input parameter needed to estimate the surrogate LD50 mix and the tox per fraction comparison are summarized for FEL02.

Table 9.6.6.2-1 Calculation of surrogate LD50 for the mixture of active substances

A.s.	**Concentration a.s. in mixture [g/kg]**	**Fraction a.s. in mixture**	**LD_{50} a.s. [µg a.s./bee]**	**Fraction a.s./ LD_{50} a.s.**	**Surrogate LD_{50} [µg total a.s./bee]**	**Tox per fraction (a.s.)**	**Deviation tox per fraction (a.s.) from the tox per fraction (mix) [%]**	**LD_{50PPP} [µg total a.s./bee]**	**MDR**
Contact									
Copper	200	0.83	23.3	0.0358	26.51	27.96	5.5%	12.38	0.47
Cymoxanil	40	0.17	85.5	0.0019		511.8	1830%		
Oral									
Copper	200	0.83	25.2	0.0331	28.79	30.24	5.0%	100.7	3.5
Cymoxanil	40	0.17	100	0.0167		600.0	1984%		

Based on the calculation above, copper contributes over 90% to the acute toxicity of the mixture. MDR values (formulation endpoint divided by the calculated surrogate endpoint) show that the toxicity of the formulations is in agreement with the surrogate value based on concentration addition (value is between 0.2 and 5).

It should be noted that no EU agreed chronic endpoints are available for cymoxanil. However, metabolism studies on lettuce and potato evaluated in the EU review indicated a rapid and extensive degradation of cymoxanil. Cymoxanil was metabolised via several intermediates to glycine which was further conjugated or incorporated with or

into natural substances (e.g., carbohydrates, peptides or proteins) (DAR Cymoxanil, 2013). The data show that a DT50 of 2 days can be considered a worst-case assumption for the residue calculation in vegetation. In addition to that, metabolism study of cymoxanil in grapes, potatoes and tomatoes demonstrated that [^{14}C]-labelled cymoxanil is rapidly metabolised by grapes, potatoes and tomatoes, resulting primarily in the formation of [^{14}C]glycine with subsequent reincorporation of the radiolabel into other naturally occurring compounds closely associated with the metabolism of glycine. These results support the EU reviewed studies on potatoes and lettuce. Based on the low acute toxicity to bees and the rapid degradation in plants, cymoxanil is also not expected to contribute significantly to the chronic risk of FEL02 to bees. Therefore, the risk assessment for copper is considered to cover that for the formulation FEL02 as well.

9.6.3 Effects on bumble bees

The in-field risk of FEL02 for bumblebees was calculated as Application Rate/LD_{50}. The result of the risk assessment is presented in Table 9.6.3-1.

Table 9.6.3-1 First-tier assessment of the risk for bees for the product FEL02 for adult bumblebees (SANCO/10329/2002)

Type	Route	Application rate (g product/ha)	Exposure (g product/ha)	Endpoint (µg product/bee)	HQ	Trigger
FEL02, bumblebees						
In-field	Oral	3000	3000	107	28	50
	Contact			>500	<0.17	50

Hazard Quotient (HQ) values are below the trigger value of 50 for bumble bees therefore the risk is considered acceptable according to the assessment scheme in line with SANCO/10329/2002.

EFSA risk assessment scheme

An ~~illustrative~~ bumblebee risk assessment has been conducted according to the EFSA risk assessment scheme, including the results of the adult acute contact and oral studies performed with the formulation, although the scheme is not yet Noted and is currently under review. The assessment was conducted using the EFSA spreadsheet "EFSA Bee Tool v3". Considering the use in potatoes in the GAP, the downward spraying category was used as well as the application rate of the commercial product (3.0 kg product/ha). For the contact assessment, hazard quotients (HQ) were calculated, and for the oral assessment exposure toxicity ratios (ETR) were calculated.

Table 9.6.2.1-4 Screening assessment of the risk of FEL02 to adult bumblebees based on EFSA Guidance on risk assessment for honeybees (2013)

Species	Test design	Endpoint	Calculation factor	HQ	Trigger
Bumblebee	Contact, acute	LD_{50} >500 µg prod./bbee	1	6.0	7
Species	**Test design**	**Endpoint**	**SV**	**ETR**	**Trigger**
Bumblebee	Oral, acute	107 µg prod./bbee	11.2	**0.31**	0.036

HQ/ETR values in **bold** breach the relevant trigger

The screening assessment for the use of FEL02 in potatoes indicates that there may be unacceptable risks to bumblebees. Although the HQ value for acute contact risk is below the relevant trigger value, the ETR value for oral acute risk to adult bumblebees exceeds the trigger. Therefore, a First Tier Assessment was performed, taking into consideration the application timing in potatoes (BBCH 21-95). The resulting ETR are presented in the table below:

Table 9.6.2.1-5 Adult and larvae honeybee – First-Tier risk assessment based on EFSA Guidance on risk assessment for bees (2013)

category	scenario	BBCH	Bumblebee	
			ETR	trigger
acute	treated crop	10 - 39	**0.06**	0.036
acute	treated crop	40 - 69	**0.06**	0.036
acute	treated crop	≥ 70	0.00	0.036
acute	weeds	10 - 39	**0.18**	0.036
acute	weeds	40 - 69	**0.05**	0.036
acute	weeds	≥ 70	**0.05**	0.036
acute	field margin	10 - 39	0.00	0.036
acute	field margin	40 - 69	0.00	0.036
acute	field margin	≥ 70	0.00	0.036
acute	adjacent crop	10 - 39	0.00	0.036
acute	adjacent crop	40 - 69	0.00	0.036
acute	adjacent crop	≥ 70	0.00	0.036
acute	next crop	10 - 39	0.03	0.036
acute	next crop	40 - 69	0.03	0.036
acute	next crop	≥ 70	0.03	0.036

Values in **bold** indicate unacceptable risks

The first-tier risk assessment of the oral acute and chronic risk to bees from the use of FEL02 indicates that there may be unacceptable risks, please see also point 9.6.2.2 for further consideration in respect to bees in general. ~~However, since the risk assessment according to EFSA is only illustrative and the assessment in line with the honeybee assessment according to the SANCO/10329/2002 guidance document indicated that the risk is acceptable, no further consideration of the risk to bumblebees is required.~~

9.6.4 Effects on solitary bees

No adverse effects are expected, please refer to Section 9.6.2.2.

9.6.5 Overall conclusions

The first-tier risk assessment of the oral acute and chronic risk to bees and bumblebees from the use of FEL02 indicates that there may be unacceptable risks. However, the higher tier risk assessment shows that the risks following exposure of FEL02 to bees are acceptable at doses of up to 2.5 kg copper/ha (proposed use of FEL02 amounts to 720 g cu/ha). No higher tier data on bumblebees is available but since the risk assessment according to EFSA is only illustrative and the assessment in line with the honeybee assessment according to the SANCO/10329/2002 guidance document indicated that the risk is acceptable, no further consideration of the risk to bumblebees is required.

zRMS comments: Agreed. The first-tier risk assessment of the oral acute and chronic risk to bees and bumblebees from the use of **Cuprofix C** indicates that there may be unacceptable risks. However, the higher tier risk assessment shows that the risks following exposure of **Cuprofix C** to bees are acceptable at doses of up to 2.5 kg copper/ha (proposed use of **Cuprofix C** amounts to 720 g cu/ha). ~~No higher tier data on bumblebees is available but since the risk assessment according to EFSA is only illustrative and the assessment in line with the honeybee assessment according to the SANCO/10329/2002 guidance document indicated that the risk is acceptable, no further consideration of the risk to bumblebees is required.~~

Justification: Based on the lowest LD_{50} of 12.1 µg Cu/bee value for copper high acute risk for adult bees is concluded.
It should be noted that during the renewal of the active substance – copper two studies were performed, a semi-field study and a cage test.

The results indicated that no significant effects on the numbers of dead bees or on their behavior or brood development up to concentrations of 1.25 kg Cu/ha. Based on the available data an acceptable risk to honeybees for all intended uses with a single application rate up to 1.25kg/ha can be considered. However, the data are not considered adequate to cover multiple applications of copper. Possible adverse effects on honeybees due to multiple applications of copper are not covered by the semi-field studies. Furthermore, EFSA concluded (EFSA/2018/5152) that "*A tunnel test already considered with the confirmatory data (European Food Safety Authority, 2013a) was available where a statistically significant reduction is observed for flight intensity at the highest dose tested (2500 g Cu/ha). Data from literature provided by the applicants indicated that, chronic exposure of copper via feeding of copper solutions as an antivarroa treatment in hives did not show adverse effects on bees at dose similar to the current apiculture practices (1–2 g Cu/L preparation). Overall, with the available data, it was not possible to draw a conclusion on various aspects of the risk assessment to bees.*"
In conclusion, due to the high number of applications a long-term exposure to honeybees cannot be excluded.
Therefore, to protect bees the following restriction are proposed by zRMS-PL:
SPe 8: Dangerous to bees. To protect bees and other pollinating insects do not apply to crop plants when in flower. Do not use where bees are actively foraging. Do not apply when flowering weeds are present. Remove weeds before flowering.
The final risk mitigation measures should be decided at MSs level.

Based on the low acute toxicity to bees and the rapid degradation in plants, cymoxanil is also not expected to contribute significantly to the chronic risk of Cuprofix C to bees. Therefore, the risk assessment for copper is considered to cover that for the formulation Cuprofix C as well. Combined risk assessment. Additionally, it is clear that copper drives the toxicity of the mixture for bees (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product.

Updated 04.2024
The risk assessment to bumble bees is not to be considered only illustrative, given that this is done with the more up to date scientific background document, that some MSs and the EFSA follows. Thus, the risk would remain according to in line with the EFSA guideline. However, the guideline is not yet fully implemented and therefore the final decision remains for consideration by Member states.

Final decision should be taken into account at MSs level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Toxicity data for Copper, Cymoxanil and FEL02

Studies on the toxicity to non-target arthropods have been carried out with all supported forms of copper. Full details of these studies are provided in the respective EU DAR (France, 2018) and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of five representative formulations were evaluated as part of the EU assessment of copper compounds. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Toxicity data on cymoxanil were obtained from the EFSA Scientific Report for Cymoxanil (2008).

Six extended studies with the formulated product FEL02, including the two indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi,* two additional species *Chrysoperla carnea* and *Aleochara bilineata* and two aged residues studies with the indicator species are available and were used in the risk assessment.

Table 9.7.1-1 Endpoints and effect values for non-target arthropods – Copper

Species	Substance	Exposure System	Results*	Reference
Aphidius rhopalosiphi (adults)	Copper hydroxide	Laboratory test glass plates (2D)	LR_{50} = 0.05 kg Cu/ha	EFSA Conclusion (2018)
	Bordeaux Mixture	Laboratory test glass plates (2D)	**LR_{50} > 14.7 kg Cu/ha**	
	Tribasic copper sulphate	Laboratory test glass plates (2D)	LR_{50} > 0.1344 kg Cu/ha	
	Copper oxide	Laboratory test glass plates (2D)	LR_{50} > 39.2 kg Cu/ha	
Typhlodromus pyri (protonymphs)	Copper hydroxide	Laboratory test glass plates (2D)	LR_{50} >14.88 kg Cu/ha	EFSA Conclusion (2018)
	Copper oxychloride	Laboratory test glass plates (2D)	LR_{50} >14.89 kg Cu/ha	
	Bordeaux Mixture	Laboratory test glass plates (2D)	**LR_{50} > 13.2 kg Cu/ha**	
	Tribasic copper sulphate	Laboratory test glass plates (2D)	LR_{50} > 0.08 kg Cu/ha	
	Copper oxide	Laboratory test glass plates (2D)	LR_{50} > 26.1 kg Cu/ha	
Aphidius rhopalosiphi (adults)	Copper oxychloride WP	Extended laboratory test (3D)	Mortality: 0% at 1 kg Cu/ha 0% at 3.97 kg Cu/ha Parasitisation: -22.38% at 1.0 kg Cu/ha 10.89% at 3.97 kg Cu/ha	EFSA Conclusion (2018)
Aphidius rhopalosiphi (adults)	Tribasic copper sulphate	Extended laboratory test (3D)	Mortality: 0% at 0.00154 kg Cu/ha 2.5% at 0.00768 kg Cu/ha 2.5% at 0.0384 kg Cu/ha 5.0% at 0.192 kg Cu/ha 2.5% at 0.960 kg Cu/ha Parasitisation: -29.8% at 0.00154 kg Cu/ha -72.6% at 0.00768 kg Cu/ha -40.4% at 0.0384 kg Cu/ha -13.8% at 0.192 kg Cu/ha 30.5% at 0.960 kg Cu/ha	EFSA Conclusion (2018)
Aphidius rhopalosiphi (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 10% at 3.213 kg Cu/ha Fecundity: -7.4% at 3.213 kg Cu/ha	EFSA Conclusion (2018)
Typhlodromus pyri (protonymphs)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: -7.4% at 3.213 kg Cu/ha Fecundity: 16.9% at 3.213 kg Cu/ha	EFSA Conclusion (2018)

Species	Substance	Exposure System	Results*	Reference
Typhlodromus pyri (protonymphs)	Tribasic copper sulphate SC	Extended laboratory test (3D)	Mortality: 1.8% at 0.015 kg Cu/ha 3.5% at 0.06 kg Cu/ha 13.9% at 0.25 kg Cu/ha 3.5% at 1.01 kg Cu/ha 0.0% at 4.032 kg Cu/ha Fecundity: -7.3% at 0.015 kg Cu/ha -17.1% at 0.06 kg Cu/ha -11% at 0.25 kg Cu/ha 12.2% at 1.01 kg Cu/ha 31.7% at 4.032 kg Cu/ha	EFSA Conclusion (2018)
Chrysoperla carnea (larvae)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 12.5% at 1.922 kg Cu/ha Fecundity: 0% at 1.922 kg Cu/ha	EFSA Conclusion (2018)
Chrysoperla carnea (larvae)	Copper oxychloride WP	Extended laboratory test (3D)	Mortality: 4.8% at 0.5 kg Cu/ha 21.4% at 1.0 kg Cu/ha 11.9% at 2.0 kg Cu/ha 23.8% at 4.0 kg Cu/ha 40.5% at 8.0 kg Cu/ha Fecundity: 1.7% at 0.5 kg Cu/ha 16.7% at 1.0 kg Cu/ha 7.9% at 2.0 kg Cu/ha 15.3% at 4.0 kg Cu/ha 6.7% at 8.0 kg Cu/ha	EFSA Conclusion (2018)
Chrysoperla carnea (larvae)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 55.6% at 0.56 kg Cu/ha Fecundity: 71.1% at 0.56 kg Cu/ha	EFSA Conclusion (2018)
Trichogramma cacoeciae (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Parasitisation: 6.4% at 0.59 kg Cu/ha	EFSA Conclusion (2018)
Trichogramma cacoeciae (adults)	Copper oxychloride WP	Extended laboratory test (3D)	Parasitisation: -42.9% at 2.02 kg Cu/ha	EFSA Conclusion (2018)
Diaeretiella rapae (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 14.8% at 0.59 kg Cu/ha Parasitisation: 52.5% at 0.59 kg Cu/ha	EFSA Conclusion (2018)
Poecilus cupreus (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 0% at 0.59 kg Cu/ha Predation: 8.0% at 0.59 kg Cu/ha	EFSA Conclusion (2018)
Pardosa amen-	Tribasic copper	Extended laboratory	Mortality:	EFSA Conclu-

Species	Substance	Exposure System	Results*	Reference
tata (adults)	sulphatte SC	test (3D)	2.9% at 0.0202 kg Cu/ha Predation: 4.39% at 0.2688 kg Cu/ha	sion (2018)
Coccinella septempunctata (larvae)	Copper oxychloride WP	Extended laboratory test (3D)	Mortality: 17.5% at 0.58 kg Cu/ha Fecundity: -149% at 0.58 kg Cu/ha	EFSA Conclusion (2018)
Coccinella septempunctata (larvae)	Tribasic copper sulphatte SC	Extended laboratory test (3D)	Mortality: 20.88 % at 0.0067 kg Cu/ha Fecundity: 43.8 % at 0.1344 kg Cu/ha	EFSA Conclusion (2018)
Field or semi-field tests				
None				

* Positive percentages relate to adverse effects. **Bold values are used in the risk assessment**

Table 9.7.1-2 Endpoints and effect values for non-target arthropods – Cymoxanil

Species	Substance	Exposure System	Results*	Reference
Typhlodromus pyri	Cymoxanil 50 WP	Laboratory test glass plates	**LR_{50} > 0.480 kg a.s./ha**	EFSA Conclusion (2008)
Aphidius rhopalosiphi	Cymoxanil 50 WP	Laboratory test glass plates	**LR_{50} > 0.480 kg a.s./ha**	EFSA Conclusion (2008)

Bold values are used in the risk assessment

Table 9.7.1-3 Endpoints and effect values relevant for the risk assessment for non-target arthropods – FEL02

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri(protonymphs)	FEL02	Extended laboratory test, bean leaf discs, 7 d exposure	LR_{50} > 3 kg prod./ha **ER_{50} = 1 kg prod./ha*** (11.5% effect)	Fallowfield, 2011 KCP 10.3.2.2/01
Aphidius rhopalosiphi(adults)	FEL02	Extended laboratory test, leaves seedling barley, 48 h exposure	LR_{50} > 3 kg prod./ha **ER_{50} > 3 kg prod./ha*** (8.3% effect)	Stevens, 2012 KCP 10.3.2.2/02
Chrysoperla carnea	FEL02	Extended laboratory test, bean leaves, 13 - 25 d exposure	**LR_{50} > 9.6 kg prod./ha**	Moll, 2018 KCP 10.3.2.2/03
Aleochara bilineata	FEL02	Extended laboratory test, soil, 28 d exposure	**LR_{50} > 13.8 kg prod./ha**	Schmitzer, 2018 KCP 10.3.2.2/04
Typhlodromus pyri (protonymphs)	FEL02	Extended laboratory study, aged residue in bean leaves, 14 d exposure	No statistically significant effect at **9.6 kg prod./ha** (mortality and reproduction) after 14 days (-5.7% effect)	Leopold, 2020 KCP 10.3.2.2/05
Aphidius rhopalosiphi	FEL02	Extended laboratory study, aged residue in beans leaves, 48h exposure	No statistically significant effect at **9.6 kg prod./ha** (mortality and reproduction) after 14 days (21.3% effect)	Leopold, 2020 KCP 10.3.2.2/06

* ER_{50} value for reproduction**; bold values are used in the risk assessment**

9.7.1.1 Justification for new endpoints

Additional extended laboratory studies with aged residues of the formulation FEL02 in bean plants are available and are used in the present risk assessment.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

The in-field risk assessment is performed for the use in potatoes (only proposed use) taking into consideration the worst-case application pattern of 6 applications at a dose rate of 3 kg product per ha applied with an interval of 7 days. In the EFSA Peer Review for Copper Compounds (2017) it was discussed whether the use of MAF_{soil} for applications at BBCH < 20 is more appropriate than the use of MAF for leaf substrates, considering available evidence that copper accumulates in soil. Since the GAP proposed use for potatoes at BBCH 21-95, this approach was not considered as relevant in the present risk assessment. Therefore, the foliar MAF of 3.2 is considered appropriate for the risk assessment for copper, cymoxanil and FEL02. The resulting PER are presented in the tables below.

Table 9.7.2.1-1 First-tier assessment of the in-field risk for the active substance copper to non-target arthropods due to the use of FEL02 in potatoes

Intended use	Potatoes		
Active substance/product	copper		
Application rate [kg a.s./ha]	6 × 0.6		
MAF	3.2		
Test species **Tier I**	**LR_{50} (lab.)** **[kg Cu/ha]**	**$PER_{in\text{-}field}$** **[kg Cu/ha]**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	> 14.7	1.92	< 0.131
Aphidius rhopalosiphi	> 13.2		< 0.145

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50% effect.

Table 9.7.2.1-2 First-tier assessment of the in-field risk for the active substance cymoxanil to non-target arthropods due to the use of FEL02 in potatoes

Intended use	Potatoes		
Active substance/product	cymoxanil		
Application rate [kg a.s./ha]	6 × 0.12		
MAF	3.2		
Test species **Tier I**	**LR_{50} (lab.)** **[kg Cym/ha]**	**$PER_{in\text{-}field}$** **[kg Cym/ha]**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	> 0.480	0.384	< 0.800
Aphidius rhopalosiphi	> 0.480		< 0.800

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50% effect.

The Tier-1 in-field HQ values based on the active substances copper and cymoxanil (Tables 9.7.2.1-1 and 9.7.2.1-2) are below the relevant trigger value of 2, indicating an acceptable in-field risk for non-target arthropods to the active substances. Four extended studies with the formulated product FEL02, including the two indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* and two additional species *Chrysoperla carnea* and *Aleochara bilineata* are available and therefore the higher tier risk assessment was also performed.

Table 9.7.2.1-3 Higher tier assessment of the in-field risk to non-target arthropods due to the use of FEL02 in potatoes

Intended use	Potatoes		
Active substance/product	FEL02		
Application rate [kg product/ha]	6 × 3.0		
MAF	3.2		
Test species Higher-tier	**ER_{50}[1]/LR_{50}[2] [kg FEL02/ha]**	**$PER_{in\text{-}field}$ [kg FEL02/ha]**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	1.0(1)	9.6	**9.6**
Aphidius rhopalosiphi	> 3.0(1)		**< 3.2**
Chrysoperla carnea	> 9.6(2)		< 1
Aleochara bilineata	> 13.8(2)		< 0.696
Typhlodromus pyri (Aged residues, 14 DAA)	> 9.6**		< 1
Aphidius rhopalosiphi (Aged residues, 14 DAA)	> 9.6**		< 1

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50% effect.
** No statistically significant effects on mortality and reproduction at 9.6 kg FEL02/ha rate.

Based on the extended studies with the indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* an in-field risk to NTA could not be excluded. Therefore, additional aged residues studies with both indicator species as well as extended tests with *Chrysoperla carnea* and *Aleochara bilineata* were performed. As shown in Table 9.7.2.1 3, all HQ based on the additional studies were below 1, indicating an acceptable risk. There is no unacceptable off-field risk, as demonstrated under 9.7.2., and no statistically significant effects on mortality and reproduction of the indicator species were seen when exposed to FEL02 at a dose rate of 9.6 kg/ha aged for 14 days, which demonstrates a potential for re-colonisation of the in-field environment by affected NTA populations within one year.

9.7.2.2 Risk assessment for off-field exposure

The off-field risk assessment is performed for the use in potatoes (only proposed use) taking into consideration the worst-case application pattern of 6 applications at a dose rate of 3 kg product per ha applied with an interval of 7 days. In the EFSA Peer Review for Copper Compounds (2017) it was discussed whether the use of MAF_{soil} for applications at BBCH < 20 is more appropriate than the use of MAF for leaf substrates, considering available evidence of copper accumulation in soil. Since the GAP proposed use for potatoes at BBCH 21-95 this approach was not considered as relevant in the present risk assessment. Therefore, the foliar MAF of 3.2 is considered appropriate for the risk assessment for copper, cymoxanil and FEL02. The resulting PER are presented in the tables below.

Table 9.7.2.2-1 First-tier assessment of the off-field risk for the active substance Copper to non-target arthropods due to the use of FEL02 in potatoes (arable crops)

Intended use	Potatoes						
Active substance/product	Copper						
Application rate [kg/ha]	6 × 0.6						
MAF	3.2						
vdf	5 (2-D)						
Test species Tier I	**LR_{50} (lab.) [kg Cu/ha]**	**Drift rate [%]**	**vdf**	**$PER_{off\text{-}field}$ [kg Cu/ha]**	**CF**	**$PER_{off\text{-}field}$ corrected [kg Cu/ha]**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 13.2	1.64	5	0.0063	10	0.0630	0.005
Aphidius rhopalosiphi	> 14.7						0.004

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50% effect.

Table 9.7.2.2-2 First-tier assessment of the off-field risk for the active substance Cymoxanil to non-target arthropods due to the use of FEL02 in potatoes (arable crop)

Intended use	Potatoes						
Active substance/product	Cymoxanil						
Application rate [kg/ha]	6 × 0.12						
MAF	3.2						
vdf	5 (2-D)						
Test species Tier I	**LR_{50} (lab.) [kg Cym/ha]**	**Drift rate [%]**	**vdf**	**$PER_{off\text{-}field}$ [kg Cym/ha]**	**CF**	**$PER_{off\text{-}field}$ corrected [kg Cym/ha]**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 0.480	1.64	5	0.0013	10	0.0126	0.026
Aphidius rhopalosiphi	> 0.480						0.026

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50% effect.

The Tier-1 off-field HQ values based on the active substances copper and cymoxanil (Tables 9.7.2.2-1 and 9.7.2.2-2) are below the relevant trigger value of 2, indicating no off-field risk for non-target arthropods to the active substances. Four extended studies with the formulated product FEL02, including the two indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* and two additional species *Chrysoperla carnea* and *Aleochara bilineata* are available and therefore the higher tier risk assessment was also performed.

Table 9.7.2.2-3 Higher tier assessment of the off-field risk to non-target arthropods due to the use of FEL02 in potatoes

Intended use	Potatoes						
Active substance/product	FEL02						
Application rate [kg product/ha]	6 × 3.0						
MAF	3.2						
vdf	5 (2-D) / 1 (3-D)						
Test species Higher-tier	**ER_{50}[1]/LR_{50}[2] [kg FEL02/ha]**	**Drift rate [%]**	**vdf**	**$PER_{off\text{-}field}$ [kg FEL02/ha]**	**CF**	**$PER_{off\text{-}field}$ corrected [kg FEL02/ha]**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	1.0(1)	1.64	5	0.0315	5	0.1574	0.157
Aphidius rhopalosiphi	> 3.0(1)		1	0.1574		0.7872	0.262
Chrysoperla carnea	> 9.6(2)		5	0.0315		0.1574	0.016
Aleochara bilineata	> 13.8(2)		5	0.0315		0.1574	0.011

MAF: Multiple application factor; PER: Predicted environmental rate; Criteria values shown in bold breach the relevant trigger.

In the higher tier assessment, the $PER_{off\text{-}field}$ corrected were below the HQ of 1 for all test species, demonstrating an acceptable risk to non-target arthropods when FEL02 is used as proposed in potatoes.

9.7.2.3 Additional higher-tier risk assessment

No additional higher-tier risk assessment needed.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Based on an acceptable of field risk and a potential for recolonisation from the off-field environment to the in-field environment within one year, the risk of FEL02 to non-target arthropods, when used as proposed, is concluded to be acceptable without mitigation measures.

zRMS comments: zRMS agrees with the Applicant's assessment with This assessment indicates that Cuprofix C poses low risk in-field and off-field for non-target arthropods following application according to the proposed use patterns. It can therefore be concluded that the in-field and off-field risk to non-target arthropods is low for the representative uses.

Updated 04.2024r.

According to the AT comment: Please use the endpoints for the risk assessment of copper that were also used in the EFSA renewal.

Opinion zRMS: In addition, it should be taken into account that during the ecotox expert meeting for copper compounds it was suggested that for soil the total amount applied in the season should be used since it cannot be ensured that dissipation occurs between applications. The experts agreed to use the total amount applied in the year in the risk assessment for soil NTA. Therefore, in -field risk assessment with MAFsoil of 1 as the worst case was provided by zRMS in the table below.

First-tier assessment of the in-field risk for non-target arthropods due to the use of FEL02

Intended use	Potatoes
Active substance/product	Copper
Application rate [g Cu/ha]	6 x 600
MAF	1 (soil)

Test species Higher-tier	**LR_{50} (lab.) [g Cu/ha]**	**$PER_{in\text{-}field}$ (g Cu /ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
T. pyri	>13200	3600	0.27
A. rhopalosiphi	>14700		0.24

*** During the ecotox expert meeting it was suggested that for soil the total amount applied in the season should be used since it cannot be ensured that dissipation occur between applications. The experts agreed to use the total amount applied in the year in the risk assessment for soil NTA.

The $HQ_{in\text{-}field}$ is below trigger of 2, indicating an acceptable risk for soil NTA.

First-tier assessment of the off-field risk for non-target arthropods due to the use of FEL02

Intended use	Potato				
Active sub-stance/product	Copper				
Application rate [g/ha]	6 x 600				
MAF	3.2 foliar				
vdf	10 (Tier 1)				
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**
Typhlodromus pyri (2-D)	>13200	1.64%	0.003	10	0.002
Aphidius rhopalosiphi (2-D)	>14700				0.002
MAF	1 soil				
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**
Typhlodromus pyri (2-D)	>13200	1.64	0.006	10	0.0045
Aphidius rhopalosiphi (2-D)	>14700				0.004

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.

First-tier assessment of the off-field risk for non-target arthropods due to the use of FEL02

Intended use	Potato				
Active sub-stance/product	Copper				
Application rate [g/ha]	6 x 600				
MAF	3.2 foliar				
vdf	5 (Tier 1)				
Test species Tier I	**LR_{50} (lab.) [g/ha]**	**Drift rate**	**$PER_{off-field}$ [g/ha]**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri (2-D)	>13200	1.64%	0.006	10	0.0045
Aphidius rhopalosiphi (2-D)	>14700				0.004
MAF	1 soil				
Test species Tier I	**LR_{50} (lab.) [g/ha]**	**Drift rate**	**$PER_{off-field}$ [g/ha]**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri (2-D)	>13200	1.64	0.012	10	0.009
Aphidius rhopalosiphi (2-D)	>14700				0.008

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.

The calculations of the risk assessment off-field for two indicator species were performed by zRMS-PL. The off-field risk assessment with MAF_{soil} of 1 as the worst case was provided by zRMS (based on VDF = 10 and 5). Based on the results off-field risk to non-target arthropods is considered as acceptable according to the proposed use patterns.
The $HQ_{off-field}$ is below trigger of 2, indicating an acceptable risk for soil NTA.

Updated 04.2024r.

There are some studies formulation ATOFEL02. In our opinion - due to the same content of the active substance inside FEL02 and ATOFEL02 (cooper as Bordeaux mixture 200 g/kg and cymoxanil 40 g/kg) and the same type of formulation (water-dispersible granule - WG formulation) it could be used in risk assessment in ecotoxicology point of view. Due to the AT and CZ comments, the Applicant should provide a comparison of the formulations of ATOFEL 02 and FEL02 including Part C (considering the new more strict rules by EFSA also applied at a.s. level). This approach should be considered at MSs level.

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 different copper compounds and with cymoxanil. Full details of these studies are provided in the respective EU DARs and related documents. Studies performed with the formulation can be found in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of FEL02 were not evaluated as part of the EU assessments of cymoxanil and copper. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Table 9.8.1-1 Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – Copper compounds

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Copper chloride	Mixed into substrate / 28 d, chronic 3.9 % peat content	NOEC = 8.4 mg Cu/kg dw	EFSA Conclusion (2018)
Folsomia candida	Copper chloride	Mixed into substrate 28 d, chronic 1.4-37 % peat content	EC_{10} = 31 mg/kg dw	EFSA Conclusion (2018)
Hypoaspis aculeifer	Copper chloride	Mixed into substrate 21 d, chronic 3.9 % peat content	EC_{10} = 179 mg/kg dw	EFSA Conclusion (2018)
Field studies				
A 10 year field study on earthworms population has been conducted on grassland with copper applications every year. After 10 years of treatment with copper, the NOEC of the study is the dose rate 4 kg Cu/ha (EFSA Conclusion, 2018)				
Litter bag test				
Not conducted				

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

Table 9.8.1-2 Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – Cymoxanil

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Cymoxanil	Acute	LC50 > 1000 mg/kg dw	EFSA Conclusion (2008)
Eisenia fetida	Cymoxanil	Chronic	6.6 mg/kg soil, based on product containing Cymoxanil and another active ingredient	EFSA Conclusion (2008)

Table 9.8.1-3 Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – FEL02

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	ATOFEL 02	Chronic, 56 d, mixed into soil, 10% peat	NOEC = 525 mg product/kg soil dw (126 mg total a.s./kg soil dw)	McCormac, 2012 KCP 10.4.1.1/01
Hypoaspis aculeifer	FEL02	Chronic, 14 d, mixed into soil, 5% peat	Mortality, NOEC ≥ 1000 mg/kg soil dw Reproduction, NOEC ≥ 1000 mg/kg soil dw (240 mg total a.s./kg soil)	Lührs, 2018a KCP 10.4.2.1/01
Folsomia candida	FEL02	Chronic, 28 d, mixed into soil, 5% peat	Mortality, NOEC ≥ 1000 mg/kg soil dw Reproduction, NOEC ≥ 1000 mg/kg soil dw (240 mg total a.s./kg soil)	Lührs, 2018b KCP 10.4.2.1/02
Field studies				
None				
Litter bag test				
None				

9.8.1.1 Justification for new endpoints

The EU agreed endpoints on earthworms and other non-target soil organisms (mesofauna) are used in the present risk assessment. Additional studies conducted with the formulation FEL02 on earthworms and other non-target soil organisms (mesofauna) are available and are also used in the risk assessment.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Point 8.7.2, **Table 8.7.2-4**.

A need to include natural background levels of copper originating from geogenic copper and previous anthropogenic copper inputs from a variety of sources in the soil exposure assessment was identified (EFSA, 2013). This requirement to include sources other than the regulated use is exceptional, possibly uniquely required for copper, so a standard soil exposure assessment is not possible.

European monitoring programs provided a comprehensive overview of copper levels in agricultural soils. Concentrations suitable for use in soil exposure assessments, including sources other than the regulated use, were identified. Accumulated PEC_{soil} values were calculated for repeated annual applications. More details on the predicted environmental concentrations (standard field calculations) in soil (PEC_{soil}) for copper in soil are presented in Part B.8, Point 8.7. For details on the assumptions, please refer to dRR Part B8, Environmental Fate.

Table 9.8.2.1-1 First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FEL02 in Potatoes

Product/active substance	NOEC / EC_{10} [mg a.s./kg dw]	PEC_{soil} [mg/kg dw]	TER_{LT} (criterion TER ≥ 5)
Chronic effects on earthworms			
Cymoxanil	6.6	0.129	51
Copper	8.4	38[a,b]	**0.22**
		25.4[a,c]	**0.33**
		19[a,d]	**0.44**
FEL02	126*	38.1[a,b]	**3.3**
		25.5[a,c]	**4.9**
		19.1[a,d]	6.6
Combination toxicity	-	38.1[a,b]	**0.22****
		25.5[a,c]	**0.33****
		19.1[a,d]	**0.44****
Chronic effects on *Folsomia candida*			
Copper	31	38[a,b]	**0.82**
		25.4[a,c]	**1.2**
		19[a,d]	**1.6**
FEL02	≥ 240*	38.1[a,b]	≥ 6.3
		25.5[a,c]	≥ 9.4
		19.1[a,d]	≥ 13
Chronic effects on *Hypoaspis aculeifer*			
Copper	179	38[a,b]	**4.7**
		25.4[a,c]	7.1
		19[a,d]	9.4
FEL02	≥ 240*	38.1[a,b]	≥ 6.3
		25.5[a,c]	≥ 9.4
		19.1[a,d]	≥ 13

TER values shown in bold fall below the relevant trigger.
*Expressed as total a.s. ** Calculated assuming concentration addition according to $TERcombi=1/((1/TER_{a.s.1}) + (1/TER_{a.s.2})$
a Overall $PEC_{soil,\ accumulation}$ over 7 years = Background monitoring value + C_{low} + $PEC_{soil,\ initial}$ at 5 cm
b based on background monitoring value of 26 mg Cu/kg
c based on background monitoring value of 13.4 mg Cu/kg
d based on background monitoring value of 7 mg Cu/kg

The TER for both cymoxanil and FEL02 are above the relevant trigger indication an acceptable risk. The risk of copper to *Hypoaspis aculeifer* is also acceptable as the TER based on the refined monitoring data is above the trigger as well. However, as the long-term first tier TER values for copper are lower than the trigger for earthworms and *Folsomia candida* a refinement of the risk of copper is presented under 9.8.2.2. Based on the toxicity of the active substances respectively and assuming concentration addition, the long-term first tier CombiTER values are all below the rigger of 5. Therefore, further consideration of the risk of a combination of the actives is presented under 9.8.2.2, based on the refinement presented for copper and taking into account that copper is the driver of the risk.

Metabolites

In line with EFSA conclusion for cymoxanil (2008), due to the rapid degradation of cymoxanil the major metabolites IN-U3204, IN-W3595 and IN-JX915 were assumed to have been formed in the test systems of the studies performed with cymoxanil or with the formulation FEL02. The risk assessment for cymoxanil is therefore considered to cover that of the metabolites as well. Since no data on the toxicity to *Folsomia candida* and *Hyposaspis aculeifer*

are available, the endpoints obtained with the formulation have been considered as a worst-case approach. The resulting TER values are presented in the table below.

Table 9.8.2.1-2 First-tier assessment of the chronic risk of metabolites for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FEL02 in Potatoes

Product/active substance	NOEC / EC_{10} [mg a.s./kg dw]	PEC_{soil} [mg/kg dw]	TER_{LT} (criterion TER ≥ 5)
Chronic effects on earthworms			
Cymoxanil	6.6	0.129	51
Cymoxanil*	21	0.129	163
Chronic effects on *Folsomia candida*			
Cymoxanil*	≥ 39	0.129	302
Chronic effects on *Hypoaspis aculeifer*			
Cymoxanil*	≥ 39	0.129	302

TER values shown in bold fall below the relevant trigger.
*based on endpoints obtained with FEL02

As Tier I calculations indicate an acceptable risk to earthworms and other non-target soil organisms based on exposure to cymoxanil, the risk of its relevant soil metabolites to earthworms and other non-target soil organisms is considered acceptable as well.

9.8.2.2 Higher-tier risk assessment

So far, the following soil invertebrate species have been tested in the laboratory: most often the lumbricid species *Eisenia fetida* and *E. andrei* but also several species belonging to the invertebrate mesofauna: the springtail *Folsomia candida*, the predatory mite *Hypoaspis aculeifer* (see **Table 9.8.2.1-1**), and the enchytraeid *Enchytraeus crypticus* (App. 1+2 KCP 10.4.1.2/04, Caetano et al., 2015). Referring to the information presented above it seems that earthworms are the most sensitive species among those tested so far. However, in higher-tier tests only earthworms and, partly, enchytraeids have been studied (App. 1 KCP 10.4.1.2/08 and KCP 10.4.1.2/09. Menezes-Oliveira et al., 2011, 2013). In terms of sensitivity all data gained so far indicate that earthworms react most sensitively to the exposure to copper, meaning that they are the main invertebrate group to be considered in risk assessment.

However, there are more good reasons to focus higher tier, in particular, field studies on the effects of copper in the soil compartment on earthworms: in temperate regions they are in many, especially agricultural (crop sites, grasslands) soils the dominant soil invertebrate group in terms of their ecological functions. In comparison to most other soil organisms lumbricid earthworms are relatively large and provide in many soils the highest biomass. Ecologically, they are divided in three ecological groups (App. 1 KCP 10.4.1.2/06. Bouché, 1977): litter dwellers (epigeics) (1) live at or close to the soil surface in the organic matter such as leaf litter. Actually, the well-known test species *Eisenia fetida* and *E. andrei* belong to this group. Mineral dwellers such as the ("endogeics") (2) live in horizontal burrows in the mineral soil. The globally widely distributed species *Aporrectodea caliginosa* belongs to this group. Vertical burrowers (anecics) (3) live in deep vertical burrows. Best example for this group is *Lumbricus terrestris* which act as "ecosystem engineers", i.e., organisms which "directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing they modify, maintain and create habitats" (App. 1, KCP 10.4.1.2/07. Jones et al., 1997). Earthworms provide an impressive list of ecological services, especially at agricultural sites, where at least several species provide several ecosystem services, such as nutrient cycling, drainage, and regulating greenhouse gas emissions. Probably from a human point of view their ability to stimulate crop growth is their the most important contribution (e.g. Van Groenigen et al. 2014, see App. 1, KCP 10.4.1.2/10), but their positive influence on other services such as water drainage, soil aggregate stability, distribution of microbial populations or being a relevant food source for many predators should also not be forgotten.

The field study (App. 1 + 2, KCP 10.4.1.2/01. Klein, 2015) was performed to evaluate the effects of copper on the earthworm fauna in Central Europe. Copper hydroxide was applied over a period of 10 years on two investigation sites with three different doses (T1: 4 kg/ha/year; T2: 8 kg/ha/year; T3: 40 kg/ha/year). The collected data on earth-

worm abundance, biomass, and earthworm species were evaluated using different statistical methods.

In an addendum to the final report of the field study, the applied statistical methods are described and discussed in detail (App. 1+2, KCP 10.4.1.2/02. Klein, 2019. Addendum 1 to final report). A summary of the basics is given below:

- Analysis of variance (ANOVA) and Analysis of covariance (ANCOVA):
  - analysis calculated and each treatment compared to the control using a two-sided Dunnett's t-test at the 5% significance level
  - robust and sensitive way to analyse for potential significant treatment effects
  - procedure recommended by ISO (ISO 11268-3, ISO 2014) and by De Jong et al. (2006). App. 1, KCP 10.4.1.2/05.
- Principal response curve (PRC):
  - a common multivariate analysis, a special type of redundancy analysis (time as covariate, interaction between time and treatment as environmental factor to show differences from the control), evaluation of extent and course of development of the earthworm abundance compared to the control taking into account the time factor and random changes
  - univariate analysis of the PRC scores of the first axis to identify differences between individual sampling points
  - time as a covariate, aims to translate the responses from a large number of taxa into a smaller number of components that can be interpreted as representing the response of the whole community
  - method to be used to refine the interpretation of effects on the population level
  - procedure listed as viable method in ISO 11268-3 (ISO 2014) and recommend by De Jong et al. (2010)[11] for the analysis of non-target arthropod field studies
- Linear mixed models (LMM):
  - also includes time to the interpretation of results
  - its ability to detect significant treatment effects is limited due to the restriction of normal distributed data
  - Tukey test (results comparable to ANOVA/ANCOVA)
  - LSD test: over-conservative due to expected and observed alpha inflation increasing the overall chance of a type I error to theoretically 14% instead of 5%. According to Environment Canada (2005)[12], the LSD test should only be used for a small pre-selected selection of all possible comparisons to avoid this inflation of false positives (type I error).

Significant effects on earthworms were observed in the highest treatment only (40 kg/ha/year), while the two lower treatments showed only individual and isolated differences compared to the water-control treatment. These isolated cases (for some species or groupings) were e.g. significant reductions in abundance and biomass in the two lower treatments (T1: 4 kg Cu/ha/year; T2: 8 kg Cu/ha/year) which were detected at different sampling dates, but which were not observed on consecutive sampling dates. It seemed that these significant reductions appeared sporadically but disappeared again in later samplings. Similar observations are considered in long-term studies as normal sporadic changes in earthworm species abundance and has been confirmed by the PRC analysis at community level (and the linear mixed model). Due to the erratic nature of significances observed in T1 and T2, those effects were not considered caused by the treatment with copper. As agreed by the expert panel, these effects are not significant at the community level (see EU Dossier Vol. 3, B.9 (AS), p. 454-455).

The results of the study after 8 years of application were reviewed by an independent expert panel (Dr. K.C. Brown, Prof. Dr. P. van den Brink; Dr. C.A.M.van Gestel). **All three experts supported a NOEC of 8 kg/ha/year**.

According to the RMS, additional statistical analysis using the LMM provided in the study report (App. 1+2, KCP 10.4.1.2/01, Klein, 2015) show that specific effects were observed during 2011 and 2013 also in the two lower treatments (T1 and T2). The LMM was performed using two methods: 1) Tukey and 2) LSD. The LMM was applied

[11] De Jong, F.M.W, Bakker, F.M, Brown, K, Jilesen, C.J.T.J, Posthuma-Dodeman, C.J.A.M., Smit, C.E., Van der Steen, J.J.M. & Van Eekelen, G.M.A. (2010) Guidance for summarizing and evaluating field studies with nontarget arthropods, National Institute for Public Health and the Environment, The Netherlands.

[12] ENVIRONMENT CANADA (2005) Guidance Document on Statistical Methods. EPS, l/RM/46. Ottawa, ON, Canada.

to investigate effects abundances and biomass at the samplings for individual species, ecotypes and other groupings. The Tukey test only identified significant effects for the highest treatment (40 kg/ha/year), while the LSD method detected several significant effects in the T1 as well as the T2 treatment. For both methods the significance level was set at alpha = 5%. With regard to the results of the LMM statistical evaluation, the RMS proposes a no observed adverse effect concentration (NOAEC) of 4 kg/ha/year.

As described above, the LSD test is over-conservative as it is prone to alpha-inflation, which results in false positives seeing significant effects where in reality no effects are. In case of this field study, the theoretical chance of a type I error increases from the selected 5% to 14.3% when performing all possible pairwise comparisons for a given taxon on the data set (26 sampling occasions, 26 comparisons). As described above, Environment Canada restricts the use of the LSD test procedure.

In conclusion, the results of the statistical evaluation with the LMM and the LSD test should not be considered for the derivation of the NOE(A)C of this earthworm field study. As only individual, isolated significant effects were observed at the T1 and the T2 treatment levels based on the other reliable and recommended statistical methods, **a NOEC of 8 kg Cu/ha/year is plausible**.

In addition to the field study (App. 1+2, KCP 10.4.1.2/01. Klein, 2015), a long-term laboratory study with earthworms and soil from the investigated sites of the field study was performed (Wagenhoff, 2019; App. 1+2 KCP 10.4.1.1/02). This study was designed to determine the effects of Cu-level and soil properties in different Cu-loaded soils originating from two field sites on adult mortality, body weight change and on reproduction of field-collected adult *Aporrectodea caliginosa* SAVIGNY (Annelida, Lumbricidae), an earthworm species which is known to be sensitive to high soil Cu concentrations. The test organisms originated from the same field sites as the soils and a crossover design was used: earthworms from both field sites were exposed to soils of both field sites. According to the study director, this was the first attempt to study chronic effects in *A. caliginosa*. Therefore, no guidance and experience were available.

The findings observed during the course of the study have been found related to missing guidance on how to conduct such a study and maintain *A. caliginosa* for an extended period in the laboratory environment. No adverse effects could be derived from the presence of copper in the field sampled soils.

The following observations were made during the study:

- The Cu concentration in the sampled topsoils (0-5 cm) were at a similar level (control soils: Niefern: 26.5 mg/kg soil dw; Heiligenzimmern: 25.9 mg/kg soil dw; treated soils: Niefern: 135.2 mg/kg soil dw; Heiligenzimmern: 142.2 mg/kg soil dw). In the treated plots, Cu had been applied three times per year at a nominal rate of 8 kg Cu/ha/year for the past 14 years. However, the soil samples differed in ecologically relevant physicochemical parameters (WHC_{max}, soil texture (% sand, silt and clay), content of organic matter).
- Adult mortality was not affected by the Cu-treated soils compared to the control soils after 112 days. Mortality in the treated soils was lower than in the control soils where a maximum mortality of 20% was reached after 112 days.
- During the exposure phase an increasing number of the adult worms were observed to have entered a stage of quiescence. After 112 days of exposure to the test soils, almost half of the worms had entered the quiescent stage. The presence of copper did not have an effect on the appearance of quiescence. A continuous loss of biomass was also observed during the 112 days of exposure in each of the treatment groups. Loss of biomass and the increasing number of worms entering a stage of quiescence indicated adverse changes in the test soil environment. The test conditions were most likely mainly influenced by the fluctuation and the decrease of the moisture content of the test soils.
- The adult biomass change was influenced by the following factors: origin of worms, treatment of soil. A third factor, the origin of the soil, did not solely affect the earthworm biomass. Earthworms originating from Niefern had a higher initial biomass than Heiligenzimmern worms and showed a higher biomass loss. In the Cu-treated soils a higher biomass loss was observed than in the control soils.
- The number of juveniles was affected by the following factors: origin of soil (higher reproductive output in Heiligenzimmern soils) and treatment of soil (higher reproductive output in Cu-treated soils), but not solely affected by the factor origin of worms. There was a significant two-factor interaction between treatment of soil and origin of soil (difference in juvenile numbers between Cu and control treatment more pronounced in the Heiligenzimmern soil) and between treatment of soil and origin of worms (difference in juvenile numbers between Cu and control treatment more pronounced in the Heiligenzimmern worms) as well as an interaction between all three factors. Higher reproductive output in Cu-treated soils compared to control soils can most probably not be attributed to the presence of higher Cu concentrations in the treated soils but rather to differences among physicochemical soil parameters between Cu-treated and control soils (e.g. wa-

ter availability, water potential).

It can be concluded that the field-aged copper concentrations of 135 and 142 mg/kg soil dw, which resulted from an application of 8 kg Cu/ha/year for the past 14 years (3 times/year), did not cause any adverse effects on *A. caliginosa*.

This lab study supports the derivation of the NOEC of 8 kg Cu/ha/year based on the long-term field study.

Short-term effects of Cu fungicide (Cu oxychloride) on enchytraeid and earthworm communities were investigated under field conditions (Amossé et al., 2018; App. 1+2: KCP 10.4.1.2/03). The Cu fungicide was applied at two doses (0.75 and 7.5 kg Cu/ha). At both concentrations no effect was observed on the earthworm population. With regard to the EFSA opinion (EFSA PPR Panel 2017), this corresponds to negligible effects (i.e., reduction up to 10%). Thus, **this study also supports the NOEC of 8 kg Cu/ha/year** derived from the long-term field study (App. 1+2, KCP 10.4.1.2/01; Klein, 2015).

In addition to the above derived NOEC of 8 kg Cu/ha/year, regulatory acceptable concentrations (RAC) for copper exposure to earthworms have been derived for the major regulatory zones of Europe and three types of land coverage by Oorts & Peeters (2019); App. 1: KCP 10.4.1/01.

These RACs are based on an evaluation of a quality-screened database on chronic toxicity of Cu to earthworms (Oorts, 2015; App. 1: KCP 10.4.1/02). 62 reliable EC_{10}/NOEC values for long-term effects on earthworms (*Eisenia andrei*, *Eisenia fetida*, *Lumbricus rubellus*, *Aporrectodea caliginosa*, *Dendrobaena rubida*, *Octolasion cyaneum*) were selected. Some of the data had to be corrected for the type of Cu application (freshly spiked soils vs. aged Cu contamination) using a lab-to-field factor of 4. Geometric mean normalized NOEC/EC_{10} values for the most sensitive endpoint could be calculated only for 3 different earthworm species (*Eisenia andrei*, *Eisenia fetida* and *Lumbricus rubellus*). The lowest geometric mean normalized NOEC/EC_{10} value from each of three species was selected as the regulatory acceptable concentration (RAC) for effects of Cu on earthworms. Without information on soil properties of a site of interest, the lowest species mean value for a reasonable worst-case soil with eCEC of 8 cmolc/kg, i.e. 159 mg Cu/kg, is selected as an appropriate regulatory acceptable concentration (RAC) value for risk assessment.

