REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **Nordox 75 WG**

Chemical active substance:

Copper (I) oxide (Cu_2O), 750 g/kg

Interzonal

NATIONAL ASSESSMENT

Poland

(Authorization in accordance to Art. 43)

Applicant: Nordox AS

Submission date: 31/01/2022

Evaluation date: December 2022

MS Finalisation date: dd/mm/yyyy

Version history

When	What
31/01/2022	Original version from the applicant Nordox AS for Art. 43 submission. All new data and information are marked in yellow.
12/2022	zRMS version for comments
03/2023	zRMS final version

Table of Contents

Submission and Evaluation of Copper compounds under Art.43 of 1107/2009

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 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. **This has been recognized by EFSA, the RMS and several MS (see comments from DE and IT in the Peer review Report), and the EU Commission has mandated EFSA with the development with a Copper specific guidance (Mandate No. 2019-0036).**
Art.43 submissions and their evaluation by MS are unfortunately due before this GD will be available. The current EFSA conclusion 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 bio-availability; 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, BPD). While COM directed EFSA in their mandate to take advantage of those methodologies, TF members have to anticipate their use and in their proposed assessments of the critical areas of concern identified in the EFSA conclusion. This should be reviewed once the new GD is available and no use should be cancelled until then.

Submission and Evaluation of Copper compounds under Art.43 of 1107/2009

General observation: Copper compounds should not be considered as Candidate for Substitution (CfS).

The implementing Regulation (EU) 2018/1981 is renewing the approval of the active substance Copper compounds as candidate for substitution (CfS), in accordance with Regulation (EC) 1107/2009. Whereas (12) considers that Copper compounds are persistent and toxic in accordance with points 3.7.2.1 and 3.7.2.3 of Annex II to Regulation (EC) 1107/2009 (PBT assessment), and fulfil the condition set in the second indent of point 4 of Annex II to Regulation (EC) 1107/2009.
The EUCuTF disagrees with the approval as CfS. The conditions in Annex to Regulation (EC) 1107/2009 lack the exemption of inorganic compounds like Copper minerals from the PBT assessment as it has been established under other chemical legislations like REACh and BPD. As laid down in those legislations, the term persistence is meaningless for an element or mineral, due to its natural occurrence. Persistence per se is therefore not a relevant parameter and consequently a PBT assessment is not carried out for inorganic compounds under REACh and BPD. The recent mandate from COM to EFSA directs the development of a guidance towards methods and procedures available under those legislations better adapted for the assessment of inorganic compounds, where the relevant parameter is their bioavailability. This should include an exempt statement regarding the PBT assessment to harmonize the assessment of the same compounds under different legislations.
It should be noted that persistence of minerals is considered not