As the bioavailability of copper is influenced by the soil properties, European data for soil properties from the Land Use and Cover Area frame Statistical survey (LUCAS) database were extracted to calculate adapted RACs (Europe: 21980 data points). Four typical soil properties (pH, organic carbon content, clay content, CEC) were considered as they are strongly variable among soils across Europe.

Distributions of RAC values for the EU and the regulatory zones (North, Centre and South) were calculated non-parametrically because of the high amount of data points available and the 10^{th}, 50^{th} (median) and 90^{th} percentiles, together with minimum and maximum values, of the copper RAC data are reported (**Tables 9.8.2.2-1** and **9.8.2.2-2** below). In addition, the distribution of RAC values for specific land cover types (fruit trees, vineyards and olive groves) was also calculated. The 10^{th} percentile (P10) should be selected as a conservative value to protect most terrestrial scenarios.

With regard to the three major zones of Europe, the RACs increase from north to south at the P10 and median level. The P10 RAC for Europe was calculated to be 111 mg/kg soil dw. The RAC for the northern zone is slightly lower with 94 mg Cu/kg soil dw. The highest RAC was calculated for the southern zone (132 mg Cu/kg soil dw). The RACs (P10) for the three relevant types of land coverage are higher than the regionally adapted RACs ranging between 143 and 165 mg Cu/kg soil dw. The RACs for vineyards and olive groves are almost identical (164 and 165 mg Cu/kg soil dw).

In the long-term field study ((App. 1+2 KCP 10.4.1.2/01; Klein, 2015) the NOEC was determined to be 8 kg Cu/ha/year. Soil samples from the upper soil layer (0–5 cm) contained approximately 130 mg Cu/kg soil dw at both study sites, which is in good agreement with the RACs for the three major regulatory zones (94–132 mg Cu/kg soil dw) as well as with the three types of land coverage (143–165 mg Cu/kg soil dw).

Table 9.8.2.2-1 Distributions of regulatory acceptable concentrations (RAC) for Cu in soil (mg Cu/kg soil dw) in the whole of Europe and major regulatory zones

Zone	# of data points	Min	P10	Median	P90	Max
EU	21980	46	**111**	215	374	1158

Zone	# of data points	Min	P10	Median	P90	Max
North	5129	46	**94**	175	367	1158
Centre	8133	46	**107**	219	383	1034
South	8609	46	**132**	230	369	753

Table 9.8.2.2-2 Distributions of regulatory acceptable concentrations (RAC) for Cu in soil for the land coverages under scrutiny

Land cover	# of data points	Min	P10	Median	P90	Max
Vineyards	326	46	**164**	239	345	468
Olive groves	409	46	**165**	252	345	528
Fruit trees	279	46	**143**	227	369	523

Given that laboratory derived toxicity data for earthworms and other non-target soil macro-organisms showed that earthworms were more sensitive to copper than the other tested macro-organisms, it is considered that the NOEC of 8 kg Cu/ha/year determined from the long-term field study performed on earthworms is protective of other non-target soil macro-organisms.

Combination toxicity

The risk of FEL02 was shown to be acceptable in the Tier I risk assessment. However, a long-term risk to earthworms resulting from copper and therefore also for the combined risk assessment of cymoxanil and copper could not be excluded. As shown in the table below the long-term toxicity of the formulation is driven by copper, but not to an extend that would warrant to solely perform the risk assessment for copper.

Table 9.8.2.2-3 Calculation of surrogate LD50 for the mixture of active substances

Active substance	Concentration a.s. in mixture [g/kg]	Fraction a.s. in mixture	NOEC / EC_{10} [mg a.s./kg dw]	Fraction a.s./ endpoint	Surrogate NOEC / EC_{10} [mg a.s./kg dw]	Tox per fraction (a.s.)	Deviation tox per fraction (a.s.) from the tox per fraction (mix) [%]
Copper	200	0.83	6.6	0.099	8.03	9.64	25%
Cymoxanil	40	0.17	8.4	0.025		48.2	393%

However, the Tier 1 assessment shows a very low risk of cymoxanil to earthworms and due to the difference in degradation/ dissipation behaviour of both substances the long-term risk as a result of applications with FEL02 as proposed is clearly driven by copper. This is shown in the table below, where the TER combi is compared to the TER values determined for the active substances separately.

Table 9.8.2.2-4 First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FEL02 in Potatoes

Product/active substance	NOEC / EC_{10} [mg a.s./kg dw]	PEC_{soil} [mg/kg dw]	TER_{LT} (criterion TER ≥ 5)	Difference with TERcombi [%]
Chronic effects on earthworms				
Cymoxanil	6.6	0.129	51	23145
				15470
				11573
Copper	8.4	38[a,b]	**0.221**	0.43
		25.4[a,c]	**0.331**	0.64
		19[a,d]	**0.442**	0.86
Combination of cymoxanil and copper			**TER combi**[e]	
			0.220	-
			0.329	-
			0.438	-

TER values shown in bold fall below the relevant trigger.
a Overall $PEC_{soil,\ accumulation}$ over 7 years = Background monitoring value + C_{low} + $PEC_{soil,\ initial}$ at 5 cm
b based on background monitoring value of 26 mg Cu/kg
c based on background monitoring value of 13.4 mg Cu/kg
d based on background monitoring value of 7 mg Cu/kg
e Calculated assuming concentration addition according to $TERcombi=1/((1/TER_{a.s.1}) + (1/TER_{a.s.2})$

Even taking into account the lowest PECsoil for copper, the difference between the TER for copper and the TER-combi is only 0.86% while the TER for cymoxanil is 11573% higher than the TERcombi. Taking into account that the toxicity of the formulation is lower than that of copper alone based on the available experimental data, and that copper is the driver of the toxicity of the formulation, the higher Tier risk assessment for copper is considered to cover that for the formulation FEL02. Since the risk to earthworms is considered safe up to and including 8 kg Cu/ha/year, the risk of combined exposure of earthworms to cymoxanil and copper resulting from applications of FEL02 is acceptable.

9.8.3 Overall conclusions

The risks of FEL02 to earthworms and other non-target soil macro-organisms are considered to be acceptable when the product is applied according to the proposed GAP.

zRMS comments: The Applicant provided chronic tests on *Eisenia fetida*, and also on *Folsomia candida* and *Hypoaspis aculeifer* for formulation **Cuprofix C**, according to requirements set out in Reg. 284/2013. The studies have been accepted by RMS.

The risk assessment provided by the applicant and was verified by zRMS.
The PEC soil calculations for copper evaluated by e-fate experts in updated Section 8 was taken into account. The Cu added to soil as plant protection product only.
The predicted environmental concentrations are estimated to be higher, if background values of copper in soil will be added. The 7 years', 10 years' 20 years' period was considered and additionally, the natural copper background (median and 90th percentile values) was taken into consideration.
The national background values which cover environmental and land use conditions in the member states should be used for decision-making in terms of authorisations on national level and that outcomes of national risk assessments, if are available.

First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) for Cuprofix C (expressed as a copper) for proposed GAP.

Intended use	Endpoints NOEC/EC_{10}	All crops

Chronic effects on earthworms								
Product/active substance	**$NOEC_{corr}$ (mg Cu/kg dw)**	**Background Monitoring Value [B]**	**PEC_{soil} (mg/kg dw) 7 years [1]**	**PEC_{soil} (mg/kg dw) 10 years [2]**	**PEC_{soil} (mg/kg dw) 20 years [3]**	**TER_{LT} 7 years**	**TER_{LT} 10 years**	**TER_{LT} 20 years**
Copper	8.4	13	46.6	61	109	**0.18**	**0.14**	**0.077**
		26	59.6	74	122	**0.14**	**0.11**	**0.069**
		15	48.6	63	78	**0.17**	**0.13**	**0.11**
Chronic effects on other soil macro- and mesofauna – *Folsomia candida*								
Product/active substance	**$EC_{10\ corr}$ (mg Cu/kg dw)**	**Background Monitoring Value [B]**	**PEC_{soil} (mg/kg dw) 7 years [1]**	**PEC_{soil} (mg/kg dw) 10 years [2]**	**PEC_{soil} (mg/kg dw) 20 years [3]**	**TER_{LT} 7 years**	**TER_{LT} 10 years**	**TER_{LT} 20 years**
Copper	31	13	46.6	61	109	**0.67**	**0.51**	**0.28**
		26	59.6	74	122	**0.52**	**0.42**	**0.25**
		15	48.6	63	78	**0.64**	**0.49**	**0.4**
Chronic effects on other soil macro- and mesofauna – *Hypoaspis aculeifer*								
Product/active substance	**$NOEC_{corr}$ (mg Cu/kg dw)**	**Background Monitoring Value [B]**	**PEC_{soil} (mg/kg dw) 7 years [1]**	**PEC_{soil} (mg/kg dw) 10 years [2]**	**PEC_{soil} (mg/kg dw) 20 years [3]**	**TER_{LT} 7 years**	**TER_{LT} 10 years**	**TER_{LT} 20 years**
Copper	179	13	46.6	61	109	**3.8**	**2.93**	**1.64**
		26	59.6	74	122	**3.0**	**2.42**	**1.47**
		15	48.6	63	78	**3.68**	**2.84**	**2.29**

TER values shown in bold fall below the relevant trigger.

[B] overall median value, 90th percentile, overall mean value in European arable

[1] Overall PECsoil, accumulation = Background monitoring value (overall median value, 90th percentile value and overall mean value) + Clow + PECsoil, initial over 7 years

[2] Overall PECsoil, accumulation = Background monitoring value (overall median value, 90th percentile value and overall mean value) + Clow + PECsoil, initial over 10 years

[3] Overall PECsoil, accumulation = Background monitoring value (overall median value, 90th percentile value and overall mean value + Clow + PECsoil, initial over 20 year

The TER_{LT} values are below trigger value of 5, indicating further refinment for earthworms and soil macro-organism for all proposed uses of Cuprofix C.

Refinement risk assessment:

Several studies were assessed during the RAR, it was concluded an acceptable risk to earthworms at the maximum dose rate of 4 kg Cu /ha per year. During expert meeting (Report from Pesticides Peer Review Meeting 169, 09-10 October 2017, Copper compounds) it was concluded that earthworms seem to be the most sensitive group. In the same time the risk for soil – macroorganism was not able to be ruled out (TER_{LT} below 5). Considering all the available information, zRMS-PL is of the same opinion as RMS in RAR, and considers that the long-term risk of copper compounds would be acceptable for an annual dose rate not higher than 4 kg Cu/ha per year for all soil macroorganism.

Spe 1: To protect soil organisms do not apply this or any other product containing copper for an annual dose rate higher than 4 kg Cu/ha per year.

The proposal for extrapolating the results of the multiyear field study with earthworms to other soil macroorganisms was not supported by the experts at the Peer Review Meeting 169 (October 2017, Copper compounds), although from the available data, earthworms seem to be the most sensitive group. Therefore, the requirement of further data to refine the risk for *Folsomia candida* and *Hypoaspis aculeifer* should be dealt at national level. The risk assessment for earthworms and mesofauna (Collembola, mites) should be considered at MSs level.

Cymoxanil

The TER for both cymoxanil and product Cuprofix C are above the relevant trigger indication an acceptable risk for earthworms. zRMS agreed that taking into account that the toxicity of the formulation is lower than that of copper alone based on the available experimental data, and that copper is the driver of the toxicity of the formulation, the higher Tier risk assessment for copper is considered to cover that for the formulation Cuprofix C for earthworms and other macroorganisms.

Updated 04.2024r.
The risk assessment for earthworms and mesofauna (Collembola, mites) should be considered at MSs level. However, the proposal for extrapolating the results of the multiyear field study with earthworms to other soil macro-organisms was not supported by the experts at the Peer Review Meeting 169 (October 2017, Copper compounds), although from the available data, earthworms seem to be the most sensitive group. Therefore, the requirement of further data to refine the risk for *Folsomia candida* and *Hypoaspis aculeifer* should be dealt at national level.

Updated 04.2024r.

There are some studies formulation ATOFEL02. In our opinion - due to the same content of the active substance inside FEL02 and ATOFEL02 (cooper as Bordeaux mixture 200 g/kg and cymoxanil 40 g/kg) and the same type of formulation (water-dispersible granule - WG formulation) it could be used in risk assessment in ecotoxicology point of view. Due to the AT and CZ comments, the Applicant should provide a comparison of the formulations of ATOFEL 02 and FEL02 including Part C (considering the new more strict rules by EFSA also applied at a.s. level). This approach should be considered at MSs level.

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 Copper, its lead formulation, Cymoxanil and Cymoxanil/Famoxadone 20SC. Full details of these studies are provided in the EU DARs and related documents and EFSA Scientific Report (2008) respectively.

Effects on soil microorganisms of FEL02 were not evaluated as part of the EU assessment of Copper. However, the provision of further data on FEL02 is not considered essential, because according to the EU assessment there is no risk for soil-microorganisms from Copper.

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

Table 9.9.1-1 Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-transformation				
N-transformation	Tribasic copper sulphate SC	28 d	No effects at 11.6 kg Cu/ha (15.47 mg Cu/kg)	EFSA Conclusion (2018)
N-transformation	FEL02	28 d	No effects at 120 mg FEL02/kg soil dry weight (90 kg FEL02/ha) Corresponding to 28.8 mg total a.s./kg soil dry weight (21.6 kg total a.s./ha)	McVean, 2022 KCP 10.5/01
N-transformation	Cymoxanil a.s.	28 d	−15.5 % effect at day 28 at 1.6 mg a.s./kg d.w.soil (1.2 kg a.s./ha)	EFSA Scientific Report 167 (2008)
N-transformation	Cymoxanil/Famoxadone 20SC	28 d	−0.3 % effect at day 28 at 0.016 mL prod./kg d.w.soil (1.44 kg a.s./ha)	EFSA Scientific Report 167 (2008)
Field studies				
A multi-field site study was carried out in three sites in France. Up to four months after treatment with Copper Hydroxide WP (8 × 2 kg Cu/ha and 48 kg Cu/ha) there were no effects on the CO_2 evolution and nitrogen mineralization. There was no either evidence of significant effects on evolved CO_2 and nitrogen nitrification after a 28-day incubation in the presence of ground vine leaves, based on soils contaminated with Hydroxide WP at 16 kg and 48 kg Cu/ha.				EFSA Conclusion (2018)

9.9.1.1 Justification for new endpoints

A laboratory study with FEL02 on soil micro-organisms was conducted and is used in the risk assessment.

9.9.2 Risk assessment

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

The relevant PEC_{Soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7.2-4 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see Point 9.8).

Table 9.9.2-1 Assessment of the risk for effects on soil micro-organisms due to the use of FEL02 in tomatoes

Intended use	Potatoes		
Application rate	6 × 0.6 kg Cu/ha		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25%**	**PEC_{soil} [a] [mg/kg dw]**	**Risk acceptable?**
Cymoxanil a.s.	1.6	0.129 mg/kg dw	Yes (MoS=12.4)
Tribasic copper sulphate SC	15.47 mg Cu/kg (at 28 d)	38[b] mg/kg dw	**No** (MoS = 0.407)

	(11.6 kg Cu/ha)	25.4[c] mg/kg dw	**No** (MoS = 0.609)
		19[d] mg/kg dw	**No** (MoS = 0.814)
FEL02	28.8 mg total a.s./kg (at 28 days)	~~38.1[e] mg total a.s./kg dw~~ 46.664[e] mg/kg dw	~~No (MoS = 0.755)~~ **No (MoS = 0.617)**
		~~25.5[f] mg total a.s./kg dw~~ 61.064[f] mg/kg dw	~~No (MoS = 1.128)~~ **No (MoS = 0.47)**
		~~19.1[g] mg total a.s./kg dw~~ 109.064[g] mg/kg dw	~~No (MoS = 1.506)~~ **No (MoS = 0.26)**
		4.9[h] mg total a.s./kg dw	Yes (MoS = 5.877)

a Overall $PEC_{soil,\ accumulation}$ over 7 years = Background monitoring value + C_{low} + $PEC_{soil,\ initial}$ at 5 cm for Cu
b based on background monitoring value of 26 mg/kg for Cu
c based on background monitoring value of 13.4 mg/kg for Cu
d based on background monitoring value of 7 mg/kg for Cu
~~e based on the PECsoil for cymoxanil + background monitoring value of 26 mg/kg for Cu~~
~~f based on the PECsoil for cymoxanil + background monitoring value of 13.4 mg/kg for Cu~~
~~g based on the PECsoil for cymoxanil + background monitoring value of 7 mg/kg for Cu~~
g based on the PECsoil for cymoxanil + PECinitial for Cu

eOverall $PEC_{soil,\ accumulation}$ = Background monitoring value + C_{low} + $PEC_{soil,\ initial}$ over 7 years and PECs for cymoxanil
fOverall $PEC_{soil,\ accumulation}$ = Background monitoring value + C_{low} + $PEC_{soil,\ initial}$ over 10 years and PECs for cymoxanil
gOverall $PEC_{soil,\ accumulation}$ = Background monitoring value + C_{low} + $PEC_{soil,\ initial}$ over 20 years and PECs for cymoxanil

As presented in the table above, the risk of cymoxanil resulting from applications with FEL02 to microorganism is acceptable. The risk of FEL02 is also acceptable if the PECinitial for copper is taken into account. However, a risk of copper and of FEL02 cannot be excluded, when the PEC values for copper based on monitoring data are taken into consideration. The risk of the formulation is therefore clearly driven by copper. Further consideration of the risk of copper is presented below.

Weight of evidence approach

Copper is essential for the nitrification in soil. Copper deficiency in soil is biologically detectable by the fact that addition of copper to the soil increases the nitrifying power (Lees, 1947)[13]. The rate of nitrification is directly influenced by the availability of Copper. Copper has a very high affinity for organic matter and is strongly bound (Adriano 1986[14], Sloof et al 1989[15]). Smolders et al (2001)[16] indicated that soil pH is the main variable determining the potential nitrification rate of non-contaminated soils. Increasing the soil pH by liming increases the amount of copper held or adsorbed by clay and organic matter, thereby, decreasing the Copper availability (Sutradhar et al., 2017)[17]. The inhibition of Copper to soil nitrification is less on an organic soil than on a mineral soil (Lees, 1947).

[13] H. Lees (1947): The effects of zinc and copper on soil nitrification. Biochem. J. 42(4): 534-538.
[14] D.C. Adriano (1986): Trace elements in the terrestrial environment. Springer Verlag, New York.
[15] W. Sloof, R.F.M.J. Clevan, J.A. Janus, J.P.M. Ros (1989). Integrated criteria document copper. Report No. 758474009. National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands.
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[17] A. K. Sutradhar, D. E. Kaiser, C. J. Rosen, J. A. Lamb (2017): Copper for Crop production. FS-6790-B. University of Minnesota. Nutrient Management.

The effect of copper on the nitrification rate is not regulated by total copper concentration but by free copper concentrations (Braam & Klapwiik, 1981)[18]. Experiments at different pH showed a linear correlation between nitrification capacity and free copper concentration (Braam & Klapwiik, 1981). Due to the known sensitivity of the nitrification and nitrogen mineralization process to physicochemical characteristics of soils, bioassays seem to have limited applicability for Copper toxicity assessment in contaminated soils (Moya et al., 2017)[19]. Oorts et al. (2006)[20] indicated that in field-contaminated soil, toxicity thresholds of Copper for nitrifying microorganisms are higher than in metal-spiked soils. Sauvé et al. (1999)[21] showed that microbiological processes of field-collected soil depend more on soil properties than on copper inhibition. In conclusion the outcome of the available laboratory soil micro-organism tests may not reflect the scenario in the field.

According to SANCO/10329/2002 the decisive parameter in the risk assessment is the magnitude of effect compared to the untreated control, and the time-course of recovery. According to Annex VI of 91/414/EEC the critical level is 25% after 100 days. As a matter of fact, the maximum concentrations with effects below 25% after 100 days are not available from laboratory soil micro-organism tests with Copper compounds. The available experimental effect concentrations of 15.47 mg Cu/kg (test item: Tribasic copper sulphate) and 33.3 mg Cu/kg (test item: Bordeaux mixture RSR DISPERSS) for 28 days and 57 days, respectively are below the maximum predicted environmental concentration in soil (PEC_{soil}) of 150.4 mg Cu/kg, resulting in a potential risk. However, it is very likely that the effect concentrations for 100 days would be above the maximum PEC_{soil} value, depending on pH and organic matter of the soil tested. It is also assumed that the PEC_{soil} value is related to the total copper concentration and not to the free copper concentration (water extractable copper), which is the relevant concentration for the inhibition of nitrification. Thus, it is likely that the maximum PEC_{soil} for bioavailable copper is lower than 150.4 mg Cu/kg. Moreover, the micro-organism test with Bordeaux mixture RSR DISPERSS represents a worst-case, since mid-loamy sand was used as test substrate.

A 4-months-field study with Copper Hydroxide WP was performed (EFSA, 2018), where no effects on nitrogen mineralization were detected. The application rate was up to 48 kg Cu/ha/year, exceeding the maximum application rate of 6 kg Cu/ha/year. Based on a lack of effect, an annual application of 6.0 kg Cu/ha is not expected to cause adverse effects on soil microbial function and so the risks following the proposed use are acceptable.

Metabolites

No metabolites are relevant for copper. In line with EFSA conclusion for cymoxanil (2018), due to the rapid degradation of cymoxanil the major metabolites IN-U3204, IN-W3595 and IN-JX915 were assumed to have been formed in the test system of the study performed with cymoxanil. Therefore, as nitrification was not affected at a concentration about 14 times higher than the predicted pec soil for cymoxanil, the risk of relevant metabolites of cymoxanil to soil micro-organisms is considered acceptable as well.

9.9.3 Overall conclusions

The risk of cymoxanil resulting from applications with FEL02 to microorganism is acceptable. The risk of FEL02 is also acceptable if the PECinitial for copper is taken into account. However, a risk of copper and of FEL02 cannot be excluded, when the PEC values for copper based on monitoring data are taken into consideration. The risk of the formulation is therefore clearly driven by copper. Following a WoE approach the risks of copper to soil micro-organisms are acceptable at doses equivalent off up to 6.0 kg Cu/ha. Therefore, it can be concluded that the risk of FEL02 is also acceptable, as the yearly applied rate expressed as copper is only 720 g Cu/ha.

[18] F. Braam, A. Klapwijk (1981): Effect of copper on nitrification in activated sludge. Water Research. 15(9). pp. 1093-1098
[19] H. Moya, J. Verdejo, C. Yánez, J. E. Álvaro, S. Sauvé and A. Neaman (2017): Nitrification and nitrogen mineralization in agricultural soils contaminated by copper mining activities in Central Chile. Journal of Soil Science and Plant Nutrition. 17(1), 205-213
[20] K. Oorts, H. Bronckaers, E. Smolders (2006): Discrepancy of the microbial response to elevated copper between freshly spiked and long-term contaminated soils. Environ. Toxicol. Chem. 25, 845-853.
[21] Sauvé, S., Dumestre, A., McBride, M., Gillett, J.W., Berthelin, J., Hendershot, W. (1999). Nitrification potential in field-collected soils contaminated with Pb or Cu. Appl. Soil Ecol. 12, 29-39

zRMS comments: The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002). The relevant PEC_{soil} for risk assessment covering the proposed use pattern are taken from Section 8 (Environmental Fate). **The risk of cymoxanil resulting from applications with Cuprofix C to microorganism is acceptable. The risk of Cuprofix C is also acceptable if the $PEC_{initial}$ for copper is taken into account. However, a risk of copper and of Cuprofix C cannot be excluded, when the PEC values for copper based on monitoring data are taken into consideration. The risk of the formulation is therefore clearly driven by copper. The risk of the formulation is therefore clearly driven by copper**. However, a multi-field, site study was carried out in three sites in France. Up to four months after treatment with Copper Hydroxide WP (8 x 2 kg Cu/ha and 48 kg Cu/ha) there were no effects on the CO_2 evolution and nitrogen mineralization. There was no either evidence of significant effects on nitrogen nitrification after a 28-day incubation in the presence of ground vine leaves, based on soils contaminated with Copper Hydroxide WP at 16 kg and 48 kg Cu/ha.

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 Copper and Cymoxanil representative formulations. Full details of these studies are provided in the respective EU DARs and related documents, in the EFSA Scientific Report for Cymoxanil as well as in Appendix 2 of this document.

One study on non-target terrestrial plants of FEL02 were also taken into consideration. Data submitted 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 processes. Justifications are provided below.

Table 9.10.1-1 Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – Copper compounds

Species	Substance	Exposure System	Results	Reference
6 species	5 different copper-based test items	21 d Vegetative vigour	ER_{50} > 2000 g/ha	EFSA Conclusion (2018)

Table 9.10.1-2 Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – Cymoxanil

Species	Substance	Exposure System	Results	Reference
Beta vulgaris (Chenopodiaceae) *Glycine max* (Fabaceae) *Helianthus annuus* (Compositeae) *Cucumis sativus* (Curcurbitacae) *Lolium perenne* (Poaceae) *Avena sativa* (Poaceae) *Allium cepa* (Liliaceae)	Cymoxanil 45 WG	21 d Vegetative vigour	ER_{50} > 240 g/ha	EFSA Conclusion (2018)

Table 9.10.1-3 Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants - FEL02

Species	Substance	Exposure System	Results	Reference
O*ats (Avena sativa)* Oil seed rape *(Brassica napus)* Sunflower *(Helianthus annuus)*	FEL02 (Cuprofix C Disperss; Copper: 200 g/kg, Cymoxanil: 40 g/kg)	Seedling emergence	ER_{50} > 3000 g prod./ha (600 g Cu/ha + 120 g Cym/ha)	Friedrich, 2012 KCP 10.6.2/01
Winter wheat *(Triticum aestivum)* Onion (*Allium cepa*) Sugar beet (*Beta vulgaris*) Rape (*Brassica napus*) Cucumber (*Cucumis sativus*) Soybean (*Glycine max*)	FEL02 (Copper: 200 g/kg, Cymoxanil: 40 g/kg)	Seedling emergence	ER_{50} > 18 kg prod./ha (3600 g Cu/ha + 720 g Cym/ha)	McVean, K., 2022d KCP 10.6.2/02
Winter wheat *(Triticum aestivum)* Onion (*Allium cepa*) Sugar beet (*Beta vulgaris*) Rape (*Brassica napus*) Cucumber (*Cucumis sativus*) Soybean (*Glycine max)*	FEL02 (Copper: 200 g/kg, Cymoxanil: 40 g/kg)	Vegetative vigour	ER_{50} > 18 kg prod./ha (3600 g Cu/ha + 720 g Cym/ha)	McVean, K., 2022e KCP 10.6.2/03

9.10.1.1 Justification for new endpoints

Copper and Cymoxanil present endpoints from non-target plant studies performed with their lead formulations, which are not the most relevant endpoints for the risk assessment of this presented dossier. Thus, it is fully justified to use the endpoint from non-target plant study with FEL02.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based on 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.

FEL02 is a fungicide aimed to be applied in potatoes crop in a total of 6 applications of 3.0 kg p.c./ha. Therefore, a risk assessment is performed in the table below. For non-target terrestrial plants, the exposure rate is calculated using the same multiple application factor (MAF) and drift rate as used for the off-field risk assessment for non-target arthropods.

Table 9.10.2.2-1 Assessment of the risk for non-target plants due to the use of FEL02 in potatoes

Intended use		Potatoes (arable crop)			
Active substance/product		FEL02			
Application rate [kg product/ha]		6 × 3.0 (FEL02)			
MAF		3.2			
Test species	**Type of study**	**ER_{50} [kg FEL02/ha]**	**Drift rate [%]**	**$PER_{off\text{-}field}$ [kg FEL02/ha]**	**TER criterion: TER ≥ 5**
O*ats (Avena sativa)* Oil seed rape *(Brassica napus)* Sunflower *(Helianthus annuus)*	Seedling emergence	> 3	1.64	0.157	> 19
Winter wheat *(Triticum aestivum)* Onion (*Allium cepa*) Sugar beet (*Beta vulgaris*) Rape (*Brassica napus*) Cucumber (*Cucumis sativus*) Soybean (*Glycine max*)	Seedling emergence	>18	1.64	0.157	> 114
Winter wheat *(Triticum aestivum)* Onion (*Allium cepa*) Sugar beet (*Beta vulgaris*) Rape (*Brassica napus*) Cucumber (*Cucumis sativus*) Soybean (*Glycine max*)	Vegetative vigour	>18	1.64	0.157	> 114

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

The tier-2 TER values exceed the relevant trigger value, indicating no risk for non-target plants following all intended uses of FEL02.

Whilst the Tier-2 risk assessment showed acceptable risk to the formulation FEL02, due to the special nature of the copper it contains and its potential to accumulate in soils, a higher tier risk assessment based on a literature review was also undertaken for copper.

9.10.2.3 Higher-tier risk assessment

In a literature review (Hoare, 2015)[22] (App. 1, 10.6.2/01) a worst-case approach of exposure to copper was made on the assumption that copper would be applied to a vineyard for 100 years at a rate of six applications per year at 1.0 kg Cu/ha. Referencing the drift values according to Ganzelmeier et al. (2000), the predicted total added copper to the soil in the off-crop areas 3 m outside of the vineyard under this scenario was calculated to be approximately 52 mg/kg above the background level.

In order to calculate the total added copper in the off-crop area several assumptions have been made.

- An annual field application rate of 6 × 1.0 kg Cu/ha.
- The basic drift value (after Ganzelmeier) will be used for six applications (70th percentile) at 1m distance for

[22] Hoare, A. (2015). Toxicity of copper to plants: a literature-based assessment of the risks to non-target plants from the use of copper fungicides. EU Copper Task Force.

field crops (1.64%) and at 3m distance for vines, late application (6.41%).

- All loadings are cumulative, i.e. copper will accumulate in the soil with no dissipation between successive applications via run-off, vertical movement or by plant uptake.
- Soil depth and density will be 5 cm and 1500 kg/m³ respectively.

The total added copper was calculated for 10, 25, 50 and 100 years of successive applications of copper, see the table below.

Table 9.10.2.3-1 Theoretical increase in off-field soil-copper concentration after 10, 25, 50 and 100 years of successive applications of copper at a rate of 6 kg/ha/y

Number of years of successive application	Total added copper [mg/kg]	
	Field crops	Vineyards
10	1.312	5.128
25	3.280	12.82
50	6.560	25.64
100	13.12	51.28

The worst-case increase in total off-field soil-copper concentration was determined to be approximately 51 mg/kg after 100 years of successive applications to vines.

As a conservative estimate, the soil-copper concentration of historically untreated areas is 32 mg/kg. After 100 years of continued application on vineyards at a rate of 6 kg/ha/yr the soil-copper concentration in these areas has been estimated to increase to approximately 84 mg/kg.

This level is below the suggested threshold limit of 100 mg/kg.

It must be remembered that this is a worst-case prediction. No account has been taken of the potential removal of the added copper via leaching, run-off or plant uptake, and crucially, no account has been made for the aging process which reduces the bio-availability of copper.

Overall, it is concluded that even after 100 years of continued application, the application of copper-based fungicides at rates of up to 6 kg/ha/y poses acceptable risks to non-target plants growing in off-crop areas.

The literature review (Hoare, 2015) therefore brings evidence that for applications up to 6 kg Cu/ha/y the risk to non-target plants is acceptable, even after accumulation of copper in soil.

zRMS comments: The calculated TER values are above the Annex VI trigger of 5 based on ER_{50} values from seedling emergence and vegetative vigour and $PER_{off\text{-}field}$, indicating acceptable risk to non-target plants.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risks following exposure of copper to non-target plants are acceptable at annual doses of up to 6 kg Cu/ha.

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

No further testing on, or assessment of risk to, other terrestrial organisms is considered necessary as this is considered to have been addressed in the previous sections.

9.12 Monitoring data (KCP 10.8)

The spectrum of the biological activity of the product is well represented by the results and the risk assessments of this dossier. Therefore, further data from monitoring are not considered relevant for the risk assessment.

9.13 Classification and Labelling

Table 9.13-1 Proposals of classification for the preparation

Compounds	Toxicity	Classification (CLP) 8th ATP to the Regulation (EC) No 1272/2008	
		Hazard category [a]	Code H[b]
Copper (NRD)	**Acute** LC_{50} = 0.0344 mg a.s./L (*O. mykiss*) LC_{50} = 0.0266 mg a.s./L (*D. magna*)	Aquatic acute 1 (M-factor: 10)	H400: Very toxic to aquatic life
	Chronic EC_{10} = 0.00112 mg a.s./L (*A. transmontanus*) NOEC = 0.0076 mg a.s./L (*D. magna*) E_rC_{50} = 0.02229 mg a.s./L (*S. capricornutum*)	Aquatic chronic 1 (M-factor: 10)	H410: Very toxic to aquatic life with long lasting effects
Cymoxanil (NRD)	**Acute** LC_{50} = 29 mg a.s./L (*L. macrochirus*) EC_{50} = 27 mg a.s./L (*D. magna*)	-	-
	Chronic NOAEC = 0.044 mg a.s./L (*O. mykiss*) NOEC = 0.067 mg a.s./L (*D. magna*) NOE_bC = 0.034 mg a.s./L (A. flosaquae)	Aquatic chronic 1 (M-factor: 1)	H410: Very toxic to aquatic life with long lasting effects
FEL02[c]	**Acute** LC_{50} = 47 mg f.p./L (*O. mykiss*) EC_{50} = 0.35 mg f.p./L (*D. magna*) E_rC_{50} = 0.22 mg f.p./L (*P. subcapitata*)	Aquatic acute 1	H400: Very toxic to aquatic life
	Chronic NOE_rC= 0.16 mg f.p./L (*P. subcapitata*)	Aquatic chronic 2	H411: Toxic to aquatic life with long lasting effects
FEL02[d]	**Acute** 20% x 10 = 200% (i.e. ≥25%)	Aquatic acute 1	H400: Very toxic to aquatic life
	Chronic 20% x 10 + 4% x 1 = 204% (i.e. ≥25%)	Aquatic chronic 1	H410: Very toxic to aquatic life with long lasting effects

a.s. Active substance
f.p. Formulated product
NRD Not readily biodegradable
[a] Refer to Table 4.1.0 (a) of 8th ATP to the Regulation (EC) No 1272/2008 (p. 191)
[b] Refer to Table 4.1.4 of 8th ATP to the Regulation (EC) No 1272/2008 (p. 201)
[c] Based on the product data
[d] Based on the summation method using the M-factors derived for the two active substances, according to Regulation (EC) No 1272/2008

Implications for labelling resulting from ecotoxicological assessment according to Regulation (EC) No 1272/2008:

Hazard pictograms

Signal word

Warning

Hazard statement

H410: Very toxic to aquatic life with long lasting effects

Precautionary statement prevention

P273

Precautionary statement response

P391

Precautionary statement disposal

P501

zRMS comments: Agreed.

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 Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2/01	Van Sprang, P.	2019	RESPONSE TO EFSA COMMENTS ON THE AQUATIC EFFECTS ASSESSMENT FOR CU - EXTENSION EU Copper Task Force, not available not available GLP/GEP: no Published: no	N	EUCuTF(*)
KCP 10.2/02	Oorts, K and Verdonck, F	2019	RELEVANCE OF STANDARD ASSESSMENT FACTORS FOR RISK ASSESSMENT OF THE ESSENTIAL ELEMENT COPPER EU Copper Task Force, CuPPP20170705 not available GLP/GEP: no Published: no	N	EUCuTF(*)
KCP 10.2/03	[redacted]	2019	MODELLING OF THE FUNGURAN-OH EFFECTS ON ONCHORHYNCHUS MYKISS POPULATIONS EU Copper Task Force, not available not available GLP/GEP: no Published: no	N	EUCuTF(*)
KCP 10.2/04	Vangheluwe, M.	2019	REVISED PNEC SEDIMENT COPPER FOR THE SEDIMENT EFFECTS ASSESSMENT FOR CU : EXTENDING THE DATABASE WITH ADDITIONAL SPECIES EU Copper Task Force, not available not available GLP/GEP: no Published: no	N	EUCuTF(*)

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	[redacted]	2012a	CUPROFIX C DISPERSS (FEL02): DETERMINATION OF ACUTE TOXICITY TO RAINBOW TROUT (ONCORHYNCHUS MYKISS) United Phosphorus Ltd., BR0587/B [redacted] GLP: yes Published: no	Y	UPL EU
KCP 10.2.1/02	Hutchinson, K.A., Sharpe, A.D.	2012b	CUPROFIX C DISPERSS (FEL02): DETERMINATION OF ACUTE TOXICITY TO DAPHNIA MAGNA United Phosphorus Ltd., BR0586/B Brixham Environmental Laboratory, Brixham, UK GLP: yes Published: no	N	UPL EU
KCP 10.2.1/03	Hutchinson, K.A., Sharpe, A.D.	2012c	CUPROFIX C DISPERSS (FEL02): DETERMINATION OF TOXICITY TO THE GREEN ALGA PSEUDOKIRCHNERIELLA SUBCAPITATA United Phosphorus Ltd., BR0585/B Brixham Environmental Laboratory, Brixham, UK GLP: yes Published: no	N	UPL EU
KCP 10.3.1.1.1/01	Vinall, S.	2011	ATOFEL02 (COPPER 200 G/KG CYMOXANIL 40 G/KG WG) - LABORATORY BIOASSAYS TO DETERMINE THE ACUTE CONTACT AND ORAL TOXICITY TO THE HONEYBEE APIS MELLIFERA United Phosphorus Ltd., UP-11-13 Mambo-Tox Ltd., Southampton, UK GLP: yes Published: no	N	UPL EU
KCP 10.3.1.2/01	Ruhland, S.	2018	CHRONIC TOXICITY OF COPPER 20% + CYMOXANIL 4% WG TO THE HONEY BEE APIS MELLIFERA L. UNDER LABORATORY CONDITIONS UPL Europe Ltd., 17 48 BAC 0058 BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	UPL EU

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.2/02	Colli, M	2018a	Chronic oral effects of copper oxychloride 50% WP to adult worker honey-bees *Apis mellifera* L., 10-day feeding laboratory test BT215/17 Biotechnologie BT srl, Italy GLP: Y Published: No	N	EUCuTF(*)
KCP 10.3.1.1.1/02	McVean, K	2022a	COPPER (FROM BORDEAUX MIXTURE) 20 % + CYMOXANIL 4 % WG ACUTE ORAL TOXICITY TEST ON THE BUMBLEBEE *BOMBUS TER-RESTRIS* UPL Europe Ltd., IUO20269 NOACK LABORATORIEN GMBH GLP: yes Published: no	N	UPL EU
KCP 10.3.1.1.2/01	McVean, K	2022b	COPPER (FROM BORDEAUX MIXTURE) 20 % + CYMOXANIL 4 % WG ACUTE CONTACT TOXICITY TEST ON THE BUMBLEBEE BOMBUS TERRESTRIS UPL Europe Ltd., IUT20269 NOACK LABORATORIEN GMBH GLP: yes Published: no	N	UPL EU
KCP 10.3.1.3/02	Scheller, K.	2018b	COPPER 20% + CYMOXANIL 4% WG- REPEATED EXPOSURE OF HONEY BEE (APIS MELLIFERA L.) LARVAE UNDER LABORATORY CONDITIONS (IN VITRO) UPL Europe Ltd., 17 48 BLC 0092 BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	UPL EU
KCP 10.3.1.3/01	Colli, M	2018b	Effects of copper oxychloride 50% WP to honeybees *Apis mellifera* L. Larval toxicity test, repeated exposure. BT216/17 Biotechnologie BT srl, Italy GLP: Y Published: No	N	EUCuTF(*)

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2/01	Fallowfield, L.	2011	ATOFEL02 (CUPROFIX C DISPERSS) - A RATE-RESPONSE EXTENDED LABORATORY BIOASSAY OF THE EFFECTS OF FRESH FOLIAR RESIDUES ON THE PREDATORY MITE, TYPHLODROMUS PYRI (ACARI: PHYTOSEIIDAE) United Phosphorus Ltd., UP-11-6 Mambo-Tox Ltd., Southampton, UK GLP: yes Published: no	N	UPL EU
KCP 10.3.2.2/02	Stevens, J.	2012	ATOFEL02 (CUPROFIX C DISPERSS) - A RATE-RESPONSE EXTENDED LABORATORY BIOASSAY OF THE EFFECTS OF FRESH RESIDUES ON THE PARASITIC WASP APHIDIUS RHOPALOSIPHI (HYMENOPTERA, BRACONIDAE) United Phosphorus Ltd., UP-11-5 Mambo-Tox Ltd., Southampton, UK GLP: yes Published: no	N	UPL EU
KCP 10.3.2.2/03	Moll, M.	2018	FEL02 (COPPER 20% + CYMOXANIL 4% WG): EFFECTS ON THE LACEWING CHRYSOPERLA CARNEA, EXTENDED LABORATORY STUDY - DOSE RESPONSE TEST - UPL Europe Ltd., 130061047 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	UPL EU
KCP 10.3.2.2/04	Schmitzer, St.	2018	FEL02 (COPPER 20% + CYMOXANIL 4% WG): EFFECTS ON THE REPRODUCTION OF ROVE BEETLES ALEOCHARA BILINEATA - EXTENDED LABORATORY STUDY - - DOSE RESPONSE TEST - UPL Europe Ltd., 130061071 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	UPL EU

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1/01	McCormac, A.	2012	ATOFEL 02 (COPPER 200 G/KG CYMOXANIL 40 G/KG WG) - DETERMINATION OF CHRONIC (SUB-LETHAL) TOXICITY TO THE EARTHWORM EISENIA FETIDA IN AN ARTIFICIAL SOIL SUBSTRATE United Phosphorus Ltd., UP-11-8 Mambo-Tox Ltd., Southampton, UK GLP: yes Published: no	N	UPL EU
KCP 10.4.1/01	Oorts K. and Peeters B.	2019	DISTRIBUTION OF RAC VALUES FOR EFFECT OF CU TO SOIL INVERTEBRATES IN EUROPE. ARCHE CONSULTING, BELGIUM. RESEARCH REPORT SUBMITTED TO THE EUROPEAN COPPER TASK FORCE. European Union 2,4-D Task Force 2012, not available not available GLP/GEP: no Published: no	N	EUCuTF(*)
KCP 10.4.1/02	Oorts, K.	2015	ENVIRONMENTAL HAZARD ASSESSMENT OF CU IN SOIL - EFFECTS ON EARTHWORMS ARCHE (Assessing Risks of Chemicals), CuPPP20150701 not available GLP/GEP: no Published: no	N	EUCuTF(*)
KCP 10.4.1.1/02	Wagenhoff, E.	2019	LABORATORY STUDY ON THE SENSITIVITY OF FIELD-CAUGHT EARTHWORMS APORRECTODEA CALIGINOSA (ANNELIDA, LUMBRIDIDAE) TO COPPER IN GRASSLAND SOILS COLLECTED AT TWO FIELD SITES IN SOUTH-WESTERN GERMANY: A CROSSOVER EXPERIMENT EU Copper Task Force, S18-00119 Eurofins Agroscience Services EcoChem GmbH GLP: yes Published: no	N	EUCuTF(*)

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.2/01	Klein, O.	2015	A FIELD STUDY TO EVALUATE THE EFFECTS OF COPPER ON THE EARTHWORM FAUNA IN CENTRAL EUROPE European Copper Task Force, Petit-Lancy, Switzerland, 20031343/G1-NFEw Eurofins Agroscience Services EcoChem GmbH GLP: yes Published: no	N	EUCuTF(*)
KCP 10.4.1.2/02	Klein, O.	2019	ADDENDUM TO FINAL REPORT: A FIELD STUDY TO EVALUATE THE EFFECTS OF COPPER ON THE EARTHWORM FAUNA IN CENTRAL EUROPE - STATISTICAL ANALYSIS OF A LONG-TERM EARTHWORM FIELD STUDY EU Copper Task Force, 20031343/G1-NFEw Eurofins Agroscience Services EcoChem GmbH GLP: yes Published: no	N	EUCuTF(*)
KCP 10.4.1.2/03	Amossé, J., Bart, S., Pery, A.R.R., Pelosi, C.	2018	SHORT-TERM EFFECTS OF TWO FUNGICIDES ON ENCHYTRAEID AND EARTHWORM COMMUNITIES UNDER FIELD CONDITIONS not available, not available Ecotoxicology, 27(3), pp. 300-312 GLP/GEP: no Published: yes	N	-
KCP 10.4.1.2/04	Caetano, A., L., Ribeiro Marques, C., Goncalves, F., Ferreira da Silva, E., Pereira, R.	2015	COPPER TOXICITY IN A NATURAL REFERENCE SOIL: ECOTOXICOLOGICAL DATA FOR THE DERIVATION OF PRELIMINARY SOIL SCREENING VALUES not available, not available Ecotoxicology, 25(1), pp. 163-177 GLP/GEP: no Published: yes	N	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.2/05	de Jong, F.M.W., van Beelen, P., Smit, C.E., Monforts, M.H.M.M.	2006	A GUIDANCE DOCUMENT OF THE DUTCH PLATFORM FOR THE ASSESSMENT OF HIGHER TIER STUDIES. GUIDANCE FOR SUMMARIZING EARTHWORM FIELD STUDIES. not available, not available National Institute for Public Health and the Environment, The Netherlands, RIVM 601506006/2006, p. 47 GLP/GEP: no Published: yes	N	-
KCP 10.4.1.2/06	Bouché, M.B.	1977	STRATEGIES LOMBRICIENNES. SOIL ORGANISMS AS COMPONENTS OF ECOSYSTEMS not available, not available Ecological Bulletins, 25, pp. 122-132 GLP/GEP: no Published: yes	N	-
KCP 10.4.1.2/07	Jones, C. G., J. H. Lawton, and M. Shachak	1997	POSITIVE AND NEGATIVE EFFECTS OF ORGANISMS AS PHYSICAL ECOSYSTEM ENGINEERS. not available, not available Ecology, 78(7), pp. 1946-1957 GLP/GEP: no Published: yes	N	-
KCP 10.4.1.2/08	Menezes-Oliveira, V. B., Scott-Fordsmand, J. J., Rocco, A., Soares, A., and Amorim, M. J.B.	2011	INTERACTION BETWEEN DENSITY AND CU TOXICITY FOR ENCHYTRAEUS CRYPTICUS AND EISENIA FETIDA REFLECTING FIELD SCENARIOS. not available, not available Science of The Total Environment, 409, pp. 3370-3374 GLP/GEP: no Published: yes	N	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.2/09	Menezes-Oliveira, V. B., Scott-Fordsmand, J. J., Soares, A. MVM, and Amorim, M. J. B.	2013	EFFECTS OF TEMPERATURE AND COPPER POLLUTION ON SOIL COMMUNITY-EXTREME TEMPERATURE EVENTS CAN LEAD TO COMMUNITY EXTINCTION. not available, not available Environmental Toxicology and Chemistry, 32, pp. 2678-2685 GLP/GEP: no Published: yes	N	-
KCP 10.4.1.2/10	Van Groenigen, J. W., I. M. Lubbers, H. M. J. Vos, G. G. Brown, G. B. De Deyn, van Groenigen, K. J.	2014	EARTHWORMS INCREASE PLANT PRODUCTION: A META-ANALYSIS. not available, DOI: 10.1038/srep06365 Scientific Reports, 4, p. 6365 GLP/GEP: no Published: yes	N	-
KCP 10.4.2.1/01	Lührs, U.	2018a	FEL02 (COPPER 20% + CYMOXANIL 4% WG): EFFECTS ON REPRODUCTION OF THE PREDATORY MITE HYPOASPIS ACULEIFER IN ARTIFICIAL SOIL UPL Europe Ltd., 130061089 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	UPL EU
KCP 10.4.2.1/02	Lührs, U.	2018b	FEL02 (COPPER 20% + CYMOXANIL 4% WG): EFFECTS ON REPRODUCTION OF THE COLLEMBOLA FOLSOMIA CANDIDA IN ARTIFICIAL SOIL UPL Europe Ltd., 130061016 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	UPL EU

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.5/01	McVean, K	2022c	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Soil Micro-Organisms: Nitrogen Transformation Test UPL Europe Ltd., TBN19940 NOACK LABORATORIEN GMBH GLP: yes Published: no	N	UPL EU
KCP 10.6.2/01	Friedrich, S.	2012	TERRESTRIAL (NON-TARGET) PLANT TEST WITH CUPROFIX 30 DISPERSS NC: SEEDLING EMERGENCE AND SEEDLING GROWTH TEST United Phosphorus Ltd., 12 10 48 008 P BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	UPL EU
KCP 10.6.2/01	Hoare, A.	2015	TOXICITY OF COPPER TO PLANTS: A LITERATURE BASED AS-SESSMENT OF THE RISKS TO NON-TARGET PLANTS FROM THE USE OF COPPER FUNGICIDES EU Copper Task Force, KL/14/002/01 not available GLP/GEP: no Published: no	N	EUCuTF(*)
KCP 10.6.2/02	McVean, K.	2022d	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test TNK20269 NOACK LABORATORIEN GMBH GLP: yes Published: no	N	UPL EU
KCP 10.6.2/03	McVean, K.	2022e	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Terrestrial Plant Test: Vegetative Vigour Test TNW20269 NOACK LABORATORIEN GMBH GLP: yes Published: no	N	UPL EU

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 Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

UPL EU = UPL Europe Ltd.

EUCuTF = EU Copper Task Force,

(*) UPL is a full member of the EU Copper Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of copper compounds

UPL is a full member of the Cymoxanil AIR4 Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of cymoxanil

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

Updated 04.2024r.

There are some studies formulation ATOFEL02 (Batch no:8.335.3). In our opinion - due to the same content of the active substance inside FEL02 and ATOFEL02 (copper as Bordeaux mixture 200 g/kg and cymoxanil 40 g/kg) and the same type of formulation (water-dispersible granule - WG formulation) it could be used in risk assessment in ecotoxicology point of view. Due to the AT and CZ comments, the Applicant should provide a comparison of the formulations of ATOFEL 02 and FEL02 including Part C (considering the new more strict rules by EFSA also applied at a.s. level). This approach should be considered at MSs level. Perhaps at the level of national registrations in different countries, additional data will be required.

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

Effects on birds of FEL02 were not evaluated as part of the respective EU assessments of Copper and cymoxanil. However, further data on FEL02 is not considered essential as toxicity to birds was assessed for a broad range of Copper compounds including a Bordeaux mixture formulation, which is similar to the copper compound used in FEL02 (please refer to Part C of the dRR) and toxicity to birds was assessed for cymoxanil. Combination toxicity was addressed in the risk assessment. Additional formulation data are therefore not necessary, and all relevant data were assessed in the EU review.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

The RA shows that copper is the driver of toxicity and risk to birds. A position paper was submitted for the renewal of approval of copper which provided evidence that, owing to the homeostatic control of dietary uptake of copper, the risks to birds was acceptable providing that doses were limited to a total of 5 kg Cu/ha during the breeding season (EFSA Conclusion, 2018).

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

The acute oral toxicity study with the preparation is summarised in dRR, Part B, Section 6, Mammalian Toxicology, KCP 7.1.1/01. The oral LD_{50} of ATO FDH01 was determined as > 2000 mg product/kg bw. FDH01 is a formulation containing 20.3% Copper, 4.43% Cymoxanil and 2.21% Famoxadone and is considered to be appropriate for the risk assessment for FEL02, please refer to Part B, Section 4 of the dRR.

Updated 04.2024r.

The acute oral toxicity study with the preparation FEL02 is not provided by Applicant. The oral LD_{50} of FDH01 was provided as > 2000 mg product/kg bw. FDH01 is a formulation containing 20.3% Copper, 4.43% Cymoxanil and 2.21% Famoxadone. This formulation contains the same amount of active substances and one additional substance. Therefore, it can probably the worst case. The comparative assessment in Part C between FDH01 and FEL02 was not provided by Applicant. However, in this case copper clearly driving the risk assessment due to its high toxicity compared to Cymoxanil. In this context, a risk assessment for cooper is relevant and cover risk assessment for formulation. According to the information included in Part B6, the exact composition of the tested material is no longer available. However, it is thought that the tested composition is sufficiently close to the composition of FEL02, to be used for classification in Europe. The final decision should be considered by MSs level.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

The RA shows that copper is the driver of toxicity and risk to birds. A weight of evidence paper was submitted for the renewal of approval of copper and this provides an evidence that owing to homeostatic control, the risk to mammals is acceptable except for lagomorphs (EFSA Conclusion, 2018). A weight-of-evidence approach is provided in this dossier that the risks to the lagomorph are also acceptable.

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

According to the EFSA Conclusion (2018), the risk assessments performed for birds and aquatic organisms are considered to cover that of amphibians and reptiles. Therefore, further studies are not considered necessary.

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

Effects on aquatic organisms of FEL02 were not evaluated as part of the EU assessment of copper. Additional toxicity data for FEL02 have been generated for fish, daphnia and algae.

A 2.2.1.1 Study 1

Comments of zRMS:

The study is considered as valid.
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% (oxygen concentrations over the whole test 87 – 108%).

The study limitation: In the study the supply was then delivered to a temperature controlled header tank in the test laboratory which was set to the nominal test temperature of 15 ± 1°C. It should be 10-14% for *O.mykiss* according to OECD 203. The deviation with no impact on the quality and integrity of the study.

Analytical results – cymoxamil

Nominal conc of Cuprofix C Disperss (FEL02) sample (mg/L)	Nominal cymoxanil concentration (mg/L)	Measured cymoxanil concentration		Measured concentration as a percentage of nominal (%)
		0 hour (mg/L)	96 hour (mg/L)	
Control	-	<0.12	n/a	-
1.9	0.078	0.054	n/a	70
4.3	0.18	0.14[a]	n/a	80
9.3	0.38	0.34	n/a	90
21	0.86	0.68	n/a	79
45	1.9	1.4	n/a	75
100	4.1	3.3	n/a	81

All measurements are quoted to 2 significant figures and percentages to the nearest integer

The limit of quantification of cymoxanil in the study was 0.12 µg/L

[a] Mean of triplicate analyses: 0.15, 0.14, 0.13 mg/L

Analytical results – copper content

Nominal conc of Cuprofix C Disperss (FEL02) sample (mg/L)	Measured copper content in sample		Geometric mean measured copper concentration (mg/L)	Percentage loss of copper from 0 - 72 hours (%)
	0 hour (mg/L)	96 hour (mg/L)		
Control	0.0010	0.0050	0.0022	-
1.9	0.31	0.25	0.28	19
4.3	0.80	0.60	0.69	25
9.3	1.6	1.1	1.3	30
21	3.6	3.2	3.4	11
45	7.6	5.2	6.3	31
100	17	9.6	13	43

All measurements are quoted to 2 significant figures and percentages to the nearest integer
The limit of quantification of copper in the study was 0.001 mg/L

However, the chemical analysis for the product Cuprofix C and fish revealed, that the concentration of cymoxanil was below LOQ at the end of the test concentrations. Hence, it is concluded the toxicity endpoints from this studies may be questionable due to cannot be determined as exposure of the test compound was not maintained throughout the study. On the other hand, the combined risk assessment confirmed that it is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product and the copper concentration were properly maintained throughout the studies for fish. ~~The reliable endpoint for the study and risk assessment for plant product protection CUPROFIX C and fish should be considered at MSs level.~~ In our opinion – the final conclusion for this study in this specific situation should be considered by MSs level.

Toxicity endpoints:

Results based on nominal Cuprofix C Disperss (FEL02) concentrations	Time	LC50	95% confidence limits	Calculation method
	24 hour	*	*	*
	48 hour	82 mg/L	64 – 140 mg/L	MAA
	72 hour	59 mg/L	41 – 106 mg/L	MAA
	96 hour	47 mg/L	35 – 65 mg/L	MAA

96 hour NOEC was 21 mg/L and the LOEC was 45 mg/L

Results based on measured copper concentration	Time	LC50	95% confidence limits	Calculation method
	24 hour	*	*	*
	48 hour	11 mg/L	8.7 – 18 mg/L	MAA
	72 hour	8.1 mg/L	6.0 – 13 mg/L	MAA
	96 hour	6.7 mg/L	5.2 – 8.8 mg/L	MAA

96 hour NOEC was 3.4 mg/L and the LOEC was 6.3 mg/L

Results based on initial measured cymoxanil concentrations	Time	LC50	95% confidence limits	Calculation method
	24 hour	*	*	*
	48 hour	2.7 mg/L	2.0 – 4.8 mg/L	MAA
	72 hour	1.9 mg/L	1.3 – 3.4 mg/L	MAA
	96 hour	1.5 mg/L	1.1 – 2.1mg/L	MAA

96 hour NOEC was 0.68 mg/L and the LOEC was 1.4 mg/L

MAA = Moving average angle
* = Could not be calculated

Reference:	KCP 10.2.1/01
Report	Cuprofix C Disperss (FEL02): Determination of acute toxicity to rainbow trout (*Oncorhynchus mykiss*), [redacted], report No. BR0587/B, study number 11-0134/D
Guideline(s):	OECD Guideline No. 203 (1992)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The acute toxicity of Cuprofix C Disperss (FEL02) to rainbow trout (*Oncorhynchus mykiss*) was determined in fresh water using a static system at nominal concentrations of 0 (control), 1.9, 4.3, 9.3, 21, 45 and 100 mg formulation/L over 96 hours. Seven fish each were prepared for each experimental group. The test item was tested at 6 concentrations and in addition one group without the test item was prepared and acted as a negative control.

The LC_{50} value after 96 hours was determined to be 47 mg/L (nominal concentration), corresponding to 6.7 mg/L Copper (mean measured concentration) and to 1.5 mg/L Cymoxanil (initial measured concentration). The NOEC value for mortality after 96 hours was determined to be 21 mg/L (nominal concentration), corresponding to 3.4 mg/L Copper (mean measured concentration) and to 0.683 mg/L Cymoxanil (initial measured concentration). The LOEC value for mortality after 96 hours was 45 mg/L (nominal concentration), corresponding to 6.3 mg/L Copper (mean measured concentration) and to 1.4 mg/L Cymoxanil (initial measured concentration).

The NOEC value for sublethal effects after 96 hours was determined to be 9.3 mg/L (nominal concentration), corresponding to 3.4 mg/L Copper (mean measured concentration) and to 0.342 mg/L Cymoxanil (initial measured concentration). The LOEC value for sublethal effects after 96 hours was 21 mg/L (nominal concentration), corresponding to 3.4 mg/L Copper (mean measured concentration) and to 0.683 mg/L Cymoxanil (initial measured concentration).

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss (FEL02)
Lot / Batch no.	10.340.3
Active ingredient content / Purity	Bordeaux mixture: 20.7% metal Copper and 4.1% Cymoxanil
Characteristics	Green free flowing granules
Density (if liquid)	-
Storage conditions	Ambient temperature
Stability (expiry date)	07.03.2011
Vehicle / control(s)	Control: untreated water Toxic reference item: none

Test System

Species	*Oncorhynchus mykiss* Walbaum (Salmoniformes: Salmonidae)
Age and Size	Mean fish size was 49 mm (range 46 – 52 mm) and the mean weight 1.55 g (range 1.00 – 1.75 g)
Source	[redacted]

Acclimatisation period	None
Food or Diet	Proprietary brand of high protein pelleted fish food.
Test Conditions	
Temperature	14.8 – 15.8°C
pH value	7.29 – 7.69
Dissolved O_2 concentration	> 60% of air saturation
Hardness	53 mg $CaCO_3$/L
Photoperiod	Light/dark cycle of 16/8 hours
Light intensity	Artificial lighting
Aeration of the test water	The test media were slightly aerated during the test.
Loading	0.54 g fish/L test solution
Study Design and Methods	
In-life dates	28.11.2011 – 16.12.2011
Conducted at	[redacted]
Test duration	96 h
Test design	Static
Test concentrations	1.9, 4.3, 9.3, 21, 45 and 100 mg/L
Test groups / Replicates	1 replicate/treatment (and control); 7 fish per replicate
Treatment	The acute toxicity of Cuprofix C Disperss (FEL02) to rainbow trout (*Oncorhynchus mykiss*) was determined in fresh water using a static system at nominal concentrations of 1.9, 4.3, 9.3, 21, 45 and 100 mg formulation/L over 96 hours. In addition, one group without the test item was prepared and acted as a negative control.
Observations	Records of mortality and sublethal effects of exposure were made after 3, 24, 48, 72 and 96 hours.
Analytical verification	The copper concentration of the test solutions was measured for samples collected at 0 and 96 hours using the ICP-MS method. Samples were acidified by the addition of 2% concentrated nitric acid the stored under refrigeration. The concentration of cymoxanil in the test solutions was measured in samples collected at 0 and 96 hours using HPLC-MS method.
Statistics	The LC_{50}-values are the estimated test item concentrations causing 50% mortality of the test organisms. The LC_{50} value was calculated according to Stephan (1977). The NOEC (mortality) was established based on the highest test concentration at which no mortality above the allowed control mortality was observed.

RESULTS AND DISCUSSIONS

Analytical results

The results of the analytical measurements are summarised in **Table A 2.2.1.1-1**. The measured Copper concentration in the test solutions at the start of the study ranged from 0.31 to 16.9 mg/L, at the end of the test Copper concentration ranged from 0.250 to 9.62 mg/L. The Copper content in the control was determined to be 0.0010 and 0.0050 mg/L at the start and end of the test respectively. The measured concentrations at the start of the test were within the range of 78.9 – 89.9% of the nominal concentrations. At test end, the measured concentrations were within a range of 46.4 – 73.6% of the nominal concentrations.

The measured Cymoxanil concentration in the test solutions at the start of the study ranged from 70 to 90% of the nominal Cymoxanil concentration. The Cymoxanil concentration in the nominal 4.3 mg/L test solution was analysed in triplicate and showed good consistency. The limit of quantification of Cymoxanil was 0.123 µg/L.

At the end of the test the Cymoxanil analysis was unsuccessful due to a loss of sensitivity in the analytical method; as such it was not possible to quantify the Cymoxanil concentration in the test solutions at 96 hours.

On the basis of the analytical data the nominal Cuprofix C Disperss (FEL02) concentrations, the geometric mean measured Copper content and the initial measured Cymoxanil concentrations were used for the calculation and reporting of results.

Table A 2.2.1.1-1 Analytical results for Copper and Cymoxanil over a period of 96 hours

Nominal concentration [mg/L]			Measured concentrations					
			Copper [mg/L] *			Cymoxanil [mg/L] **		
Cuprofix C Disperss	Copper	Cymoxanil	0 h	96 h	Geometric mean	0 h	96 h	Geometric mean
0	0	0	0.001	0.005	0.002	< 0.123	nd	nd
1.9	0.393	0.078	0.310	0.250	0.280	0.054	nd	nd
4.3	0.890	0.177	0.795	0.600	0.690	0.142	nd	nd
9.3	1.93	0.381	1.56	1.10	1.3	0.342	nd	nd
21	4.35	0.861	3.59	3.18	3.4	0.683	nd	nd
45	9.32	1.85	7.56	5.22	6.3	1.38	nd	nd
100	20.7	4.10	16.9	9.62	13	3.33	nd	nd

* limit of quantification (LOQ) of 0.001 mg/L for Copper
** limit of quantification (LOQ) of 0.12 µg/L for Cymoxanil
nd = not determined

Biological 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% (oxygen concentrations over the whole test 87 – 108%).

Table A 2.2.1.1-2 Observed mortality of rainbow trout exposed to Cuprofix C Disperss for 96 hours in a static acute test

Nominal Concentration [mg formulation/L]	Cumulative Mortality (Number of dead/Number introduced)			
	24 hr	48 hr	72 hr	96 hr
0 (control)	0/7	0/7	0/7	0/7
1.9	0/7	0/7	0/7	0/7
4.3	0/7	0/7	0/7	0/7
9.3	0/7	0/7	0/7	1/7
21	0/7	0/7	0/7	0/7
45	0/7	0/7	2/7	3/7
100	1/7	5/7	6/7	7/7

No mortality or visible sublethal effects were observed in the control and in the test item treatment groups up to and including 4.3 mg/L. One fish died in the 9.3 mg/L test item treatment group, however, as no fish died in the next higher treatment group, this was considered to be not treatment-related. All fish died in the highest test item treatment group.

No sublethal effects were observed in the two lowest test item treatment groups. Sublethal effects observed in the other test item treatment groups were sounding and dark discolouration.

CONCLUSIONS

The LC_{50} value after 96 hours was determined to be 47 mg/L (nominal concentration), corresponding to 6.7 mg/L Copper (mean measured concentration) and to 1.5 mg/L Cymoxanil (initial measured concentration). The NOEC value for mortality after 96 hours was determined to be 21 mg/L (nominal concentration), corresponding to 3.4 mg/L Copper (mean measured concentration) and to 0.683 mg/L Cymoxanil (initial measured concentration). The LOEC value for mortality after 96 hours was 45 mg/L (nominal concentration), corresponding to 6.3 mg/L Copper (mean measured concentration) and to 1.4 mg/L Cymoxanil (initial measured concentration).

The NOEC value for sublethal effects after 96 hours was determined to be 9.3 mg/L (nominal concentration), corresponding to 3.4 mg/L Copper (mean measured concentration) and to 0.342 mg/L Cymoxanil (initial measured concentration). The LOEC value for sublethal effects after 96 hours was 21 mg/L (nominal concentration), corresponding to 3.4 mg/L Copper (mean measured concentration) and to 0.683 mg/L Cymoxanil (initial measured concentration).

A 2.2.1.2 Study 2

Comments of zRMS:

The study is considered as valid. The validity criteria were met as the mortality in the control was not more than 10% after 48 hours (actual mortality: 0%), the dissolved oxygen concentration at the end of the test was > 3 mg/L of air saturation (actual concentration: > 7.3 mg/L).

Analytical results – copper content

Nominal conc of Cuprofix C Disperss (FEL02) sample (mg/L)	Measured copper content in sample		Geometric mean measured copper concentration (mg/L)	Percentage loss of copper from 0 - 48 hours (%)
	0 hour (mg/L)	48 hour (mg/L)		
Control	0.0020	0.0010	0.0014	50
0.088	0.022	0.014	0.018	36
0.19	0.034	0.026	0.030	24
0.43	0.080	0.054	0.066	33
0.93	0.17	0.11	0.13	36
2.1	0.39	0.24	0.30	39
4.5	0.85	0.41	0.59	51

All measurements are quoted to 2 significant figures and percentages to the nearest integer
The limit of quantification of copper in the study was 0.001 mg/L

Analytical results – cymoxamil

Nominal conc of Cuprofix C Disperss (FEL02) sample (mg/L)	Nominal cymoxanil concentration (µg/L)	Measured cymoxanil concentration		Measured concentration as a percentage of nominal (%)
		0 hour (µg/L)	48 hour (µg/L)	
Control	-	<0.12	n/a	-
0.088	3.6	2.2	n/a	60
0.19	7.8	4.6[a]	n/a	59
0.43	18	10	n/a	57
0.93	38	23	n/a	60
2.1	86	<49	n/a	-
4.5	185	63	n/a	34

All measurements are quoted to 2 significant figures and percentages to the nearest integer
The limit of quantification of cymoxanil in the study was 0.12 µg/L

n/a Not applicable
[a] Mean of triplicate analyses: 4.2, 4.6, 5.0 mg/L

However, the chemical analysis for the product Cuprofix C and Daphnia revealed, that the concentration of cymoxanil was below LOQ at the end of the test concentrations. Hence, it is concluded the toxicity endpoints from this studies may be questionable due to cannot be determined as exposure of the test compound was not maintained throughout the study. On the other hand, the combined risk assessment confirmed that it is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product and the copper concentration were properly maintained throughout the studies for Daphnia. ~~*The reliable endpoint for the study and risk assessment for plant product protection CUPROFIX C and fish should be considered at MSs level*~~ *In our opinion – the final conclusion for this study in this specific situation should be considered by*

	MSs level. **Toxicity endpoints:**

Results based on nominal Cuprofix C Disperss (FEL02) concentrations	**Time**	**EC50**	**95% confidence limits**	**Calculation method**
	24 hour	*	*	*
	48 hour	0.35 mg/L	0.27 – 0.45 mg/L	**MAA**
	48 hour NOEC was 0.19 mg/L and the LOEC was 0.43 mg/L			
Results based on geomentric mean measured copper concentrations	**Time**	**EC50**	**95% confidence limits**	**Calculation method**
	24 hour	*	*	*
	48 hour	0.062 mg/L	0.049 – 0.077 mg/L	**MAA**
	48 hour NOEC was 0.030 mg/L and the LOEC was 0.066 mg/L			
Results based on initial cymoxanil concentrations	**Time**	**EC50**	**95% confidence limits**	**Calculation method**
	24 hour	*	*	*
	48 hour	8.7 µg/L	6.9 – 11 µg/L	**MAA**
	48 hour NOEC was 4.6 µg/L and the LOEC was 10 µg/L			

MAA = Moving Average Angle
* = Could not be calculated

Reference:	KCP 10.2.1/02
Report	Cuprofix C Disperss (FEL02): Determination of acute toxicity to *Daphnia magna*, Hutchinson, K.A. & Sharpe, A.D., 2012b, report No. BR0586/B, study number 11-0134/C
Guideline(s):	OECD Guideline No. 202 (2004)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The acute toxicity of Cuprofix C Disperss (FEL02) to *Daphnia magna* was determined in fresh water using a static system at nominal concentrations of 0 (control), 0.088, 0.19, 0.43, 0.93, 2.1 and 4.5 mg formulation/L over 48 hours. Four groups of 5 daphnids with an age of less than 24 hours were used for each treatment group. Records of immobility were made 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.

The EC_{50} value (48 h) for the formulation based on nominal concentrations amounted to 0.35 mg/L (95% confidence limits: 0.27 – 0.45 mg/L), corresponding to a mean measured Copper concentration of 0.062 mg a.s./L and to an initial measured Cymoxanil concentration of 8.7 µg a.s./L. The NOEC was determined to be 0.19 mg/L (nominal concentration), corresponding to a mean measured Copper concentration of 0.03 mg a.s./L and to an initial measured Cymoxanil concentration of 4.6 µg a.s./L. The LOEC was determined to be 0.43 mg/L (nominal concentration), corresponding to a mean measured Copper concentration of 0.066 mg a.s./L and to an initial measured Cymoxanil concentration of 10 µg a.s./L.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss (FEL02)
Lot / Batch no.	10.340.3
Active ingredient content / Purity	Bordeaux mixture (20.7 % metal Copper) and 4 % Cymoxanil)
Density (if liquid)	-
Storage conditions	Room temperature
Stability (expiry date)	07.03.2013
Vehicle / control(s)	Control: dilution water Toxic reference item: none

Test System

Species	*Daphnia magna* Straus, Clone V
Age	Less than 24 hours
Culture medium	US EPA 'Moderately Hard Water' Daphnia medium (Ref2)
Supplier	Obtained from continuous laboratory cultures held at Brixham Laboratory
Acclimatisation period	None
Food or Diet	Mixed algae diet of *Chlorella vulgaris*, strain CCAP 211/12 and *Pseudokirchneriella subcapitata*, strain CCAP 278/4.

Test Conditions

Temperature	20 ± 1°C
pH value	8.30
Dissolved O_2 concentration	7.30 - 7.94 mg/L
Hardness	152.7 mg/L $CaCO_3$
Photoperiod	Light/dark cycle of 16/8 hours
Light intensity	Not stated
Aeration of the test water	The test solutions were not aerated
Loading	Not stated

Study Design and Methods

In-life dates	30.11.2011 - 16.12.2011
Conducted at	Brixham Environmental Laboratory, AstraZeneca UK Limited, Freshwater Quarry, Brixham, TQ5 8BA, UK
Test duration	48 h
Test design	static
Test concentrations	0 (control), 0.088, 0.19, 0.43, 0.93, 2.1 and 4.5 mg formulation/L
Test groups / Replicates	Four replicates per test concentration (volume of the test medium 250 mL)
Treatment	The acute toxicity of Cuprofix C Disperss (FEL02) to *Daphnia magna* was determined in fresh water using a static system at nominal concentrations of 0 (control), 0.088, 0.19, 0.43, 0.93, 2.1 and 4.5 mg formulation/L over 48 hours. Four groups of 5 daphnids with an age of less than 24 hours were used for each treatment group.
Observations	Records of immobility were made 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.
Analytical verification	The copper concentration of the test solutions was measured for samples collected at 0 and 48 hours using the ICP-MS method. Samples were acidified by the addition of 2% concentrated nitric acid the stored under refrigeration. The concentration of cymoxanil in the test solutions was measured in samples collected at 0 and 48 hours using high performance liquid chromatography method.
Statistics	The 48 h EC_{50} are the estimated concentrations where 50% of the daphnids were immobilised after 48 hours, respectively. The values for EC_{50} were determined according to Stephan (1977). The NOEC was established based on the highest concentration at which the immobilisation is not higher than the allowed control immobilisation (≤ 10% immobilisation).

RESULTS AND DISCUSSIONS

Analytical results

The measured Copper concentration in the test solutions at the start of the study ranged from 0.022 to 0.85 mg/L, at the end of the test the copper concentration ranged from 0.014 to 0.41 mg/L. The measured concentrations at the start of the test were within the range of 88.1 – 122.2% of the nominal concentrations. At test end, the measured concentrations were within a range of 38.2 – 77.8% of the nominal concentrations. The Copper content in the control was determined to be 0.002 and 0.001 mg/L at the start and end of the test respectively. The results of the analytical measurements are summarised in **Table A 2.2.1.2-1.**

Table A 2.2.1.2-1 Analytical results for Copper and Cymoxanil over a period of 48 h

Nominal concentration [mg/L]			Measured concentrations					
			Copper [mg/L] *			Cymoxanil [µg/L] **		
Cuprofix C Disperss	Copper	Cymoxanil	0 h	48 h	Geometric mean	0 h	48 h	Geometric mean
0	0	0	0.002	0.001	0.001	< 0.123	nd	nd
0.088	0.018	0.0036	0.022	0.014	0.018	2.2	nd	nd
0.19	0.039	0.0078	0.034	0.026	0.030	4.6	nd	nd
0.43	0.089	0.018	0.080	0.054	0.066	10	nd	nd
0.93	0.193	0.038	0.170	0.110	0.130	23	nd	nd
2.1	0.435	0.086	0.390	0.240	0.300	49	nd	nd
4.5	0.932	0.185	0.850	0.41	0.590	63	nd	nd

* limit of quantification (LOQ) of 0.001 mg/L for Copper
** limit of quantification (LOQ) of 0.123 µg/L for Cymoxanil
nd = not determined

The measured Cymoxanil concentration in the test solutions at the start of the study ranged from 34 to 60% of the nominal Cymoxanil concentration. The Cymoxanil concentration in the nominal 0.19 mg/L test solution was analysed in triplicate and showed good consistency. Analytical spikes at the start of the test showed good consistency and an adjustment for recovery was not deemed necessary. The limit of quantification of Cymoxanil was 0.123 µg/L. At the end of the test the Cymoxanil analysis was unsuccessful due to a loss of sensitivity in the analytical method; as such it was not possible to quantify the Cymoxanil concentration in the test solutions at 48 hours.

On the basis of the analytical data the nominal Cuprofix C Disperss (FEL02) concentrations, the geometric mean measured Copper content and the initial measured Cymoxanil concentrations were used for the calculation and reporting of results.

Biological test results

The validity criteria were met as the mortality in the control was not more than 10% after 48 hours (actual mortality: 0%), the dissolved oxygen concentration at the end of the test was > 3 mg/L of air saturation (actual concentration: > 7.3 mg/L).

The results for immobility are presented in **Table A 2.2.1.2-2**. In the control and in the three lowest test concentrations up to and including 0.19 mg/L no immobile daphnids were observed. At test concentrations of 0.43, 0.93, 2.1 and 4.5 mg/L, 80, 100, 100 and 100% immobilisation, respectively, was observed after 48 hours of exposure.

Table A 2.2.1.2-2 Observed immobility of *Daphnia magna* exposed to Cuprofix C Disperss for 48 hours in a static acute test

Nominal concentration [mg formulation/L]	Immobility			
	24 h		48 h	
	No immobile	% immobile	No immobile	% immobile
0 (control)	0	0	0	0
0.088	0	0	0	0
0.19	0	0	0	0
0.43	0	0	16	80
0.93	3	15	20	100
2.1	4	20	20	100
4.5	3	15	20	100

20 animals (4 replicates of 5 animals each) were introduced per concentration

At 24 hours two daphnids in the 2.1 mg/L test item treatment group were observed to be floating and one daphnid was slow moving. In the 4.5 mg/L test item treatment group three daphnids were observed to be slow moving. There was no immobility or other symptoms of toxicity observed in the control.

CONCLUSIONS

The EC_{50} value (48 h) for the formulation based on nominal concentrations amounted to 0.35 mg/L (95% confidence limits: 0.27 – 0.45 mg/L), corresponding to a mean measured Copper concentration of 0.062 mg a.s./L and to an initial measured Cymoxanil concentration of 8.7 µg a.s./L. The NOEC was determined to be 0.19 mg/L (nominal concentration), corresponding to a mean measured Copper concentration of 0.03 mg a.s./L and to an initial measured Cymoxanil concentration of 4.6 µg a.s./L. The LOEC was determined to be 0.43 mg/L (nominal concentration), corresponding to a mean measured Copper concentration of 0.066 mg a.s./L and to an initial measured Cymoxanil concentration of 10 µg a.s./L.

A 2.2.1.3 Study 3

Comments of zRMS:	The study is considered as valid. The validity criteria were met as the biomass in the control increased by a factor of at least 16 (actual increase: 63), the coefficient of variation for specific growth rate was not more than 35% (actual coefficient of variation: 19%) and the coefficient of variation of average growth in the control replicates was not more than 7% (actual coefficient of variation: 5%). The shift of the pH value in the control was not more than 1.5 units.

Analytical results – cymoxamil

Nominal conc of Cuprofix C Disperss (FEL02) sample (mg/L)	Nominal cymoxanil concentration (µg/L)	Measured cymoxanil concentration		Measured concentration as a percentage of nominal (%)
		0 hour (µg/L)	72 hour (µg/L)	
Control	-	<0.123	n/a	-
0.010	0.41	0.19	n/a	46
0.025	1.0	0.58[a]	n/a	58
0.064	2.6	1.5	n/a	58
0.16	6.6	1.8	n/a	27
0.40	16	9.7	n/a	61
1.0	41	23	n/a	56

All measurements are quoted to 2 significant figures and percentages to the nearest integer
The limit of quantification for cymoxanil in the study was 0.12 µg/L

[a] Mean of triplicate analyses: 56, 61, 58 mg/L

Analytical spikes:
0 hours, Mean recovery: 23% (24, 19, 26%)

However, the chemical analysis for the product Cuprofix C and algae revealed, that the concentration of cymoxanil was below LOQ at the end of the test concentrations. Hence, it is concluded the toxicity endpoints from this studies may be questionable due to cannot be determined as exposure of the test compound was not maintained throughout the study. On the other hand, the combined risk assessment confirmed that it is clear that copper drives the toxicity of the mixture for all organism groups (%TU >90%). The risk assessment for copper alone is considered sufficient to cover the risk of exposure to the product and the copper concentration were properly maintained throughout the studies for algae. ~~The reliable endpoint for the study and risk assessment for plant product protection CUPROFIX C and fish should be considered at MSs level~~ In our opinion – the final conclusion for this study in this specific situation should be considered by MSs level.

Toxicity endpoints:

Results based on geometric mean measured concentrations of copper

Based on yield at the end of the test, the results obtained were:

	Copper (mg/L)	95% Confidence Interval (mg/L)
NOEC	0.0040	-
LOEC	0.011	-
E_yC50	0.0083	0.0069 – 0.0092
E_yC20	0.0050	0.0011 – 0.0062
E_yC10	0.0032	0.0005 – 0.0055

Based on growth rate over the test period, the results obtained were:

	Copper (mg/L)	95% Confidence Interval (mg/L)
NOEC	0.018	-
LOEC	0.38	-
E_rC50	0.023	0.018 – 0.048
E_rC20	0.0033	0.0031 – 0.0036
E_rC10	0.0028	0.0025 – 0.0030

Results based on geometric mean measured concentrations of copper

Based on yield at the end of the test, the results obtained were:

	Copper (mg/L)	95% Confidence Interval (mg/L)
NOEC	0.0040	-
LOEC	0.011	-
E_yC50	0.0083	0.0069 – 0.0092
E_yC20	0.0050	0.0011 – 0.0062
E_yC10	0.0032	0.0005 – 0.0055

Based on growth rate over the test period, the results obtained were:

	Copper (mg/L)	95% Confidence Interval (mg/L)
NOEC	0.018	-
LOEC	0.38	-
E_rC50	0.023	0.018 – 0.048
E_rC20	0.0033	0.0031 – 0.0036
E_rC10	0.0028	0.0025 – 0.0030

Results based on initial measured cymoxanil concentration

Based on yield at the end of the test, the results obtained were:

	Cymoxanil (µg/L)	95% Confidence Interval (µg/L)
NOEC	0.58	-
LOEC	1.5	-
E_yC50	1.1	0.95 – 1.3
E_yC20	0.72	0.018 – 0.88
E_yC10	0.38	0.0 – 0.81

Based on growth rate over the test period, the results obtained were:

	Cymoxanil (µg/L)	95% Confidence Interval (µg/L)
NOEC	1.8	-
LOEC	9.7	-
E_rC50	3.7	2.0 - 15
E_rC20	1.1	0.96 – 1.3
E_rC10	0.81	0.68 – 0.93

Reference:	KCP 10.2.1/03
Report	Cuprofix C Disperss (FEL02): Determination of toxicity to the green alga *Pseudo-kirchneriella subcapitata*, Hutchinson, K.A. & Sharpe, A.D., 2012c, Report No: BR0585/B, Study number 11-0134/B
Guideline(s):	OECD Guideline No. 201 (2011)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The toxicity of Cuprofix C Disperss (FEL02) to *Pseudokirchneriella subcapitata* was determined in a static system at nominal concentrations of 0.01, 0.025, 0.064, 0.16, 0.40 and 1 mg formulation/L over 72 hours. Test media were inoculated with 0.5×10^4 cells/mL. Six replicates for the controls and three replicates for the test item treatment

groups were established. Algal growth was measured every day by electronic particle counting. Growth was expressed as rate of increase in cell numbers per day and as yield. Active substance concentrations were analysed from samples taken at the start and at the end of the test.

The EC_{50} for yield was 0.049 mg/L, corresponding to 0.008 mg Copper/L (mean measured concentration) and to 1.1 µg/L Cymoxanil (initial measured concentration). The NOEC for yield was 0.025 mg/L, corresponding to 0.004 mg/L Copper (mean measured concentration) and to 0.58 µg Cymoxanil/L (initial measured concentration).

The EC_{50} for growth rate was 0.22 mg/L, corresponding to 0.023 mg/L Copper (mean measured concentration) and to 3.7 µg/L Cymoxanil (initial measured concentration). The NOEC for growth rate was 0.16 mg/L, corresponding to 0.018 mg a.s./L (mean measured total Copper) and to 1.8 µg Cymoxanil/L (initial measured concentration).

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss (FEL02)
Lot / Batch no.	10.340.3
Active ingredient content / Purity	20.7% Copper and 4% Cymoxanil
Characteristics	Green free flowing granules
Density (if liquid)	-
Storage conditions	Room temperature
Stability (expiry date)	07.03.2013
Vehicle / control(s)	Control: culture medium Toxic reference item: none

Test System

Species	*Pseudokirchneriella subcapitata* Hindák (Sphaeropleales: Selenastraceae) Strain: SAG 61.81
Source	Brixham Environmental Laboratory culture from strain CCAP 278/4
Culture medium	Modified ISO growth medium
Acclimatisation period	None
Initial cell density	0.5×10^4 cells/mL

Test Conditions

Temperature	22 ± 2°C
pH value	At start: 7.98 - 8.08; at end: 7.81 - 8.01
Dissolved O_2 concentration	Not stated
Hardness	Not stated
Photoperiod	Continuous light
Light intensity	6333 lux (by cosine receptor) ± 6%

Study Design and Methods

In-life dates	28.11.2011 - 16.12.2011
Conducted at	Brixham Environmental Laboratory, AstraZeneca UK Limited, Freshwater Quarry, Brixham, Devon, TQ5 8BA, UK
Test duration	72 hours
Test design	static
Test concentrations	0.01, 0.025, 0.064, 0.16, 0.40 and 1 mg formulation/L
Test groups / Replicates	Six replicates for the controls and three replicates for the test item

	treatment groups were established.
Treatment	The toxicity of Cuprofix C Disperss (FEL02) to *Pseudokirchneriella subcapitata* was determined at nominal concentrations of 0.01, 0.025, 0.064, 0.16, 0.40 and 1 mg formulation/L. Test media were inoculated with 0.5×10^4 cells/mL.
Observations	Algal growth was measured every day by electronic particle counting. Growth was expressed as rate of increase in cell numbers per day and as yield.
Analytical verification	Active substance concentrations were analysed from samples taken at the start and at the end of the test.
Statistics	The data for cell counts were statistically analysed using an in-house software package, PCADA, where appropriate procedures were applied to test for significant differences ($p < 0.05$) between the control and test concentrations. EC_{50}, EC_{20} and EC_{10} values and associated 95% confidence intervals were determined using the US EPA program ICPIN (version 2, June 1993).

RESULTS AND DISCUSSIONS

The validity criteria were met as the biomass in the control increased by a factor of at least 16 (actual increase: 63), the coefficient of variation for specific growth rate was not more than 35% (actual coefficient of variation: 19%) and the coefficient of variation of average growth in the control replicates was not more than 7% (actual coefficient of variation: 5%). The shift of the pH value in the control was not more than 1.5 units.

Analytical results

The measured concentrations of the active substances Copper and Cymoxanil are summarised in **Table A 2.2.1.3-1**. The measured Copper concentration in the test solutions at the start of the study ranged from 0.002 to 0.15 mg/L, at the end of the test the Copper concentration ranged from 0.003 to 0.025 mg/L. The Copper content in the control was determined to be < LOQ and 0.001 mg/L at the start and end of the test respectively.

Table A 2.2.1.3-1 Analytical results for Copper and Cymoxanil over a period of 72 h

Nominal concentration [mg/L]			Measured concentrations					
			Copper [mg/L] *			Cymoxanil [µg/L] **		
Cuprofix C Disperss	Copper	Cymoxanil	0 h	72 h	Geometric mean	0 h	72 h	Geometric mean
0	0	0	< LOD	< LOD	-	< LOQ	nd	nd
0.01	0.002	0.00041	0.002	0.003	0.002	0.19	nd	nd
0.025	0.005	0.001	0.004	0.004	0.004	0.58	nd	nd
0.064	0.013	0.0026	0.011	0.012	0.011	1.5	nd	nd
0.16	0.033	0.0066	0.028	0.011	0.018	1.8	nd	nd
0.40	0.083	0.016	0.069	0.021	0.038	9.7	nd	nd
1.0	0.207	0.410	0.15	0.025	0.061	23	nd	nd

* limit of detection (LOD) of 0.001 mg/L for Copper
** limit of quantification (LOQ) of 0.123 µg/L for Cymoxanil
nd = not determined

The measured Cymoxanil concentration in the test solutions at the start of the study ranged from 28 to 59% of the nominal concentration. The Cymoxanil concentration in the nominal 0.025 mg/L test solution was analysed in triplicate and showed good consistency. Analytical spikes at the start of the test showed good consistency and an adjustment for recovery was not deemed necessary. The limit of quantification of Cymoxanil was 0.123 µg/L. At the end of the test the Cymoxanil analysis was unsuccessful due to a loss of sensitivity in the analytical method; as such it was not possible to quantify the Cymoxanil concentration in the test solutions at 72 hours.

On the basis of the analytical data the nominal Cuprofix C Disperss (FEL02) concentrations, the geometric mean measured Copper content and the initial measured Cymoxanil concentrations were used for the calculation and reporting of results.

Biological results

The mean cell densities after 24, 48 and 72 hours are summarised in **Table A 2.2.1.3-2**. Some growth inhibition was observed in the two lowest test item treatment groups (inhibition of maximum 10.3%). At the highest test concentration of 1 mg/L, there was 100% growth inhibition. 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 9.32% for the nominal concentration of 0.01 mg/L, to a maximum value of 100% for the highest concentration (1 mg/L).

Table A 2.2.1.3-2 Mean cell concentrations of *Pseudokirchneriella subcapitata* after exposure to Cuprofix C Disperss

Nominal Concentration [mg formulation/L]	Mean Cell Concentration [× 10^4 cells/mL] after			Inhibition relative to control after 72 h [%]
	24 h	48 h	72 h	
0	2.21	7.95	31.1	-
0.01	0.117	8.09	28.2	9.32
0.025	0.00001 *	7.68	27.9	10.3
0.064	0.00001 *	4.73	8.17	73.7
0.16	0.00001 *	4.06	5.82	81.3
0.40	0.015	2.03	2.51	91.9
1.00	0.00001 *	0.474	0.00001 *	100

Initial cell density was 0.49 x 104/mL
* Apparent negative particle density amended to 0.00001 for statistical analyses

Algal growth in terms of mean growth rate showed a 100% reduction at the highest test item treatment groups and a 0% reduction at 0.01 mg/L (**Table A 2.2.1.3-3**).

Table A 2.2.1.3-3 Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to Cuprofix C Disperss for 72 hours

Nominal Concentration [mg formulation/L]	Mean growth rate (0 – 72 hours) [10^4 cells/mL/day]	Inhibition relative to control [%]
0	1.4	-
0.01	1.4	0
0.025	1.3	7.14
0.064	0.94	32.9
0.16	0.83	40.7
0.40	0.52 *	62.9
1.00	0 *	100

* Significantly different to the control (p = 0.05)

The microscopic observations, made at test end, showed that for the control and all test concentrations, the cells appeared normal.

Table A 2.2.1.3-4 EC_{10}, EC_{50}, LOEC and NOEC values for growth rate and yield of *Pseudokirchneriella subcapitata* after 72 hours of exposure to Cuprofix C Disperss in a static test system

Growth Function	EC_{10} [mg/L] *	EC_{50} [mg/L] *	LOEC [mg/L]	NOEC [mg/L]
Cuprofix C Disperss (FEL02)				
Yield	0.17 (0.0003 – 0.034)	0.049 (0.042 – 0.054)	0.064	0.025
Growth rate	0.035 (0.029 – 0.040)	0.22 (0.16 – 0.61)	0.40	0.16
Copper (based on geometric mean measured concentrations)				
Yield	0.003 (0.001 – 0.006)	0.008 (0.007 – 0.009)	0.011	0.004
Growth rate	0.003 (0.003 – 0.003)	0.023 (0.018 – 0.048)	0.38	0.018
Cymoxanil (based on initial measured concentrations) **				
Yield	0.38 (0.0 – 0.81)	1.1 (0.95 – 1.3)	1.5	0.58
Growth rate	0.81 (0.68 – 0.93)	3.7 (2.0 – 15)	9.7	1.8

* Values in parentheses are 95 % confidence intervals
** value for Cymoxanil given in µg/L

CONCLUSIONS

The EC_{50} for yield was 0.049 mg/L, corresponding to 0.008 mg Copper/L (mean measured concentration) and to 1.1 µg/L Cymoxanil (initial measured concentration). The NOEC for yield was 0.025 mg/L, corresponding to 0.004 mg/L Copper (mean measured concentration) and to 0.58 µg Cymoxanil/L (initial measured concentration).

The EC_{50} for growth rate was 0.22 mg/L, corresponding to 0.023 mg/L Copper (mean measured concentration) and to 3.7 µg/L Cymoxanil (initial measured concentration). The NOEC for growth rate was 0.16 mg/L, corresponding to 0.018 mg a.s./L (mean measured total Copper) and to 1.8 µg Cymoxanil/L (initial measured concentration).

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

Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms with the formulation were not performed, since it is possible to extrapolate from data obtained with the active substances.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Further testing on aquatic organisms with the formulation was not performed, since it is possible to extrapolate from an indoor microcosm study with Copper Hydroxide WP (500 g copper/kg) submitted for the EU review.

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

Effects on bees of FEL02 were not evaluated as part of the EU assessment of copper. Additional toxicity data for FEL02 have been generated for acute oral and contact toxicity.

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.1.1 Study 1

Comments of zRMS:	The study is considered as valid. This study was evaluated according to OECD

	213. The study met the relevant validity criteria. The following endpoints are considered valid for use in the risk assessment: 1. Oral exposure: 96h LD_{50} = 51.6 µg product/bee; corresponding to 0.491 µg Cymoxanil/bee and 2.45 µg Copper/bee. 2. Contact exposure: 48h LD_{50} > 419.6 µg product/bee; corresponding to > 83.9 µg copper/bee and > 16.8 µg cymoxanil/bee. **Validity criteria:** For the definitive bioassays to be deemed valid, the protocol indicated that mortality in the control treatment after 48 h should not exceed 10%. Also, for validation of the toxic reference treatment, bees from the same hive should give a LD_{50} after 24-h of between 0.10 and 0.35 µg a.i./bee for the oral test and between 0.10 and 0.30 µg a.i./bee for the contact test (Gough *et al*., 1994). These criteria were met.

Reference:	KCP 10.3.1.1.1/01
Report	ATOFEL02 (Copper 200 g/kg Cymoxanil 40 g/kg WG) – Laboratory bioassays to determine the acute contact and oral toxicity to the honeybee, *Apis mellifera*., Vinall, S., 2011, UP-11-13
Guideline(s):	OECD 213 and 214 (1998)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

ATOFEL02 does not cause adverse effects on *Apis mellifera* L. after contact exposure. Therefore, the LD_{50} for contact toxicity was set above the tested dose. The contact LD_{50} value (48 h) was determined to be > 419.6 µg/bee, corresponding to > 83.9 µg Copper/bee and > 16.8 µg Cymoxanil/bee.

In the oral toxicity test, ATOFEL02 had adverse effects on *Apis mellifera* L. in a number of treatment rates. The oral LD_{50} value (96 h) based on the actual consumed dose was determined to be 51.6 µg/bee, corresponding to 0.491 µg Cymoxanil/bee and 2.45 µg Copper/bee.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	ATOFEL02
Lot / Batch no.	8.335.3
Active ingredient content / Purity	200 g/kg Copper and 40 g/kg Cymoxanil (nominal) 200 g/kg Copper and 38.3 g/kg Cymoxanil (analysed)
Characteristics	Greyish-green granules
Density (if liquid)	-
Storage conditions	Not stated
Stability (expiry date)	03.08.2012
Vehicle / control(s)	Control: topical test: a) 0.05% solution of Farmon blue in purified water; b) purified water; oral test: 50% w/v solution of sucrose in purified water Toxic reference item: Dimethoate technical

Test System

Species	*Apis mellifera* L.
Age	Adult worker bees
Source	Roselea Apiaries, East Wellow, Hampshire, UK
Acclimatisation period	Not stated
Food	Bees were fed *ad libitum* with a 50% w/v solution of sucrose in water from the glass feeding-vial.

Test Conditions

Temperature	24.1 - 25.5°C
Relative Humidity	46 - 63%
Photoperiod	Darkness, except during assessment

Study Design and Methods

In-life dates	05.07.2011 - 12.08.2011
Conducted at	Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southhampton SO16 7NP, UK
Test duration	48 hours
Test concentrations	22.2, 39.9, 71.8, 129.7, 233.3 and 419.6 µg/bee, corresponding to 5.3, 9.5, 17.1, 30.9, 55.6 and 100 µg a.s./bee (sum of active substances)
Test vessels	Cages were made of stainless-steel netting of 2.0 - 3 mm mesh size. These cages were cylindrical, measuring 140 mm deep by 40 mm in diameter, and were closed at both ends with bungs of polyurethane foam.
Treatment	The 48-hour acute contact and oral toxicity of ATOFEL02 to young adult worker bees was determined by topical application and offering test item-treated sugar solution under laboratory conditions. In the oral toxicity test, the different doses were prepared by mixing a definite amount of stock solution with a definite amount of a 50% (w/v) aqueous sugar solution to give the test concentrations of 22.2, 39.9, 71.8, 129.7, 233.3 and 419.6 µg/bee, corresponding to 5.3, 9.5, 17.1, 30.9, 55.6 and 100 µg a.s./bee (sum of active substances). The study included 6 test item treatment groups, a control and 1 reference item group with three replicates for the test item treatment groups and 5 replicates for the control. A single replicate contained a group of 10 bees. Before the test items administration, the bees were starved for not longer than two hours. Quantities of 200 µL (20 µL/bee) of treated solutions were offered to each cage of 10 bees for six 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. The test item was dispersed in a 0.05% solution of Farmon Blue (a wetting agent) in water. A 0.05% v/v solution of Farmon Blue in water and a water only treatment were applied as controls. Untreated 50% w/v sugar solution was provided as sustenance for the bees throughout the test. Following a range-finding test, a definitive limit-rate test was carried out with one rate of ATOFEL02 at 419.6 µg/bee, equivalent to 100 µg a.s./bee (sum of active substances). There were five replicate cages of 10 bees each (i.e. 50 bees in total) per treatment. Dimethoate was used as toxic reference item at a dose range of 0.1 - 0.2 µg a.s./bee for both the oral and contact tests.
Observations	Assessments of bee mortality for each test were made over 48 h. The results were used to determine the median lethal dose (LD_{50}) for the test item after 24 and 48 h.
Statistics	No statistical analysis of the data was required.

RESULTS AND DISCUSSIONS

The results obtained for Dimethoate indicated that the 24-h LD_{50} values for contact and oral modes of application were 0.154 and 0.152 µg a.s./bee, respectively, and these are in line with published values. This demonstrated that the bees were of a suitable sensitivity.

Oral toxicity test

Mortality in the control was 4% after 48 h (validity criteria 10 %). Due to an increase in mortality of > 10% being observed between the 24 and 48 h assessments, the test was extended for a further 48 h. After 96 h, mortality in the control was still 4%, compared with 17 - 100% mortality in the respective test item treatment groups.

The mean consumption in the individual treatments was actually equivalent to 49.7, 40.2, 29.7, 18.0, 9.8 and 5.5 µg a.s./bee for the nominal 100, 55.6, 30.9, 17.1, 9.5 and 5.3 µg a.s./bee treatment rates, respectively. It is evident from these values that the treated sugar solution was relatively unpalatable to the bees at the highest test item treatment group and, to a lesser extent, at the second highest test item treatment group. Nevertheless, sufficient doses were consumed to cause ≥ 97% mortality.

Table A 2.3.1.1.1.1-1 Mortality of honeybees exposed to ATOFEL02 for 96 hours in an oral toxicity test

Treatment group [µg a.s./bee]	Mean dose consumed [µg a.s./bee] [1]	Acute oral mortality [%] after [2]	
		48 h	96 h
Control (sugar solution)	-	4	4
5.3	5.5	17	17
9.5	9.8	37 *	43 *
17.1	18.0	73 *	73 *
30.9	29.7	97 *	100 *
55.6	40.2	100 *	100 *
100	49.7	97 *	97 *

[1] Based on the combined measured content of the two active substances. Derived from mean weight of test solution consumed per cage of 10 bees, corrected for density of the test solutions (treated 50% w/v sugar solution).
[2] mortality of the treated group is corrected according to Abbott (1925)
* significantly different compared to the control (Fisher's Exact Test, $p < 0.05$)

The statistical analysis showed statistically significant differences between the effects of the test item and the effects obtained in the untreated control group (Fisher's Exact Test, $p > 0.05$) for all test item treatment groups except for the lowest treatment group. The results are summarised in the Table below. The oral LD_{50} (48 h) was determined to be 56.2 µg/bee, corresponding to 2.25 µg Cymoxanil/bee and 11.2 µg Copper/bee. The oral LD_{50} (96 h) was determined to be 51.6 µg/bee, corresponding to 0.491 µg Cymoxanil/bee and 2.45 µg Copper/bee.

Contact toxicity test

Mortality in the control was 0% after 48 h. The statistical analysis showed no statistically significant difference between the effects of the test item and the effects obtained in the untreated control group (Fisher's Exact Test, $P > 0.05$). Corrected mortality in the treatment group after 48 hours was 0%. Therefore, the contact LD_{50} (48 h) was determined to be > 419.6 µg/bee, corresponding to > 83.9 µg Copper/bee and > 16.8 µg Cymoxanil/bee. The results are summarised in the table below.

Table A 2.3.1.1.1.1-2 Mortality of honeybees exposed to ATOFEL02 for 48 hours in a contact toxicity test

Treatment group	Acute contact mortality [%] after	
	24 h	48 h
Control (water)	0	0
Control (Farmon blue)	0	8
419.6 µg ATOFEL02/bee	0	2 (0) *

* value in parentheses represent mortality corrected for the Farmon blue control according to Abbott (1925)

CONCLUSIONS

ATOFEL02 does not cause adverse effects on *Apis mellifera* L. after contact exposure. Therefore, the LD_{50} for contact toxicity was set above the tested dose. The contact LD_{50} value (48 h) was determined to be > 419.6 µg/bee, corresponding to > 83.9 µg Copper/bee and > 16.8 µg Cymoxanil/bee.

In the oral toxicity test, ATOFEL02 had adverse effects on *Apis mellifera* L. in a number of treatment rates. The oral LD_{50} value (96 h) based on the actual consumed dose was determined to be 51.6 µg/bee, corresponding to 0.491 µg Cymoxanil/bee and 2.45 µg Copper/bee.

A 2.3.1.1.1.2 Study 2

Comments of zRMS:

The study is considered as valid. This study was evaluated according to OECD 247 (217). The study met the relevant validity criteria. The following endpoints are considered valid for use in the risk assessment:

Mortality of Bumblebee after 48 hours

	Test item		Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG
	Test system		*Bombus terrestris*
			Mortality [%] ± SD
Control			0 ± 0
Test item [µg Cu/bbee]	nominal	actual[1)]	
	4.27	4.34	0 ± 0
	9.39	9.77	0 ± 0
	20.7	21.8	**66.7 ± 48.0**
	45.5	47.7	**83.3 ± 37.9**
	100	102	**100 ± 0.0[2)]**
	LD_{50} with confidence intervals	48 h	21.4 (8.82 - 51.6) µg Cu/bbee
	NOED	48 h	9.39 µg Cu/bbee
Reference item [µg a.i./bbee]	nominal	actual[1)]	
	3.5	3.62	100 ± 0.0[2)]

1) = Food uptake was taken into account 2) = Mortality determined after 24 h
bold = Statistically significant inhibition compared to the control

Validity criteria:
The study is considered to be valid as the validity criteria according to the test guideline were
fulfilled (control mortality ≤ 10%, mean mortality in the toxic reference group ≥ 50%).

Reference:	KCP 10.3.1.1.1/02
Report	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Acute Oral Toxicity Test on the Bumblebee *Bombus terrestris*, McVean, K., 2022, IUO20269
Guideline(s):	OECD 247 (2017)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The acute oral effects of FEL02 to the bumblebee *Bombus terrestris* were determined according to OECD 247 (2017) in 2022. The test was conducted with the nominal test item doses of 100 - 45.5 - 20.7 - 9.39 - 4.27 µg Cu/bbee (spacing factor 2.2). 50 % aqueous sucrose solution was used as control. Dimethoate with an application dose of 3.5 µg a.i./bbee was used as reference item. 50 replicates for the control and 30 replicates for the test and reference item treatments were tested. Each replicate contained one bumblebee. The mortality and sublethal effects in the test item, reference and control treatments were assessed after 4, 24 and 48 hours exposure period.

The recoveries of Cu and Cymoxanil in the test item in the highest stock solution, the lowest and highest application solutions were between 70% to 98%, indicating the correct preparation of the test item solution.

Biological results are based on nominal test item doses.

Under laboratory test conditions FEL02 induced significant mortality on *Bombus terrestris* at nominal doses ≥ 20.7 µg Cu/bbee, corresponding to 21.8 µg Cu/bbee consumed dose. Therefore, the NOED was determined to be 9.39 µg Cu/bbee. The 24 hours and 48 hours LD_{50} values were determined to be 21.4 µg Cu/bbee.

MATERIALS AND METHODS

Test Item

Designation	FEL02
Lot / Batch no.	0722137
Active ingredient content / Purity	200 g/kg Copper and 40 g/kg Cymoxanil (nominal) 200 g/kg Copper and 41 g/kg Cymoxanil (analysed)
Characteristics	Greyish-green granules
Density (if liquid)	-
Storage conditions	18 - 25 °C, dark, in the tightly closed original container
Stability (expiry date)	17.05.2024
Vehicle / control(s)	Control: 50% w/v solution of sucrose in purified water Toxic reference item: Dimethoate (87.7% purity)

Test System

Species	*Bombus terrestris*
Age	Adult bumblebees
Source	Biobest Group N.V. (Belgium)
Acclimatisation period	The bumblebees were adapted to test conditions (including single housing) for approximately 24 hours.
Food	Bumblebees were fed *ad libitum* with a 50% w/v solution of sucrose in water from the glass feeding-vial.

Test Conditions

Temperature	25°C
Relative Humidity	65 - 83%
Photoperiod	Darkness, except during assessment

Study Design and Methods

In-life dates	30.08.2022 – 01.09.2022
Conducted at	Noack Laboratorien GmbH, Käthe-Paulus-Str. 1, 31157, Sarstedt, Germany
Test duration	48 hours
Test concentrations	100 - 45.5 - 20.7 - 9.39 - 4.27 µg Cu/bbee (spacing factor 2.2)
Test vessels	NICOT® queen breeding cages were used as test containers. The bumblebees were kept in individual cages. To allow olfactory and visual contact between the bumblebees, the cages were placed next to each other. Plastic syringes without tip were used as feeders.

Treatment	In the oral toxicity test, the different doses were prepared by mixing a definite amount of stock solution with a definite amount of a 50% (w/v) aqueous sugar solution to give the appropriate test concentrations. The study included 5 test item treatment groups, a control and 1 reference item group with 30 replicates for the test item treatment groups and 50 replicates for the control. A single replicate contained a single Bumblebee. The bumblebees were starved for 2.5 - 3.5 hours prior to application. After the starvation period each bumblebee was provided with 40 µL application solution. As feeders, plastic injection syringes without tip were used. The consumption of untreated and treated sucrose solution was determined by weighing the feeders before and after the feeding duration of maximum 4 hours. Dimethoate was used as toxic reference item at a dose of 3.5 µg a.s./bee for both the oral and contact tests.
Observations	Food consumption was determined after a maximum of 4 hours by weighing the feeding syringes. Mortality and sublethal effects (affected, moribund) was determined after 4, 24 ± 2 and 48 ± 2 h. The room temperature and humidity were recorded every hour with a datalogger.
Statistics	Statistical analysis Kruskal-Wallis One Way Analysis of Variance on Ranks (α=0.05) was carried out to determine statistically significant differences compared to the control. A Normality Test (Shapiro-Wilk) was run before and failed. The LD values were based on the nominal test item doses. The LD50 value (incl. confidence limits) was calculated by Probit analysis using linear max. likelihood regression. The confidence limits were determined according to Fieller´s theorem. Sytstat and ToxRat Professional, TOXRAT® SOLUTION GMBH was used for the calculations.

RESULTS AND DISCUSSIONS

All validity criteria of OECD guideline 247 were met as:

- No mortality was found in the control (should be ≤ 10 %) at the end of the test.
- Mortality in the toxic reference substance group was 100% (should be ≥ 50 %) at the end of the test.

Oral toxicity test

No mortality occurred in the control treatment. The statistical analysis showed statistically significant differences between the effects of the test item and the effects obtained in the untreated control group (Kruskal-Wallis One Way Analysis of Variance on Ranks (α=0.05)) for all test item treatment groups except for the lowest two treatment groups in which no mortality occurred. The results are summarised in the Table below. The oral LD_{50} (48 h) was determined to be 21.4 (95% c.i.: 8.82-51.6) µg Copper/bee.

Table A 2.3.1.1.1.2-1 Mortality of bumblebees exposed to FEL02 for 48 hours in an oral toxicity test

	Nominal test dose [µg Cu/bbee]	Consumed test doses [µg Cu/bbee]	Mean Mortality [%] 4 h	24 h	48 h
Control	---		0	0	0
Test item FEL02	4.27	4.34	0	0	0
	9.39	9.77	0	0	0
	20.7	21.8	0	66.7*	66.7*

	45.5	47.7	0	83.3*	83.3*
	100	102	0	100*	---
Reference item Dimethoate	3.5	3.62	0	100	---

* Statistically significant inhibition compared to the control

CONCLUSIONS

Under laboratory test conditions Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG induced significant mortality on *Bombus terrestris* at nominal doses ≥ 20.7 µg Cu/bbee, corresponding to 21.8 µg Cu/bbee consumed dose. Therefore, the NOED was determined to be 9.39 µg Cu/bbee. The 24 hours and 48 hours LD_{50} values were determined to be 21.4 µg Cu/bbee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

An acute contact toxicity study performed with FEL02 with honeybees is provided in support of the assessment and described in A 2.3.1.1.1.1. The acute contact toxicity test with Bumblebees is summarised below.

A 2.3.1.1.2.1 Study 1

Comments of zRMS:

The study is considered as valid. This study was evaluated according to OECD 246 (217). The study met the relevant validity criteria. The following endpoints are considered valid for use in the risk assessment:

Mean Mortality in [%]

n = 50 (controls and test item treatment), n = 30 (reference item treatment)

		Mean Mortality [%]		
		4-5 h	24 h	48 h
Control	Demineralised water	0 ± 0	0 ± 0	0 ± 0
	Triton X® 100 (0.1 %)	0 ± 0	0 ± 0	0 ± 0
Test item [µg Cu/bbee]	100	0 ± 0	0 ± 0	2.00 ± 14.1
Reference item [µg a.i./bbee]	10	0 ± 0	96.7 ± 18.3	100 ± 0

Validity criteria:

Validity Criteria	required	actual
Mean mortality in the control treatment	≤ 10 %	demineralised water: 0 % Triton X® 100 (0.1 %): 0 %
Mean mortality in the reference treatment	≥ 50 %	100 %

Reference:	KCP 10.3.1.1.2/01
Report	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Acute Contact Toxicity Test on the Bumblebee Bombus terrestris, McVean, K., 2022, IUT20269
Guideline(s):	OECD 246 (2017)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The acute contact effects of FEL02 (batch-No. 0722137) on the bumblebee *Bombus terrestris* were determined according to the guideline OECD 246 (2017) in 2022.

The test was conducted with the nominal limit test item dose of 100 µg Cu/bbee. Demineralised water and demineralised water with 0.1 % Triton X® 100 were used as controls. Dimethoate with an application dose of 10 µg a.i./bbee was used as reference item. 50 replicates for the controls and the limit test item treatment and 30 replicates for the reference item treatment were tested. Each replicate contained one bumblebee. The mortality and sublethal effects in the test item, reference and control treatments were assessed after 4, 24 and 48 hours exposure period.

The concentration of the active ingredient Cymoxanil of the test item FEL02 was determined by analysis of the test item stock solution and control solution (Triton X® 100 solution (0.1 %)) *via* LC-MS/MS. The recovery of the test item in the stock solution was 106 % of the nominal concentration, indicating the correct preparation of the test item solution. Biological results are based on nominal test item concentrations.

The concentration of the active ingredient copper of the test item FEL02 was determined by analysis of the test item stock solution and control solution (Triton X® 100 solution (0.1 %)) *via* IVP-OES. The recovery of the test item in the stock solution was 93.8 % of the nominal concentration, indicating the correct preparation of the test item solution. Biological results are based on nominal test item concentrations.

Under laboratory test conditions FEL02 did not induce significant mortality on *Bombus terrestris* at the nominal limit test item dose of 100 µg Cu/bbee. Therefore, the NOED was determined to be nominal ≥ 100 µg Cu/bbee (equivalent to ≥ 500 µg FEL02/bbee). The 24 hours and 48 hours LD_{50} values were determined to be > 100 µg Cu/bbee (equivalent to > 500 µg FEL02/bbee).

MATERIALS AND METHODS

Test Item

Designation	FEL02
Lot / Batch no.	0722137
Active ingredient content / Purity	200 g/kg Copper (Bordeaux mixture) and 40 g/kg Cymoxanil (nominal) 200 g/kg Copper (Bordeaux mixture) and 41 g/kg Cymoxanil (analysed)
Characteristics	Greyish-green granules
Density (if liquid)	-
Storage conditions	18 - 25 °C, dark, in the tightly closed original container
Stability (expiry date)	17.05.2024
Vehicle / control(s)	Control: Demineralised water Solvent control: Demineralised water with 0.1 % Trito X® Toxic reference item: Dimethoate, 87.7% purity

Test System

Species	*Bombus terrestris*

Age	Adult bumblebees
Source	Biobest Group N.V. (Belgium)
Acclimatisation period	The bumblebees were adapted to test conditions (including single housing) for > 8 hours.
Food	During keeping and adaption the bumblebees were fed *ad libitum* with 50 % sucrose solution.
Test Conditions	
Temperature	25°C
Relative Humidity	62 - 83%
Photoperiod	Darkness, except during assessment
Study Design and Methods	
In-life dates	31.08.2022 - 02.09.2022
Conducted at	Noack Laboratorien GmbH, Käthe-Paulus-Str. 1, 31157, Sarstedt, Germany
Test duration	48 hours
Test concentrations	500 µg/bee, corresponding to 100 µg Cu/bbee and 120 µg a.s./bbee (sum of active substances)
Test vessels	NICOT® queen breeding cages were used as test containers. The bumblebees were kept in individual cages. To allow olfactory and visual contact between the bumblebees, the cages were placed next to each other. Plastic injection syringes without tip were used as feeders.
Treatment	Test item, controls and reference item were applied onto the thorax of each bumblebee. A drop of 2 µL was applied with a micro piston pipette after paralysation of the bumblebee with CO_2 (approximately 3 sec.). The test item was dispersed in a 0.1 % solution of Triton X® 100 in water. A 0.01% solution of Triton X® 100 in water and a water only treatment were applied as controls. Untreated 50% w/v sugar solution was provided as sustenance for the bumblebees throughout the test. Following a range-finding test, a definitive limit-rate test was carried out with one rate of FEL02 at 500 µg/bee, equivalent to 120 µg a.s./bee (sum of active substances). There were 50 replicate cages of 1 bumblebee each in the controls and 30 each in te test item treatment. Dimethoate was used as toxic reference item at a dose of 10 µg a.i./bumblebee.
Observations	Assessments of bee mortality were made over 48 h. The results were used to determine the median lethal dose (LD_{50}) for the test item after 24 and 48 h.
Statistics	No statistical analysis was conducted since no test item related differences in mortality were found between the controls and the test item treatment.

RESULTS AND DISCUSSIONS

As a result of the application with the reference item dimethoate at 10 µg a.i./bbee 100% mortality occurred. This demonstrated that the bumblebees were of a suitable sensitivity.

Mortality in the control, the solvent control and in the test item treatment was 0% after 48 h. Therefore, the contact LD_{50} (48 h) was determined to be > 500 µg test item/bbee, corresponding to > 100 µg Copper/bbee and > 20 µg Cymoxanil/bee. The results are summarised in the table below.

Table A 2.3.1.1.2.1-1 Mortality of Bumblebees exposed to FEL02 for 48 hours in a contact toxicity test

		Mean Mortality [%]		
		4-5 h	24 h	48 h
Control	Demineralised water	0 ± 0	0 ± 0	0 ± 0

	Triton X® 100 (0.1 %)	0 ± 0	0 ± 0	0 ± 0
Test item [µg/bbee]	500	0 ± 0	0 ± 0	2.00 ± 14.1
Reference item [µg a.i./bbee]	10	0 ± 0	96.7 ± 18.3	100 ± 0

All validity criteria of OECD guideline 246 were met as:

- No mortality was found in the control and the solvent control (should be ≤ 10 %) at the end of the test.
- mortality in the toxic reference substance group was 100% (should be ≥ 50 %) at the end of the test.

CONCLUSIONS

Under laboratory test conditions FEL02 did not induce significant mortality on *Bombus terrestris* at the nominal limit test item dose of 500 µg Test item/bbee. The 24 hours and 48 hours LD_{50} values were determined to be > 500 µg test item/bbee, corresponding to > 100 µg Copper/bbee and > 20 µg Cymoxanil/bee.

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

A 2.3.1.2.1 Study 1

Comments of zRMS:	The study is considered as valid. This study was evaluated according to OECD 245 (217). The study met the relevant validity criteria. **Validity of the study** Mortality in the controls: 3.3% mean mortality in control group AC and 6.7% in the viscosifier control group BC; validity criterion was met Mortality in the reference group: 96.7% mean mortality after 10 days of exposure; validity criterion was met **Agreed toxicity endpoints:** The LDD_{50} was determined to be 5.51 µg consumed product/bee/day (equivalent to 1.32 µg consumed a.s./bee/day) and the LC_{50} to be 0.2213 g product/kg food (equivalent to 0.0532 g a.s./kg food), respectively. The NOEDD was determined to be 2.15 µg consumed product/bee/day (equivalent to 0.516 µg consumed a.s./bee/day) and the NOEC to be 0.0678 g product/kg food (equivalent to 0.0163 g a.s./kg food), respectively.

Reference:	KCP 10.3.1.2/01
Report	Chronic toxicity of Copper 20% + Cymoxanil 4% WG to the honey bee *Apis mellifera* L. under laboratory conditions, Ruhland, S., 2018, 17 48 BAC 0058
Guideline(s):	Yes (OECD 245 (2017))
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a 10-day chronic toxicity feeding test, 2 days old worker honey bees (*Apis mellifera* L. subspecies *iberiensis*) were exposed to a daily application of Copper 20% + Cymoxanil 4% WG diluted in the bee food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The chronic toxicity of the test item was determined at nominal doses of 41.6, 16.6, 6.65, 2.66, 1.06 and 0.426 µg product/bee/day (equivalent to 10.0, 3.99, 1.60, 0.639, 0.255 and 0.102 µg a.i./bee/day), corresponding to concentrations of 1.0588, 0.4235, 0.1694, 0.0678, 0.0271 and 0.0108 g product/kg food (equivalent to 0.2541, 0.1016, 0.0407, 0.0163, 0.0065 and 0.0026 g a.i./kg food). Effective doses were 19.4, 7.48, 4.92, 2.15, 0.828 and 0.364 µg consumed product/bee/day (equivalent to 4.65, 1.79, 1.18, 0.516, 0.199 and 0.0873 µg consumed a.i./bee/day).

Additionally, honey bees were treated with Dimethoate EC 400 as toxic standard at a nominal dose of 27.3 ng a.i./bee/day. Untreated 50% (w/v) aqueous sucrose solution and 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan served as controls.

The LDD_{50} was determined to be 5.51 µg consumed product/bee/day (equivalent to 1.32 µg consumed a.s./bee/day) and the LC_{50} to be 0.2213 g product/kg food (equivalent to 0.0532 g a.s./kg food), respectively. The NOEDD was determined to be 2.15 µg consumed product/bee/day (equivalent to 0.516 µg consumed a.s./bee/day) and the NOEC to be 0.0678 g product/kg food (equivalent to 0.0163 g a.s./kg food), respectively.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Copper 20% + Cymoxanil 4% WG
Lot / Batch no.	15.351.3
Active ingredient content / Purity	Copper 20% (nom.) 20.4% (analysed) Cymoxanil 4% (nom.) 4.0% (analysed)
Characteristics	WG (water dispersible granules)
Density (if liquid)	-
Storage conditions	Ambient
Stability (expiry date)	17.12.2017
Vehicle / control(s)	Control: 50% (w/v) sucrose solution / Viscosifier control: 0.1% (w/v) xanthan Toxic reference item: Dimethoate EC 400

Test System

Species	*Apis mellifera* L.
Age	Adult worker bees (max. 2 days old)
Source	Beekeeper Joaquin Cordero, Paseo del Moro No. 19, 41370 Cazalla (Sevilla), Spain. All bees used in the test derived from a healthy, disease free and queen-right bee colony (colony no.: 32SE340/10). The bees were taken from a hive that had not received treatments with chemical substances for at least one month.
Pre-treatment culturing conditions	Brood combs with capped cells were taken from one outside hive (D -2). Sufficient food supply was ensured either by honey and pollen which is on the same brood comb or by an additional comb containing food. These frames were placed without adult worker bees in a “five comb hive body” and incubated under controlled environmental conditions in a climatic chamber at 33 ± 2°C in darkness (until D -1). Afterwards, the newly hatched worker bees were transferred into the test cages in groups of 10 bees/cage. For the following 24 ± 2 h (until D 0), bees were held in the test cages at 33 ± 2°C and 50 - 70% RH and provided with sucrose solution for acclimatisation to the test conditions. Moribund and dead bees were rejected and replaced by healthy bees that were held in spare cages before starting the test.

Food	50% (w/v) sucrose solution. Young worker bees were provided continuously with pollen, water, treated or untreated test solution via syringes.
Test Conditions	
Temperature	31.9 - 34.1°C
Relative Humidity	59.6 - 69.8%
Photoperiod	Constant darkness throughout the test (diffuse artificial light only during handling and assessments)
Study Design and Methods	
In-life dates	14.11.2017 - 24.11.2017
Conducted at	BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Test duration	10 days
Test vessels	Aluminium cages with the dimensions: 95 mm × 60 mm × 70 mm; with holes in the lateral walls for ventilation and two glass plates (one in front and one in the back) for observation of the bees.
Treatment	The application of the respective item dose took place daily for a period of successional 10 days. Test solutions were freshly prepared every day right before administration of food. Test item: 41.6, 16.6, 6.65, 2.66, 1.06, 0.426 µg prod./bee/d (nom.) 19.4, 7.48, 4.92, 2.15, 0.828, 0.364 µg prod./bee/d (con.) Control: 50% (w/v) aqueous sucrose solution and 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan Reference: 27.3 ng a.s./bee/day (nom.) 16.0 ng a.s./bee/day (consumed) 3 Replicates per treatment group with 10 bees per cage
Observations	Mortality and behaviour were recorded daily at about the same time of the day (every 24 h ± 2 h), starting 24 ± 2 hours after start of the test period (initial feeding). Additionally, behavioural abnormalities were recorded daily at the same time as the assessments of mortality according to the following categories: healthy/normal, moribund (M), affected in terms of uncoordinated movements (A), cramping (C), apathetic (Ap), vomiting (V). Any other behavioural abnormalities were noted and clearly described, if observed.
Analytical verification	For verification of the exposure concentration, the highest test item solution (AT) and the lowest test item solution (FT) ast well as the control solution (BC) were sampled in duplicate as specimens for analysis and retention directly after preparation on each day of application. The determination of the active ingredient in sucrose solution was conducted by an in-house developed method using atomic absorption spectroscopy (AAS).
Statistics	The statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (2015). For statistical calculation of the mortality results the Step-down Cochran-Armitage test was used ($\alpha = 0.05$; one sided greater). For calculation of the LDD_{50} and LC_{50} Probit analysis (linear maximum likelihood regression) was used. The following endpoints were determined: - mean daily intake per bee - the NOEDD/NOEC (No observed effect dietary dose/ concentration) - LDD_{50} and LC_{50} (Median lethal dietary dose/concentration)

RESULTS AND DISCUSSIONS

After 10 days, a mortality of 3.3% in control group AC was observed. In the viscosifier control group BC a mortality of 6.7% was recorded. Taking into account the actual food uptake and the evaporated amount of feeding solution the bees effectively consumed doses of 19.4, 7.48, 4.92, 2.15, 0.828 and 0.364 µg product/bee/day which caused mortalities of 100.0, 90.0, 36.7, 3.3, 6.7 and 6.7%, respectively after 10 days. Mortalities in the 19.4, 7.48 and 4.92 µg consumed product/bee/day treatment groups were statistically significantly increased compared to the control.

On the last day of the test, treatment related behavioural abnormalities could be observed in both middle test item groups. Bees were described as being affected in terms of uncoordinated movements.

The recovery rates of copper ranged between 97% and 105% in the highest and between 101% and 108% in the lowest test item dose (samples taken on each day of application). No test item has been detected in the control sample.

The effective reference dosage in the study was 16.0 ng a.s./bee/day, which caused a mean mortality of 96.7%.

In the test item group, the food consumption ranged between 17.7 and 33.5 mg solution per bee per day which is 45.0% to 85.4% of the expected amount (control AC: on average 41.3 mg/bee/day = 105.1%, viscosifier control BC: on average 35.6 mg/bee/day = 90.8%) with a tendency of higher food uptake in the lower test item dosages. The results are summarised in the following table:

Table A 2.3.1.2.1-1 Mean mortality, behaviour of bees and toxicity of Copper 20% + Cymoxanil 4% WG after 10 days in a chronic toxicity feeding test

Treatment group	Treatment group ID	Daily dose [µg product/bee/day]		Concentration	After 10 days		
		nominal	consumed	[g product/ kg food]	Mean mortality		Number of bees with behavioural abnormalities**
					absolute [%]	Corrected [%]	
Control	AC	-	-	-	3.3	-	0 out of 29
Viscosifier control	BC	-	-		6.7	-	0 out of 28
Test item	AT	41.6	19.4	1.0588	100.0*	100.0	-
	BT	16.6	7.48	0.4235	90.0*	89	0 out of 3
	CT	6.65	4.92	0.1694	36.7*	32.1	3 out of 19
	DT	2.66	2.15	0.0678	3.3	0.0	1 out of 29
	ET	1.06	0.828	0.0271	6.7	0.0	0 out of 28
	FT	0.426	0.364	0.0108	6.7	0.0	0 out of 28
		[ng a.s./bee/day]		[mg a.s./kg food]			
Reference item	AR	27.3	16.0	0.696	96.7	96.6	1 out of 1

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values and corrected for evaporation

corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); test item group was corrected for mortality of untreated control group BC, reference item group for mortality of control group AC; negative values are treated as "0"

* Statistically significant difference in pairwise comparison between treatment and untreated viscosifier control (BC) (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

** Number of bees with behavioural abnormalities referring to number of remaining bees

Table A 2.3.1.2.1-2 Endpoints determined after 10 days in a chronic toxicity feeding test

	Endpoints	**10 d**
Test item doses	LDD_{50} [µg consumed product/bee/day][1]	5.51 (4.92 – 6.05)
	LDD_{50} [µg consumed a.s./bee/day][1*]	1.32 (1.18 – 1.45)
	NOEDD [µg consumed product/bee/day][2]	2.15
	NOEDD [µg consumed a.s./bee/day][2*]	0.516
Test item concentrations	LC_{50} [g product/kg food][1]	0.2213 (0.1832 – 0.2673)
	LC_{50} [g a.s./kg food][1*]	0.0532 (0.0440 – 0.0642)
	NOEC [g product/kg food][2]	0.0678
	NOEC [g a.s./kg food][2*]	0.0163

[1] Median lethal dietary dose/concentration (95% cl lower-upper) was calculated using Probit analysis (linear max. likelihood regression)
[2] No observed effect dietary dose/concentration was calculated using Step-down Cochran-Armitage Test Procedure (α = 0.05; one sided greater)
* sum ob both active substances

CONCLUSIONS

The chronic oral toxicity of Copper 20% + Cymoxanil 4% WG on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

The LDD_{50} was determined to be 5.51 µg consumed product/bee/day (equivalent to 1.32 µg consumed a.s./bee/day) and the LC_{50} to be 0.2213 g product/kg food (equivalent to 0.0532 g a.s./kg food), respectively.

The NOEDD was determined to be 2.15 µg consumed product/bee/day (equivalent to 0.516 µg consumed a.s./bee/day) and the NOEC to be 0.0678 g product/kg food (equivalent to 0.0163 g a.s./kg food), respectively.

A 2.3.1.2.2 Study 2

Comments of zRMS:	The study is considered as valid. The study met the relevant validity criteria. **The test was considered valid because the following criteria were satisfied:** - The average mortality for the control did not exceed 15% at the end of the test (actual value: 3.3%); - The average mortality in the reference item treatment was ≥50% at the end of the test (actual value: 100%). **Deviations from the study:** Temperature and humidity occasionally deviated from the guideline norm values. As this occurred for < 2 hours/day this deviation is not considered to adversely affect the results of the study. **Agreed toxicity endpoints:** In a 10-day chronic toxicity feeding study with Copper Oxychloride 50% WP the LDD_{50} was determined to be 0.466 µg copper/bee/day and the LC50 was determined to be 10.07 mg copper/kg food, respectively. The NOEDD was determined to be 0.177 µg copper/bee/day, and the NOEC was determined to be 6.33 mg copper/kg food, respectively.

	The effects of **COPPER OXYCHLORIDE 50% WP** to adult worker honeybees (*Apis mellifera* L.) were assessed in a 10-day oral chronic toxicity test. In terms of dose related to the mean food consumption/bee/day, the **NOEDD** was determined to be 0.350 µg prod./bee/day (0.177 µg copper/bee/day), and the LDD_x values with 95% Confidence limits (CL) resulted as: • **LDD_{10}**: 0.613 µg prod./bee/day (CL: 0.474÷0.704 µg prod./bee/day), equivalent to 0.310 µg copper/bee/day; • **LDD_{20}**: 0.721 µg prod./bee/day (CL: 0.598÷0.801 µg prod./bee/day), equivalent to 0.365 µg copper/bee/day; • **LDD_{50}**: 0.920 µg prod./bee/day (CL: 0.836÷0.987 µg prod./bee/day), equivalent to 0.466 µg copper/bee/day. In terms of concentration, the **NOEC** was 12.50 mg of prod./kg diet (6.325 mg copper/kg diet), the LC_x values with 95% Confidence limits (CL) resulted as: • **LC_{10}**: 19.904 mg prod./kg diet (CL: 13.178÷25.600 mg prod./kg diet), equivalent to 10.071 mg copper/kg diet; • **LC_{20}**: 27.863 mg prod./kg diet (CL: 20.619÷33.912 mg prod./kg diet), equivalent to 14.099 mg copper/kg diet; • **LC_{50}**: 46.308 mg prod./kg diet (CL: 38.826÷54.153 mg prod./kg diet), equivalent to 23.432 mg copper/kg diet. The analytical results demonstrate that the active substances' content in the stock solutions and in the feeding solutions was in the range of ± 20% of nominal concentration. The end-points of the test were calculated with respect to the nominal concentration of the test item.

Reference:	KCP 10.3.1.2/02
Report	Chronic oral effects of copper oxychloride 50% WP to adult worker honeybees *Apis mellifera* L., 10-day feeding laboratory test, Colli, M., 2018a, Report No: BT215/17
Guideline(s):	Draft Test Guideline on Honey bee (*Apis mellifera* L.), Chronic Oral Toxicity test, 10-day feeding test in the laboratory (March 2017).
Deviations:	Temperature and humidity occasionally deviated from the guideline norm values. As this occurred for < 2 hours/day this deviation is not considered to adversely affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The purpose of this study was to assess the chronic oral toxicity of low doses of the test item to adult worker bees of *Apis mellifera* L. under laboratory conditions. In a ten-day chronic toxicity feeding test, 2-day old worker honey bees were exposed to a daily application of Copper Oxychloride 50% WP diluted in the bee food (50 % w/v aqueous sucrose solution).

The chronic toxicity of the test item was determined at nominal doses of 3.16, 6.33, 12.65, 25.30 and 50.60 mg copper/kg feeding solution. Effective doses were 0.104, 0.177, 0.357, 0.500 and 0.162 µg copper/bee/day. Bees were treated with dimethoate as the toxic standard at a nominal dose of 1 mg/kg feeding solution. Untreated feeding solution served as the control.

The 10-day LDD_{50} was determined to be 0.466 µg copper/bee/day and the LC_{50} was 23.432 mg copper/kg food.

The NOEDD was determined to be 0.177 µg copper/bee/day, and the NOEC was 6.33 mg a.s./kg food.

MATERIALS AND METHODS

Test Item

Designation	Copper oxychloride 50% WP
Lot / Batch no.	183538
Active ingredient content / Purity	50.6% as copper
Characteristics	Green powder
Storage conditions	15 – 25°C
Stability (expiry date)	July 2018
Vehicle / control(s)	Control: water

	Toxic reference item: Dimethoate
Test System	
Species	Worker honey bees *Apis mellifera* L.
Age	Max. 2 days old – young worker bees
Supplier	Hives no. 4 and 7 of the Biotecnologie BT S.r.l. breeding colonies
Acclimatisation period	24 h
Housing	Disposable cardboard cages 5×9.5×6.5 cm with a frontal transparent lid, 10 bees/cage.
Food or Diet	50% (w/v) aqueous sucrose solution
Test Conditions	
Temperature	26.3 – 33.6 °C (average 32.4ºC)
Relative humidity	28.5 – 66.6 % (average 52.6%)
Photoperiod	24 hours dark, except during observations
Study Design and Methods	
In-life dates	13.09.2017 – 27.09.2017
Conducted at	Biotecnologie BT S.R.l., Frazione Pantalla, 06059 Todi (PG), Italy
Test duration	10-days
Test design	Chronic oral toxicity test
Test concentrations	0 (control), 3.16, 6.33, 12.65, 25.65, 25.30, 50.60 mg Cu/kg f.s.
Test groups / Replicates	Three replicates of 10 worker bees each were prepared for each experimental group.
Treatment	All bees used in the test derived from healthy, disease free and queen-right bee colonies. Capped brood combs with emerging bees were used to obtain the number of bees needed for the test. The frames were incubated in a climatic chamber until hatch, under the same conditions of the test. Sufficient food supply was guaranteed by honey and pollen in the combs. The newly hatched worker bees were transferred into the test cages in groups of 10 bees/cage. One day before the start of the test, the bees were collected from the combs and distributed into the test cages and acclimatized to the test conditions for about one day (after a hatching period of one day). Bees were fed ad libitum with sucrose solution, but no additional feeding of pollen and water was necessary during acclimatization and test period. No starvation period was necessary before test start. The bees were randomly distributed within replicates. The bees were fed with 50% w/v aqueous sucrose solution including the test item or the reference item. The control treatments were fed with 50 % w/v aqueous sucrose solution. The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application and was replaced daily. The test item was dissolved in water to get a stock solution and subsequent dilutions. The water solutions were prepared freshly every day. The feeding solutions were obtained from the stock solutions with a measured quantity of 50% (w/v) aqueous sucrose solution. The feeding solutions were also prepared freshly every day and were observed homogeneous without obvious signs of precipitations throughout one feeding interval (about 24 hours).
Observations	Mortality and sub-lethal effects were recorded every 24 h ± 2 h, starting 24 ± 2 hours after the start of the test period (initial feeding). Sub-

	lethal effects were quantitatively observed. The amount of consumed feeding solution was determined daily by weighing each feeder with a calibrated precision balance before and after administration.
Analytical verification	The analytical method for the determination of the Copper Oxychloride concentration was validated according to the guidance document SANCO/3029/99 rev. 4 and POS BT 365 (last version). One sample of the lowest concentration and one sample of the highest concentration of the stock solutions were collected each day from D3 to D6 and analysed by an ICP-MS. The active substances content in the sample solutions was calculated on the basis of the calibration curve equation. The recovery was expressed as percentage and determined from the ratio between the measured concentration and the nominal content.
Statistics	The Step-down Cochran-Armitage Test Procedure (step down test to detect an increasing trend in response – alpha 0.05) was performed to verify the significance of the data and evaluate the NOEDD/NOEC values. A Weibull analysis (with linear maximum likelihood regression) was used to evaluate the LDDx and LCx values. The software ToxRat Professional 3.2.1 was used to perform the statistics.

RESULTS AND DISCUSSIONS

Food consumption and mortality

The mean feed consumption and mortality after daily exposure of bees to five concentrations of copper oxychloride 50% WP is presented in the table below. There were no sub-lethal effects observed at all treatment levels at the end of the test.

Table A 2.2.1.2-1 Food consumption and mortality of bees in a 10-day chronic oral toxicity test with Copper oxychloride 50% WP

Treatments (mg copper/kg f.s)	Mean consumption	Mean uptake of a.s.	Mortality	
	[µg test item /bee/day]	[µg a.s/bee/day]	Mean [%]	Mean corrected [%]
Control	0	-	3.3 ±5.8	-
3.16	0.21	0.106	3.3 ±5.8	0.0
6.33	0.35	0.177	6.7 ±5.8	3.45
12.65	0.71	0.359	16.7* ±11.5	13.79
25.30	0.99	0.500	63.3* ±25.2	62.07
50.60	1.27	0.643	96.7* ±5.8	96.55
Reference item (1.0 mg dimethoate/kg diet)	0.04	0.04	100 ±0	100

* mean was significantly different from the control group (Step-down Cochran-Armitage Test – alpha 0.05)

Validity Criteria

The test was considered valid because the following criteria were satisfied:

The average mortality for the control did not exceed 15% at the end of the test (actual value: 3.3%);

The average mortality in the reference item treatment was ≥50% at the end of the test (actual value: 100%).

Toxicity endpoints

The LC50 and NOEC, based on nominal concentration, and the LDD50 and NOEDD, based on the mean uptake of test item per bee are presented in the following table. Calculated values of LC20,10 and LDD20,10 are also presented.

Table A 2.2.1.2-2 Chronic oral toxicity to honey bees exposed to Copper Oxychloride 50% WP – Summary of endpoints

LC_{50} [mg copper/kg f.s]	10.071
LC_{20} [mg copper/kg f.s]	14.099
LC_{10} [mg copper/kg f.s]	23.432
LDD_{50} [µg copper/bee/day]	0.466
LDD_{20} [µg copper/bee/day]	0.365
LDD_{10} [µg copper/bee/day]	0.310
NOEC	6.33 mg copper/kg f.s
NOEDD	0.177 µg copper/bee/day

NOEDD / NOEC = No Observed Effect Dietary Dose/Concentration (calculated by using Step-down Cochran-Armitage Test Procedure; α = 0.05; one sided greater)

CONCLUSION

In a 10-day chronic toxicity feeding study with Copper Oxychloride 50% WP the LDD50 was determined to be 0.466 µg copper/bee/day and the LC50 was determined to be 10.07 mg copper/kg food, respectively.

The NOEDD was determined to be 0.177 µg copper/bee/day, and the NOEC was determined to be 6.33 mg copper/kg food, respectively.

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 Study 1

Comments of zRMS:	Study is considered as valid. This study was evaluated according to OECD 237 and OECD 239. The study met the relevant validity criteria. **The test was considered valid because the following criteria were satisfied:** **Validity of the study** Larval mortality in the control: 0.0% for larvae across all control replicates (between D3 and D8); validity criterion was met Larval mortality in the reference item: 77.8% mortality (between D3 and D8) of larvae across all replicates exposed to a total of 7.4 µg a.i./larva; validity criterion was met **Deviations from the study:** No deviation with impact on quality and integrity of the study. **Agreed toxicity endpoints:** In a larval toxicity study, where honeybee larvae (*A. mellifera* L.) were repeatedly exposed to Copper 20% + Cymoxanil 4% WG, the LD_{50} was determined to be > 100.4 µg product/larva, which is equivalent to an LC_{50} of > 634.8 mg product/kg food. The NOED was determined to be 20.1 µg product/larva and the NOEC was determined to be 127.0 mg product/kg food.

Reference:	KCP 10.3.1.3/01
Report	Copper 20% + Cymoxanil 4% WG - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (in vitro), Scheller, K., 2018a, 17 48 BLA 0003
Guideline(s):	Yes (OECD 237 Guideline for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013); Guidance Document on Honey Bee Larval

	Toxicity Test following Repeated Exposure, Series on Testing and Assessment, No. 239, OECD (2016) with adaptations based on SCHMEHL et al. (2016))
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a feeding toxicity test with repeated exposure honey bee larvae (Apis mellifera L.) were exposed to Copper 20% + Cymoxanil 4% WG diluted in the larvae's food. The toxicity of the test item was determined at total doses of 100.4, 45.2, 20.1, 9.0 and 4.0 µg product/larva. The concentrations of test item in the diet were 634.8, 285.7, 127.0, 57.1 and 25.4 mg product/kg food. Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a dose of 7.4 µg dimethoate/larva (concentration: 46.7 mg a.s./kg). Untreated diet served as control. All validity criteria of the respective test guideline were met. The LD_{50} was determined to be > 100.4 µg product/larva, which is equivalent to an LC_{50} of > 634.8 mg product/kg food. The NOED was determined to be 20.1 µg product/larva and the NOEC was determined to be 127.0 mg product/kg food.

MATERIALS AND METHODS

Test Item

Designation	Copper 20% + Cymoxanil 4% WG
Lot / Batch no.	15.351.3
Active ingredient content / Purity	Copper 20% (nom.) 20.4% (analysed) Cymoxanil 4% (nom.) 4.0% (analysed)
Characteristics	WG (water dispersible granules)
Density (if liquid)	-
Storage conditions	Kept at ambient temperature
Stability (expiry date)	17.12.2017
Vehicle / control(s)	Control: untreated artificial diet Toxic reference item: Dimethoate tech. (analyzed purity: 98.8% w/w)

Test System

Species	*Apis mellifera* L.
Age	The larvae were in first instar stage (L1, one day old) during grafting.
Source	Beekeeper Joaquin Cordero, Paseo del Moro No. 19, 41370 Cazalla (Sevilla), Spain. All larvae used in the test derived from healthy (free of clinical symptoms of any disease) and queen-right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month.
Pre-treatment culturing conditions	The bee colonies producing the larvae were held under field conditions in hives including a healthy queen. Brood in egg, larval and pupal stages as well as filled food combs (containing nectar and pollen) were present. A sufficient amount of food was present in the bee hives.
Food	50% aqueous yeast/sugar solution and 50% royal jelly.

Test Conditions

Temperature	34.0 – 34.8 °C
Relative Humidity	90.1 – 99.8%
Photoperiod	Darkness (except during assessments)

Study Design and Methods

In-life dates	20.11.2017 – 27.11.2017
Conducted at	BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Test duration	8 days: pre-grafting (in vitro): D1 to D2; grafting: D1; pre-exposure (in vitro): D1 to D2; exposure (in vitro): D3 to D6; post exposure (in vitro): D7 to D8
Test concentrations	Total doses: 100.4, 45.2, 20.1, 9.0 and 4.0 µg product/larva; concentration in the diet: 634.8, 285.7, 127.0, 57.1 and 25.4 mg product/kg food
Test vessels	Crystal polystyrene grafting cells (CNE Nicoplast, internal diameter 9 mm) were placed in 48 plates. Artificial diet A was pipetted into grafting cells, followed by placing one freshly grafted larva per cell.
Treatment	The test and reference item were mixed daily (on D3, D4 and D6) into sterile filtered aqueous sugar solution, gaining the test item base stock. Several dilutions were prepared by adding further sugar solution. After preparation of the stock solutions, the royal jelly was added at a ratio of 1:1, based on w/w, to reach the final test concentrations. Test item: 4.0 – 9.0 – 20.1 – 45.2 – 100.4 µg product/larva 25.4 – 57.1 – 127.0 – 285.7 – 634.8 mg product/kg food Active: 1.0 – 2.2 – 4.8 – 10.8 – 24.1 µg a.s./larva 6.1 – 13.7 – 30.5 – 68.6 – 152.3 mg a.s./kg food Control: untreated diet B/C (50% aqueous yeast/sugar solution) Reference: 7.4 µg a.s./larva (corresponding concentration: 46.7 mg a.i./kg food) 3 Replicates per treatment group with 12 larvae each
Observations	Number of dead larvae between D4 and D8 was described as cumulative larval mortality, daily on D4 and D8 (larvae). Notification of, e.g. unconsumed food and/or discolorations and/or undersized larvae was done on D7 and D8.
Analytical verification	The test item Base stock solution (BSt) was sampled in duplicate as specimen for analysis and retention directly after preparation on D3, D4, D5, and D6. The determination of the active ingredient in the diets was conducted by an in-house developed method using high performance liquid chromatography (HPLC) with UV-detection.
Statistics	Descriptive statistics: The Step-down Cochran-Armitage Test for determination of NOED/NOEC. LD/LC_{50} was calculated with the Trimmed Spearman-Karber procedure.

RESULTS AND DISCUSSIONS

After 120 hours of repeated oral exposure (on D8), no larval mortality was observed in the control (AC). In the test item group, larval mortalities ranged between 0.0% and 13.9%. Mortality in the reference (AR) was above 50% across all replicates, being 77.8%. Other observations like remaining food or small body size were not observed in any treatment.

Statistically significant differences in larval mortality compared with the control occurred in 13.9% of larvae exposed to 100.4 µg product/larva (corresponding concentration in the diet was 634.8 mg product/kg food) and in 11.1% of larvae exposed to 45.2 µg product/larva (corresponding concentration in the diet was 285.7 mg product/kg food). On D3, D4, D5 and D6, the recoveries of active substance copper in the Base stock solution ranged within 88-98% of the nominal concentration. No test item has been detected in the control specimen.

The study can be regarded as valid due to < 15% larval mortality in the control group (D8) and > 50% larval mortality in the reference item group, treated with 7.4 µg dimethoate/larva (D8). The results are summarised in **Table A 2.3.1.3.1-1**:

Table A 2.3.1.3.1-1 Toxicity and sublethal effects of Copper 20% + Cymoxanil 4% WG to larvae and pupae of *Apis mellifera* L. after repeated exposure

Treatment group		Dosage [µg product/larva]	Concentration [mg product/kg food]	On D8 (120 h after 1st appl.)		
				Mean cumulative mortality of larvae D3-D8 [%]		Mean other observations [%]
				absolute	corrected	
Control	AC	-	-	0.0	-	0.0
Test item	AT	100.4	634.8	13.9*	13.9	0.0
	BT	45.2	285.7	11.1*	11.1	0.0
	CT	20.1	127.0	0.0	0.0	0.0
	DT	9.0	57.1	0.0	0.0	0.0
	ET	4.0	25.4	0.0	0.0	0.0
Reference item	AR	7.4	46.7	77.8	77.8	0.0

Results are averages based on 3 replicates, containing 12 larvae each;
Corrected: Cumulative mortality corrected for control (according to SCHNEIDER-ORELLI 1947)
Absolute: Cumulative absolute mortality as counted from the results
Calculations were performed with non-rounded values
OO: Other observations (remaining food, small body size)
* Statistically significant compared to the control (BC) (Step-down Cochran-Armitage Test)

Table A 2.3.1.3.1-2 Calculated endpoints of the repeated exposure larvae toxicity test

	Endpoints	On D8 (120 h after 1st application)
Test item doses	LD_{50} [µg product/larva][2]	> 100.4
	NOED [µg product/larva][1]	20.1
Test item concentrations	LC_{50} [mg product/kg food][2]	> 634.8
	NOEC [mg product/kg food][1]	127.0

[1] Step-down Cochran-Armitage Test; α = 0.05; one sided greater
[2] Estimated following the Trimmed Spearman-Karber procedure, α = 0.05; one sided greater

CONCLUSIONS

In a larval toxicity study, where honeybee larvae (*A. mellifera* L.) were repeatedly exposed to Copper 20% + Cymoxanil 4% WG, the LD_{50} was determined to be > 100.4 µg product/larva, which is equivalent to an LC_{50} of > 634.8 mg product/kg food. The NOED was determined to be 20.1 µg product/larva and the NOEC was determined to be 127.0 mg product/kg food.

A 2.3.1.3.2 Study 2

Comments of zRMS:	Study is considered as valid. This study was evaluated according to OECD 237 and OECD 239. The study met the relevant validity criteria. **The test was considered valid because the following criteria were satisfied:** **Validity of the study** Larval mortality in the control: 0.0% for larvae across all control replicates (between D3 and D8); validity criterion was met Larval mortality in the reference item: 77.8% mortality (between D3 and D8) of larvae across all replicates exposed to a total of 7.4 µg a.i./larva; validity criterion was met

	Deviations from the study: No deviation with impact on quality and integrity of the study. **Agreed toxicity endpoints:** In a larval toxicity study, where honeybee larvae (*A. mellifera* L.) were repeatedly exposed to Copper 20% + Cymoxanil 4% WG, the LD_{50} was determined to be > 100.4 µg product/larva, which is equivalent to an LC_{50} of > 634.8 mg product/kg food. The NOED was determined to be 20.1 µg product/larva and the NOEC was determined to be 127.0 mg product/kg food.

Reference:	KCP 10.3.1.3/02
Report	Copper 20% + Cymoxanil 4% WG – Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (in vitro), Scheller, K., 2018b, 17 48 BLC 0092
Guideline(s):	Yes (OECD 239 (2016))
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

In a feeding toxicity test with repeated exposure honey bee larvae (*Apis mellifera* L.) were exposed to Copper 20% + Cymoxanil 4% WG diluted in the larvae's food. The toxicity of the test item was determined at total doses of 100.4, 45.2, 20.1, 9.0 and 4.0 µg product/larva. The concentrations of test item in the diet were 634.8, 285.7, 127.0, 57.1 and 25.4 mg product/kg food. Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a dose of 7.4 µg dimethoate/larva (concentration: 46.7 mg a.i./kg). Untreated diet served as control.

In a repeated exposure larval toxicity study with Copper 20% + Cymoxanil 4% WG, the ED50 (successful adult emergence up to D22) was determined to be > 100.4 µg product/larva, which is equivalent to an EC_{50} of > 634.8 mg product/kg food. The respective NOED was 45.2 µg product/larva and the corresponding NOEC was 285.7 mg product/kg food.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Copper 20% + Cymoxanil 4% WG
Lot / Batch no.	15.351.3
Active ingredient content / Purity	Copper 20% (nom.) 20.4% (analysed); Cymoxanil 4% (nom.) 4.0% (analysed)
Characteristics	WG (water dispersible granules)
Density (if liquid)	-
Storage conditions	Kept at room temperature
Stability (expiry date)	17.12.2017
Vehicle / control(s)	Control: fed with untreated artificial diet Toxic reference item: Dimethoate tech. (98.8 ± 0.5% (w/w)

Test System

Species	*Apis mellifera* L.
Age	The larvae are in first instar stage (L1, one day old) during grafting

Source	Beekeeper Joaquin Cordero, Paseo del Moro No. 19, 41370 Cazalla (Sevilla), Spain. All larvae used in the test derived from a healthy (free of clinical symptoms of any disease) and queen-right bee colonies. The larvae were taken from a hives that had not received treatments with chemical substances for at least one month.
Pre-treatment culturing conditions	The bee colonies producing the larvae were held under field conditions in hives including a healthy queen. Brood in egg, larval and pupal stages as well as filled food combs (containing nectar and pollen) were present. A sufficient amount of food was present in the bee hives.
Feeding	The aqueous yeast/sugar solutions as one component of the artificial diets were prepared freshly on D3 and thereafter stored in a freezer for use on D4, D5 and D6. The sugar solution was mixed with royal jelly every day before each feeding occasion. D1: 20 µL diet A (44.25% royal jelly, 55.75% yeast/sugar solution w/w) D3: 20 µL diet B (50% royal jelly; 50% yeast/sugar solution w/w) D4: 30 µL diet C (50% royal jelly; 50% yeast/sugar solution w/w) D5: 40 µL diet C (50% royal jelly; 50% yeast/sugar solution w/w) D6: 50 µL diet C (50% royal jelly; 50% yeast/sugar solution w/w)
Test Conditions	
Temperature	34.0 – 34.9 °C
Relative Humidity	D1 – D8: 90.1 – 99.8% D8 – D15: 75.7 – 79.0% D15 – D22: 59.6 – 69.8%
Photoperiod	Constant darkness throughout the test (diffuse artificial light only during handling and assessments)
Study Design and Methods	
In-life dates	15.11.2017 – 28.02.2018
Conducted at	BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Test duration	22 days Pre-grafting (in vivo): D-3 to D1 Grafting: D1 Pre-exposure (in vitro): D1 to D2 Exposure (in vitro): D3 to D6 Post exposure (in vitro): D7 to D22
Test concentrations	1.0 – 2.2 – 4.8 – 10.8 – 24.1 µg total a.s./larva 6.1 – 13.7 – 30.5 – 68.6 – 152.3 mg a.s./kg food
Test vessels	Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in 48 well plates.

Treatment	The test and reference item was mixed daily (on D3, D4, D5 and D6) into sterile filtered aqueous sugar solution (ASS), gaining the test item base stock. A sample of the test item base stock solution was taken daily in duplicates for chemical analysis. The royal jelly was added at a ratio of 1:1, based on w/w, to reach the final test concentrations. The test item was mixed into diet B/C and offered to each larva individually on four successive days. Larvae of the control treatment received untreated diet B/C. Test item: 4.0 – 9.0 – 20.1 – 45.2 – 100.4 µg total prod./larva 25.4 – 57.1 – 127.0 – 285.7 – 634.8 mg prod./kg food Active: 1.0 – 2.2 – 4.8 – 10.8 – 24.1 µg total a.s./larva 6.1 – 13.7 – 30.5 – 68.6 – 152.3 mg a.s./kg food Control: Fed with untreated artificial diet. Reference: 7.4 µg total a.s./larva 46.7 mg a.s./kg food 3 Replicates per treatment group with 12 larvae
Analytical verification	The test item Base stock solution (BSt) was sampled in duplicate as specimen for analysis and retention directly after preparation on D3, D4, D5, and D6. The determination of the active ingredient in the diets was conducted by an in-house developed method using high performance liquid chromatography (HPLC) with UV-detection.
Observations	Number of dead larvae, daily on D4 to D8 (larvae) and D15 (pupae) and D22. Bees which emerged successfully were counted. Lifeless pupae and bees were marked as dead. Notification, e.g. of larger amounts of unconsumed food and/or discolorations and/or substantially undersized larvae on D7 and D8 in order to support in the interpretation of mortality data.
Statistics	For statistical evaluation of the mortality results after test item treatment and for determination of NOEC/NOED the Step-down Cochran-Armitage Test was used. The accepted significance level was $\alpha = 0.05$. ED/EC_{50} was calculated with Probit analysis using linear maximum likelihood regression. In order to correct for control mortality, any statistical calculations were performed using mortality data instead of adult emergence data. The statistical calculations were performed with the computer program ToxRatPro 3.2.1.

RESULTS AND DISCUSSIONS

The results are summarised in the following table:

Table A 2.3.1.3.2-1 Mortality and other observations of larvae after 120 hours of repeated oral exposure (on D8)

				On D8			On D22				
Treatment group	**Test solution ID**	**Dose**	**Concentration**	**Mean mortality of larvae D3 to D8**		**Mean OO**	**Mean mortality of pupae D8-D22**		**Mean mortality of pupae & larvae D3-D22**		**Adult emergence rate**
		[µg prod./larva]	**[mg prod./kg food]**	**[%]**		**[%]**	**[%]**	**[%]**	**[%]**	**[%]**	**[%]**
				abs.	**corr.**		**abs.**	**corr.**	**abs.**	**corr.**	**abs.**
Control	AC	-	-	0.0	-	0.0	19.4	-	22.2	-	77.8
Test item	AT	100.4	634.8	13.9	13.9	0.0	48.5	36.1	55.6	42.9	44.4
	BT	45.2	285.7	11.1	11.1	0.0	25.2	7.1	36.1	17.9	63.9
	CT	20.1	127.0	0.0	0.0	0.0	22.2	3.4	22.2	0.0	77.8
	DT	9.0	57.1	0.0	0.0	0.0	16.7	0.0	19.4	0.0	80.6
	ET	4.0	25.4	0.0	0.0	0.0	16.7	0.0	16.7	0.0	83.3
Reference item	AR	7.4	46.7	77.8	77.8	0.0	50.0	37.9	94.4	92.9	5.6

Results are averages based on 3 replicates, containing 12 larvae each; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947); abs.: absolute mortality as counted from the results; negative values are set to "0"; calculation are performed with non-rounded values
OO: Other observations (e.g. remaining food)
* Statistically significant if compared to the control (Step-down Cochran-Armitage Test)

No abnormalities, like remaining food or discolorations, were observed in any of the larvae during the assessments.

On D3, D4, D5 and D6, the recoveries of the active substance copper in the Base stock solution ranged within 94-96% of the nominal concentration. No test item has been detected in the control speciemen.

Table A 2.3.1.3.2-2 Calculated endpoints of the repeated exposure larvae toxicity test

	Endpoints: Successful adult emergence	**Up to D22**
Test item doses	ED_{50} [µg product/larva][2]	> 100.4
	NOED [µg product/larva][1]	45.2
Test item concentrations	EC_{50} [mg product/kg food][2]	> 634.8
	NOEC [mg product/kg food][1]	285.7

[1] Step-down Cochran-Armitage Test; α = 0.05; one sided greater
[2] Estimated following the Probit analysis using linear maximum likelihood regression

CONCLUSIONS

In a repeated exposure larval toxicity study with Copper 20% + Cymoxanil 4% WG, the ED50 (successful adult emergence up to D22) was determined to be > 100.4 µg product/larva, which is equivalent to an EC_{50} of > 634.8 mg product/kg food. The respective NOED was 45.2 µg product/larva and the corresponding NOEC was 285.7 mg product/kg food.

A 2.3.1.3.3 Study 3

Comments of zRMS:	Study is considered as valid. This study was evaluated according to OECD 237 and OECD 239. The study met the relevant validity criteria.

	The test was considered valid because the following criteria were satisfied: - The cumulative larval mortality in the control plate(s) did not exceed 15% from D3 to D8 across replicates (actual value: 2.78%); - The adult emergence rate in the control plate(s) was ≥ 70% on D22 (exact value: 80.56%); - The larval mortality in the reference item group was ≥ 50% on D8 (exact value: 100%). **Deviations from the study:** No deviation with impact on quality and integrity of the study. **Agreed toxicity endpoints:** For larval mortality, the LD_{50} was determined to be 59.607 µg test item/larva (corresponding to 30.16 µg copper/larva), the LD_{20} was 42.79 µg test item/larva (corresponding to 21.65 µg copper/larva) and the LD_{10} was 34.359 µg test item/larva (corresponding to 17.386 µg copper/larva). The NOED was 28.00 µg test item/larva (corresponding to 14.17 µg copper/larva) For adult emergence, ED_{50} was determined to be 39.937 µg test item/larva (corresponding to 20.20 µg copper/larva), the ED_{20} was 17.437 µg test item/larva (corresponding to 8.82 µg copper/larva) and the ED_{10} was 10.074 µg test item/larva (corresponding to 5.10 µg copper/larva). The NOED was 28.00 µg test item/larva (corresponding to 14.17 µg copper/larva).

Reference:	KCP 10.3.1.3/03
Report	Effects of copper oxychloride 50% WP to honeybees *Apis mellifera* L. Larval toxicity test, repeated exposure, Colli, M., 2018b, Report No: BT216/17
Guideline(s):	OECD Test Guideline 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a chronic toxicity test, honeybee larvae (*Apis mellifera* L.) were repeatedly exposed to copper oxychloride 50% WP. The toxicity of the test item was determined at doses of 1.79, 4.48, 11.20, 28.00, 70.00 µg test item/larva (corresponding to 0.90, 2.27, 5.67, 14.17 and 35.42 µg copper/larva). The concentrations in the diet were 11.636, 29.091, 72.727, 181.818 and 454.545 µg test item/kg food.

Additionally, further honeybee larvae were exposed to the reference item Dimethoate at a dose rate of 7.39 µg dimethoate/larva as positive control. A third group of larvae served as negative control, being fed with untreated diet.

The analytical results demonstrate that the active substances' content in the stock solutions was in the range of ± 20% of nominal concentration. The end-points of the test were calculated with respect to the nominal concentration of the test item.

Assessments of larval mortality were performed on Days 4 to 8, 15 and 22. Assessment of pupal mortality and adult emergence was performed on Day 22.

For larval mortality, the LD_{50} was determined to be 59.607 µg test item/larva (corresponding to 30.16 µg copper/larva), the LD_{20} was 42.79 µg test item/larva (corresponding to 21.65 µg copper/larva) and the LD_{10} was 34.359 µg test item/larva (corresponding to 17.386 µg copper/larva). The NOED was 28.00 µg test item/larva (corresponding to 14.17 µg copper/larva)

For adult emergence, ED_{50} was determined to be 39.937 µg test item/larva (corresponding to 20.20 µg copper/larva), the ED_{20} was 17.437 µg test item/larva (corresponding to 8.82 µg copper/larva) and the ED_{10} was 10.074 µg test

item/larva (corresponding to 5.10 µg copper/larva). The NOED was 28.00 µg test item/larva (corresponding to 14.17 µg copper/larva).

No developmental or behavioral abnormality was observed during the study.

MATERIALS AND METHODS

Test Item

Designation	Copper oxychloride 50% WP
Lot / Batch no.	20151202003
Active ingredient content / Purity	50.6% as copper
Characteristics	Green powder
Storage conditions	15 – 25°C
Stability (expiry date)	July 2018
Vehicle / control(s)	Control: water Toxic reference item: Dimethoate

Test System

Species	*Apis mellifera* L.
Age	3-day old larvae
Supplier	Healthy colonies (n. 8, 9 and 12) maintained at Biotecnologie BT S.r.l.
Acclimatisation period	None
Housing	Crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm.
Food or Diet	**Day 1-2**: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight glucose and 12% weigh fructose. **Day 3**: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight glucose and 15% weight fructose. **Day 4-6**: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight glucose and 18% weigh fructose

Test Conditions

Temperature	33 – 35ºC
Relative humidity	49.3 – 96.8%
Photoperiod	24 hours dark, except during observations

Study Design and Methods

In-life dates	25.07.2016 – 15.08.2016
Conducted at	Biotecnologie BT S.R.l., Frazione Pantalla, 06059 Todi (PG), Italy
Test duration	22-days
Test design	Larval toxicity test, repeated exposure
Test concentrations	0 (control), 11.636, 29.091, 72.727, 181.818, 454.545 mg test item/kg diet
Test groups / Replicates	Three replicates of 12 larvae each were prepared for each experimental group.
Treatment	At day 1 (D1), the combs containing first instar larvae were carried

	from the hive to the laboratory. A volume of 20 µL of diet A was dropped into each cell, then one larva was grafted from a comb to the cell, onto the surface of the diet, using a grafting tool or a wetted paintbrush. All larvae were fed once a day from D1 to D6 (except at D2), and food was added even if the previous administered food was not totally consumed. Assessments of larval mortality were performed on Days 4 to 8, 15 and 22. Assessment of pupal mortality and adult emergence was performed on Day 22. From D3 to D6, the test item solutions were mixed into the diet at the respective concentration, just prior to its administration. Twelve larvae from each of three colonies were allocated on the same plate on D3. Each plate corresponded to a treatment level, to the control or to the reference item. As the test item was a water-soluble formulated product, the stock solutions were prepared in ultrapure water, then the treated diets were prepared using the stock solutions. The reference item stock solution was prepared in deionized water once and stored at about 2°C. The treated diets were prepared daily, warmed in an incubator before use.
Observations	Any dead larva was counted and then removed for sanitary reasons, from D4 to D8. No uneaten food was observed at D8. On D15, larvae that had not transformed into pupae were recorded as dead and removed, and the pupal mortality was evaluated. The total mortality and the adult emergence were evaluated on D22. At each observation time, larval mortality from D4 to D8, pupal mortality from D8 to D15 and the adult emergence on D22 was recorded as follows: a) Adult emergence rate was calculated in percentage by comparing the number of bees emerged on D22 to the number of larvae on D3 when dosing started. b) Pupal mortality was calculated in percentage comparing the number of pupae failed to emerge (including those bees without emergence on D22 and dead pupae removed from D8 to D22), to the number of bees entering pre-pupa stage on D8. c) Larval mortality was calculated in percentage comparing the number of larvae died from D3 to D7 to the number of larvae on D3 when dosing started. The total mortality (larval + pupal) was calculated. The NOED was determined on D8 and on D22 for total mortality and on D22 for adult emergence. The condition of the test system was observed on D4, D5, D6, D7, D8, D15 and D22.
Analytical verification	The analytical method for the determination of the Copper Oxychloride concentration was validated according to the guidance document SANCO/3029/99 rev. 4 and POS BT 365 (last version). One sample of the lowest concentration and one sample of the highest concentration of the stock solutions were collected each day from D3 to D6 and analysed by an ICP-MS. The active substances content in the sample solutions was calculated on the basis of the calibration curve equation. The recovery was expressed as percentage and determined from the ratio between the measured concentration and the nominal content.
Statistics	The Step-down Cochran-Armitage Test Procedure (step down test to detect an increasing trend in response – alpha 0.05) was performed in order to verify the significance of the data and evaluate the NOED and NOEC values. The Weibull analysis (with linear maximum likelihood regression) was used to evaluate the ED_x/LD_x and EC_x/LC_x values. The software ToxRat Professional 3.2.1 was used to perform the statis-

tics.

RESULTS AND DISCUSSIONS

Food consumption and mortality

The following tables show the mean larval mortality, the pupal mortality and the effects on the emergence of adults.

Table A 2.2.1.3-1 Mean larval mortality, pupal mortality and effects on emergence of adults

Dose [µg test item/ larva]	% Mean larval mortality on D8		% Mean pupal mortality D8-D22		% Mean mortality of pupae & larvae D3-D22		Adult emergence	
	Absolute	Corrected	Absolute	Corrected	Absolute	Corrected	Absolute%	%Reduction
Control	2.78	-	17.14	-	19.44	-	80.56	-
1.79	2.78	0.00	22.86	6.90	25.00	6.90	75.00	6.90
4.48	2.78	0.00	20.00	3.45	22.22	3.45	77.78	3.45
11.20	5.56	2.86	23.53	7.70	27.78	10.34	72.22	10.34
28.00	2.78	0.00	31.43	17.24	33.33	17.24	66.67	17.24
70.00	72.22*	71.43*	60.00*	51.72*	88.89*	86.21*	11.11*	86.21*
Reference item (7.39)	100	100	n/a	n/a	100	100	0	100

* = significant (Step-down Cochran-Armitage Test Procedure, alpha = 0.05).

No developmental or behavioral abnormality was observed during the study.

Validity criteria

The test was considered valid because the following criteria were satisfied:

- The cumulative larval mortality in the control plate(s) did not exceed 15% from D3 to D8 across replicates (actual value: 2.78%);
- The adult emergence rate in the control plate(s) was ≥ 70% on D22 (exact value: 80.56%);
- The larval mortality in the reference item group was ≥50% on D8 (exact value: 100%).

CONCLUSION

For larval mortality, the LD_{50} was determined to be 59.607 µg test item/larva (corresponding to 30.16 µg copper/larva), the LD_{20} was 42.79 µg test item/larva (corresponding to 21.65 µg copper/larva) and the LD_{10} was 34.359 µg test item/larva (corresponding to 17.386 µg copper/larva). The NOED was 28.00 µg test item/larva (corresponding to 14.17 µg copper/larva)

For adult emergence, ED_{50} was determined to be 39.937 µg test item/larva (corresponding to 20.20 µg copper/larva), the ED_{20} was 17.437 µg test item/larva (corresponding to 8.82 µg copper/larva) and the ED_{10} was 10.074 µg test item/larva (corresponding to 5.10 µg copper/larva). The NOED was 28.00 µg test item/larva (corresponding to 14.17 µg copper/larva).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Studies on sublethal effects with the formulation were not performed, since it is possible to extrapolate from data submitted for the EU review.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Cage and tunnel tests with the formulation were not performed, since it is possible to extrapolate from data submitted for the EU review.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

The higher-tier risk assessment demonstrated that the proposed use of FEL02 poses no unacceptable risk to honeybees at doses of up to 2.5 kg/ha. Therefore, further studies are not considered necessary

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

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

Please refer also to A 2.3.2.2.

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 Study 1

Comments of zRMS:	The study is considered as valid. This study was evaluated according to Blümel *et al*. (2000), modified for extended laboratory conditions. The study met the relevant validity criteria. **Deviations of the study:** No deviation with impact on quality and integrity of the study **Validity criteria:** For the definitive bioassay to be deemed valid, the protocol indicated that: a) mortality in the control treatment over the initial 7 days should not exceed 20%. b) mortality in the toxic reference treatment should be 50-100%. c) the mean cumulative number of eggs produced between 7 and 14 days should be equal to or exceed 4.0 per female in the control treatment. All of these criteria were met in the bioassay. **The following endpoints are considered valid for use in the risk assessment:**

Treatment	Rate (g product/ha)	Mean number eggs per female [a)]	% effect on reproduction [b)]
Control	-	7.6	-
ATOFEL02	3000	1.0 **	87.3
	1000	6.8	11.5
	333.3	6.1	20.3

a) Results for reproduction were compared by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Treatments differing significantly from the control are indicated with asterisks (** $P < 0.01$).

b) Egg production, relative to the control. A positive value indicates a decrease.

Reference: KCP 10.3.2.2/01

Report: ATOFEL02 (Cuprofix C Disperss) – A rate-response extended laboratory bioassay of the effects of fresh foliar residues on the predatory mite *Typhlodromus pyri* (Acari, Phytoseiidae), Fallowfield, L., 2011, UP-11-6

Guideline(s): Blümel *et al*. (2000), modified for extended laboratory conditions.

Deviations: No deviation with impact on quality and integrity of the study.

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of Cuprofix C Disperss on the survival and reproduction of *Typhlodromus pyri* exposed to residues of the test item were determined during 14 days. The test product was applied to leaf discs at five concentrations, the application rates of 37, 111.1, 333.3, 1000 and 3000 g product/ha. Other plants were sprayed with deionised water and a reference substance at a rate of 200 L/ha. Three replicates were prepared for control and test item treatment groups and the reference substance group, each containing 20 mites.

The LR_{50} of Cuprofix C Disperss under extended laboratory conditions using natural substrates is > 3000 g product/ha. The NOER for effects on survival was determined to be 1000 g/ha and the ER_{50} is estimated to be between 1000 and 3000 g product/ha.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss
Lot / Batch no.	8.335.3
Active ingredient content / Purity	200 g/kg Copper and 40 g/kg Cymoxanil
Characteristics	Greyish-green granules
Storage conditions	Room temperature
Stability (expiry date)	03.08.2012
Vehicle / control(s)	Control: Deionised water Toxic reference item: Perfekthion (400 g/L Dimethoate, 12 g a.s./ha)

Test System

Species	*Typhlodromus pyri*
Age	2 - 3 days old
Source	P.K. Nützlingszuchten, Welzheim, Germany
Acclimatisation period	Not stated
Food	Pollen: 1:1 v/v mixture of almond (*Prunus* sp. Var Butte) and apple (*Malus* sp.)

Test Conditions

Temperature	25 – 26°C
Humidity	62 – 69%
Photoperiod	16 h light/8 h dark
Light intensity	360 - 1860 lux

Study Design and Methods

In-life dates	06.09.2011 – 18.10.2011
Conducted at	Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK
Test duration	14 days
Test concentrations	37, 111.1, 333.3, 1000 and 3000 g product/ha
Test vessels / Exposure unit	Leaf discs cut from French bean plants
Treatment	The effects of Cuprofix C Disperss on the survival and reproduction of *Typhlodromus pyri* exposed to residues of the test item were determined during 14 days. The test product was applied to leaf discs at five concentrations, the application rates of 37, 111.1, 333.3, 1000 and 3000 g product/ha. Other plants were sprayed with deionised water and a reference substance at a rate of 200 L/ha. Three replicates were prepared for control and test item treatment groups and the reference substance group, each containing 20 mites.
Observations	The mortality of the mites was assessed 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 at day 14.
Statistics	The individual values were totalled for each replicate and the average for the three replicates was calculated. The results were compared by one-way ANOVA and Dunnett's t-test.

RESULTS AND DISCUSSION

All validity criteria were met: mortality in the control over the initial 7 days not more than 20% (actual mortality 7%), mortality in the toxic reference group should be between 50 and 100% (actual mortality 100%) and the mean cumulative number of eggs produced between 7 and 14 days in the control should be more than 4 per female (actual number 7.6).

After 7 days of exposure, observed mortality reached 7.0% in the control and 12.0 to 23.0% mortality in the test item treatment groups. The LR_{50} of Cuprofix C Disperss after 7 days of exposure was determined to be > 3000 g/ha. There was 100% mortality in the reference substance group. The NOER for effects on survival was determined to be 1000 g/ha.

Table A 2.3.2.2.1-1 Effects of Cuprofix C Disperss on mortality of *Typhlodromus pyri* after 7 days of exposure

Treatment [g formulation/ha]	Mortality 7 DAT [%]	Corrected Mortality [1] [%]	Mean number of eggs per female	Reduction in fecundity [%]
Control	7	-	7.6	-
37	13	7	n.d.	n.d.
111.1	15	9	n.d.	n.d.
333.3	15	9	6.1	20.3
1000	12	5	6.8	11.5
3000	23 *	18	1.0 **	87.3
Reference substance	100 ***	100	n/a	n/a

[1] mortality corrected according to Abbott (Abbott, 1925)
* significantly different compared to control (Fisher's Exact Test, $p < 0.05$)
** significantly different compared to control (ANOVA and Dunnett's t-Test, $p < 0.01$)
*** significantly different compared to control (Fisher's Exact Test, $p < 0.001$)
n/a not assessed due to high mortality

~~Control females produced a mean of 7.6 eggs/female. Females exposed to leaves treated with Cuprofix C Disperss produced a mean of 1.0 to 6.1 eggs/female. This corresponded to a reduction in mean egg production/female of 20.3 to 87.3% compared to mean egg production in the control. The NOER for effects on survival was determined to be 1000 g/ha.~~

Control females produced a mean of 7.6 eggs/female. Females exposed to leaves treated with Cuprofix C Disperss produced a mean of 1.0 to 6.1 eggs/female. This corresponded to a reduction in mean egg production/female of 20.3, 11.5 and 87.3% compared to mean egg production in the control. The biologically relevant reduction in fecundity is observed on a dose at 333.3 g formulation/ha (above 20%). Due to the lack of a clear dose-response relationship, a reliable NOEC toxicity endpoint could not be established. However, in our opinion the NOEC is likely to be above 111.1 g formulation/ha.

CONCLUSIONS

The LR_{50} of Cuprofix C Disperss under extended laboratory conditions using natural substrates is > 3000 g product/ha. The NOER for effects on survival was determined to be 1000 g/ha and the ER_{50} is estimated to be between 1000 and 3000 g product/ha.

A 2.3.2.2.2 Study 2

Comments of zRMS:	The study is considered as valid. This study was evaluated according to Mead-Briggs *et al.* (2009). The study met the relevant validity criteria. **Deviations of the study:** No deviation with impact on quality and integrity of the study. **Agreed toxicity endpoints:**

Mortality

Treatment	Rate (g product/ha)	% mortality at 48 h [a]	% observations of wasps settled on treated plants [b]	
			Initial 3 h	24 & 48 h
Control	-	0.0	37.3	36.7
ATOFEL02	3000	0.0	29.3	25.0
	1500	0.0	31.3	16.7 **
	750	0.0	32.7	21.7
	375	0.0	36.7	26.7
Toxic reference	-	96.7 ***	24.7	~

a) The individual treatment results were compared to the control using Fisher's Exact Test ($\alpha = 0.05$) and asterisks indicate where they differed significantly (*** $P < 0.001$).

b) For each column of results, the data were compared by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Asterisks indicate where treatments differed significantly from the control (** $P < 0.01$)

~ Indicates that assessments were not analysed due to high mortality.

Reproduction

	Rate (g product/ha)	Mean number mummies per surviving female [a]	Standard deviation	% change in reproduction, relative to control [b]
Control	-	23.3	7.8	-
ATOFEL02	3000	21.3	3.2	8.3
	1500	22.0	5.0	5.4
	750	24.9	6.5	-7.2

a) The results were compared by one-way ANOVA ($\alpha = 0.05$), but values for the test-item treatments did not differ significantly from the control.

b) The reproduction rates in the test item treatments, compared to the control. A negative value indicates an increase, a positive value a decrease, relative to the control.

Validity criteria:

The following endpoints are considered valid for use in the risk assessment:

For the test to be considered valid, the protocol indicated that mortality in the definitive bioassay within the control treatment should not exceed 10% (i.e. 3 wasps from 30) at 48 h and mortality within the toxic reference treatment should not exceed 25% within the initial 2 h but should be 50-100% at 48 h. The protocol also stated that, for the reproduction assessments, the mean number of mummies in the control treatment should be > 5.0 per female and there should not be more than two zero values in the control treatment. Although not a criterion stated in the test guideline of Mead-Briggs *et al*. (2009), for the purposes of this study a validity criterion was set such that there should be a minimum of 30% of observations of wasps settled on the treated plants in the control treatment during the initial 3 h of the behavioral assessments. All of these criteria were met.

Conclusion:

Under extended laboratory test conditions, freshly-dried residues of product had no significant adverse effects on the survival of the parasitic wasp, *Aphidius rhopalosiphi*, at treatment rates up to and including 3000 g product/ha. It was concluded, therefore, that the LR_{50} (median lethal rate) for product was higher than 3000 g product/ha. In addition, the reproductive capacity of surviving wasps was not significantly affected by the test item at treatment rates up to and including 3000 g product/ha.

Reference: KCP 10.3.2.2/02

Report	ATOFEL02 (Cuprofix C Disperss) – A rate-response extended laboratory test to determine the effects of fresh residues on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), Stevens, J., 2011, UP-11-5
Guideline(s):	Mead-Briggs *et al*. (2000), no deviations
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of ATOFEL02 on the survival and reproduction of *Aphidius rhopalosiphi* exposed to residues of the test item were determined during 48 hours. All treatments were applied at an application rate of 400 L/ha to pots of seedling barley. Nominal treatment rates were 375, 750, 1500 and 3000 g ATOFEL02/ha. Once residues had dried 5 female wasps of *A. rhopalosiphi* were confined in each arena. In the bioassay, all treatment groups were replicated six times. A toxic reference and an untreated control were included in the test. To assess the sub-lethal effects of treatment on the fecundity of the wasps, 15 surviving female wasps from the test item treatment groups with ≤ 60% mortality and from the untreated control were individually confined in fecundity cages with untreated barley plants previously infested with aphids and given 24 hours to parasitize aphids. The wasps were then removed and the number of mummies (parasitized aphids) that developed was assessed 10 days later.

Under extended laboratory test conditions, the 48-h LR_{50} of ATOFEL02 to the parasitic wasp, *Aphidius rhopalosiphi*, was > 3000 g formulation/ha. Treatment rates of ATOFEL02 up to and including 3000 g formulation/ha had no statistically adverse effects on reproduction.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss (ATOFEL02)
Lot / Batch no.	8.335.3
Active ingredient content / Purity	4% Cymoxanil and 20% Copper from Bordeaux Mixture
Characteristics	Greyish-green granules
Density (if liquid)	-
Storage conditions	Room temperature
Stability (expiry date)	03.08.2012
Vehicle / control(s)	Control: Deionised water Toxic reference item: Perfekthion (400 g/L Dimethoate; 4 g a.s./ha)

Test System

Species	*Aphidius rhopalosiphi*
Age	Less than 48 hours
Source	Katz Biotech AG, Baruth, Germany
Acclimatisation period	Female adults were used in bioassays within 48 h of their emergence
Food	1:3 v/v solution of honey in water

Test Conditions

Temperature	20 - 21°C
Humidity	67 - 76%

Photoperiod	16 h photoperiod
Light intensity	1360 - 1600 lux
Study Design and Methods	
In-life dates	26.01.2011 – 14.11.2011
Conducted at	Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK
Test duration	48 h
Test concentrations	375, 750, 1500 and 3000 g ATOFEL02/ha
Test vessels / Exposure unit	Pots of seedling barley
Treatment	The effects of ATOFEL02 on the survival and reproduction of *Aphidius rhopalosiphi* exposed to residues of the test item were determined for 48 hours. All treatments were applied at an application rate of 400 L/ha to pots of seedling barley. Nominal treatment rates were 375, 750, 1500 and 3000 g ATOFEL02/ha. Once residues had dried 5 female wasps of *A. rhopalosiphi* were confined in each arena. In the bioassay, all treatment groups were replicated six times. A toxic reference and an untreated control were included in the test. To assess the sub-lethal effects of treatment on the fecundity of the wasps, 15 surviving female wasps from the test item treatment groups with ≤ 60% mortality and from the untreated control were individually confined in fecundity cages with untreated barley plants previously infested with aphids and given 24 hours to parasitize aphids. The wasps were then removed and the number of mummies (parasitized aphids) that developed was assessed 10 days later.
Observations	The mortality of the wasps was assessed 3, 24 and 48 hours after treatment. The number of mummies was assessed 10 days later.
Statistics	Where there was treatment mortality at 48 h, this was compared to the control treatment using Fishers's Exact Test ($\alpha = 0.05$). The values for repellency assessments were angularly transformed (square root arcsine) prior to comparison of one-way analysis of variance and Dunnett's t-test ($\alpha = 0.05$). the numbers of mummies produced per female found alive after the 24-h parasitism period were analysed by one-way ANOVA. square root transformation was carried out on the data prior to analysis.

RESULTS AND DISCUSSION

The test met all validity criteria: control mortality during the first 48 hours should not exceed 13% (actual mortality of 0%), mortality in the reference treatment should be more than 50% (actual mortality 96.7%), wasps in the control treatment should produce more than 5 mummies per female over a 24-hour period with no more than 2 wasps producing zero values (actual values of 23.3 mummies per female and no wasps producing a zero value).

Table A 2.3.2.2.2-1 Effects of ATOFEL02 on mortality and reproduction of *Aphidius rhopalosiphi*

Treatment group	Mortality after 48 h [%]	Mean number of mummies per wasp	Reduction in fecundity [%] [1]
Control	0	23.3 ± 7.8	-
375 g/ha	0	n/a	n/a
750 g/ha	0	24.9 ± 6.5	-7.2
1500g/ha	0	22.0 ± 5.0	5.4
3000 g/ha	0	21.3 ± 3.2	8.3
Toxic reference	96.7 ***	n/a	n/a

[1] compared to the control, a negative value indicates an increase in reproduction, a positive value a decrease
* statistically significant compared to the control (Fisher's Exact Test, $p < 0.001$)
n/a mortality after 48 h too high to assess fecundity

None of the test item treatment groups resulted in a significant reduction in the numbers of wasps seen settling on the treated plants. After 48 h there was 0% mortality in the control as well as no mortality in all test item treatment groups. In the toxic reference treatment, 96.7% corrected mortality was observed at 48 h. The 48 h LR_{50} for ATOFEL02 was determined to be > 3000 g formulation/ha.

In the fecundity test, the mean numbers of mummies produced in the test item treatment groups with a mortality < 60% was 21.3 – 24.9 mummies per female. In the control there were 23.3 mummies per female. There were no statistically adverse effects on the reproductive performance of surviving wasps by treatment rates up to and including 3000 g/ha.

CONCLUSIONS

Under extended laboratory test conditions, the 48-h LR_{50} of ATOFEL02 to the parasitic wasp, *Aphidius rhopalosiphi*, was > 3000 g formulation/ha. Treatment rates of ATOFEL02 up to and including 3000 g formulation/ha had no statistically adverse effects on reproduction.

A 2.3.2.2.3 Study 3

Comments of zRMS:

The study is considered as valid. This study was evaluated according to Vogt et al. 2000. The study met the relevant validity criteria.
Deviations of the study: No deviation with impact on quality and integrity of the study.

According to Study Plan:	Temperature: 25 °C ± 2 °C
Deviation to the Study Plan:	Temperature temporarily > 25 °C (maximum 28 °C): End of exposure period and pre-oviposition period: on 3 days for approximately 3 - 6 hours.
Reason for the Deviation:	Technical reason
Presumed Effect on the Study:	None, only slight deviation; the test organisms were in a good condition during the oviposition period.

Agreed toxicity endpoints:
Chrysoperla carnea

Pre-imaginal mortality and reproduction of *Chrysoperla carnea*

	Rate [1] [kg/ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [eggs/female/day]	Larval hatching rate [%]
Control	--	15.0	--	20.9	99.1
FEL02	0.6	30.0 n.s.	17.6	21.3	99.1
FEL02	1.2	7.5 n.s.	-8.8	20.3	96.4
FEL02	2.4	25.0 n.s.	11.8	24.5	96.6
FEL02	4.8	10.0 n.s.	-5.9	17.8	97.9
FEL02	9.6	17.5 n.s.	2.9	24.3	97.4
Endpoint					
LR_{50}: > 9.6 kg product/ha					

1) Application rate in 200 L deionised water/ha

2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces
(Bonferroni-Chi^2 Test, α = 0.05: n.s. = not significant)

3) Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli; negative value indicates better survivorship compared to control

Validity criteria:
The following endpoints are considered valid for use in the risk assessment:

	Control Mortality:	15.0 %, validity criterion was met
	Reference Item Mortality:	88.2 % corrected mortality, validity criterion was met
	Fecundity in the Control Group:	20.9 eggs per female per day (mean number), validity criterion was met
	Fertility in the Control Group:	99.1 % larval hatching rate (mean value), validity criterion was met
	Conclusion: Under extended laboratory conditions the LR_{50} of product (Copper 20% + Cymoxanil 4% WG) is estimated to be greater than 9.6 kg product/ha in 200 L water/ha. Reproduction was > 15 eggs per female per day and the mean hatching rate was > 70 % at all dose rates. This indicates that there was no negative effect of the test item on reproductive performance of *C. carnea* up to and including 9.6 kg product/ha.	

Reference:	KCP 10.3.2.2/03
Report	FEL02 (Copper 20% + Cymoxanil 4% WG): Effects on the lacewing *Chrysoperla carnea*, extended laboratory study – dose response test -, Moll, M., 2018, 130061047
Guideline(s):	Vogt et al. 2000
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

Larvae of *Chrysoperla carnea* were exposed to dried residues of FEL02 (Copper 20% + Cymoxanil 4% WG) on treated leaf surfaces (bean leaves). Single application of 7 treatment groups (5 dose rates of the test item, control, reference item) with 40 replicates each containing 1 larva. Exposure lasted until pupae were transferred to the reproduction units for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. In addition, for the control and the test item treatment groups where the corrected mortality was < 50%, the reproduction performance, i.e. egg deposition and larval hatching rate, was determined (2 checks/week, 24 hours period each check).

The LR_{50} is estimated to be greater than 9.6 kg product/ha in 200 L water/ha. The reproductive capacity of *C. carnea* was tested at all dose rates. This indicates that there was no negative effect of the test item on reproductive performance of *C. carnea* up to and including 9.6 kg product/ha.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	FEL02 (Copper 20% + Cymoxanil 4% WG)
Lot / Batch no.	15.351.3
Active ingredient content / Purity	203 g/kg Copper and 39 g/kg Cymoxanil
Characteristics	Solid, green
Density (if liquid)	-
Storage conditions	At 20 ± 5°C, in the dark
Stability (expiry date)	29.11.2018
Vehicle / control(s)	Control: 200 L deionised water / ha Toxic reference item: Perfekthion (nominal: 400 g dimethoate/L)

Test System

Species	Lacewing (*Chrysoperla carnea*)
Age	2 - 3 days old larvae
Source	Katz Biotech AG, Baruth, Germany
Acclimatisation period	
Food	Larvae: UV-sterilised *Sitotroga cerealella* Oliv. eggs, *ad libitum* Adults: artificial diet: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheat germ and deionised water (approx. 45 mL) mixed homogeneously, *ad libitum*.

Test Conditions

Temperature	24 - 28°C
Humidity	65 - 81%
Photoperiod	16 h light/8 h dark
Light intensity	1110 - 1310 lux

Study Design and Methods

In-life dates	25.01.2018 – 13.04.2018
Conducted at	IBACON, Arheilger Weg 17, 64380 Rossdorf, Germany
Test duration	Exposure time: 13 – 25 days until the cocoons were transferred to the oviposition cages. Pre-oviposition period: 8 – 15 days (time interval from adult hatch to start of oviposition). Oviposition period: The first assessment was done 12 days after the first egg laying was observed and continued for 1 week.
Test concentrations	0, 0.6, 1.2, 2.4, 4.8 and 9.6 kg product/ha; reference item: 140 mL Perfektion/ha
Test vessels / Exposure unit	Exposure units: Detached primary leaves of bean plants (*Phaseolus vulgaris*) were cut to discs of 55 mm in diameter. These leaf cuts were treated on their upper surface. The leaf discs were placed with their treated side upwards on a wet cotton wool pad in a petri dish (Ø 60 mm) The petri dish had a hole for a wick. A Fluon treated cylinder was fixed on each leaf by two elastic bands. Post-exposure units: An acrylic cylinder (15 cm high and 10 cm in diameter) with a cotton net on the top for egg-laying and a hole (Ø 2 cm) on the bottom to provide water through a cotton plug.
Treatment	Single application of 7 treatment groups (5 dose rates of the test item, control, reference item) with 40 replicates each containing 1 larva. The larvae were exposed once to dried residues on treated leaf surfaces (bean leaves). Exposure lasted until pupae were transferred to the reproduction units for development of adults. All treatments were applied in 200 L water/ha. The spraying dilutions were sprayed onto leaves via laboratory spraying equipment, which were then air dried.
Observations	Mortality checks were carried out regularly until hatching of adult lacewings. In addition, for the control and the test item treatment groups where the corrected mortality was < 50%, the reproduction performance, i.e. egg deposition and larval hatching rate, was determined (2 checks/week, 24 hours period each check).
Statistics	Mortality data of the test item were analysed for significance using the Bonferroni-Chi^2 Test and for the reference item the Fisher's Exact Test was used. Both tests are distribution-free tests and do not require testing for normality of homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1.

RESULTS AND DISCUSSION

Table A 2.3.2.2.3-1 Pre-imaginal mortality and reproduction of *Chrysoperla carnea*

	Rate[1] [kg/ha]	Mortality[2] [%]	Mortality corr.[3] [%]	Reproduction [eggs/female/day]	Larval hatching rate [%]
Control	-	15.0	-	20.9	99.1
FEL02	0.6	30.0	17.6	21.3	99.1
FEL02	1.2	7.5	-8.8	20.3	96.4
FEL02	2.4	25.0	11.8	24.5	96.6
FEL02	4.8	10.0	-5.9	17.8	97.9
FEL02	9.6	17.5	2.9	24.3	97.4

[1] Application rate in 200 L deionised water/ha
[2] Pre-imaginal mortality after exposure to spray residues on leaf surfaces (Bonferroni-Chi^2 Test, $\alpha = 0.05$: n.s. = not significant)
[3] Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli; negative value indicates better survivorship compared to control

The reference item applied at a rate of 140 mL Perfektion/ha produced a statistically significant mortality of 90.0% (88.2% corrected mortality).

CONCLUSIONS

Under extended laboratory conditions the LR_{50} of FEL02 (Copper 20% + Cymoxanil 4% WG) is estimated to be greater than 9.6 kg product/ha in 200 L water/ha. The reproductive capacity of *C. carnea* was tested at all dose rates. This indicates that there was no negative effect of the test item on reproductive performance of *C. carnea* up to and including 9.6 kg product/ha.

A 2.3.2.2.4 Study 4

Comments of zRMS:

The study is considered as valid. This study was evaluated according to Grimm et al. 2000. The study met the relevant validity criteria.
Deviations of the study: No deviation with impact on quality and integrity of the study.

Deviations to the Study Plan

Concerning:	**Test Conditions, Temperature**
According to Study Plan:	20 °C ± 2 °C
Deviations to the Study Plan:	During reproduction phase of the experiment the temperature was slightly lower (min. 17 °C) on three occasions for max. 3 to 6 hrs.
Reason for the Deviations:	Due to technical reasons the air condition failed for a short time.
Presumed Effect on the Study:	No effect is considered, because this short term lasting and slight temperature deviation must be considered as incidental, which is also obvious by the normal reproduction rate of the control group.

Validity criteria:

Mean Number of Emerged Beetles in the Control Group:	781 beetles, validity criterion was met
Reduction of Reproduction in the Reference Item compared to the Control:	99.6 %, validity criterion was met

Agreed toxicity endpoints:

	Rate [1]	Reproduction Efficiency [mean number of emerged beetles ± Standard Deviation]	Effect on Reproduction [2] [%]
FEL02 (Copper 20% + Cymoxanil 4% WG)	0.86 kg/ha	725 ± 112 (n.s.)	+ 7.2
FEL02 (Copper 20% + Cymoxanil 4% WG)	1.73 kg/ha	785 ± 33 (n.s.)	- 0.4
FEL02 (Copper 20% + Cymoxanil 4% WG)	3.45 kg/ha	695 ± 84 (n.s.)	+ 11.0
FEL02 (Copper 20% + Cymoxanil 4% WG)	6.90 kg/ha	718 ± 40 (n.s.)	+ 8.1
FEL02 (Copper 20% + Cymoxanil 4% WG)	13.8 kg/ha	683 ± 58 (n.s.)	+ 12.6
NOER Test Item	**≥ 13.8 kg product/ha**	**LR_{50}**	**> 13.8 kg product/ha**
Control	-	781 ± 50	-
Reference Item	4.0 L/ha	3 ± 4 (*)	+ 99.6

[1] Application rate in 400 L water/ha
[2] Effect on reproduction according to the following formula: (1-Rt/Rc)*100 % calculated on the exact raw data (negative values represent an increased, positive values a decreased reproduction compared to the control)
Statistic: * = statistically significantly difference compared to the control; n.s. = not statistically significantly difference compared to the control;
Test Item: Dunnett`s multiple t-test; Reference Item: Welch pairwise t-test, one-sided smaller, α = 0.05

Reference: KCP 10.3.2.2/04

Report: FEL02 (Copper 20% + Cymoxanil 4% WG): Effects on the reproduction of rove beetles *Aleochara bilineata* – extended laboratory study – dose response test-, Schmitzer, S., 2018, 130061071

Guideline(s): Grimm et al. 2000

Deviations: No deviation with impact on quality and integrity of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): Not applicable

Executive Summary

The effects of FEL02 (Copper 20% + Cymoxanil 4% WG) on the reproduction capacity of the rove beetle Aleochara bilineata was determined in an extended laboratory study at rates of 0.86, 1.73, 3.45, 6.90 and 13.8 kg product/ha in 400 L water/ha.

Under extended laboratory conditions the reduction of reproduction capacity of the rove beetle *Aleochara bilineata* was not statistically significantly different to the control values. The NOER for reproduction was ≥ 13.8 kg product/ha. The LR_{50} for reproduction was > 13.8 kg product/ha.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation: FEL02 (Copper 20% + Cymoxanil 4% WG)

Lot / Batch no.: 15.351.3

Active ingredient content / Purity	Copper: nominal: 200 g/kg or 20% (w/w); 203 g/kg (analysed) Cymoxanil: nominal: 40 g/kg or 4% (w/w); 39 g/kg (analysed)
Characteristics	Solid, green
Density (if liquid)	-
Storage conditions	At 20 ± 5°C, in the dark
Stability (expiry date)	29.11.2018
Vehicle / control(s)	Control: 400 L deionised water / ha Toxic reference item: BAS 152 11 I; dimethoate: 400 g/L (nominal); 429 g/L (analytical)

Test System

Species	Rove beetle (*Aleochara bilinieata*)
Age	2 - 5 days old adults at test start
Source	De groene Vlieg, Duivenwaardsedijk 1; NL-3244 LG – Nieuwe Tonge
Acclimatisation period	2 - 5 days under test conditions
Host organism	*Delia antiqua* Meig. pupae
Food	Frozen midge larvae every 1-3 days *ad libitum*. First feeding was done within the first hour after the application.

Test Conditions

Temperature	Acclimatisation: 18 - 20°C Exposure: 18 - 21°C Post-exposure: 17 - 22°C
Relative humidity	Acclimatisation: 60 - 70% Exposure: 66 - 70% Post-exposure: 65 - 81%
Photoperiod	16 h light/8 h dark
Light intensity	Acclimatisation: 280 lux Exposure: 860 - 980 lux Post-exposure: 370 - 570 lux
Ventilation	The test room was ventilated to avoid accumulation of test item vapour

Study Design and Methods

In-life dates	19.01.2018 - 06.04.2018
Conducted at	IBACON, Arheilger Weg 17, 64380 Rossdorf, Germany
Test duration	The adult test organisms were exposed to the test item for 28 days. After 28 days all surviving adult beetles were removed from the substrate. The substrate and the parasitized onion fly pupae wer returned to the climatic room in the original test units for one further week an to allow the substrate dry. 35 days after application the pupae of each replicate were transferred into a separate emergence container.
Test concentrations	0, 0.86, 1.73, 3.45, 6.9 and 13.8 kg product/ha; reference item: 4 L /ha
Test vessels / Exposure unit	Plastic boxes (18.3 cm × 13.6 cm × 6 cm; length, width, height) covered with perforated plastic lids, filled with soil (600 mL LUFA 2.1 soil), moistened to about 3.5 ± 5% of its maximum water holding capacity with deionized water. The height of the moistened soil was approx. 4 cm and the soil surface area were 190 cm^2. The walls of the exposure units were protected with a plastic inlet to avoid an increase of the concentration of the spray liquid on the soil by run-off of droplets from the walls. The inlet was removed after application.

Treatment	Single application of 7 treatment groups (5 dose rates of the test item, control, reference item with 4 replicates per treatment group each containing 20 individuals (10 females and 10 males per unit). All treatments were applied in 400 L water/ha. The spraying dilutions were sprayed onto soil surface via laboratory spraying equipment, which were then air dried. Once a week ca. 500 *Delia antiqua* pupae per container were mixed into the soil at days 7, 14 and 21.
Observations	Emerging beetles were counted and removed from the emergence containers at least 3 times per week; emergence of the F1-generation was monitored until the control treatment fell below a rate of two beetles per replicate per day. At days 7, 14 and 21 during exposure of the adult beetles the water content of the soil in the exposure units was checked by reweighing the test units and adjusting the desired weight by addition of deionised water.
Statistics	Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test ($\alpha = 0.05$) and Levene's test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogenous (test item) or inhomogenenous (reference item), Dunnett's multiple t-test (test item group) or Welch pairwise t-test (reference item group), one-sided smaller, $\alpha = 0.05$, was used. The determination of the NOER was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1.

RESULTS AND DISCUSSION

Table A 2.3.2.2.4-1 Effects on reproduction of *Aleochara bilineata* exposed to FEL02 in an extended laboratory trial

	Rate[1]	Reproduction efficiency [mean number of emerged beetles ± standard deviation]	Effect on reproduction[2] [%]
Control	-	781 ± 50	-
Reference item	4.0 L/ha	3 ± 4*	+99.6
FEL02	0.86 kg/ha	725 ± 112 (n.s.)	+7.2
FEL02	1.73 kg/ha	785 ± 33 (n.s.)	-0.4
FEL02	3.45 kg/ha	695 ± 84 (n.s.)	+11.0
FEL02	6.90 kg/ha	718 ± 40 (n.s.)	+8.1
FEL02	13.8 kg/ha	683 ± 58 (n.s.)	+12.6

[1] Application rate in 400 L water/ha
[2] (1-Rt/Rc)*100% calculated on the exact raw data (negative values represent an increased, positive values a decreased reproduction compared to the control)
* statistically significantly different compared to the control; n.s. not statistically significantly different compared to the control Test item: Dunnett's multiple t-test; reference item: Welch pairwise t-test, one-sided smaller, $\alpha = 0.05$

CONCLUSIONS

Under extended laboratory conditions the reduction of reproduction capacity of the rove beetle *Aleochara bilineata* was not statistically significantly different to the control values. The NOER for reproduction was ≥ 13.8 kg product/ha. The LR_{50} for reproduction was > 13.8 kg product/ha.

A 2.3.2.2.5 Study 5

Comments of zRMS:

The study is considered as valid. This study was evaluated according to Blümel *et al*. (2000). The study met the relevant validity criteria.
Deviations of the study: No deviation with impact on quality and integrity of the study.

Validity criteria: All validity criteria of the respective test guideline were met.

Agreed toxicity endpoints:
Typhlodromus pyri

Effects on mortality and reproduction of *Typhlodromus pyri* exposed to aged residues of product (FEL02) - Second bioassay: test start 14 days after application

	Rate[1]	Mortality (%)[2]	Mortality corrected (%)[3]	Reproduction (eggs/female)[4]	Effect on reproduction [%][5]
Control	-	20.0	-	6.0	-.
Cuprofix C Disperss (FEL02)	9.6 kg/ha	14.0 n.s.	-7.5	6.4 n.s.	-5.7

1) Application rate in 400 L tap water/ha; application was done in the field under outdoor conditions
2) Mortality: after 7 days of exposure to aged spray residues on leaf surfaces (Chi² 2x2 Table Test, one-sided greater, α = 0.05: n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative value indicates better survivorship compared to the control.
4) Reproduction: mean number of eggs/female, (Student t-test, one-sided smaller, α = 0.05: n.s. = not significant)
5) Negative values indicate a better reproductive performance compared to the control

Conclusion:

After an aging period of 14 days under natural conditions with rain-protection, a single application of 9600 g Cuprofix (FEL02)/ha in 400 L tap water/ha did not cause unacceptable effects on survival and reproduction of adult *Typhlodromus pyri.* Both effects were below the ESCORT 2 trigger value of 50 %.

Reference:	KCP 10.3.2.2/05
Report	Cuprofix C Disperss (FEL02): Effects on the Predatory Mite *Typhlodromus pyri*, Extended Laboratory Study - Aged Residue Test – Leopold, J., 2020, 149941060
Guideline(s):	Blümel et al. 2000 and Oomen 1988
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of aged residues of Cuprofix C Disperss (FEL02) applied in beans plants in field conditions on the mortality and reproduction capacity of the predatory mite *Typhlodromus pyri* was determined in two bioassays: the first starting on the day of application and the second starting 14 days after the application.

A single tested application rate of 9600 g product/ha, the reference item (dimethoate) at 50 mL/ha and the control (tap water) in a volume of 400 L water/ha was used.

After an aging period of 14 days under natural conditions with rain-protection, a single application of 9600 g Cuprofix C Disperss (FEL02)/ha did not cause unacceptable effects on survival and reproduction of adult *Typhlodromus pyri.* Both effects were below the ESCORT 2 trigger value of 50%.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss (FEL02)
Lot / Batch no.	0718322
Active ingredient content / Purity	Copper: 19.5 % w/w (195 g/kg, analysed) Cymoxanil: 4.21 % w/w (42.1 g/kg, analysed)
Characteristics	Solid, green
Density (if liquid)	-
Storage conditions	At 20 ± 5°C, in the dark (at test facility)
Stability (expiry date)	22.01.2022
Vehicle / control(s)	Control: 400 L tap water/ha Toxic reference item: DANADIM PROGRESS; dimethoate: 385 g/kg (38.5% w/w); corresponding to 408 g/L (40.8% w/v)

Test System

Species	Predatory mites (*Typhlodromus pyri* Scheuten)
Age	Protonymphs, not older than 24 hours
Source	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimatisation	Under test conditions
Food	A mixture of pine (*Pinus sp*.) and birch (*Betula sp*.) pollen (3:1) ad libitum on the day of the test start and on each assessment day except for the last one resp. at least every four days.
Water	Tap water, available from a small strip of filter paper

Test Conditions

Temperature	First bioassay: 24 - 26 °C Second bioassay: 24 - 26 °C
Relative humidity	First bioassay: 67 - 74 % Second bioassay: 67 - 74 %
Photoperiod	16 h light/8 h dark
Light intensity	First bioassay: 330 - 370 lux Second bioassay: 320 - 470 lux

Study Design and Methods

In-life dates	29.06.2020 – 30.07.2020
Conducted at	IBACON, Arheilger Weg 17, 64380 Rossdorf, Germany

Test duration	14 days for the control and the test item treatment group if reproduction was performed after 7 days.
Test rate / Application	Control, 9600 g product/ha (corresponding to 24.0 g product/L) and reference item (50 mL p.c./ha, corresponding to 125 µL p.c./L). The items were sprayed onto bean plants grown in the field conditions, with the equipment (PSG-System 4" (Fa. Schachtner, D-71640 Ludwigsburg) with an extension tube including 5 spraying nozzles) and air dried afterwards outdoors under natural conditions. An application rate of 4 mg/cm^2 ± 10% (corresponding to 400 L spray liquid/ha) was used.
Rain protection	The treated plants were protected against rain by a transparent, light permeable roof for the whole aging period of both bioassays.
Test vessels / Exposure unit	Test units: Leaf discs obtained from field treated bean plants (*Phaseolus vulgaris* 'Nassau', grown under field conditions (non-GLP)). After the application outside and drying and aging of the spray residues, leaves were detached and cut to discs with a diameter of approximately 45 mm and used as test unit. Test container: The test unit was placed with its treated side upward on a wet cotton wool in a petri dish. The petri dish was constantly filled with tap water during the trial. A small strip of filter paper (approximately 5 mm x 25 mm) was partly laid onto the leaf (approximately 5 mm distance) and extended onto the cotton wool pad (approximately 20 mm distance). This provides a water source for the mites. A glue barrier was added to prevent the mites from escaping.
Treatment	Single application of 9600 g product/ha, reference item (50 mL product/ha) and control group (tap water) was made in bean plants at growth stage BBCH 17-19 in field conditions. The residues were allowed to dry in natural outdoor conditions. After the application leaves were cut from the treated bean plants and transferred to the laboratory. The test was divided in two bioassays: The first bioassay was carried out with freshly dried residues on the day of the application. The second bioassay with aged residues was started on day 14 after application. Ten replicates with 10 mites were used in each treatment group.
Observations	Mortality: Number of living, dead and escaped mites was counted in the first week on day 3 and day 7 (all bioassays) after test initiation. Dead mites were removed, escaped mites were considered as dead. Reproduction: Number of eggs laid and number of live and dead juvenile stages per female was counted and removed afterwards on 3 assessment days from day 7 on with a maximum interval of 3 days up to day 14 (inclusive). Eggs laid until day 7 inclusive were removed from the test arena and were not counted. The reproduction assessment was performed where the corrected mortality (Mcorr) was ≤ 50 %. No reproduction assessment was performed for the reference item.
Statistics	Mortality data were analysed for significance using the Chi² 2x2 Table Test (one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior analysis. Reproduction was tested for normal distribution and homogeneity of variance using the Shapiro Wilk`s test ($\alpha = 0.01$) and the Levene´s test ($\alpha = 0.01$). Afterwards, reproduction data were analysed using the Student t-test (normally distributed and homogeneous) or Welch t-test (normally distributed and heterogeneous). Both tests were carried out one-sided smaller with $\alpha = 0.05$. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

Table A 2.3.2.2.5-1 Effects on mortality and reproduction of *Typhlodromus pyri* exposed to residues of Cuprofix C Disperss (FEL02) - First bioassay: test start on the day of application

	Rate[1]	Mortality (%)[2]	Mortality corrected (%)[3]	Reproduction (eggs/female)[4]	Effect on reproduction [%]
Control	-	11.0	-	6.3	-
Reference item	50 mL/ha	83.0*	80.9	-	100
Cuprofix C Disperss (FEL02)	9.6 kg/ha	40.0*	32.6	0.0*	-

1) Application rate in 400 L tap water/ha; application was done in the field under outdoor conditions
2) Mortality: after 7 days of exposure to freshly dried spray residues on leaf surfaces (Chi² 2x2 Table Test, one-sided greater, α = 0.05: * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of eggs/female, (Welch t-test, one-sided smaller, α = 0.05: * = significant)

Table A 2.3.2.2.5-2 Effects on mortality and reproduction of Typhlodromus pyri exposed to aged residues of Cuprofix C Disperss (FEL02) - Second bioassay: test start 14 days afterapplication

	Rate[1]	Mortality (%)[2]	Mortality corrected (%)[3]	Reproduction (eggs/female)[4]	Effect on reproduction [%][5]
Control	-	20.0	-	6.0	-.
Cuprofix C Disperss (FEL02)	9.6 kg/ha	14.0 n.s.	-7.5	6.4 n.s.	-5.7

1) Application rate in 400 L tap water/ha; application was done in the field under outdoor conditions
2) Mortality: after 7 days of exposure to aged spray residues on leaf surfaces (Chi² 2x2 Table Test, one-sided greater, α = 0.05: n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli; negative value indicates better survivorship compared to the control.
4) Reproduction: mean number of eggs/female, (Student t-test, one-sided smaller, α = 0.05: n.s. = not significant)
5) Negative values indicate a better reproductive performance compared to the control

CONCLUSIONS

After an aging period of 14 days under natural conditions with rain-protection, a single application of 9600 g Cuprofix C Disperss (FEL02)/ha in 400 L tap water/ha did not cause unacceptable effects on survival and reproduction of adult *Typhlodromus pyri.* Both effects were below the ESCORT 2 trigger value of 50 %.

A 2.3.2.2.6 Study 6

Comments of zRMS:

The study is considered as valid. This study was evaluated according to Mead-Briggs *et al.* 2000 and Mead-Briggs *et al.* 2010. The study met the relevant validity criteria.
Deviations of the study: No deviation with impact on quality and integrity of the study.

Validity criteria: All validity criteria of the respective test guideline were met.

Agreed toxicity endpoints:
Aphidius rhopalosiphi

Effects on mortality and on the parasitisation efficiency of the parasitoid *Aphidius rhopalosiph*i exposed to aged residues of product FEL02 - Second bioassay: test start 14 days after application

	Rate[1]	Mortality (%)[2]	Mortality corrected (%)[3]	Reproduction (mummies/female)[4]	Effect on reproduction [%][5]
Control	-	7.5		46.7	-
FEL02	9.6 kg/ha	15.0 n.s.	8.1	36.8 n.s.	21.3

1) Application rate in 400 L water/ha; application was done in the field under outdoor conditions
2) Mortality: after 48 hours of exposure to spray residues on leaf surfaces (Fisher's Exact Binomial Test, α = 0.05, one-sided greater; n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of parasitised aphids/female,
(Student t-test, α = 0.05, one-sided smaller; n.s. = not significant)
5) Calculated on the exact raw data

	CONCLUSIONS After an aging period of 14 days under natural conditions, a single application of 9600 g FEL02/ha in 400 L tap water/ha did not cause unacceptable effects on mortality and reproduction of adult *Aphidius rhopalosiphi*. Both effects were below the ESCORT 2 trigger value of 50 %.

Reference:	KCP 10.3.2.2/06
Report	Cuprofix C Disperss (FEL02): Effects on the Parasitoid *Aphidius rhopalosiphi*, Extended Laboratory Study - Aged Residue Test - Leopold, J., 2020, 149941003
Guideline(s):	Mead-Briggs *et al.* 2000 and Mead-Briggs *et al.* 2010
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of aged residues of Cuprofix C Disperss (FEL02) applied in beans plants in field conditions on the mortality and reproduction capacity of the parasitoid *Aphidius rhopalosiphi* was determined in two bioassays: the first starting on the day of application and the second starting 14 days after the application. A single tested application rate of 9600 g product/ha, the reference item (dimethoate) at 50 mL/ha and the control (tap water) in a volume of 400 L water/ha was used.

The organisms were exposed to the dried residues for a period of 48 hours, following by a 24-h parasitation period. The number of aphid mummies was assessed after a 10 days period.

After an aging period of 14 days under natural conditions, a single application of 9600 g Cuprofix C Disperss (FEL02)/ha in 400 L tap water/ha did not cause unacceptable effects on mortality and reproduction of adult *Aphidius rhopalosiphi.* Both effects were below the ESCORT 2 trigger value of 50 %.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	Cuprofix C Disperss (FEL02)
Lot / Batch no.	0718322
Active ingredient content / Purity	Copper: 19.5 % w/w (195 g/kg, analysed) Cymoxanil: 4.21 % w/w (42.1 g/kg, analysed)
Characteristics	Solid, green
Density (if liquid)	-
Storage conditions	At 20 ± 5°C, in the dark (at test facility)
Stability (expiry date)	22.01.2022
Vehicle / control(s)	Control: 400 L tap water/ha Toxic reference item: DANADIM PROGRESS; dimethoate: 385 g/kg (38.5% w/w); corresponding to 408 g/L (40.8% w/v)

Test System

Species	*Aphidius rhopalosiphi* (DeStefani-Perez)
Age	Adults, not older than 48 hours
Source	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimatisation	Approximately 1 - 2 days under test conditions (see 6.4) in hatching chambers
Food	A 10 % fructose solution (10 g fructose ad 100 g tap water), provided on a cotton wool pad *ad libitum* during the acclimatisation, and sprayed (ad libitum) on the treated leaves and left to dry shortly before each bioassay and approximately 3 hours after the parasitation.

Test Conditions

Temperature	First bioassay: 19 - 22°C Second bioassay: 18 - 21°C
Relative humidity	First bioassay: 68 - 79% (acclimatisation, exposure) Second bioassay: 67 - 79% (acclimatisation, exposure) 72 - 74% (post-parasitisation period; within the test units)
Photoperiod	16 h light/8 h dark

Light intensity	First bioassay: 1190 – 1200 lux (acclimatisation, exposure) Second bioassay: 1060 – 1120 lux (acclimatisation, exposure) 860 – 2200 lux (parasitisation period) 9720 – 16320 lux (post-parasitisation period)
Ventilation	Exposure units were ventilated with a small pump (sucking air)

Study Design and Methods

In-life dates	29.06.2020 – 30.07.2020
Conducted at	IBACON, Arheilger Weg 17, 64380 Rossdorf, Germany
Test duration	Exposure time: approximately 48 hours Parasitation period: 24 hours Post-parasitation period: 10 days
Test rate / Application	Control, 9600 g product/ha (corresponding to 24.0 g product/L) and reference item (corresponding to 50 mL product/ha). The items were sprayed onto bean plants grown in the field conditions, with the equipment (PSG-System 4" (Fa. Schachtner, D-71640 Ludwigsburg) with an extension tube including 5 spraying nozzles) and air dried afterwards outdoors under natural conditions. An application rate of 4 mg/cm^2 ± 10% (corresponding to 400 L spray liquid/ha) was used
Rain protection	The test item treated plants were protected against rain with a thin plastic sheeting (acrylic, transparent, thickness 1.8 mm) during the whole aging period.
Test vessels / Exposure unit	Hatching chambers: Glass tubes with a length of approximately 15 cm and a diameter of 1.5 cm at the large and 0.5 cm at the small opening. Exposure units: Comprising 2 untreated glass plates (13 cm x 13 cm) which were held apart by an untreated aluminium frame (13 cm x 1.5 cm x 1 cm per side) and held together with at least 2 clamps. 3 sides of the frame had 6 ventilation holes (approximately 1 cm in diameter) covered with a cloth. The 4th side of the frame had 1 small hole (approximately 1 cm in diameter) for inserting and feeding the test organisms. The hole on the 4th side was closed with a well-fitting stopper until the tubes with the food were connected. The entire lower glass plate was covered with a thin, slightly moistened paper tissue and on this tissue approximately 1 – 4 treated bean leaves (leaves were cut from field treated bean plants (*Phaseolus vulgaris* 'Nassau') were spread inside the exposure cage with the upper surface upwards. The area covered by the leaves was the same among all treatment groups. Post-exposure units (parasitisation and post-parasitisation period): Untreated pots (13 cm in diameter) with barley seedlings (*Hordeum vulgare* 'Sunshine'; 13 - 25 seedlings, 10 days old) infested with 100 - 200 host aphids of all developmental stages (*Rhopalosiphum padi*; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). The cylinder had two holes (70 x 195 mm) which were closed with a fine gauze to improve the ventilation and another hole (approximately 2 cm in diameter) closed with cotton wool for the introduction of the parasitoids. The top of the cylinder was closed with a fine gauze. The soil surface was covered with a thin layer of quartz sand.

Treatment	Single application of 9600 g product/ha, reference item (corresponding to 50 mL product/ha) and control group (tap water) was made in bean plants at growth stage BBCH 17-19 in field conditions. The residues were allowed to dry in natural outdoor conditions. After the application leaves were cut from the treated bean plants and transferred to the laboratory. The test was divided in two bioassays: the first bioassay (test item, control, reference item) was carried out with freshly dried residues on the day of the application. The second bioassay (test item, control) with aged residues was started on day 14 after application. During the exposure period, 4 replicates per treatment of 10 parasitoids (7 females and 3 males) were used. During the post-exposure period, 20 replicates of 1 female each was used per treatment.
Observations	Mortality and behaviour: Observations of mortality were recorded approximately 2, 24 and 48 hours after test initiation. The number of parasitoids alive, affected, moribund and dead were recorded. Moribund parasitoids were counted as dead. Settling of the Parasitoids: To determine whether residues of the test item were repellent to the wasps, observations on the position of the individual insects were made during the initial 2.5 h after their release. Additionally settling behaviour was assessed 24 and 48 h after the start of the test. Reproduction: Number of aphid mummies was counted 10 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hour parasitisation period (second bioassay: n = 20). The number of live, dead or not found females after the 24 hours parasitisation period was documented in the raw data and reported in the final report. The reproduction assessment was performed where mortality (Mcorr) was ≤ 50 %. No reproduction testing was performed with the reference item.
Statistics	Mortality data were analysed for significance using the Fisher's Exact Binomial Test, which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis (pairwise comparison, one-sided greater, $\alpha = 0.05$). Reproduction data were tested for normal distribution and homogeneity using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). Because reproduction data were normally distributed and homogenous the Student t-test (pairwise comparison, one-sided smaller, $\alpha = 0.05$) was used. The percent values of wasps settled on the plants were angularly transformed (square root arcsine) prior to analysis. The transformed data were tested for normal distribution and homogeneity using the Shapiro-Wilk's test or Kolmogorov- Smirnov-test and the Levene's test ($\alpha = 0.01$ for all pretests). Afterwards the Student t-test (normally distributed and homogeneous data) or Welch t-test (normally distributed and heterogeneous data) were used. All pairwise comparisons were carried one-sided smaller with $\alpha = 0.05$. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

Table A 2.3.2.2.6-1 Effects on mortality and on the parasitisation efficiency of the parasitoid *Aphidius rhopalosiphi* exposed to residues of Cuprofix C Disperss (FEL02) - First bioassay: test start on the day of application

	Rate[1]	Mortality (%)[2]	Mortality corrected (%)[3]
Control	-	7.5	-
Reference item	50 mL/ha	100.0*	100.0
Cuprofix C Disperss (FEL02)	9.6 kg/ha	90.0*	89.2

1) Application rate in 400 L tap water/ha; application was done in the field under outdoor conditions
2) Mortality: after 48 hours of exposure to spray residues on leaf surfaces (Fisher's Exact Binomial Test, α = 0.05, one-sided greater; * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli

Table A 2.3.2.2.6-2 Effects on mortality and on the parasitisation efficiency of the parasitoid *Aphidius rhopalosiph*i exposed to aged residues of Cuprofix C Disperss (FEL02) - Second bioassay: test start 14 days afterapplication

	Rate[1]	Mortality (%)[2]	Mortality corrected (%)[3]	Reproduction (mummies/female)[4]	Effect on reproduction [%][5]
Control	-	7.5		46.7	-
Cuprofix C Disperss (FEL02)	9.6 kg/ha	15.0 n.s.	8.1	36.8 n.s.	21.3

1) Application rate in 400 L water/ha; application was done in the field under outdoor conditions
2) Mortality: after 48 hours of exposure to spray residues on leaf surfaces (Fisher's Exact Binomial Test, α = 0.05, one-sided greater; n.s. = not significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of parasitised aphids/female,
(Student t-test, α = 0.05, one-sided smaller; n.s. = not significant)
5) Calculated on the exact raw data

CONCLUSIONS

After an aging period of 14 days under natural conditions, a single application of 9600 g Cuprofix C Disperss (FEL02)/ha in 400 L tap water/ha did not cause unacceptable effects on mortality and reproduction of adult *Aphidius rhopalosiphi.* Both effects were below the ESCORT 2 trigger value of 50 %.

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

The risk assessment demonstrated that the proposed use of FEL02 poses no unacceptable risk to non-target arthropods. Therefore, further studies are not considered necessary.

A 2.3.2.4 KCP 10.2.3.4 Field studies with non-target arthropods

The risk assessment demonstrated that the proposed use of FEL02 poses no unacceptable risk to non-target arthropods. Therefore, further studies are not considered necessary.

A 2.3.2.5 KCP 10.2.3.5 Other routes of exposure for non-target arthropods

Other routes of exposure for non-target arthropods are not considered relevant. According to EFSA Conclusion (2018), Copper is a non-systemic-like compound.

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

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of FEL02 were not evaluated as part of the EU assessment of copper. Additional data on sub-lethal effects for FEL02 have been generated.

In addition, a laboratory study on the sensitivity of field-caught earthworms *Aporrectodea caliginosa* (Annelida, Lumbricidae) to Copper in grassland soils collected at two field sites in south-western Germany is submitted with the assessment.

A 2.4.1.1.1 Study 1

Comments of zRMS:

The study is considered as valid. This study was evaluated according to OECD 222 (2004). The study met the relevant validity criteria.
Deviations of the study: No deviation with impact on quality and integrity of the study. There were no protocol deviations.

Validity criteria: All validity criteria of the respective test guideline were met.

Validity criteria

According to OECD (2004), for the test to be deemed valid:

- control treatment mortality should not exceed 10% at 28 DAT (the actual level was 4%),
- the average post-treatment weight of the worms in the control treatment should not decrease by more than 20% (the actual level was an increase of 26%),
- the number of juveniles recorded in the control treatment should be at least 30 per replicate (the actual minimum level recorded in an individual control arena was 127),
- the coefficient of variation for the results of reproduction in the control treatment replicates should not exceed 30% (the actual CV for the controls was 16.3%).

Thus, all of these criteria were met.

Agreed toxicity endpoints:
Eisenia fetida

Percentage mortality of the adult earthworms at 28 DAT. At the start of the test, 10 adult worms were introduced into each replicate arena (n = 8 for control; n = 4 for each test item concentration).

Treatment	Test item concentration [mg test item/kg soil dry weight]	% mortality 28 DAT [1]		Corrected % mortality [2]	% change in worm fresh weight [3]		
Control	-	4		-	26	(6.6)	
ATOFEL02	525	0		0	42	(14.3)	
	292	23	**	20	72	(20.0)	***
	162	3		0	45	(4.4)	
	90	15		12	54	(12.9)	**
	50	8		4	38	(5.9)	

1) Mortality after 28 days. The 28 DAT results for individual treatments were compared to the control using Fisher's Exact Test ($\alpha = 0.05$) and asterisks indicate where they differed significantly (** $P < 0.01$), but note that several worms were found to have escaped from the arenas. There were no signs of skin lesions or altered behaviour in the adult worms at 28 DAT for any of the treatment concentrations.

2) Data corrected for the mean control mortality using Abbott's formula.

3) The mean (and Standard Deviation) for percentage change in worm weights in replicate arenas between 0 and 28 DAT. A positive value indicates an increase in fresh weight. The test item and control results were compared by one-way ANOVA ($\alpha = 0.05$), and asterisks indicate where there were significant differences (** $P < 0.01$, *** $P < 0.001$) but note that these corresponded to further weight gains compared to control.

The numbers of juvenile worms produced by 56 DAT. The percentage reduction in the reproductive performances of each treated group relative to the control group is also given.

Treatment	Test item concentration [mg test item/kg soil dry weight]	Mean number (Coeff. Var.) of juveniles per replicate [1)]		% decrease in numbers of juveniles, relative to control [2)]
Control	-	169	(16.3)	-
ATOFEL02	525	144	(13.6)	15
	292	154	(50.0)	9
	162	140	(12.6)	17
	90	153	(19.4)	10
	50	223	(31.8)	-32

1) The mean number of juveniles produced per replicate. Values for the Coefficient of Variation are given in parentheses. The individual test item treatment results for reproduction were compared to control by one-way ANOVA ($\alpha = 0.05$), but there were no significant differences.

2) A positive value indicates a decrease, and a negative value an increase in reproduction, relative to the control.

Reference:	KCP 10.4.1.1/01
Report	ATOFEL 02 (Copper 200 g/kg Cymoxanil 40 g/kg WG) – Determination of chronic (sub-lethal) toxicity to the earthworm *Eisenia fetida* in an artificial soil substrate, McCormac, A., 2012, UP-11-8
Guideline(s):	OECD 222 (2004)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effect of ATOFEL02 was examined in *Eisenia fetida* over a test period of 56 days. The test item was mixed into artificial soil at nominal concentrations of 0 (control), 50, 90, 162, 292 and 525 mg formulation/kg dry soil weight. 8 replicates of 10 adult earthworms with a body weight between 300 and 600 mg were used for the controls and 4 replicates for the test item treatment groups. A toxic reference item was tested in a separate bioassay run within 12 months of this study.

ATOFEL 02 did not result in significant mortality of adult earthworms (*Eisenia fetida*) at treatment rates up to and including 525 mg/kg soil dry weight, the maximum rate tested. The biomass of the exposed adult worms was not significantly affected at treatment rates up to and including 525 mg/kg soil dry weight. No behavioural abnormalities were observed for worms in treatment rates up to and including 525 mg/kg soil dry weight. In terms of reproduction, the numbers of juveniles produced were not affected at treatment rates up to and including 525 mg/kg soil. Taking into account mortality and changes in biomass, behaviour and reproductive capacity, the NOEC for ATOFEL 02 was therefore 525 mg/kg soil dry weight.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	ATOFEL02
Lot / Batch no.	8.335.3

Active ingredient content / Purity	200 g/kg Copper and 40 g/kg Cymoxanil
Characteristics	Greyish green granules
Density	-
Storage conditions	Room temperature
Stability (expiry date)	03.08.2012
Vehicle / control(s)	Control: 170 mL purified water was mixed with 500 g dry soil Toxic reference item: Delsene 50 Flo (500 g/L suspension concentrate of Carbendazim)

Test System

Species	*Eisenia fetida*
Age	Approx. 6 months old with a visible clitellum
Weight	300 – 600 mg fresh weight
Source	Blades Biological, Edenbridge, Kent, UK
Acclimatisation period	6 days prior to treatment application
Food	Hydrated oat flakes were replenished weekly for the first four weeks of the bioassay

Test Conditions

Temperature	20.0 – 21.0°C
Photoperiod	16 h photoperiod
Light intensity	680 – 710 lux
Test soil	Artificial soil with 10% w/w peat, 20% w/w kaolin clay, 0.4% w/w calcium carbonate, 69.6% w/w horticultural silver sand. pH was adjusted to approximately 6.0 using calcium carbonate. Soil had a final moisture content of 50% of its pre-determined water-holding capacity.

Study Design and Methods

In-life dates	07.10.2011 – 08.12.2011
Conducted at	Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK
Test duration	56 days
Test concentrations	50, 90, 162, 292 and 525 mg formulation/kg dry soil weight
Test vessels / Exposure unit	Polystyrene boxes (17.1 cm × 11.3 cm in area, by 6 cm deep) with ventilated lids
Treatment	The effect of ATOFEL02 was examined in *Eisenia fetida* over a test period of 28 days. The test item was mixed into artificial soil at nominal concentrations of 0 (control), 50, 90, 162, 292 and 525 mg formulation/kg dry soil weight. 8 replicates of 10 adult earthworms with a body weight between 300 and 600 mg were used for the controls and 4 replicates for the test item treatment groups. A toxic reference item was tested in a separate bioassay run within 12 months of this study.
Observations	Worms were assessed for mortality and sublethal effects (body weight change, behavioural abnormalities) after 28 days of exposure and earthworm body weights were assessed at day 0. After a further 28 days (i.e. 56 days after application) the number of juvenile worms that had developed was recorded.
Statistics	The percentage mortality of the adult worms was calculated for each treatment both before and after correction for mean control mortality, using Abbott's formula. The 28 DAT mortality data for each treatment concentration was compared to that in the control treatment by Fisher's Exact (α = 0.05). The data on overall change in mean-weight per replicate and numbers of juveniles were analysed by ANOVA and Dunnett's test (α = 0.05).

RESULTS AND DISCUSSION

The results for the adult worms originally introduced are summarised below. No significant mortality was observed at 28 DAT for treatment rates of ATOFEL02 up to and including the maximum test item treatment rate of 525 mg/kg soil dry weight. Treatment rates of ATOFEL02 up to and including 525 mg/kg soil dry weight did not lead to a significant reduction in adult worm biomass at 28 DAT compared to the untreated control. Worms in treatment rates up to and including 525 mg/kg soil dry weight appeared healthy and showed no abnormal behaviour.

Table A 2.4.1.1.1-1 Subchronic toxicity of ATOFEL02 to earthworms

Treatment [mg formulation/ kg dry soil]	Mortality after 28 days [%] [1]	Mean body weight change [%] [2]	Mean number of juveniles at 56 DAT [3]	Decrease in numbers of juveniles [%] [4]
Control	4	26 (6.6)	169 (16.3)	-
50	8 (4)	38 (5.9)	223 (31.8)	-32
90	15 (12)	54 (12.9) **	153 (19.4)	10
162	3 (0)	45 (4.4)	140 (12.6)	17
292	23 (20) **	72 (20.0) ***	154 (50.0)	9
525	0 (0)	42 (14.3)	144 (13.6)	15

[1] Values in parentheses represent the corrected mortality using Abbott's formula for the test item treatment groups
[2] based on fresh body weight compared to start of the test; values in parentheses represent standard deviation. Positive value indicates an increase in fresh weight.
[3] values in parentheses represent coefficient of variation
[4] a positive value indicates a decrease, and a negative value an increase in reproduction relative to control
DAT = days after treatment
** significantly different to control ($p < 0.01$)
*** significantly different to control ($p < 0.00$)

There were no statistically significant reductions in juvenile numbers, compared to the control, at treatment concentrations up to and including 525 mg product/kg soil dry weight. Thus, the NOEC for reproduction was 525 mg product/kg soil dry weight.

The EC_{50} of the toxic reference was found to lie between 1 and 3 mg Carbendazim/kg soil dry weight.

CONCLUSIONS

ATOFEL 02 did not result in significant mortality of adult earthworms (*Eisenia fetida*) at treatment rates up to and including 525 mg/kg soil dry weight, the maximum rate tested. The biomass of the exposed adult worms was not significantly affected at treatment rates up to and including 525 mg/kg soil dry weight. No behavioural abnormalities were observed for worms in treatment rates up to and including 525 mg/kg soil dry weight. In terms of reproduction, the numbers of juveniles produced were not affected at treatment rates up to and including 525 mg/kg soil. Taking into account mortality and changes in biomass, behaviour and reproductive capacity, the NOEC for ATOFEL 02 was therefore 525 mg product/kg soil dry weight.

A 2.4.1.1.2 Study 2

Comments of zRMS:	Accepted as additional information.

Reference:	KCP 10.4.1.1/02
Report	Laboratory study on the sensitivity of field-caught earthworms *Aporrectodea caliginosa* (Annelida, Lumbricidae) to Copper in grassland soils collected at two field sites in south-western Germany: a crossover experiment, Wagenhoff, E., 2019, Report no. S18-00119
Guideline(s):	No specific guideline available
Deviations:	No deviation with impact on quality and integrity of the study.

GLP:	Yes (certified laboratory)
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The purpose of this study was to determine the effects of Copper-level and soil properties in different Copper-loaded soils originating from two field sites on adult mortality, body weight change and on reproduction of field-collected adult *Aporrectodea caliginosa* SAVIGNY.

The study was conducted using a full 2 × 2 × 2-factorial design with the following three factors, each one with two levels:

- factor 1: treatment of soil (Copper-treated vs. control),
- factor 2: origin of soil (Niefern vs. Heiligenzimmern),
- factor 3: origin of earthworms (Niefern vs. Heiligenzimmern),

resulting in 8 treatment groups. Each treatment group consisted of four replicates and 20 adult earthworms (i.e. five animals per single replicate). Since the adult worms continuously lost biomass and a high proportion entered a quiescence stage after 112 days, the exposure phase was terminated, i.e. the last biological assessment was performed at day 112.

The following endpoints were assessed: Mortality, biomass, and the percentage of animals in a quiescence stage (identified by the formation of an estivation chamber in which the inactive worm perseveres in a coiled position). They were determined every 28 days until day 112; reproductive output (i.e. number of cocoons and juveniles produced) was determined after 112 days.

Generally, mortality of the introduced adult worms in the different treatment groups, including the Copper-treated test soils, remained on a low level throughout the whole exposure phase with a maximum of 20% mortality after 112 days. During progression of the exposure phase, especially from day 56 onwards, an increasing number of the adult worms entered the stage of quiescence in each of the treatment groups. During the course of exposure to the test soils, there was a continuous loss of mean biomass in each of the treatment groups until day 112; the main drop of biomass was observed at the day 84 and day 112 assessment (see figure below). Only after 28 days an increase of biomass was observed in six of the treatment groups, mainly in the Copper-treated soils. After 112 days of exposure to the test soils, mean loss of biomass in each treatment group ranged between 20.7% and 44.8%. The initial difference in individual worm biomass from the two different field sites (i.e. Niefern worms with a higher mass of 85.0 mg compared to Heiligenzimmern worms) decreased during the exposure phase in the laboratory; after 112 days of exposure to the different test soils the mean worm weights from both field sites were nearly the same (i.e. Niefern worms with a higher mass of 2.1 mg only compared to Heiligenzimmern worms). As parameter of reproduction the number of juveniles was evaluated statistically only.

MATERIALS AND METHODS

Test Item

Designation	Copper
Lot / Batch no.	Not stated
Purity	Not stated
Stability (expiry date)	Not stated

Test System

Species	*Aporrectodea caliginosa*
Source	Copper-untreated plots of the two different field sites of a long-term field study (S13-02262) in South-Western Germany (Niefern and Heiligenzimmern)
Age	Adult earthworms

Feeding	At the start of exposure, the test soils contained plant material originating from the vegetation (organic matter content ranged between 5.10 and 7.24% of dry mass) and therefore no additional food was provided within the first 28 days. From day 28 onwards, however, the worms were additionally fed with finely ground air-dried cow manure. Every 28 days, per replicate an amount of 3–5 g air-dried cow manure was mixed into the test soil (day 28: 3.0 g, day 56: 3.0 g, day 84: 5.0 g; total amount: 11.0 g).
Test Conditions	
Temperature	17.4 – 19.6ºC
Photoperiod	Start: 46.1 – 60.4%, End: 38 – 53.6%
Light intensity	16 hours light / 8 hours dark
Study Design and Methods	
In-life dates	08.11.2017 - 23.03.2018
Test facility	Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany
Test concentrations	Niefern: (control) 26.5 and 135.2 mg/kg soil dw); Heiligenzimmern: (control) 25.9 and 142.2 mg/kg soil dw
Test organism assignment and treatment	The test organisms were sorted by hand from Copper-untreated plots of the same two different field sites (Niefern and Heiligenzimmern) of the long-term field study (S13-02262), where also the test soils from the control had been collected. Collection of the worms was conducted on 15 November 2017. Until the start of the exposure, the worms were maintained in the test facility in untreated soil from the field site where they originated. On the day of the start of the exposure phase, the worms were washed, rinsed and blotted dry, and they were weighed individually (the initial weight of each worm was recorded). The weight range of the worms was between 392 and 701 mg (mean ± SD: 549 ± 76 mg) for Niefern and between 304 and 656 mg (mean ± SD: 464 ± 81 mg) for Heiligenzimmern, respectively. Groups of five test organisms were distributed randomly throughout all treatment groups. All organ-isms used for the test were healthy and showed the presence of a clitellum. After placement of the worms onto the surface of the test soils, the test units were closed with the lids allowing ventilation and then incubated under the specified test condition.
Dose preparation	Copper had been applied three times per year at a nominal rate of 8 kg Cu/ha/year in the past 14 years. The soils from both field sites and treatments (Niefern/control, Niefern/Copper-treated, Heiligenzimmern/control, and Heiligenzimmern/Copper-treated) were frozen, dried, homogenized and used as test soils during the exposure phase.

Measurements and observations	The temperature in the climate chamber was recorded continuously with appropriate, calibrated equipment. The climatic chamber was ventilated during the study. The illumination was measured once during light hours to be between 550 and 800 lux (target: 400 to 800 lux). At the start of the exposure phase and after 121 days, soil samples from each treatment group were taken and the pH was measured using a calibrated electrode (in 0.01 M CaCl2 solution). The water content of the test soils was determined at the start of the exposure phase and after 112 days. Soil samples from each treatment group were taken and weighed before and after drying overnight at 105°C. Every 28 days, the test units were emptied on a metallic tray and the adult earthworms were sorted from the soil while their reaction to a gentle mechanical stimulus at the anterior end was tested. At the beginning of the exposure phase, the earthworms were weighed individually. Every 28 days, the total weight of all surviving earthworms per replicate was determined. Prior to weighing, the worms were washed, rinsed and blotted dry. After 112 days of exposure, the juvenile earthworms and the cocoons were sorted from the soil by hand and the total number of juveniles and cocoons per replicate was recorded.
Statistics	Statistics was performed for the day 112-assessment only. The level of significance was set to $\alpha = 0.05$ for each of the tests. Prior to hypothesis testing, data were analysed for normality and variance homogeneity. Normality was checked using Shapiro-Wilk test or Kolmogorov-Smirnov test and by visual inspection of the respective histogram of residuals. Homogeneity of variances was checked using Brown-Forsythe test. Mean biomass of the worms from both field sites at test start were analysed for a difference using Student's t-test (two-sided). Mortality data and the frequency of adult worms in quiescence (expressed as % of surviving worms) for each treatment group were analysed for significant differences for all meaningful pair-wise comparisons (i.e. comparisons between two treatment groups which differed within the two levels of one factor only) using multiple Fisher's exact test with Bonferroni-Holm adjustment (two-sided). A three-way ANOVA was used to analyse the data of juvenile production and biomass change after 112 days of exposure (in order to fulfil the criteria of normality and variance homogeneity percent-ages of body weight change were arcsine-square root transformed beforehand) on the explanatory value of the simple three main factors [1] origin of soil, [2] treatment of soil, and [3] origin of worms and for their different interactions. In case that one factor significantly explained part of the variance on $\alpha = 0.05$-level, the post-hoc Holm-Sidak test was used to detect differences between the two levels of that factor. For data evaluation the statistical programme SIGMAPLOT 13 (© 2014) was used. Bonferroni-Holm adjustment (adult mortality and occurrence of diapausing worms) was performed with a self-programmed MS-Excel-file.

RESULTS AND DISCUSSION

Characteristics of the test soils

The two test soils originating from the same field site (control vs. Copper-treated) both for Niefern and Heiligenzimmern differed in terms of WHC_{max}, soil texture (% sand, silt and clay) and content of organic matter. This means that the two soils from each of the same field site cannot be regarded as identical soils which differ in the concentration of Copper only (**Table A 2.4.1.1.1-1**).

Table A 2.4.1.1.1-1 Characteristics of the test soils

Test soil	WHC max (%)	pH ($CaCl_2$)	pH (H_2O)	Sand (%)[1)]	Silt (%)[1)]	Clay (%)[1)]	Classification[1)]	TOC (% C)	OM (%)	CEC (mmol/ 100 g)	CEC (meq/ 100g)
Niefern/ control	79.8	5.3	6.1	24.2	72.7	3.1	silt loam	3.2	5.4	16.1	4.0
Niefern/ Copper	89.0	5.3	5.9	12.9	79.4	7.8	silt loam	3.0	5.2	16.4	3.9
Heiligen-zimmern/ control	77.0	6.8	7.2	17.8	52.1	30.1	silty clay loam	4.3	7.5	15.4	15.5
Heiligen-zimmern/ Copper	80.5	6.6	7.2	14.2	47.1	38.8	silty clay loam	4.0	6.98	14.0	15.5

WHC_{max}: maximum water-holding capacity; TOC: total organic carbon; OM: organic matter; CEC: cation exchange capacity; OM: organic matter
[1)] according to USDA

Copper Residues in the Test Soils

Total concentrations of Copper in the test soils of both field sites were almost equivalent both for the control soils (background concentrations) and for the Copper-treated soils (**Table A 2.4.1.1.1-2**).

Table A 2.4.1.1.1-2 Copper residues in the test soils (given as mean of the analytical and retain sample)

Test soil	Concentration of total Copper (mg/kg soil dry weight)
Niefern/control	26.5
Niefern/Copper	135.2
Heiligenzimmern/control	25.9
Heiligenzimmern/Copper	142.2

Adult Mortality

Generally, mortality of the introduced adult worms in the different treatment groups, including the Copper-treated test soils, remained on a low level throughout the whole exposure phase with a maximum of 20% mortality after 112 days.

Table A 2.4.1.1.1-3 Cumulated mortality of adult Aporrectodea caliginosa after 28, 56, 84 and 112 days of exposure to the test soils

Origin of soil	Treatment of soil	Origin of worms	Cumulated mortality [%]			
			Day 28	Day 56	Day 84	Day 112
Niefern	Control	Niefern	5.0	10.0	10.0	20.0
Niefern	Control	Heiligenzim-mern	0.0	0.0	0.0	10.0
Niefern	Copper	Niefern	0.0	5.0	5.0	5.0
Niefern	Copper	Heiligenzim-mern	0.0	0.0	0.0	0.0
Heiligenzim-mern	Control	Heiligenzim-mern	0.0	0.0	0.0	0.0
Heiligenzim-mern	Control	Niefern	0.0	0.0	0.0	0.0
Heiligenzim-mern	Copper	Heiligenzim-mern	0.0	0.0	0.0	0.0

Origin of soil	Treatment of soil	Origin of worms	Cumulated mortality [%]			
			Day 28	Day 56	Day 84	Day 112
Heiligenzim-mern	Copper	Niefern	0.0	5.0	10.0	15.0

All pairwise comparisons (i.e. comparisons between two treatment groups which differed within the two levels of one factor only) did not show statistically significant differences even before Bonferroni-Holm adjustment was applied to keep the global $\alpha = 0.05$-level (multiple Fisher's exact tests: two-sided, all $p > 0.05$). This means that neither the exposure to Copper nor to the soil of the field site from which the worms originated have had a negative effect on adult survival after 112 days.

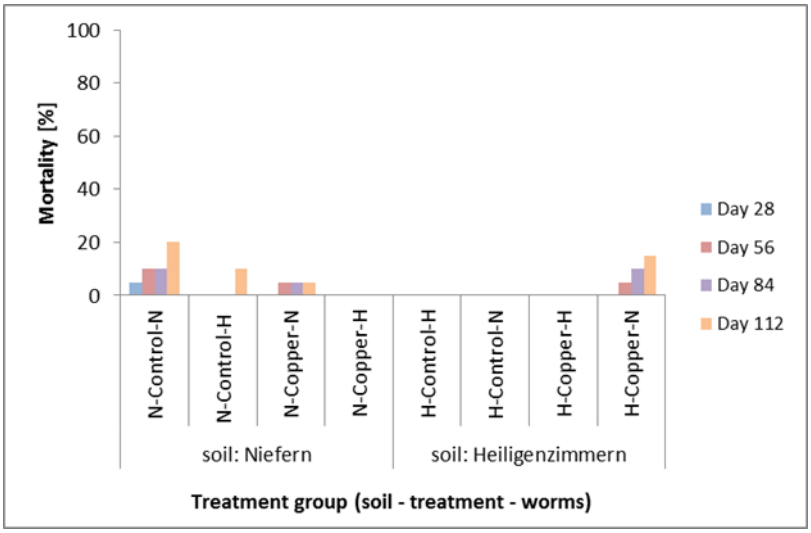

Figure A 2.4.1.1.1-1 Mortality of adult *Aporrectodea caliginosa* for each of the eight treatment groups after 28, 56, 84 and 112 days of exposure to the test soils (naming of treatment groups: N-Control-N: Niefern soil, control treatment, Niefern worms, etc.)

Entering into quiescence stage

During progression of the exposure phase, especially from day 56 onwards, an increasing number of the surviving adult worms entered a stage of quiescence in each of the treatment groups (see **Table A 2.4.1.1.1-4**). After 112 days of exposure to the test soils, almost half of the worms had entered the quiescent stage. Twenty-seven out of 72 surviving worms (37.5%) with origin Niefern entered quiescence compared to 45 individuals out of 78 surviving worms (57.7%) with origin Heiligenzimmern.

Table A 2.4.1.1.1-4 Appearance of quiescence in adult Aporrectodea caliginosa after 28, 56, 84 and 112 days of exposure to the test soils, given as absolute number of worms in quiescence and as % of surviving worms (the two treatment groups highlighted in bold differed significantly in the proportion of diapausing adult worms on global $\alpha = 0.05$-level after Bonferroni-Holm correction)

Origin of soil	Treatment of soil	Origin of worms	Number of adult worms in quiescence (absolute number of worms and % of surviving worms in brackets)			
			Day 28	Day 56	Day 84	Day 112
Niefern	Control	Niefern	0 (0%)	0 (0%)	5 (28%)	6 (38%)
Niefern	Control	Heiligenzimmern	0 (0%)	0 (0%)	3 (15%)	8 (44%)
Niefern	Copper	Niefern	0 (0%)	1 (5%)	2 (11%)	9 (47%)
Niefern	**Copper**	**Heiligenzim-mern**	0 (0%)	0 (0%)	3 (15%)	**7 (35%)**

Origin of soil	Treatment of soil	Origin of worms	Number of adult worms in quiescence (absolute number of worms and % of surviving worms in brackets)			
			Day 28	Day 56	Day 84	Day 112
Heiligenzim-mern	Control	Heiligenzimmern	0 (0%)	0 (0%)	0 (0%)	13 (65%)
Heiligenzim-mern	Control	Niefern	0 (0%)	0 (0%)	5 (25%)	4 (20%)
Heiligenzim-mern	**Copper**	**Heiligenzim-mern**	0 (0%)	1 (5%)	1 (5%)	**17 (85%)**
Heiligenzim-mern	Copper	Niefern	0 (0%)	1 (5%)	6 (33%)	8 (47%)
all soils combined			0 (0%)	3 (2 %)	25 (16%)	72 (48%)

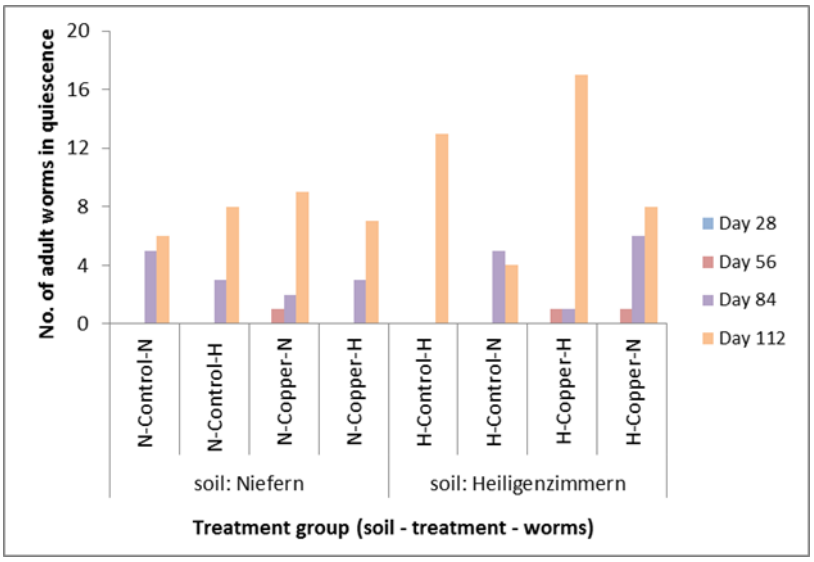

Figure A 2.4.1.1.1-2 Appearance of the quiescence stage in adult *Aporrectodea caliginosa* after 28, 56, 84 and 112 days of exposure to the test soils (naming of treatment groups: N-Control-N: Niefern soil, control treatment, Niefern worms, etc.)

Adult Biomass

At test start, biomass of the worms originating from Niefern was significantly higher compared to the worms originating from Heiligenzimmern (Student's t-test: two-sided, $\alpha = 0.05$).

During the course of exposure to the test soils, there was a continuous loss of mean biomass in each of the treatment groups until day 112; the main drop of biomass was observed at the day 84 and day 112 assessment (see figure below). Only after 28 days an increase of biomass was observed in six of the treatment groups, mainly in the Copper-treated soils. After 112 days of exposure to the test soils, mean loss of biomass in each treatment group ranged between 20.7% and 44.8%. The initial difference in individual worm biomass from the two different field sites (i.e. Niefern worms with a higher mass of 85.0 mg compared to Heiligenzimmern worms) decreased during the exposure phase in the laboratory; after 112 days of exposure to the different test soils the mean worm weights from both field sites were nearly the same (i.e. Niefern worms with a higher mass of 2.1 mg only compared to Heiligenzimmern worms).

Three-way ANOVA (normality and variance homogeneity had been confirmed beforehand: Kolmogorov-Smirnov test: $p = 0.303$, Brown-Forsythe test: $p = 0.248$) revealed a significant simple main effect for the factors origin of worms ($F = 16.421$, $df = 1$, $p < 0.001$) and treatment of soil ($F = 8.698$, $df = 1$, $p = 0.007$) on % biomass change (arcsine-square root transformed) of the adult worms between day 0 and day 112. Irrespective of the factors origin of soil and treatment of soil, biomass loss was higher in the worms from Niefern as in the worms from Heiligenzimmern (Holm-Sidak test: $p < 0.001$). Irrespective of the factors origin of soil and origin of worms, biomass loss was higher in the control soil as in the Copper-treated soil (Holm-Sidak test: $p = 0.007$). There was no significant simple main effect of the factor origin of soil on % biomass change of the adult worms between day 0 and day 112

(F = 1.135, df = 1, p = 0.297). Moreover, there was no significant interaction for each combination of two factors and for the combination of all three factors (all combinations: p > 0.05).

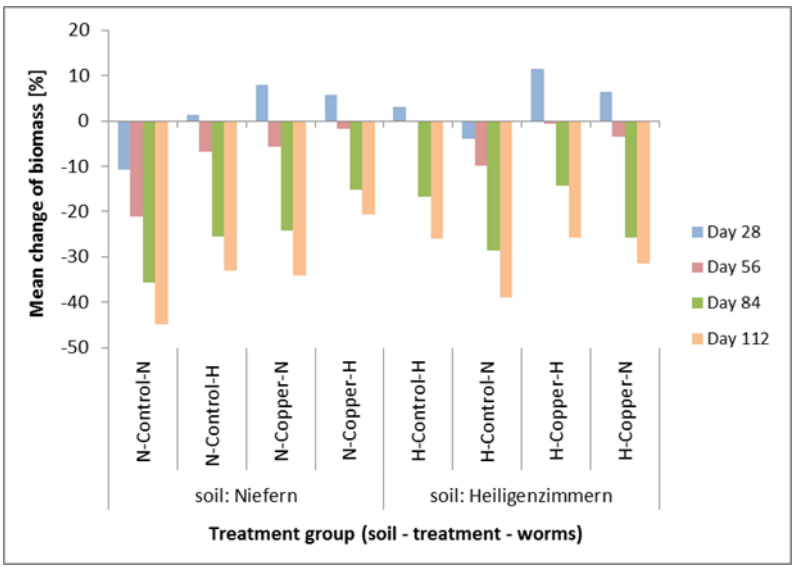

Figure A 2.4.1.1.1-3 Biomass development (mean % change per treatment group) of adult *Aporrectodea caliginosa* after 28, 56, 84 and 112 days of exposure to the test soils (naming of treatment groups: N-Control-N: Niefern soil, control treatment, Niefern worms, etc.)

Reproduction

Variance homogeneity was confirmed by Brown-Forsythe test (p = 0.06). The test on normality, however, failed (Kolmogorov-Smirnov test: p < 0.05) but nevertheless a parametric test was used. The results on juvenile production for each treatment group are shown in the figure 4 below:

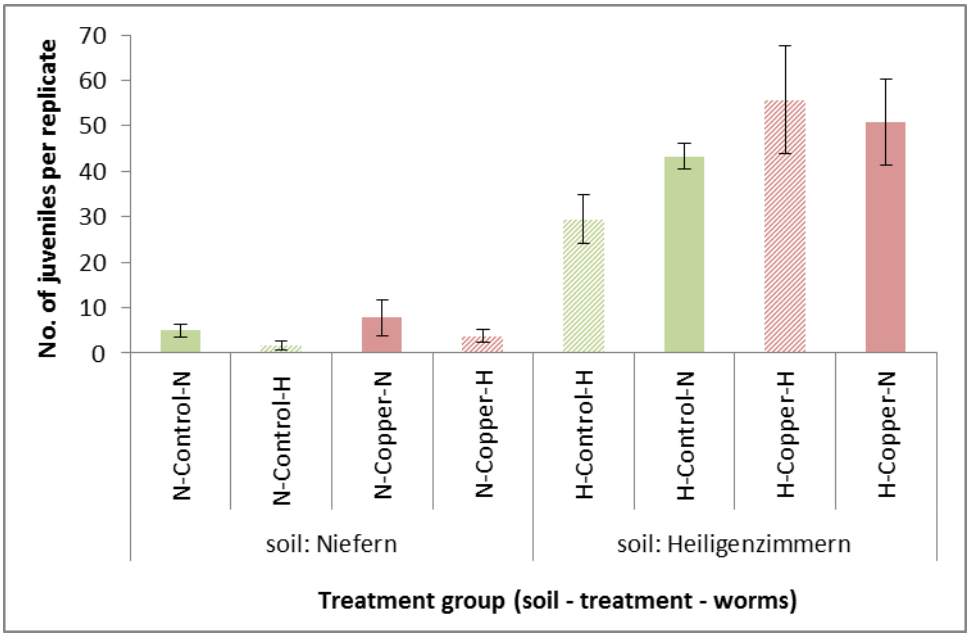

Figure A 2.4.1.1.1-4 Number of juveniles per replicate (mean ± sd) of *Aporrectodea caliginosa* after 112 days of exposure to the test soils (green columns: control soils, red columns: Copper-treated soils; filled columns: Niefern worms, dashed columns: Heiligenzimmern worms; naming of treatment groups: N-Control-N = Niefern soil / control treatment / Niefern worms, etc.)

Three-way ANOVA revealed a significant simple main effect for the factors *origin of soil* (F = 361.899, df = 1, p < 0.001) and *treatment of soil* (F = 20.695, df = 1, p < 0.001) on juvenile production after day 112 (see figure below). Irrespective of the factors *treatment of soil* and *origin of worms*, reproductive output was higher in the soil

from Heiligenzimmern as in the soil from Niefern (Holm-Sidak test: $p < 0.001$). Irrespective of the factors *origin of soil* and *origin of worms*, reproductive output was higher in the Copper-treated soil as in the control soil (Holm-Sidak test: $p < 0.001$). There was no significant simple main effect of the factor *origin of worms* on reproductive output after 112 days ($F = 3.574$, $df = 1$, $p = 0.071$).

There was no significant two-way interaction between the factors *origin of soil* and *origin of worms* (*soil x worms*: $F = 0.031$, $df = 1$, $p = 0.861$).

However, there was a significant two-way interaction between the factors *origin of soil* and *treatment of soil* (*soil x treatment*: $F = 11.742$, $df = 1$, $p = 0.002$); irrespective of the *origin of worms*, the mean number of juveniles of the worms in the control soil and Copper-treated soil from Niefern was quite similar (difference between means: 2.4) whereas in the Heiligenzimmern soils the mean number of juveniles was markedly higher in the Copper-treated soil as in the control soil (difference between means: 16.9).

There was another significant two-way interaction between the factors *treatment of soil* and *origin of worms* (*worm x treatment*: $F = 4.524$, $df = 1$, $p = 0.044$): irrespective of the *origin of soil*, reproductive output in the Copper-treated soils was similar for worms from Niefern and from Heiligenzimmern (Holm-Sidak test: $p > 0.05$) whereas in the control soils reproductive output of the worms from Niefern was significantly higher than that of the worms from Heiligenzimmern (Holm-Sidak test: $p = 0.009$).

Finally, there was a significant three-way interaction between the tested factors (*soil x treatment x worms*: $F = 5.309$, $df = 1$, $p = 0.030$).

CONCLUSIONS

Concentrations of Copper.

The test item concentration in the test soils of both field sites were almost equivalent both for the control soils (background concentrations) and for the Copper-treated soils, indicating similar conditions among the two soil origins with regard to Copper concentrations.

Soil parameters.

The two test soils of the same field site (control vs. Copper-treated) both for Niefern and Heiligenzimmer differed in ecologically relevant physiochemical soil parameters, mainly in terms of WHC_{max}, soil texture (% sand, silt and clay) and content of organic matter. This in turn means that possible differences in earthworms' performance and response to the Copper-treated and control soil of the same field site could not be ascribed to the presence of higher or lower Copper concentrations solely but might also be influenced or mimicked by differences in physiochemical soil parameters (WHC_{max}, water potential, texture, organic matter, etc.). There was a decline of the water content in all test soils between day 0 and day 112, which was most probably caused by evaporation during day 98 and day 112 and by the addition of dry cow manure as food for the worms.

Adult mortality

No difference was detected between the treatment groups. That is, adult survival was not negatively affected by the presence of increased Copper concentrations, equivalent to 8 kg Cu/ha/year (i.e. 135.2 and 142.2 mg Cu/kg soil dry weight), in the test soils even after 112 days of exposure. Moreover, adult mortality in this test was on a rather low level (< 20%) in all treatment groups (including Copper-treated soils) even after 112 days; after 56 days, adult mortality was within the accepted range (≤ 10%) for the control group within a study using *E. fetida* in artificial soil to be valid (OECD 222, 2016).

Entering into quiescence stage

During the exposure phase an increasing number of the adult worms were observed to have entered a stage of quiescence. After 112 days of exposure to the test soils, almost half of the worms had entered the quiescent stage and therefore the last biological assessment was performed at day 112. The presence of Copper at 8 kg/ha/year in the test soils did not have an effect on the appearance of quiescence in the test organisms in the laboratory.

Adult biomass

During the course of the study a continuous loss of earthworm biomass of *A. caliginosa* was observed in each of the treatment groups accompanied by an increasing number of worms entering a stage of quiescence, indicating adverse changes in the test soil environment. One reason for this to happen could have been the fluctuation and finally the decrease of the moisture content of the test soils. Adult biomass change was affected by the factors *origin of worms* (higher biomass loss in Niefern worms, which had higher biomass at test start than Heiligenzimmern worms) and *treatment of soil* (higher biomass loss in control soils), but not solely affected by the factor *origin of soil*. There was no interaction between any combinations of the three factors.

Reproduction

The number of juveniles was affected by the factors origin of soil (higher reproductive output in Heiligenzimmern soils) and treatment of soil (higher reproductive output in Copper-treated soils), but not solely affected by the factor origin of worms. There was a significant two-factor interaction between treatment of soil and origin of soil (difference in juvenile numbers between Copper and control treatment more pronounced in the Heiligenzimmern soil) and between treatment of soil and origin of worms (difference in juvenile numbers between Copper and control treatment more pronounced in the Heiligenzimmern worms) as well as an interaction between all three factors. Higher reproductive output in Copper-treated soils compared to control soils can most probably not be attributed to the presence of higher Copper concentrations in the treated soils but rather to differences among physiochemical soil parameters between Copper-treated and control soils (e.g. water availability, water potential).

This study was conducted with field sampled soils and earthworm and to our knowledge is one of the first attempts to test chronic effects on *A. caliginosa* in the lab. Any findings observed during the course of the study have been found related to missing guidance on how to conduct such a study and maintain *A. caliginosa* for an extended period in the laboratory environment.

However, it can be concluded, that no adverse effects could be derived from the presence of copper in the field sampled soils and therefore it can be concluded that the field aged copper concentrations of 135 and 142 mg/kg did not cause any adverse effects on *A. caliginosa.*

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

2.4.1.2.1 Study 1

Comments of zRMS:	Accepted as additional information. Due to the high relevance of the original study Klein, 2015, to demonstrate a safe use in the risk assessment of non-target soil meso- and macrofauna, the statistical re-analysis of the Klein (2015) data by Klein (2019) is evaluated and discussed at the EU level before being used in zonal or national application processes like it was done in the past with Klein, 2015.

Reference:	KCP 10.4.1.2/01
Report	A Field Study to Evaluate the Effects of Copper on the Earthworm Fauna in Central Europe, European Copper Task Force, Petit-Lancy, Switzerland, 20031343/G1-NFEw
Guideline(s):	Not applicable – Expert opinion on statistical evaluation
Deviations:	No (not applicable)
GLP:	Not applicable – Expert opinion on statistical evaluation
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable.

2.4.1.2.2 Study 2

Comments of zRMS:	Accepted as additional information. Due to the high relevance of the original study Klein, 2015, to demonstrate a safe use in the risk assessment of non-target soil meso- and macrofauna, the statistical re-analysis of the Klein (2015) data by Klein (2019) is evaluated and discussed at the EU level before being used in zonal or national application processes like it was done in the past with Klein, 2015.

Reference:	KCP 10.4.1.2/02
Report	Addendum to Final Report: A Field Study to Evaluate the Effects of Copper on the Earthworm Fauna in Central Europe: Statistical Analysis of a long-term earthworm field study, Klein, O., 2019, Addendum 1 to Final Report 20031343/G1-NFEw
Guideline(s):	Not applicable – Expert opinion on statistical evaluation
Deviations:	No (not applicable)
GLP:	Not applicable – Expert opinion on statistical evaluation
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable.

Executive Summary

The assessment of the earthworm population in the long-term earthworm field study 20031343/G1-NFEw was evaluated with different statistical methods, including ANOVA/ANCOVA, pairwise comparisons, principal response curve analysis (PRC), and linear mixed model analysis (LMM).

An analysis of variance (ANOVA, SAS) and an analysis of covariance (ANCOVA, SAS) was calculated and each treatment was compared to the control using a two-sided Dunnett's t-test at the 5% significance level.

Additionally, a common multivariate analysis was run (principal response curve (PRC), CANOCO). The results show the extent and course of development of the earthworm abundance compared to the control taking into account the time factor and random changes. PRCs are a special type of redundancy analysis, which use the time as covariate and the interaction between time and treatment as environmental factor to show differences from the control.

Furthermore, the analysis with a linear mixed model system (LMM, SAS) was performed.

Statistical analysis using a classical approach with ANOVA / ANCOVA test procedures followed by Dunnett's significance tests for the copper treatment data applied in different rates is a robust and sensitive way to analyse for potential significant treatment effects. This procedure is also recommended in the ISO guideline (ISO 11268-3, ISO 2014) and by De Jong et al. (2006) for the statistical evaluation of earthworm field studies.

The PRC analysis involves time in the analysis as a covariate and aims to translate the responses from a large number of taxa into a smaller number of components that can then be interpreted as representing the response of the whole community. Due to the large set of data and the time effect, it makes sense to use this approach to refine the interpretation of effects on the population level. This method has also the advantage of considering information from all species (even low-frequency taxa) found at the field site in the statistical evaluation, in contrast to the other statistical methods that can only be used to analyse taxa with a certain minimum abundance and that are thus typically limited to the analysis of the 2 or 3 dominant species. The PRC analysis is also mentioned as a viable method for statistical analysis in the ISO-11268-3 guideline (ISO 2014). It is also recommended for the analysis of non-target arthropod field studies (De Jong et al. 2010).

The analysis using Linear Mixed Models aims also to include the time factor to the interpretation of the results but its ability to detect significant treatment effects is limited due to the restriction of normal distributed data. Using the Tukey test procedure it produces results comparable to the ANOVA / ANCOVA approach. The use of the LSD test procedure is over conservative due to the expected and observed alpha inflation increasing the overall chance of a Type I error (of falsely claiming an effect, when there is in fact none) to theoretically 14% instead of 5%. According to Environment Canada (2005), the LSD test should only be used for a small pre-selected selection of all possible comparisons to avoid this inflation of false positives (type I errors).

STUDY DESIGN AND METHODS

Information on the study design and methods is given in the summary of the final study report in the Draft Renewal Assessment Report "Copper Compounds – Volume 3 – B.9 (AS)" (version: May 2018) in chapter B.9.4.1.2.

The statistical methods which were applied for the evaluation of the study are summarized in the table below.

Table A 2.4.1.2.2-1 Statistical test approaches and their significance tests

Test	Significance tests
ANOVA / ANCOVA (copper treatment)	Dunnett's t-test (α = 0.05, two sided), irrespective of outcome of pre-tests on normality and homogeneity of variance
Pairwise comparison (toxic reference)	[a)] Student t-test (α = 0.05, two sided). [d)] Satterthwaite t-test (α = 0.05, two sided). [b)] pair-wise U-Test (Wilcoxon) with Exact-Statement (α = 0.05, two-sided).
Linear mixed model	Tukey Test: advantage is that the test is more robust and that the risk of type 1 errors is low (stays at alpha = 5%). Least Significance Difference Test (LSD Test): advantage to be very sensitive, but the risk of type 1 errors is high (in this test design, alpha reached 14%).
CANOCO PRC analysis (copper treatments and toxic reference)	Copper treatment: Permutation test for test item treatments [a)] Dunnett Test (α = 0.05) of PRC scores [c)] Jonckheere-Terpstra Test (α = 0.05, two sided) of PRC scores. Toxic reference item: Permutation test for reference treatment [a)] pooled t-Test. [b)] pair-wise Mann-Whitney-U Test (α = 0.05, exact) [c)] Satterthwaite t-test (α = 0.05).

[a)] data normally distributed with variance homogeneity
[b)] data without normality
[c)] data without normality or without variance homogeneity
[d)] data normally distributed without variance homogeneity

Explanation of the applied statistical methods

ANOVA/ANCOVA

The statistical evaluation using ANOVA and ANCOVA procedures can be seen as the classical approach. This method is recommended in the ISO guideline ISO 11268-3 (ISO, 2014) and by De Jong et al. (2006) for the evaluation of earthworm field studies.

The ANOVA was applied on the pre-treatment counts and weights. The pooled estimate of residual error variance obtained was used to compare each treatment to the control using a two-sided Dunnett's t-test at 5% significance level.

As the test organism is naturally distributed over the field site and that the distribution of earthworms depends amongst others on site-inherent factors (e.g. soil conditions, soil moisture regime, soil compaction etc.) an ANCOVA was selected. These site-inherent factors do not change in this spatial scale at the field site in a short time. Therefore, the spatial distribution of earthworms at trial start had to be considered in order to eliminate these influences. Otherwise, these influences could interfere with possible effects of the test item. The covariance analysis should correct the comparison of the investigated criterion in a way that important influencing variables which do not have any relation to the treatment effect are eliminated. Thus, an ANCOVA could work out more decisively a possible treatment effect.

An analysis of covariance (ANCOVA) was performed on the post-treatment numbers, using the pre-treatment numbers (data before any treatment from the first pre-treatment sampling) as covariate, and on the post-treatment weights, using the pre-treatment weights (data before any treatment from the first pre-treatment sampling) as covariate. Additionally, an analysis of covariance was performed using the replicate dependent numbers and weights as covariates. These analyses were followed by an F-test for significance at the 5 % level to elucidate two questions: first, if the pre-treatment numbers/weights influence the post-treatment number/weights, and second, if the replicates influence the numbers/weights. If the covariate was found to be significant, an analysis of covariance was selected, whereas if the covariate was found to be non-significant an analysis of variance was selected. For both, counts and weights, the pooled estimate of residual error variance obtained from the selected form of analysis (ANOVA or ANCOVA) was used to compare each treatment to the control using a two-sided Dunnett's t-test at the 5% significance level (Dunnett, 1955).

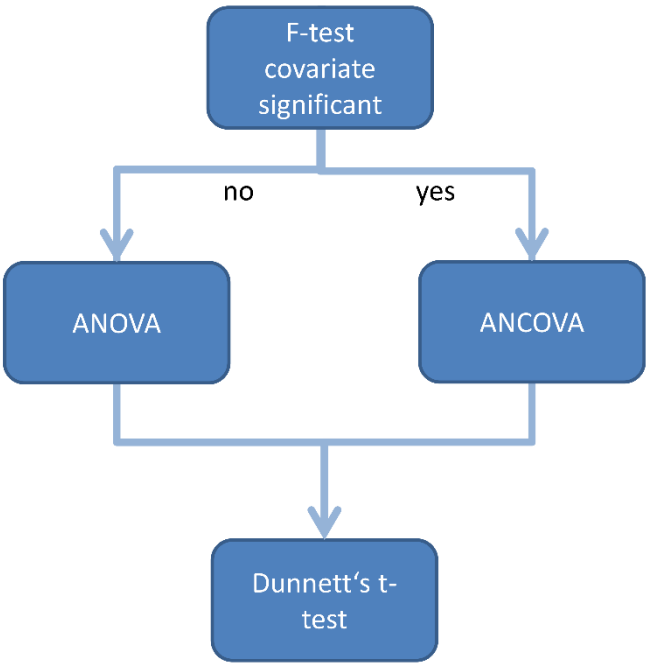

Figure A 2.4.1.2.2-1 Decision tree for ANOVA/ANCOVA test procedure

2.4.1.2.3 Study 3

Comments of zRMS:	Accepted as additional information. Please be aware that any supporting literature should be attached also to submission fully as original publication. Study limitations: ▪ There are some information necessary for transparency and reproducibility missing related to the test item (batch number, purity, content a.i., expiration data) and the test organisms *F. candida* (number of specimens or soil added per test vessel, number of replicates). However, as the reproduction tests on *E.andrei, E.crypticus* and *F. candida* after all were performed according to ISO 11268-2(2012), ISO 16387 (2004) and ISO 11267 (1999) (with some adjustments). General conclusion/comments: The study authors stated that the results are in contrast to other studies, mainly explainable by the mode of contamination, the number of tested contaminants, test design, i.e. controlled laboratory versus different field conditions. The final results based on a short-term, one-month study with a one-time application. This is in contrast to the present product application (accord. to GAP multiple uses) and to other long-term field studies like Klein (2015) highlighting that effects on *Oligochaeta* might appear after several years of copper-fungicide application and thus copper accumulating in soil by time. Besides, that copper effects on *Oligochaeta* might appear after several year of application, in a long-time perspective, is one reason why the continuation of the Klein (2015) field study by the EUCuTF is so valuable to understand which effects might occur or vanish by time, although still such short-term and intermediate field- and lab. RMS opinion is that the sttudy can increase the understanding of copper (compounds) effecting *Oligochaeta*, e.g. in short-term or mechanistic understanding.

Reference:	KCP 10.4.1.2/03
Report	Short-term effects of two fungicides on enchytraeid and earthworm communities under field conditions. Ecotoxicology, Amossé, J., Bart, S., Pery, A.R.R., Pelosi, C., 2018, https://doi.org/10.1007/s10646-018-1895-7
Guideline(s):	ISO 11268-3, 2014a

Deviations:	No (not applicable)
GLP:	Yes (certified laboratory).
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable.

Executive summary

The purpose of this study was to investigate the patterns of diversity and community structure of earthworms and enchytraeids in response to pesticide exposure (i.e., two commercial formulations) under field conditions. During the procedure the effects of different concentrations of two fungicide formulations, i.e., Cuprafor Micro (composed of 500 g kg^{-1} copper oxychloride) and Swing Gold (composed of 50 g l^{-1} epoxiconazole and 133 g l^{-1} dimoxystrobin) were tested on two families of terrestrial oligochaetes (*Lumbricidae* and *Enchytraeidae*) after 1 month of exposure. The experimental trial consisted of four replicates of 6 treatments (including the control) randomly located. The exposure period was 1 month.

The following endpoints were assessed: density, diversity indices and some ecological and functional traits (i.e., ecological categories for earthworms, proportion of r-strategists for enchytraeids) of each family. They were determined at the end of the test procedure (1 month).

Along with the feeding activity, the enchytraeid density, diversity and communities were not different in the control and the contaminated plots. The copper fungicide (at 0.75 and 7.5 kg Cu ha^{-1}) and the treatment with the pesticide mixture (Cuprafor Micro at 0.75 kg Cu ha^{-1} and Swing Gold at the recommended dose) did not affect *Oligochaeta* communities compared with the control, except the Shannon index for earthworms in the mixture of both fungicides. Responses of the two annelid families to the tested pesticides were different with higher effects observed on the diversity and the community structure of earthworms compared with enchytraeids. This study allowed detecting early changes on oligochaete populations after pesticide application.

MATERIALS

Test materials:	
Test item:	Cuprafor Micro
Source:	Industrias Quimicas del Valles
Purity:	500 g/kg copper oxychloride, Cu_2Cl (OH)3
Date of expiry:	
Test concentrations:	
Test item:	Luvisol, Versailles, France
Treatment groups:	Control (T), Cuprafor Micro at 0.75 kg Cu/ha (C1) —equal to one of the three to four copper applications per year in an agronomical context and 7.5 kg Cu/ha (C10).
Test organisms:	
Species	*Lumbricidae* and *Enchytraeidae*
Source	Not applicable
Age	Not applicable
Feeding	
Environmental conditions:	
Air temperature	mean air temperature of 11.1°C
Soil temperature	15.9°C in average of all plots
Relative humidity	the cumulated rainfall during the procedure was 54 mm
Photoperiod	Field study
Soil	Luvisol (loam texture (USDA), OM content 11%, pH (H_2O) 7.5 and Cu_{tot} 25.2 mg kg−1)

STUDY DESIGN AND METHODS

In-life phase: April – May 2016

Test organism assignment and treatment

This is a field study. The field plots were treated in April 2016 using a manual sprayer (capacity of twenty liters). Before pesticide application, the vegetation was cut as short as possible and the residues were removed with a lawn mower.

Dose preparation

The pesticides were diluted within eight litres of water and applied homogeneously on each plot. A volume of eight litres of water was also spiked in the control plots.

Measurements and observations

The climate was oceanic and the temperate and rainfall data were recorded daily at the weather station at 500 m from the study site, La lanterne, Versailles. Soil temperature and moisture were checked at the experimental site to ensure earthworm sampling conditions.

Soil temperature was measured in the field with an electronic digital thermometer at 10 cm of soil depth. For soil moisture, soil cores were sampled with a metal cylinder (5 cm internal diameter) at two soil depths i.e., 10 cm for enchytraeids (i.e., 25.7% in average of all plots) and 20 cm (i.e., 22.6%) for earthworms. Soil moisture was then measured in the laboratory after drying soil samples for 72 h at 105°C.

One month after pesticide application (i.e., in May 2016), earthworms were extracted by using an expellant solution of allyl isothiocyanate diluted with isopropanol (propan-2-ol) and water to obtain a 0.1 g l^{-1} solution. In each of the 24 plots, four sampling points were done. For each sampling point, twice 3.2 L of the expellant solution were poured in a metal frame of 0.16 m^2 surface (0.4 × 0.4 m). After 20 min during which emerging earthworms were retrieved, a block of soil (i.e., 40 × 40 × 20 cm) was excavated in the same squares and the last earthworms were extracted manually.

Earthworms were stored in a 4% formaldehyde solution. Adult, sub-adult individuals and juveniles were identified at the species level. In cases where species-level identification was impossible (e.g., no discrimination characters between juveniles of *Aporrectodea longa* and *Aporrectodea giardi*), juvenile individuals were allocated to species proportionally to the number of adults and sub-adults. All individuals were counted, weighted, and classified according to three ecological categories defined by Bouché (1977), i.e., epigeic, endogeic and anecic.

Enchytraeids were sampled in each plot using a split soil corer (diameter of 5 cm) at 10 cm depth. Each sample was transferred separately into a plastic bag and stored at 4 °C. Enchytraeids were extracted using wet funnel extractors under a light from incandescent light bulbs (40 W). Soil samples were heated up from 17 to 43 °C on their upper surface for 3 h. All individuals were kept in Petri dishes with tap water and counted. Adult and sub-adult individuals were identified at the species level under a light microscope. Not Identified (NI) enchytraeids (e.g., dead specimens) were also counted. The total enchytraeid density, the density of each species and the proportion of r-strategist species were determined.

The global rate and the vertical distribution of the feeding activity were measured and calculated using the bait lamina method (ISO 18311, 2014b).

Statistics

For each plot, measurement endpoints for the group of annelids (i.e., total density, species density, epigeic, anecic and endogeic density, proportion of r-strategist enchytraeids) were calculated from the sum of the four samples and expressed as density (ind m^{-2}). Mean values of each variable were then averaged on the four replicates of each treatment. The differences in diversity indices, i.e., species richness, Shannon and Pielou's evenness, and feeding activity between all treatments were assessed on log transformed data (log(x + 1)) using parametric tests (one way ANOVA followed by a multiple comparison Dunnett test, Hothorn et al. 2017 (multcomp.glht)) if the homogeneity of variance (Bartlett-test, Snedecor and Cochran 1989) and the normality of residuals (Shapiro test) were respected. Non-parametric tests (Kruskal–Wallis test followed by a multiple comparison kruskalmc test, Giraudoux 2017 (pgirmess.kruskalmc)) were used if these conditions were not respected. At each multiple post-hoc test, adjusted p-values based on Bonferroni's corrections were applied (Bland and Altman 1995). All statistical analyses were done with n = 4. The level of significance was fixed at $p < 0.05$. Minimum Detectable Differences (MDDs) were calculated for key species and ecological groups of earthworms according to Brock et al. (2015). They were expressed as percentage (% MDD, 4 replicates) of the control after back-transformation of the data.

The correlations between enchytraeid and earthworm variables, and between annelid variables and feeding activity were tested using Pearson or Kendall coefficient of correlation (for normal and non-normal distribution of the data,

respectively). Given the high number of tests, Bonferroni's corrections to p-values were also applied. Relationships between earthworm and enchytraeid communities were assessed in the different treatments using Mantel tests (Mantel 1967) using vegan (Oksanen et al. 2015) on Bray–Curtis dissimilarity transformation matrices ($p < 0.05$, 23 permutations).

All analyses were carried out with R statistical software (R Development Core Team 2016).

RESULTS AND DISCUSSION

Enchytraeids

A total of 5637 enchytraeids were collected from all plots. The mean density of total enchytraeids varied from 24,574 (in C10) to 36,733 ind m^{-2} (in M) without any significant difference between treatments (Fig. 2) Similarly, no difference was observed for the diversity metrics (i.e., species richness, Shannon index, proportion of r-strategists, and evenness) between plots treated with or without pesticides. Species richness was positively correlated ($r = 0.348$, p-value = 0.025) with the enchytraeid density. A total of 21 enchytraeid species were identified in the six treatments. The most abundant species was the r-strategist *Enchytraeus buchholzi*, followed by *Fridericia galba* and then *Fridericia isseli*. The density of each species was not significantly different between treatments (**Table A 2.4.1.2.3-1**).

Table A 2.4.1.2.3-1 Enchytraeid and earthworm densities, diversity metrics and community composition (n = 4, ±standard deviation) in the six treatments

Soil faunal group	Variable	T	C1	C10	D1	D10	M
Enchytraeids	Density (ind m^{-2})	**29667 ± 11519**	**27948 ± 10458**	**24574 ± 5430**	29857 ± 13684	30653 ± 8163	**36733 ± 14726**
	Species richness	**9.8 ± 1**	**10 ± 2.5**	**9.8 ± 1.7**	10.5 ± 1	9.8 ± 1.5	**10 ± 2.9**
	Shannon index	**6.61 ± 0.75**	**6.92 ± 1.38**	**6.32 ± 1.07**	5.61 ± 1.21	6.06 ± 1.26	**6.12 ± 1.91**
	Evenness	**0.83 ± 0.06**	**0.84 ± 0.03**	**0.81 ± 0.01**	0.73 ± 0.07	0.79 ± 0.09	**0.61 ± 0.11**
	r-strategists (%)	**25.8 ± 9.5**	**26.1 ± 5.3**	**33.5 ± 14.8**	33.7 ± 22.1	32 ± 8.5	**33.7 ± 20.1**
Earthworms	Density (ind m^{-2})	**231 ± 147**	**211 ± 84**	**264 ± 131**	214 ± 109	127 ± 46	**231 ± 126**
	Biomass (ind m^{-2})	**86.8 ± 32.4**	**79.5 ± 28.4**	**93.6 ± 34.7**	78.9 ± 27.1	48.3 ± 14	**83.1 ± 33.9**
	Species richness	**7 ± 1.4**	**5.8 ± 1**	**6.3 ± 1.7**	7.8 ± 1	2.8 ± 0.5	**5.5 ± 1**
	Shannon index	**3.07 ± 0.74**	**2.45 ± 0.28**	**2.48 ± 0.51**	3.11 ± 0.61	1.17 ± 0.08	**2.13 ± 0.28**
	Evenness	**0.52 ± 0.09**	**0.51 ± 0.14**	**0.55 ± 0.09**	0.57 ± 0.09	0.16 ± 0.05	**0.44 ± 0.04**
	Epigeic (ind m^{-2})	**12.9 ± 13**	**10.2 ± 4.5**	**7.4 ± 3.9**	8.2 ± 4.1	0 ± 0	**3.1 ± 2.9**
	Endogeic (ind m^{-2})	**184 ± 124**	**168 ± 70**	**211 ± 121**	178 ± 94	124 ± 45	**198 ± 107**
	Anecic (ind m^{-2})	**34 ± 13**	**34 ± 18**	**47 ± 18**	28 ± 17	3 ± 1	**31 ± 17**

Treatments are: control (T), Cuprafor Micro at 0.75 kg Cu ha−1 (C1) and 7.5 kg Cu ha−1 (C10), Swing Gold at tne (D1) and at ten (D10) times the recommended dose, and a mixture of Cuprafor Micro 0.75 kg Cu ha−1 and Swing Gold at the recommended dose (M)

Oligochaeta

A total of 3274 earthworms were collected from all plots. The mean density of total earthworms ranged from 127 (in D10) to 264 ind m^{-2} (in C10) (Fig. 2) and the mean biomass ranged from 48.3 (in D10) to 93.6 g m^{-2} (in C10). Density and biomass of earthworms were highly correlated ($r = 0.941$, $p < 0.001$). No significant difference was observed between treatments (Table 1).

The most abundant species was the endogeic *Aporrectodea icterica* followed by *Lumbricus terrestris* and then *Aporrectodea caliginosa.* The density of endogeic earthworms was not significantly different between treatments. Epigeic, earthworms were found in all treatments with copper. The anecic density was significantly lower in the D10 treatment compared with the control.

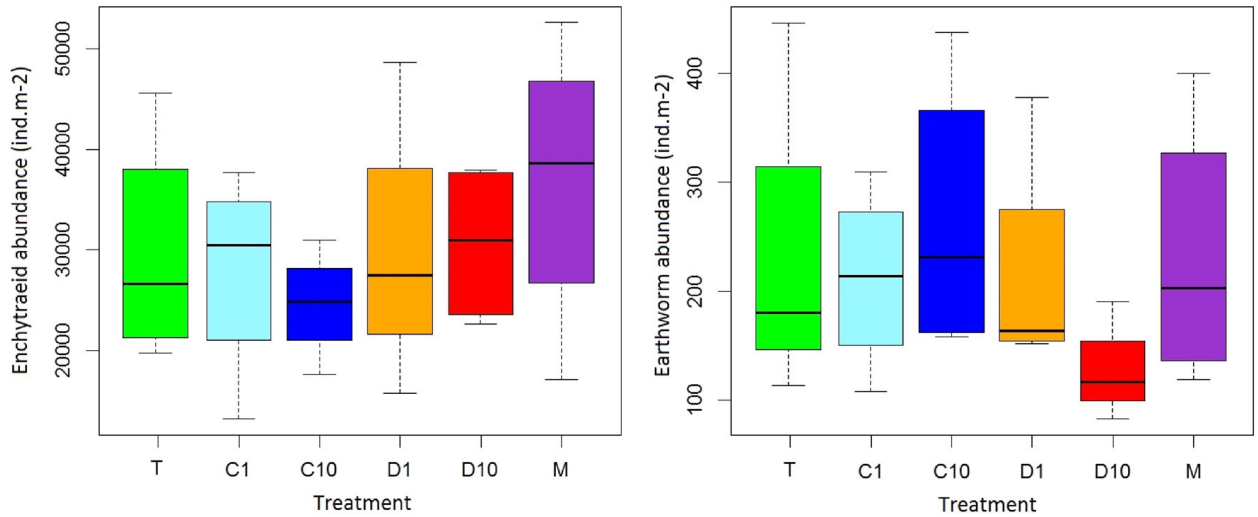

Figure A 2.4.1.2.3-1 Total densities of enchytraeids (on the left) and earthworms (on the right) per treatment. Treatments are: control (T), Cuprafor Micro at 0.75 kg Cu ha−1 (C1) and 7.5 kg Cu ha−1 (C10), Swing Gold® at one (D1) and at ten (D10) times the recommended dose, and a mixture of Cuprafor Micro 0.75 kg Cu ha^{-1} and Swing Gold at the recommended dose (M)

Annelid community patterns

No significant correlation was observed between earthworms and enchytraeid species richness, functional groups (ecological categories for earthworms and percentage of r-strategists for enchytraeids). Moreover, mantel tests did not reveal any significant relationship between enchytraeid and earthworm communities in treated and non-treated soils, except a positive relationship between enchytraeid and earthworm communities in C10 (r = 0.743, p-value = 0.042). Earthworm and enchytraeid communities were not different in the control (T) and the other treatments.

Feeding activity

The feeding rate varied from 16.7% (in C10) to 24.1% (in C1), but no significant difference was observed between treatments. In the first three centimeters of soil, the feeding rate was higher in C1 compared with the other treatments. No relationship was found between the density of each annelid families and the feeding activity (r = 0.088, p-value = 0.551 for enchytraeids; r = −0.227, p-value = 0.123 for earthworms).

CONCLUSION

Enchytraeids

It was found in the study that enchytraeids were not affected by a pesticide formulation with copper (Cuprafor Micro) whatever the fungicide concentrations.

Earthworms.

Concerning the copper fungicide, no effect was observed on earthworm populations. Based on the results, it can be concluded that copper at the tested concentrations had no short-term impact on Oligochaeta populations.

Feeding activity

In the study, enchytraeid density, diversity and community structure did not change after copper pesticide application. This suggested that no habitat competition occurred between earthworms and enchytraeids.

This study revealed contrasting patterns among annelid groups (i.e., earthworms and enchytraeids) in response to pesticide exposure.

Overall conclusion

Based on the EFSA's opinion (EFSA PPR Panel 2017), it was considered that effects of tested pesticides on enchytraeids are negligible (i.e., reduction up to 10%) to small (i.e., reduction above 10% and below 35% four weeks after pesticide application) compared with the control. The magnitude of effects is considered to allow for internal

recovery of enchytraeids populations and would have no consequences on the provision of ecosystem services (EFSA PPR Panel 2017).

No effects of the copper fungicide were observed concerning earthworm populations at concentrations of 0.75 kg Cu/ha and 7.5 kg Cu/ha. With regard to the EFSA opinion (EFSA PPR Panel 2017), this corresponds to negligible effects (i.e., reduction up to 10%).

2.4.1.2.4 Study 4

Comments of zRMS:	Accepted as additional information. Please be aware that any supporting literature should be attached also to submission fully as original publication. There are some information necessary for transparency and reproducibility missing related to the test item (batch number, purity, content a.i., expiration data) and the test organ-isms *F. candida* (number of specimens or soil added per test vessel, number of replicates). However, as the reproduction tests on *E. andrei, E. crypticus* and *F. candida* after all were performed according to ISO 11268-2(2012), ISO 16387 (2004) and ISO 11267 (1999) (with some adjustments).

Reference:	KCP 10.4.1.2/04
Report	Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values. Ecotoxicology, Caetano, A., L., Ribeiro Marques, C., Goncalves, F., Ferreira da Silva, E., Pereira, R., 2015, DOI 10.1007/s10646-015-1577-7
Guideline(s):	ISO 11268-2 (2012, *E. andrei)*; ISO 16387 (2004, *E. crypticus*)
Deviations:	No (not applicable)
GLP:	
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable.

Executive summary

The main objective of the present work is to generate ecotoxicological data for Cu in different terrestrial species (microorganisms, invertebrates and plants), endpoints and functions, using a Portuguese natural soil (PTRS1). The obtained dataset will be used to derive a SSV range for Cu based on the Assessment Factor approach. Furthermore, metal bioavailability will be taken into consideration in these estimations, by integrating a lab/field factor (formerly named as leaching/aging factor) to the toxicity values achieved in soil-spiking experiments, hence harmonizing with toxic effects in field.

Two replicates per concentration were prepared in the reproduction tests with *E. andrei*, and three in the potworm assay. All the controls were run with five replicates. The exposure period was 1 month.

The following endpoints were assessed: reproduction. They were determined at the end of the test procedure (1 month).

MATERIALS

Test materials:

Test item:	Copper (II) sulfate pentahydrate ($CuSO_4*5H_2O$)
Source:	Merck Ensure
Purity:	
Date of expiry:	

Test concentrations:

Test item:	PTRS1 Soil, non-impacted, non-industrial
Source:	Ervas Tenras (Pinhel, Guard, center of Portugal)
Conductivity	4.8 ± 0.02 mS/cm
Organic matter	6.5 ± 0.004%

Water Holding Capacity (WHC)	23.9 ± 1.84%
Treatment groups	

Test organisms:

Species	The earthworm *E. andrei (Oligochaeta: Lumbricidae),* the potworm *E. crypticus (Oligochaeta: Enchytraeidae)*
Source	From a culture kept in the laboratory, under environmental conditions
Age	Age-synchronized
Feeding	The earthworms were fed every 2 weeks with oatmeal previously hydrated with deionized water and cooked for 5 min. The potworms were fed twice a week with a small amount of grounded oat.

Environmental conditions:

Air temperature

Soil temperature

Relative humidity

Photoperiod

Soil

STUDY DESIGN AND METHODS

Test organism assignment and treatment

For the tests with invertebrates, the soil was air-dried and then sieved through a 4 mm sieve, and the 4 mm fraction was de-faunated through two freeze-thawing cycles (48 h at -20°C followed by 48 h at 25°C), before the beginning of the assays.

The earthworms selected for the test presented a developed clitellum and were pre-weighed to an individual fresh weight between 250 and 600 mg. The organisms were acclimatized in PTRS1 soil for 24 h and then introduced into each test container with 500 g of dry soil, hence totaling ten individual replicates. During the test, the worms were weekly fed with 5 g of de-faunated horse manure (see previous sub-section) per box.

Ten potworms with 12–14 mm size were introduced in each test vessel containing 20 g of dry soil. The adults were exposed during 28 days. Rolled oats were placed on the soil surface weekly to feed them.

Dose preparation

The stock solution was prepared with Milli-Q water (hereinafter referred as deionized water), in order to obtain the different ranges of concentrations to be tested (0 mg Cu Kg-1 soil dw corresponded no the negative controls; **Table A 2.4.1.2.4-1**). These concentrations were defined based on the results of range finding tests performed with the test organisms, besides taking into consideration the recommendations set in the OECD (2008) guideline. The amount of deionized water required to adjust soil water content to 45% of its maximum water holding capacity (WHCmax) was used to dilute the stock solution for the tests with invertebrates. Prior to the test start, the spiked soil was allowed to equilibrate for 48 h.

In order to discard the potential effect of sulfate on the highest concentrations of copper sulfate, controls with calcium sulfate ($CaSO_4 \times 2H_2O$) were additionally performed at 2303.2, 366.3 mg of $CaSO_4 \times 2H_2OK$/g soil dw for *Eisenia andrei* and *Enchytraeus crypticus*, respectively.

Table A 2.4.1.2.4-1 Copper concentrations used in the ecotoxicological assessment [mg Cu/Kg soil dw]

Biochemical parameters	***E. andrei***	***E. crypticus***
0.0	0.0	0.0
80.7	35.0	150.0
96.9	40.2	172.5
116.2	46.2	198.3
139.5	60.1	238.0
167.4	78.2	285.6
200.9	101.6	342.7

241.1	132.2	411.3
289.3	171.8	493.6
347.2	223.4	592.3
416.6	256.9	681.1
500.0	295.4	783.3
600.0	339.7	900.8

Measurements and observations

Adult earthworms were removed from the test containers after 28 days of exposure. No mortality of adult organisms was recorded during this period. The produced cocoons were left in the soil until 56 days of experiment. At the end of this period, the juveniles from each test container were counted after making them float in a water bath at 50 – 60°C. At the end of the test, the potworms were killed with alcohol, colored with Bengal red and counted according to the Ludox Flotation Method.

Statistics

The number of juveniles produced by earthworms and potworms were compared to the respective controls by a one-way ANOVA (SigmaPlot 11.0 for Windows). The Kolmogorov-Smirnov test was applied to check data normality, whereas homoscedasticity of variances was checked by the Levene's test. Whenever the ANOVA assumptions were not met, a Kruskal–Wallis analysis was performed (SigmaPlot 11.0 for Windows). If statistically significant differences were determined, the post hoc Dunnett's (for parametric one-way ANOVA) or the Dunn's test (for non-parametric ANOVA) were carried out to perceive which concentrations were significantly different from the respective control. The no observed-effect-concentration (NOEC) and low-observed effect-concentration (LOEC) values were determined based on the outcomes of the post hoc tests. The metal concentration producing a 20% (EC_{20}) and a 50% (EC_{50}) reduction in the tested endpoints was calculated after fitting the data to a logistic model for the reproduction of invertebrates, using the STATISTICA software version 7.0.

RESULTS AND DISCUSSION

A 100% survival was recorded for E. andrei adults in all treatments. No mortality was observed for *E. crypticus* adults in the control. However, an average of 16 % mortality was obtained in the lowest tested concentration, while it was between 70 and 100% in higher Cu concentrations (411.3–900.8 mg Cu/Kg soil dw).

A significant impairment on the reproduction of all invertebrates was recorded under Cu exposure (F = 11.3, d.f. = 16,12, p\0.05 for *E. andrei*; F = 15.9, d.f. = 22,12, p\0.05 for *E. crypticus*). The LOEC for *E. andrei* and *E. crypticus* was 132.2 and 150.0 mg Cu/Kg soildw, respectively, and no juveniles were produced by potworms above 681.1 mgKg-1 soil dw (**Figure and Table A 2.4.1.2.4-1**).

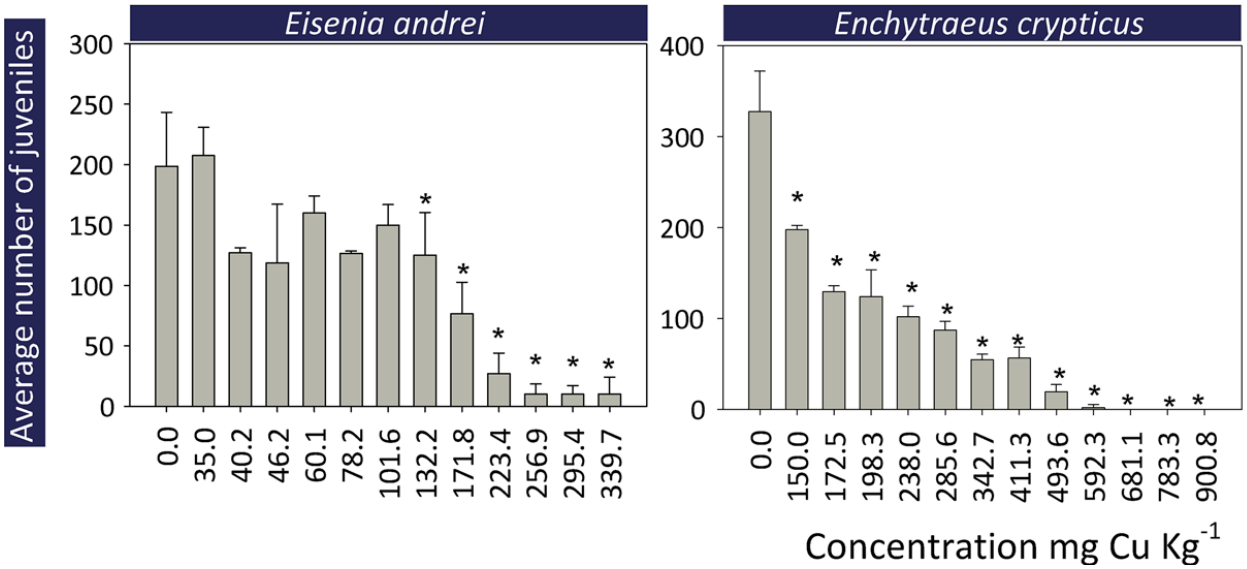

Figure A 2.4.1.2.4-1 Reproductive output of *Eisenia andrei*, *Enchytraeus crypticus* exposed to the natural soil PTRS1 spiked with increasing Copper concentrations. Error bars indicate the standard error and asterisks sign out significant differences between the treatment and the control (0.0 mg Cu Kg/dw) (p<0.05)

CONCLUSION

The results accomplished in this study strengthened the toxicity of Cu reported in the literature for different soil organisms and endpoints. The estimated EC_{20} (65.8 to \150.0 mg kg^{-1} soil dw) and EC_{50} (130.9–191.6 mg/kg soil dw) values were similar for both invertebrates (**Table A 2.4.1.2.4-2**). But based on the latter point estimate, the species can be ranked along a decreasing sensitivity order: *E. andrei, E. albidus*. This ranking is in agreement with previous studies, pointing out the influence of different exposure routes on metal uptake by soil invertebrates. In this context, soft-body invertebrates are normally exposed to metals through pore-water and dietary intake. Consequently, Cu toxicity to soft-body invertebrates tends to be more pronounced.

Table A 2.4.1.2.4-2 Toxicity data obtained for copper (mg Cu/ Kg soil dw) in PTRS1 soil on invertebrates.

Test organism	Test duration	NOEC	LOEC	EC_{20}	EC_{50}
Eisenia andrei	56 days	101.6	132.2	73 (34.94 – 111.14)	130.9 (91.69 – 170.14)
Enchytraeus crypticus	28 days	<150	150	150	165.1 (146.84 – 183.27)

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

Laboratory and field studies on effects of different Copper salts on soil macro-organisms other than earthworms are available and were evaluated in the EU review. Based on the available data it was concluded in the DAR that soil macro-organisms are more tolerant to Copper than earthworms and that the risk to soil macro-organisms is acceptable. Additional toxicity data for FEL02 have been generated for *Folsomia candida* and *Hypoaspis aculeifer*.

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 1

Comments of zRMS:	The study is considered as valid. This study was evaluated according to OECD 226. The study met the relevant validity criteria. **Deviations of the study:** No deviation with impact on quality and integrity of the study. **Validity criteria:** All validity criteria of the respective test guideline were met.

Validity Criteria of the Study

Control Mortality:	Mean mortality was 4%, validity criterion was met.
Control Reproduction:	The number of juvenile mites per replicate was 117 to 194, validity criterion was met.
Coefficient of Variation of the Control Reproduction:	Was 14.7%, validity criterion was met.

The coefficient of variation (CoV) was calculated according to the following equation:

$$v = \frac{s}{\bar{x}} \times 100$$

v = Coefficient of variation
s = standard deviation of the control reproduction
$\bar{x}$ = mean of control reproduction

Agreed toxicity endpoints:
Hypoaspis aculeifer

Mortality

Mortality of adult *Hypoaspis aculeifer* after 14 days

Treatment group	number of surviving females per replicate								mean mortality [%]	standard deviation [%]	signifi-cance[1]
	1	2	3	4	5	6	7	8			
control	10	10	10	9	9	9	10	10	4	± 5	-
62.5	10	9	10	9	-	-	-	-	5	± 6	n.s.
125	10	10	9	10	-	-	-	-	3	± 5	n.s.
250	10	10	10	9	-	-	-	-	3	± 5	n.s.
500	10	10	10	10	-	-	-	-	0	± 0	n.s.
1000	10	9	9	10					5	± 6	n.s.

The results represent rounded values calculated from the exact raw data
The test item dosages are given as mg test item/kg artificial soil dry weight
[1] Fisher's Exact Test, one-sided greater, α = 0.05
- not applicable
n.s. not statistically significantly different compared to the control

Reproduction

Reproduction of *Hypoaspis aculeifer* after 14 days

Treatment group	number of juveniles per replicate[1]								mean	standard deviation	% of control	signifi-cance[2]
	1	2	3	4	5	6	7	8				
control	117	192	167	185	157	176	194	174	170	± 25	-	-
62.5	<u>191</u>	180	188	201	-	-	-	-	190	± 9	112	n.s.
125	187	155	166	189	-	-	-	-	174	± 17	102	n.s.
250	180	191	186	187	-	-	-	-	186	± 4	109	n.s.
500	173	169	197	169	-	-	-	-	177	± 14	104	n.s.
1000	132	151	164	175	-	-	-	-	155	± 19	91.2	n.s.

The results represent rounded values calculated from the exact raw data
The test item dosages are given as mg test item/kg artificial soil dry weight
[1] mean of two counts; numbers underlined are median of three counts
[2] Dunnett's t-test, α = 0.05, one-sided smaller
- not applicable
n.s. not significantly different compared to the control

Conclusion:

The No Observed Effect Concentration (NOEC) of product FEL02 (Copper 20% + Cymoxanil 4% WG) for mortality and reproduction of *Hypoaspis aculeifer* was determined to be ≥1000 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for mortality and reproduction was estimated to be >1000 mg test item/kg soil.
The EC values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10}, EC_{20} and EC_{50} were estimated to be >1000 mg test item/kg soil.

Reference:	KCP 10.4.2.1/01
Report	FEL02 (Copper 20% + Cymoxanil 4% WG): Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil, Lührs, U., 2018, 130061089
Guideline(s):	OECD 226
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

Hypoaspis aculeifer were exposed to FEL02 (Copper 20% + Cymoxanil 4% WG) for a period of 14 days. The test item was mixed homogeneously into artificial soil which was filled in glass vessels before the predatory mites were introduced on top of the soil. Concentrations of 62.5, 125, 250, 500 and 1000 mg/kg soil (dw) and one control were tested. 4 replicates per test item concentration and 8 replicates for the control, with 10 female predatory mites each were tested. Assessment of adult mortality and reproduction was performed after 14 days.

The NOEC of FEL02 for mortality and reproduction of *H. aculeifer* was determined to be ≥ 1000 mg test item/kg soil. The LOEC for mortality and reproduction was estimated to be > 1000 mg test item/kg soil. The EC values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10}, EC_{20} and EC_{50} were estimated to be > 1000 mg test item/kg soil.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	FEL02 (Copper 20% + Cymoxanil 4% WG)
Lot / Batch no.	15.351.3
Active ingredient content / Purity	Copper: 200 g/kg (nominal), 203 g/kg (analysed) Cymoxanil: 40 g/kg (nominal), 39 g/kg (analysed)
Characteristics	Water dispersible granule (WG), solid, green
Density (if liquid)	-
Storage conditions	At 20 ± 5°C, in the dark
Stability (expiry date)	29.11.2018
Vehicle / control(s)	Control: Untreated (and moistened with deionised water) Toxic reference item: Perfekthion: Deimethoate; 400 g/L (nominal), 405.2 g/L (analysed)

Test System

Species	Predatory mite *Hypoaspis aculeifer*
Age	Adult females, approximately 8 days after reaching the adult stage (29 days after placing adult females in clean rearing vessels over a period of 3 days)
Source	Cultured by ibacon
Acclimatisation period	None
Food	One spatula of cheese mites (*Tyrophagus putrescentiae*) *ad libitum* at test start and on day 2, 5, 7, 9 and 12.

Test Conditions

Temperature	18 – 22.0°C
Photoperiod	16 h light / 8 h dark

Light intensity	400 - 800 lux
Test soil	Artificial soil based on OECD 226; initial pH 5.8 to 6.0; water content at experimental start 19.8% to 20.7% (50.7% to 53.0% of the maximum water holding capacity); at experimental end 18.2% to 19.9% (46.5% to 51.0% of the maximum water holding capacity of 39% of the dry weight).

Study Design and Methods

In-life dates	31.01.2018 – 16.02.2018
Conducted at	ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Test duration	14 days
Test concentrations	Control, 62.5, 125, 250, 500 and 1000 mg FEL02/kg soil
Test vessels / Exposure unit	Glass vessels
Treatment	*Hypoaspis aculeifer* were exposed to FEL02 for a period of 14 days. The test item was mixed homogeneously into artificial soil which was filled in glass vessels before the predatory mites were introduced on top of the soil. Concentrations of 62.5, 125, 250, 500 and 1000 mg and one control were tested. 4 replicates per test item concentration and 8 replicates for the control, with 10 female predatory mites each were tested. Assessment of adult mortality and reproduction was performed after 14 days.
Observations	Number of surviving adult female predatory mites 14 days after test initiation was recorded (counted after extraction). The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Number of juvenile mites at day 14 after application was counted after extraction.
Statistics	Standard procedures, Fisher's Exact Test (mortality), Dunnett's t-test (reproduction)

RESULTS AND DISCUSSION

Table A 2.4.2.1.1-1 Effect of FEL02 (Copper 20% + Cymoxanil 4% WG) on the predatory mite *Hypoaspis aculeifer* in a 14-day reproduction study

FEL02 [mg/kg soil dry weight]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (14 day) [%]	4	5	3	3	0	5
Statistical significance[1]	-	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 14)	170	190	174	186	177	155
Reproduction in [%] of control (day 14)	-	112	102	109	104	91.2
Statistical significance[2]	-	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significantly different compared to the control
[1] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2] Dunnett's t-test, $\alpha = 0.05$, one-sided smaller

No behavioural abnormalities were observed in any of the treatment groups.

CONCLUSIONS

The NOEC of FEL02 (Copper 20% + Cymoxanil 4% WG) for mortality and reproduction of *Hypoaspis aculeifer* was determined to be ≥ 1000 mg test item/kg soil. The LOEC for mortality and reproduction was estimated to be > 1000 mg test item/kg soil. The EC values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10}, EC_{20} and EC_{50} were estimated to be > 1000 mg test item/kg soil.

A 2.4.2.1.2 Study 2

Comments of zRMS:

The study is considered as valid. This study was evaluated according to OECD 232. The study met the relevant validity criteria.
Deviations of the study: There were no deviations to the study plan.

Validity criteria: All validity criteria of the respective test guideline were met.

Validity Criteria of the Study

Control Mortality:	Mean mortality was 4%, validity criterion was met.
Control Reproduction:	Mean number of juvenile Collembola per replicate was 401 to 506, validity criterion was met.
Coefficient of Variation of the Control Reproduction:	Was 7.7%, validity criterion was met.

The coefficient of variation was calculated according to the following equation:

$$v = \frac{s}{\bar{x}} \times 100$$

v = Coefficient of variation
s = standard deviation of the control reproduction
$\bar{x}$ = mean of control reproduction

Agreed toxicity endpoints:

Folsomia candida

Effect of FEL02 (Copper 20% + Cymoxanil 4% WG) on Collembola (*Folsomia candida)* in a 28-day reproduction study

FEL02 (Copper 20% + Cymoxanil 4% WG) [mg/kg soil dry weight]	Control	15.63	31.25	62.5	125	250	500	1000
Mortality (day 28) [%]	4	0	5	13	5	3	10	3
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	441	438	430	429	455	430	451	387
Reproduction in [%] of control (day 28)	-	99.3	97.5	97.2	103	97.5	102	87.9
Statistical significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Endpoints [mg/kg soil dry weight]			
NOEC (mortality)	≥ 1000		
LOEC (mortality)	>1000		
NOEC (reproduction)	≥ 1000		
LOEC (reproduction)	>1000		
EC Values (reproduction) [3)]	EC_{10} > 500	EC_{20} >1000	EC_{50} >1000
95% confidence limits	n.d.	n.d.	n.d.

n.s. = not significantly different compared to the control
[2)] Dunnett's t-test, α = 0.05, one-sided smaller
- not applicable
[1)] Fisher's Exact Test, α = 0.05, one-sided greater
[3)] estimated values
n.d. not determinable

Conclusion:
FEL02 (Copper 20% + Cymoxanil 4% WG) caused no significant effects on mortality and reproduction of *Folsomia candida* up to and including the highest test concentration of 1000 mg test item/kg soil. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil. The EC values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10} was estimated to be >500 mg test item/kg soil and EC_{20} and EC_{50} were estimated to be >1000 mg test item/kg soil.

Reference:	KCP 10.4.2.1/02
Report	FEL02 (Copper 20% + Cymoxanil 4% WG): Effects on Reproduction of the Collembola *Folsomia candida* in artificial Soil, Lührs, U., 2018, 130061016
Guideline(s):	OECD 232
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

Folsomia candida were exposed to FEL02 (Copper 20% + Cymoxanil 4% WG) for a period of 28 days. The test item was mixed homogeneously into artificial soil which was filled in glass vessels before the Collembola were introduced on top of the soil. Concentrations of 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg FEL02 (Copper 20% + Cymoxanil 4% WG)/kg soil and one control were tested. 4 replicates per test item concentration and 8 replicates for the control, with 10 Collembola each were tested. Assessment of adult mortality and reproduction was performed after 28 days.

The NOEC of FEL02 for mortality and reproduction of *Folsomia candida* was determined to be ≥ 1000 mg test item/kg soil. The LOEC for mortality and reproduction was estimated to be > 1000 mg test item/kg soil.

The EC values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10}, EC_{20} and EC_{50} were estimated to be > 1000 mg test item/kg soil.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	FEL02 (Copper 20% + Cymoxanil 4% WG)
Lot / Batch no.	15.351.3
Active ingredient content / Purity	Copper: 200 g/kg (nominal), 203 g/kg (analysed) Cymoxanil: 40 g/kg (nominal), 39 g/kg (analysed)
Characteristics	Water dispersible granule (WG), solid, green
Density (if liquid)	-
Storage conditions	At 20 ± 5°C, in the dark
Stability (expiry date)	29.11.2018
Vehicle / control(s)	Control: Untreated (and moistened with deionised water) Toxic reference item: Boric acid 100.3%

Test System

Species	Collembolan *Folsomia candia* (Willem 1902)
Age	10 – 12 days
Source	Bred at ibacon
Acclimatisation period	The synchronised individuals were fed with granulated dry yeast and kept under breeding conditions until test start.
Food	After the introduction of the test organisms (day 0), and after 14 days, approximately 2 mg (half small spoon spatula) of granulated dried yeast was spread over the soil surface

Test Conditions

Temperature	18 – 22.0°C
pH	Test start: 5.8 – 6.0; test end: 5.8 – 6.0
Water content	Test start: 19.8% - 20.7% (50.7% - 53.0% of the maximum water holding capacity); test end: 17.0% - 19.3% (43.6% - 49.5% of the maximum water holding capacity)
Photoperiod	16 h light / 8 h dark
Light intensity	400 - 800 lux
Ventilation	All vessels including the additional containers were ventilated on days 2, 4, 7, 9, 11, 14, 16, 18, 21 23 and 25 by opening the lids for a short period.
Test soil	Artificial soil based on OECD 232; 5% Sphagnum-peat, 20% Kaolin clay, 74.8% fine quartz-sand, 0.2% Calcium carbonate, maximum water holding capacity of 39% of the dry weight.

Study Design and Methods

In-life dates	31.01.2018 – 01.03.2018
Conducted at	ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
Test duration	28 days
Test concentrations	Control, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg FEL02 (Copper 20% + Cymoxanil 4% WG)/kg soil
Test vessels / Exposure unit	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g ± 1.0 g artificial soil dry weight. The height of the soil layer in the containers was 2 to 2.5 cm.
Treatment	*Folsomia candida* were exposed to FEL02 for a period of 28 days. The test item was mixed homogeneously into the soil which was filled in glass vessels before the Collembola were introduced on top of the soil. 4 replicates per test item concentration and 8 replicates for the control, with 10 Collembola each were tested.
Observations	The numbers of living adult Collembola at day 28 after application were recorded. Missing adult Collembola were recorded as dead as it is assumed that missing adult Collembola had died and degraded during the test period. Surviving Collembola were observed for any abnormal behaviour or conditions at day 28 after application. The number of juvenile Collembola at day 28 after application were recorded. pH and water content were determined according to ISO 11465 and ISO 10390 ($CaCl_2$) at test start and test end.
Statistics	Mortality data were statistically analysed using Fisher's Exact Binominal Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogenous, the further statistical evaluation was performed using Dunnett's t-test (multiple comparison; $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values for reproduction were calculated by Logit Analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ToxRat® Solutions GmbH.

RESULTS AND DISCUSSION

Table A 2.4.2.1.2-1 Effect of FEL02 on Collembola (*Folsomia candida*) in a 28-day reproduction study

FEL02 [mg/kg soil dry weight]	**Control**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	4	0	5	13	5	3	10	3
Significance[1]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	441	438	430	429	455	430	451	387
Reproduction in [%] of control (day 28)	-	99.3	97.5	97.2	103	97.5	102	87.9
Statistical significance[2]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significantly different compared to the control
[1] Fisher's Exact Test, α = 0.05, one-sided greater
[2] Dunnett's t-test, α = 0.05, one-sided smaller

A mortality of up to 13% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 4% of the Collembola died (Fisher's Exact test, α = 0.05, one-sided greater).

Reproduction of the Collembolan exposed to FEL02 (Copper 20% + Cymoxanil 4% WG) was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg soil (Dunnett's t-test, α = 0.05, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups. The reference item boric acid showed statistically significant treatment related effects on reproduction at a concentration of ≥ 48.8 mg boric acid/kg soil and above. The EC_{50} for reproduction was 104.9 mg boric acid/kg soil.

CONCLUSIONS

The NOEC of FEL02 for mortality and reproduction of *Folsomia candida* was determined to be ≥ 1000 mg test item/kg soil. The LOEC for mortality and reproduction was estimated to be > 1000 mg test item/kg soil.

The EC values were not determined by statistical analysis since there was no adequate concentration response. However, the EC_{10}, EC_{20} and EC_{50} were estimated to be > 1000 mg test item/kg soil.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No additional testing with FEL02 is considered necessary, as data are available from the EU-review.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered as valid. This study was evaluated according to OECD 216. The study met the relevant validity criteria. Deviations of the study: There were no deviations to the study plan. Validity criteria: All validity criteria of the respective test guideline were met. Variation between control replicates was < 15%. In this study variations of nitrate-N contents between control replicates were 2 % at day 0, 7 and 28 and 5 % at day 14. **Agreed toxicity endpoints:**

Effects of FEL02 on soil nitrogen transformation in a silty sand soil

NO_3-Nitrogen [mg/kg soil dry weight], mean values					
	Control	18 mg FEL02/kg soil dry weight		90 mg FEL02/kg soil dry weight	
Sampling	Nitrate-N content	Nitrate-N content	Deviation[a)]	Nitrate-N content	Deviation[a)]
Day 0	20.1	19.2	4	20.7	-3
Day 7	32.1	30.4	5	34.8	-8
Day 14	42.0	39.2	7	46.0	-10
Day 28	55.7	57.6	-3	55.6	0
NO_3-Nitrogen formation rate [mg/kg soil dry weight/day] [b]					
	Control	18 mg FEL02/kg soil dry weight		90 mg FEL02/kg soil dry weight	
Sampling	Nitrate-N formation	Nitrate-N formation	Deviation [a)]	Nitrate-N formation	Deviation [a)]
Day 0 – 7	1.72	1.61	-6.67	2.01	+17.5
Day 0 – 14	1.56	1.43	-8.68	1.81	+15.5
Day 0 – 28	1.27	1.37	+7.87	1.25	-2.0

a percent deviation to control, negative value indicates inhibitory effect, positive value indicates stimulating effect
b related to successive intervals between samplings

Conclusion:
The test item Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG did not cause stimulation or inhibition ≥ 25 % of the nitrate formation rate compared to the control after 7, 14 and 28 days of exposure at both application rates, 18 and 90 kg product/ha. Thus, Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG is not expected to cause any long term detrimental effects on nitrogen turnover in soil under normal conditions.

Reference:	KCP 10.5/01
Report	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Soil Micro-Organisms: Nitrogen Transformation Test, McVean, K., 2022, TBN19940 TBN19940
Guideline(s):	OECD Test Guideline 216 (2000)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a soil microbial activity study, the effect of FEL02 (batch No. 0720163; WG formulation containing 200 g/kg Copper (Bordeaux mixture) and 40 g/kg Cymoxanil) on nitrogen transformation in soil was investigated in a silty sand soil. The test was terminated after 28 days. Three treatment groups (control and test item at two concentrations: 24 mg FEL02/kg soil dry weight and 120 mg FEL02/kg soil dry weight (corresponding to 18 kg FEL02/ha and 90 kg FEL02/ha, respectively) with three replicates each were incubated at 20°C ± 3°C in the dark.

The nitrogen transformation rate, the nitrate-N formation rate and the mineral nitrogen content were determined. In order to stimulate nitrogen transformation, the soil was amended with lucerne meal (concentration in soil: 0.5% of soil dry weight). On days 0, 7, 14 and 28 soil nitrogen content was determined. The study was terminated after confirming that the measured activity deviated less than 25% from the control.

Regarding the nitrogen content at day 28 and the nitrate formation rate from day 0 – 28 no differences of ≥25% from the control were found for both tested rates. Therefore, the test item was found to have no long-term influence on the nitrogen turnover.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item	
Designation	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG
Product code	FEL02
Lot / Batch no.	0720163
Active ingredient content / Purity	Bordeaux mixture (Metal Copper 20%) and cymoxanil (4%)
Characteristics	Green, solid, water dispersible granules (WG)
Density (if liquid)	Not relevant
Storage conditions	18 - 25 °C, dark, in the tightly closed original container
Stability (expiry date)	11.06.2022
Vehicle / control(s)	Control: Deionised water Toxic reference item: Cyanoguanidine
Test System	
Soil source	A field fresh, silty sand soil (LUFA standard soil 2.3) was used in the study. The selected sampling site was not treated with crop protection products for a minimum of one year before sampling. Also, no organic fertilizer were applied for at least six months.
Soil parameter	Silty sand (acc. to German DIN classification), clay 7.1%, silt 35.5%, sand 57.3%, Microbial biomass = 2. 2.80% of total organic carbon, pH = 5.9
Test Conditions	
Temperature	19.7°C to 21.6°C
Photoperiod	In the dark
Study Design and Methods	
In-life dates	04.05.2022 – 01.06.2022
Conducted at	Noack Laboratorien GmbH; Käthe-Paulus-Str. 1, 31157, Sarstedt, Germany
Test duration	28 days
Test vessels / Exposure unit	Plastic boxes (volume 1.0 L)
Test concentrations	Low Dose: 24 mg FEL02/kg soil dry weight (corresponding to the maximum application rate) High Dose: 120 mg FEL02/kg soil dry weight (corresponding to 5 times the maximum application rate)

Treatment	The test item was weighed out for each application rate, dispersed in demineralised water and applied to the surface of the soil. Afterwards, the soil was mixed with a handheld blender to ensure a homogeneous distribution of the test item in the soil and divided into replicates. Compacting of the soil whilst mixing was avoided. The soil moisture content was determined. The soil was adjusted to 42 % of its MHWC with demineralised water. The amount of total inorganic nitrogen was determined at test start. The soil was checked for a detectable microbial biomass (result expressed in terms of percentage of total organic carbon). The soil amounts were amended with powdered Lucerne-grassgreen meal (0.5 % of soil dry weight). To the control soil, a corresponding amount of deionised water was added.
Observations	Measurements of inorganic nitrate were carried out after 0, 7, 14, and 28 days. The pH values and water contents were determined on day 0 and 28. The room temperature was measured and recorded continuously.
Statistics	Descriptive statistics only.

RESULTS AND DISCUSSION

The variation between the replicate control samples for soil nitrate content was within the validity criterion of 15% throughout the study (actual values: 2 % at day 0, 7 and 28 and 5 % at day 14).

The reference item (Cyanoguanidine), tested in a separate study, had significant effects on the soil nitrogen turnover (decrease of the nitrate formation rate from 0 to 29 days of 83%) in a field soil tested at a concentration of 50 g/kg soil dry weight.

Regarding the nitrogen content at day 28 and the nitrate formation rate from day 0 – 28 no differences of ≥25 from the control were found for both tested rates. Therefore, the test item was found to have no long-term influence on the nitrogen turnover.

Table A 2.5.1-2 Effects of FEL02 on soil nitrogen transformation in a silty sand soil

NO_3-Nitrogen [mg/kg soil dry weight], mean values					
	Control	**18 mg FEL02/kg soil dry weight**		**90 mg FEL02/kg soil dry weight**	
Sampling	**Nitrate-N content**	**Nitrate-N content**	**Deviation[a)]**	**Nitrate-N content**	**Deviation[a)]**
Day 0	20.1	19.2	4	20.7	-3
Day 7	32.1	30.4	5	34.8	-8
Day 14	42.0	39.2	7	46.0	-10
Day 28	55.7	57.6	-3	55.6	0
NO_3-Nitrogen formation rate [mg/kg soil dry weight/day] [b]					
	Control	**18 mg FEL02/kg soil dry weight**		**90 mg FEL02/kg soil dry weight**	
Sampling	**Nitrate-N formation**	**Nitrate-N formation**	**Deviation [a)]**	**Nitrate-N formation**	**Deviation [a)]**
Day 0 – 7	1.72	1.61	-6.67	2.01	+17.5
Day 0 – 14	1.56	1.43	-8.68	1.81	+15.5
Day 0 – 28	1.27	1.37	+7.87	1.25	-2.0

a percent deviation to control, negative value indicates inhibitory effect, positive value indicates stimulating effect
b related to successive intervals between samplings

Nitrate-N Contents and Formation Rates

Nitrate-N Contents - Day 0

Test concentration [kg product/ha]	Nitrate-N		
	Repl.	MV ± SD	CV
	[mg NO_3-N/kg DW]		[%]
Control	19.8	20.1 ± 0.46	2
	20.6		
	19.8		
18	19.5	19.2 ± 0.46	2
	18.7		
	19.5		
90	21.1	20.7 ± 0.38	2
	20.4		
	20.5		

Nitrate-N Contents and Nitrate-N Formation Rates (Day 7)

Test concentration [kg product/ha]	Nitrate-N			Nitrate-N Formation Rate (Day 0 - 7)		
	Repl.	MV ± SD	CV	Repl.	MV ± SD	CV
	[mg NO_3-N/kg DW]		[%]	[mg NO_3-N · (kg soil dry weight · d)$^{-1}$]		[%]
Control	32.4	32.1 ± 0.52	2	1.76	1.72 ± 0.08	4
	31.5			1.63		
	32.4			1.76		
18	30.8	30.4 ± 0.35	1	1.66	1.61 ± 0.05	3
	30.4			1.60		
	30.1			1.56		
90	34.8	34.8 ± 1.05	3	2.01	2.01 ± 0.15	7
	33.7			1.86		
	35.8			2.16		

Repl.: Replicate
SD: Standard deviation
MV: Mean value
CV: Coefficient of variation

Nitrate-N Contents and Nitrate-N Formation Rates (Day 14)

Test concentration [kg product/ha]	Nitrate-N			Nitrate-N Formation Rate (Day 0 - 14)		
	Repl.	MV ± SD	CV	Repl.	MV ± SD	CV
	[mg NO_3-N/kg DW]		[%]	[mg NO_3-N · (kg soil dry weight · d)$^{-1}$]		[%]
Control	40.1	42.0 ± 2.27	5	1.43	1.56 ± 0.16	10
	44.5			1.74		
	41.3			1.51		
18	39.8	39.2 ± 1.68	4	1.47	1.43 ± 0.12	8
	40.5			1.52		
	37.3			1.29		
90	46.4	46.0 ± 0.35	1	1.84	1.81 ± 0.03	2
	45.8			1.79		
	45.8			1.79		

Nitrate-N Contents and Nitrate-N Formation Rates (Day 28)

Test concentration [kg product/ha]	Nitrate-N			Nitrate-N Formation Rate (Day 0 - 28)		
	Repl.	MV ± SD	CV	Repl.	MV ± SD	CV
	[mg NO_3-N/kg DW]		[%]	[mg NO_3-N · (kg soil dry weight · d)$^{-1}$]		[%]
Control	55.6	55.7 ± 1.01	2	1.30	1.27 ± 0.04	3
	55.9			1.28		
	54.6			1.23		
18	59.0	57.6 ± 1.83	3	1.42	1.37 ± 0.06	5
	55.5			1.30		
	58.2			1.39		
90	56.9	55.6 ± 1.30	2	1.29	1.25 ± 0.05	4
	55.6			1.25		
	54.3			1.20		

Repl.: Replicate
SD: Standard deviation
MV: Mean value
CV: Coefficient of variation

CONCLUSIONS

The test item FEL02 did not cause stimulation or inhibition ≥ 25 % of the nitrate formation rate compared to the control after 7, 14 and 28 days of exposure at both application rates, 18 and 90 kg product/ha. Thus, FEL02 is not expected to cause any long-term detrimental effects on nitrogen turnover in soil under normal conditions.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 1

Comments of zRMS:

The study is considered as additional source information due to the fact that only three species were studied. This study was evaluated according to OECD 208 (2006). The study met the relevant validity criteria.

Validity criteria: All validity criteria of the respective test guideline were met.

Validity of the study

The validity criteria were met.

Seedling emergence in the control: ≥ 70 % (being 97 – 100 %)
Mean survival of emerged control seedlings: ≥ 90 % (being 100 %)
Seedlings did not exhibit visible phytotoxic effects in the control and the plants exhibit only normal variation growth and morphology for that particular species
Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix from the same source.

Toxicity endpoints:

Effects on seedling emergence on day 21 after 50% emergence

Treatment group	Effects on seedling emergence on day 21 after 50 % emergence Plant species (germination rate)			
Cuprofix C Disperss (kg test item/ha)	Day 14 (number of surviving plants)	% inhibition	Day 21 (number of surviving plants)	% inhibition
	***Avena sativa* (29[1] out of 30 seeds)**			
Control	29	0	29	0
3.0	29	0	29	0
NOER	**> 3.0**		**> 3.0**	
	***Brassica napus* (30[1] out of 32 seeds)**			
Control	29	3	29	3
3.0	30	0	30	0
NOER	**> 3.0**		**> 3.0**	
	***Helianthus annuus* (29[1] out of 32 seeds)**			
Control	29	0	29	0
3.0	29	0	29	0
NOER	**> 3.0**		**> 3.0**	

No statistically significant differences between the control and test item were calculated for seedling emergence (Fisher`s Exact Binomial Test, p > 0.05)
[1] number of emerging plants according to the determined germination rate (serves as basis for calculation of % inhibition)

Effects on shoot fresh weight on day 21 after 50 % emergence

Effects on shoot fresh weight (g) on day 21 after 50 % emergence

Treatment group	Cuprofix C Disperss (kg test item/ha) control	3.0
	Avena sativa	
Mean	**5.04**	**5.49**
SD	0.47	0.79
cv %	9.29	14.44
% inhibition	-	-9
	Brassica napus	
Mean	**6.53**	**6.99**
SD	1.01	1.10
cv %	15.45	15.77
% inhibition	-	-7
	Helianthus annuus	
Mean	**10.96**	**10.88**
SD	2.28	1.71
cv %	20.85	15.74
% inhibition	-	1

No statistically significant differences between the control and test item were calculated for shoot fresh weight (Student-t-test, $p > 0.05$)
SD: standard deviation, cv %: coefficient of variation, negative values indicate an increase

Phytotoxic effects, effects on growth and effects on plant development (BBCH growth stage) assessed on day 21 after 50 % emergence

Treatment group	Phytotoxic effects, effects on growth and effects on plant development (BBCH growth stage) Plant species		
Cuprofix C Disperss (kg test item/ha)	*Avena sativa*	*Brassica napus*	*Helianthus annuus*
	Growth inhibition (%) on day 21 after 50 % emergence[1] (mean % per treatment group)		
Control	0	0	0
3.0	0	0	0
	BBCH growth stage on day 21 after 50 % emergence		
Control	13	13-14	12
3.0	13	13-14	12

[1] Mean percent visual injuries compared to the control per treatment group

Conclusion:
No plants in either the control or the treatment groups displayed any signs of phytotoxicity throughout the test period. The NOER values based on seedling emergence and shoot fresh weight was ≥ 3.0 kg formulation/ha for each of the species tested. The LOER values based on seedling emergence and shoot fresh weight were 3.0 kg formulation/ha. The ER_{50} was in excess of the highest test item treatment rate of 3.0 kg formulation/ha.

Reference:	KCP 10.6.2/01
Report	Terrestrial (non-target) plant test with Cuprofix C Disperss: Seedling emergence and seedling growth test, Friedrich, S., 2012, 12 10 48 006 P
Guideline(s):	OECD 208 (2006)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of FEL02 on the seedling emergence and seedling growth of mono- and dicotyledonous crops (oat, oilseed rape and sunflower) were studied at a nominal application rate of 3 kg FEL02/ha. The test was carried out over a period of 21 days after 50% seedling emergence. The plants were observed weekly for seedling emergence, survival and visual phytotoxicity.

No plants in either the control or the treatment groups displayed any signs of phytotoxicity throughout the test period. The NOER values based on seedling emergence and shoot fresh weight was ≥ 3.0 kg formulation/ha for each of the species tested. The LOER values based on seedling emergence and shoot fresh weight were 3.0 kg formulation/ha. The ER_{50} was in excess of the highest test item treatment rate of 3.0 kg formulation/ha.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	FEL02
Lot / Batch no.	10.340.3
Active ingredient content / Purity	4.1% Cymoxanil and 20.7% Copper
Characteristics	Green, free flowing granules
Density	-
Storage conditions	Room temperature
Stability (expiry date)	07.03.2013
Vehicle / control(s)	Control: Deionised water Toxic reference item: none

Test System

Species	*Avena sativa, Brassica napus, Helianthus annuus*
Growth stage	BBCH 00 (at day of application)
Acclimatization	Not stated
Irrigation	Bottom watering in pot saucers daily with tap water

Test Conditions

Temperature	7 to 34°C
Humidity	25 to 77%
Photoperiod	16 h light : 8 h dark
Light intensity	311 – 352 µE/s/m²
Soil	Loamy sand (classification according to DIN 4220)

Study Design and Methods

In-life dates	18.10.2011 – 11.11.2011
Conducted at	BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany
Test duration	21 days
Test concentrations	3 kg FEL02/ha
Test vessels / Exposure unit	Non-porous plastic flower pot (Ø 15 cm); capacity/pot: 1.6 kg fresh soil; actual used amount of soil/pot: 1.4 kg; surface area: 177 cm^2
Treatment	The effects of FEL02 on the seedling emergence and seedling growth of mono- and dicotyledonous crops (oat, oilseed rape and sunflower) were studied at a nominal application rate of 3 kg FEL02/ha. The test was carried out over a period of 21 days after 50% seedling emergence. Each treatment rate and control were replicated over 6 pots for oats and 8 pots for oilseed rape and sunflower containing four plants per pot for oilseed rape and sunflower and five plants per pot for oat. The test solution was sprayed once onto the soil surface after the seeds were sown (BBCH 00).
Observations	The plants were observed weekly for seedling emergence, survival and visual phytotoxicity. At the end of the test, seedling emergence, survival (mortality), final shoot fresh weight (biomass of surviving plants) and visible detrimental effects on different parts of the plants were recorded. Analysis of the spray solution was determined. Duplicate samples were taken immediately prior to the application. Samples were analysed to determine Copper content only.
Statistics	Statistical analysis of data was performed using the software ToxRat Profession 2.10.05

RESULTS AND DISCUSSION

Analysis of the spray solutions showed that the recovery rate of Copper was 84.6%. Thus, an acceptable level of accuracy was shown for the spray solution preparation and no adjustment to the results have been made.

The test met all validity criteria as seedling emergence rate for all species was ≥ 70%, the control seedlings did not show any signs of phytotoxicity and the mean survival of emerged seedlings in the controls was > 90%.

Table A 2.6.2.1-1 Effects on seedling emergence and seedling growth of mono- and dicotyledonous plants after exposure to FEL02

Treatment [kg/ha]	Oat	Oilseed rape	Sunflower
Seedling emergence [number of surviving plants 21 days after 50% emergence] [1]			
Control	29	29	29
3.0	29 (0)	30 (0)	29 (0)
Mean shoot fresh weight [g] [1]			
Control	5.04	6.53	11.0
3.0	5.49 (-9)	6.99 (-7)	10.9 (1)

[1] Values in parentheses represent % inhibition, negative values represent increase compared to control, positive values represent decrease compared to control

No plants in the control or the treatment groups displayed any signs of phytotoxicity throughout the test period. The survival for all test species was 97 – 100%. The NOER values based on seedling emergence and shoot fresh weight was ≥ 3.0 kg formulation/ha for each of the species tested. The LOER values based on seedling emergence and shoot fresh weight were 3.0 kg formulation/ha. The ER_{50} was in excess of the highest test item treatment rate of 3.0 kg formulation/ha.

CONCLUSIONS

No plants in either the control or the treatment groups displayed any signs of phytotoxicity throughout the test period. The NOER values based on seedling emergence and shoot fresh weight was ≥ 3.0 kg formulation/ha for each of the species tested. The LOER values based on seedling emergence and shoot fresh weight were 3.0 kg formulation/ha. The ER_{50} was in excess of the highest test item treatment rate of 3.0 kg formulation/ha.

A 2.6.2.2 Study 2

Comments of zRMS:

The study is considered as valid. This study was evaluated according to OECD 208 (2006). The study met all relevant validity criteria.

Validity Criteria

The validity criteria according to the guideline were fulfilled:

- a minimum of 70 % emergence in the control
- the seedlings exhibited no visible phytotoxic effects and exhibited only normal variation in growth and morphology in the control
- the mean survival of the control plants was at least 90 % at the end of the test in the control

Environmental conditions and growing media were identical for each plant species.

Validity criteria: All validity criteria of the respective test guideline were met.

Agreed toxicity endpoints:

Winter Wheat: Inhibition [%] of Shoot Height, Shoot Fresh Weight and Rate of Emergence at Test End

Application rate [kg/product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight [mg]	Inhibition [%]	Rate of emergence[1)] [%]	Inhibition [%]
Control	32.4	-	1922	-	98	-
18	31.5	3	1814	6	100	-2

Onion: Inhibition [%] of Shoot Height, Shoot Fresh Weight and Rate of Emergence at Test End

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight [mg]	Inhibition [%]	Rate of emergence [%]	Inhibition [%]
Control	22.3	-	889	-	93	-
3.56	20.9	6	754	15	85	9
5.33	22.3	0	947	-7	85	9
8	21.8	2	947	-7	73	22
12	21.0	6	852	4	80	14
18	21.2	5	848	5	75	19

Sugar Beet: Inhibition [%] of Shoot Height, Shoot Fresh Weight and Rate of Emergence at Test End

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight[1)] [mg]	Inhibition [%]	Rate of emergence[1)] [%]	Inhibition [%]
Control	10.3	-	2712	-	98	-
18	10.7	-4	2674	1	100	-2

negative values = promoted growth

[1)] Normality test failed 1

No test item related visual phytotoxic effects were determined for all tested plant species at test end.

CONCLUSIONS

The NOER values based on seedling emergence, shoot fresh weight and shoot

	fresh weight was 18 kg formulation/ha for each of the species tested. The ER_{50} values for all species were in excess of the highest test item treatment rate of 18 kg formulation/ha.

Reference:	KCP 10.6.2/02
Report	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, McVean, K., 2022d, TNK20269
Guideline(s):	OECD 208 (2006)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of FEL02 on the seedling emergence and seedling growth of two monocotyledons (winter wheat, Poaceae; onion, Liliaceae) and four dicotyledons (sugar beet, Amaranthaceae; rape, Brassicaceae; cucumber, Cucurbitaceae; soybean, Fabaceae). The test was conducted for the plant species winter wheat, sugar beet, rape, cucumber and soybean with the limit application rate of 18 kg product/ha and for the plant species onion with the test item concentrations 18 - 12 - 8.0 - 5.33 - 3.56 kg product/ha (factor 1.5). The test was carried out over a period of 21-28 days after 50% seedling emergence. The plants were observed weekly for seedling emergence, survival and visual phytotoxicity.

No plants in either the control or the treatment groups displayed any signs of phytotoxicity throughout the test period. The NOER values based on seedling emergence, shoot height and shoot fresh weight was 18 kg formulation/ha for each of the species tested. The ER_{50} was in excess of the highest test item treatment rate of 18 kg formulation/ha.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	FEL02 (Copper 20% + Cymoxanil 4% WG)
Lot / Batch no.	0722137
Active ingredient content / Purity	4.1% Cymoxanil and 20% Copper
Characteristics	Green, free flowing granules
Density	-
Storage conditions	Room temperature
Stability (expiry date)	17.05.2024
Vehicle / control(s)	Control: Deionised water (200 L/ha) Toxic reference item: none

Test System

Species	*triticum aestivum, Allium cepa, Beta vulgaris Brassica napus, Cucumis sativus, Glycine max*
Growth stage	BBCH 00 (at day of application)
Acclimatization	Not stated
Irrigation	Bottom watering in pot saucers daily with tap water

Test Conditions

Temperature	16 to 30°C
Humidity	28 to 100%
Photoperiod	16 h light : 8 h dark
Light intensity	366 ± 28 µmol/m² sec
Soil	Loamy sand (classification according to DIN 4220)

Study Design and Methods

In-life dates	12.07.2022 – 06.10.2023
Conducted at	Noack Laboratorien GmbH, Käthe-Paulus-Str. 1, 31157 Sarstedt, Germany
Test duration	21-28 days
Test concentrations	winter wheat, sugar beet, rape, cucumber and soybean: 18 kg product/ha onion: 18 - 12 - 8.0 - 5.33 - 3.56 kg product/ha (factor 1.5).
Test vessels / Exposure unit	Plastic container (standard flower pots) with a diameter of ca. 12 cm and a surface area of approximately 113 cm2 were used.
Treatment	The control and test item solution were applied to each plant species once at start of exposure. The application apparatus of the test facility is constructed like a fixed field sprayer, under which a conveyor belt transported the test container containing test medium and seeds. Before application, the apparatus was adjusted and calibrated to guarantee the required volume of tap water (200 L/ha). For calibration dry glass plates (10 x 10 cm) were weighed. Afterwards, the glass plates received the target application spray rate and were reweighed. When five actual weights in succession without changing the adjustment were equal to the nominal mean value ± standard deviation of 0.2 g ± 10 % (actual values: test duration A: 0.213 g ± 6.3 %; test duration B: 0.211 g ± 5.6 %; test duration C: 0.206 g ± 3.1 %) the application was started.
Observations	During the observation period the plants were observed for number of emerged seedlings, visual phytotoxic effects and number of dead plants on day 7, 14 and 21 (additionally on day 28 for onion). The rating of the treated plants was done in relation to the untreated control plants. Observations included all variations, either inhibitory or stimulatory, between the treated test replicates and the untreated control replicates. Such variations were phytotoxic symptoms (e.g. chlorosis, necrosis, wilting), formative effects and growth and development rates. At the end of the study, shoot height (in cm), measured after cutting the plants, and shoot fresh weight of the shoots (in mg) were measured additionally. The room temperature and relative humidity were recorded throughout the test with a temperature and moisture datalogger. The illumination is determined twice per year
Statistics	Statistical analysis of dStudent's t-tests were carried out for the determination of statistically significant differences compared to control replicates. When running a Student's t-test, a Normality test (Shapiro-Wilk) and an Equal Variance test (Brown-Forsythe) were done first. Pvalues for both, Normality and Equal Variance test, are 0.05. For onion ANOVA was performed. The α-value (acceptable probability of incorrectly concluding that there is a difference) for Student's ttest and ANOVA is $\alpha = 0.05$. Failure of the normality test can be caused by extremely homogeneous emergence and growth patterns as opposed to higher variances in other treatments. Due to the high and even number of replicates in the control and treatment groups, the failure had no influence on the robustness of the calculations. Software used was Excel, Microsoft Corporation SigmaPlot (Windows), SPSS Incorporation

RESULTS AND DISCUSSION

The validity criteria according to the guideline were fulfilled:

- a minimum of 70 % emergence in the control
- the seedlings exhibited no visible phytotoxic effects and exhibited only normal variation in growth and morphology in the control
- the mean survival of the control plants was at least 90 % at the end of the test in the control Environmental conditions and growing media were identical for each plant species.

The spray solutions were sampled prior to application and subsequently analytically verified. The measured concentrations of Cymoxanil were 108 to 112%, indicating the correct preparation of the spray solutions.

Table A 2.6.2.2-1 Effects on seedling emergence and seedling growth of mono- and dicotyledonous plants after exposure to FEL02

Treatment [kg/ha]	Winter wheat	Sugar beet	Rape seed	cucumber	Soybean	onion
Seedling emergence [%] [1] at test end						
Control	98	98	90	90	82	93
3.6	-	-	-	-	-	85 (9)
5.3	-	-	-	-	-	85 (9)
8.0	-	-	-	-	-	73 (22)
12	-	-	-	-	-	80 (14)
18	100 (-2)	100 (-2)	75 (17)	88 (2)	83 (10)	75 (19)
Mean shoot fresh weight [mg] [1] at test end						
Control	1922	2712	3333	4311	6036	889
3.6	-	-	-	-	-	754 (15)
5.3	-	-	-	-	-	947 (-7)
8.0	-	-	-	-	-	947 (-7)
12	-	-	-	-	-	852 (4)
18	1814 (6)	2674 (-4)	3666 (-10)	4413 (-2)	5709 (5)	848 (5)
Shoot height [cm] [1] at test end						
Control	32.4	10.3	12.1	13.6	28.7	22.3
3.6	-	-	-	-	-	20.9 (6)
5.3	-	-	-	-	-	22.3 (0)
8.0	-	-	-	-	-	21.8 (2)
12	-	-	-	-	-	21.0 (6)
18	31.5 (3)	10.7 (-4)	13.2 (-9)	14.1 (-4)	29.1 (-1)	21.2 (5)

[1] Values in parentheses represent % inhibition, negative values represent increase compared to control, positive values represent decrease compared to control

Potential toxic effects of the test item were assessed on day 7, 14 and 21 (additionally on day 28 for the test plant onion) by visual observations (phytotoxic effects, number of dead plants and number of emerged seedling) and at test end by shoot height and shoot fresh weight determination. No test item related visual phytotoxic effects were determined for all tested plant species at test end.

CONCLUSIONS

The NOER values based on seedling emergence, shoot fresh weight and shoot fresh weight was 18 kg formulation/ha for each of the species tested. The ER50 values for all species were in excess of the highest test item treatment rate of 18 kg formulation/ha.

A 2.6.2.3 Study 3

227	The study is considered as valid. This study was evaluated according to OECD 208 (2006). The study met all relevant validity criteria.

Deviations from the guideline	None
Deviations from the study plan	Usage of student's t-test instead of ANOVA This deviation was considered to have no impact on quality and integrity of the study.

Validity criteria: All validity criteria of the respective test guideline were met.

Validity Criteria

The validity criteria according to the guideline were fulfilled:
- the mean growth and morphology in the control group were within the normal variation for the particular plant species
- plants in the control group exhibited no visible phytotoxic effects
- the mean survival of the plant in the control group was at least 90 % at the end of the test

For each species, all organisms were from the same source.
All test chambers or rooms used for particular species were identical and had the same conditions and contained the same amount of soil matrix, support media or substrate from the same source.

Agreed toxicity endpoints:

Winter Wheat: Inhibition [%] of Shoot Height and Shoot Fresh Weigl

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight [g]	Inhibition [%]
Control	38.0	-	20.54	-
18	37.3	2	19.86	3

Onion: Inhibition [%] of Shoot Height and Shoot Fresh Weight

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight[1)] [g]	Inhibition [%]
Control	44.9	-	44.27	-
18	46.1	-3	48.48	-10

Sugar Beet: Inhibition [%] of Shoot Height and Shoot Fresh Weight

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight [g]	Inhibition [%]
Control	14.5	-	43.33	-
18	15.1	-4	47.64	-10

Rape: Inhibition [%] of Shoot Height and Shoot Fresh Weight

Application rate [kg product/ha]	Shoot height[2)] [cm]	Inhibition [%]	Shoot fresh weight [g]	Inhibition [%]
Control	18.9	-	53.65	-
18	20.0	-6	55.49	-3

negative values = promoted growth
1) Normality test failed
2) Equal variance test failed

Cucumber: Inhibition [%] of Shoot Height and Shoot Fresh Weight

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight [g]	Inhibition [%]
Control	30.1	-	57.72	-
18	26.7	11	48.55	16

Soybean: Inhibition [%] of Shoot Height and Shoot Fresh Weight

Application rate [kg product/ha]	Shoot height [cm]	Inhibition [%]	Shoot fresh weight[1)] [g]	Inhibition [%]
Control	68.2	-	34.80	-
18	75.4	-11	38.39	-10

negative values = promoted growth
1) Normality test failed

No test item related visual phytotoxic effects were determined for all tested plant species at test end.

CONCLUSIONS

The NOER values based on shoot fresh weight and shoot fresh weight was 18 kg formulation/ha for each of the species tested. The ER_{50} values for all species were in

	excess of the highest test item treatment rate of 18 kg formulation/ha.

Reference:	KCP 10.6.2/03
Report	Copper (from Bordeaux Mixture) 20 % + Cymoxanil 4 % WG Terrestrial Plant Test: Vegetative Vigour Test, McVean, K., 2022e, TNW20269
Guideline(s):	OECD 227 (2006)
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive Summary

The effects of FEL02 on the shoot height and shoot fresh weight of two monocotyledons (winter wheat, Poaceae; onion, Liliaceae) and four dicotyledons (sugar beet, Amaranthaceae; rape, Brassicaceae; cucumber, Cucurbitaceae; soybean, Fabaceae). The test was conducted at the limit application rate of 18 kg product/ha. The test was carried out over a period of 21days. The plants were observed weekly for seedling emergence, survival and visual phytotoxicity.

No plants in either the control or the treatment groups displayed any signs of phytotoxicity throughout the test period. The NOER values based on shoot height and shoot fresh weight was 18 kg formulation/ha for each of the species tested. The ER_{50} was in excess of the highest test item treatment rate of 18 kg formulation/ha.

All validity criteria of the respective test guideline were met.

MATERIALS AND METHODS

Test Item

Designation	FEL02 (Copper 20% + Cymoxanil 4% WG)
Lot / Batch no.	0722137
Active ingredient content / Purity	4.1% Cymoxanil and 20% Copper
Characteristics	Green, free flowing granules
Density	-
Storage conditions	Room temperature
Stability (expiry date)	17.05.2024
Vehicle / control(s)	Control: Deionised water (200 L/ha) Toxic reference item: none

Test System

Species	*triticum aestivum, Allium cepa, Beta vulgaris Brassica napus, Cucumis sativus, Glycine max*
Growth stage	BBCH 12-14 (at day of application)
Acclimatization	Not stated
Irrigation	Bottom watering in pot saucers daily with tap water

Test Conditions

Temperature	16 to 27°C
Humidity	36 to 97%
Photoperiod	16 h light : 8 h dark

Light intensity	366 ± 28 µmol/m² sec
Soil	A 2:1 mixture of natural soil LUFA 2.2 (batch number: Sp2.20522, loamy sand (DIN classification)) and quartz sand (12a) was used.

Study Design and Methods

In-life dates	12.07.2022 – 06.10.2023
Conducted at	Noack Laboratorien GmbH, Käthe-Paulus-Str. 1, 31157 Sarstedt, Germany
Test duration	21 days
Test concentrations	18 kg product/ha
Test vessels / Exposure unit	Plastic container (standard flowerpots) with a diameter of ca. 12 cm and a surface area of approximately 113 cm^2 were used.
Treatment	The control and test item solution were applied to each plant species once at start of exposure. The application apparatus of the test facility is constructed like a fixed field sprayer, under which a conveyor belt transported the test container containing test medium and seeds. Before application, the apparatus was adjusted and calibrated to guarantee the required volume of tap water (200 L/ha). For calibration dry glass plates (10 x 10 cm) were weighed. Afterwards, the glass plates received the target application spray rate and were reweighed. When five actual weights in succession without changing the adjustment were equal to the nominal mean value ± standard deviation of 0.2 g ± 10 % (actual value: 0.211 g ± 5.6%), the application on the test container was started.
Observations	At test start the growth stage of the plants was documented according to BBCH code. During the observation period the plants were observed on day 7, 14 and 21 for visual phytotoxic effects and number of dead plants. The rating of the treated plants was done in relation to the untreated control plants. Observations included all variations, either inhibitory or stimulatory, between the treated replicates and the untreated controls. Such variations were phytotoxic symptoms (e.g. chlorosis, necrosis, wilting), formative effects of growth and development rates. A detailed list is given in section 15.1. At the end of the study, the shoot height (in cm), measured after cutting the plants, and the fresh weights of the shoots (in g) were measured additionally. The room temperature and relative humidity were recorded continuously throughout the test with a temperature and moisture datalogger. The illumination is determined twice per year
Statistics	NOEL of biomass growth: Student's t-tests were carried out for the determination of statistically significant differences compared to control replicates. When running a Student's t-test, a Normality test (Shapiro-Wilk) and an Equal Variance test (Brown-Forsythe) were done first. Pvalues for both, Normality and Equal Variance test, are 0.05. The α-value for Student's t-test and Welch's t-test (acceptable probability of incorrectly concluding that there is a difference) is $\alpha = 0.05$. The NOEL is defined as highest application rate at which no statistically significant effect is observed. Failure of the normality test can be caused by extremely homogeneous emergence and growth patterns as opposed to higher variances in other treatments. Due to the high and even number of replicates in the control and treatment groups, the failure had no influence on the robustness of the calculations. Software: The data for the tables in the report were computer generated and rounded for presentation from the full derived data. Consequently, if calculated manually based on the given data minor deviations may occur from these figures. Excel, Microsoft Corporation SigmaPlot (Windows), SPSS Incorporation

RESULTS AND DISCUSSION

The validity criteria according to the guideline were fulfilled:

- the mean growth and morphology in the control group were within the normal variation for the particular plant species - plants in the control group exhibited no visible phytotoxic effects

- the mean survival of the plant in the control group was at least 90 % at the end of the test

For each species, all organisms were from the same source. All test chambers or rooms used for particular species were identical and had the same conditions and contained the same amount of soil matrix, support media or substrate from the same source.

The spray solutions were sampled prior to application and subsequently analytically verified. The measured concentration of Cymoxanil was 108 %, indicating the correct preparation of the spray solution.

Table A 2.6.2.3-1 Effects on growth of mono- and dicotyledonous plants after exposure to FEL02

Treatment [kg/ha]	Winter wheat	Sugar beet	Rape seed	cucumber	Soybean	onion
Mean shoot fresh weight [g] [1] at test end						
Control	20.54	43.33	53.65	57.72	34.80	44.27
18	19.86 (3)	47.64 (-10)	55.49 (-3)	48.55 (16)	38.39 (-10)	48.48 (-10)
Shoot height [cm] [1] at test end						
Control	38.0	14.5	18.9	30.1	68.2	44.9
18	37.3 (2)	15.1 (-4)	20.0 (-6)	26.7 (11)	75.4 (-11)	46.1 (-3)

[1] Values in parentheses represent % inhibition, negative values represent increase compared to control, positive values represent decrease compared to control

Potential toxic effects of the test item were assessed on day 7, 14 and 21 by visual observations (phytotoxic effects and number of dead plants) and on day 21 by shoot height and shoot fresh weight determination. No test item related visual phytotoxic effects were determined for all tested plant species at test end.

CONCLUSIONS

The NOER values based on shoot fresh weight and shoot fresh weight was 18 kg formulation/ha for each of the species tested. The ER_{50} values for all species were in excess of the highest test item treatment rate of 18 kg formulation/ha.

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

No other studies have been conducted and no additional studies are deemed necessary.

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

The spectrum of the biological activity of the product is well represented by the results and the risk assessments of this dossier. Therefore, further data from biological primary screening or other preliminary tests are not considered relevant.

A 2.8 KCP 10.8 Monitoring data

The spectrum of the biological activity of the product is well represented by the results and the risk assessments of this dossier. Therefore, further data from monitoring are not considered relevant.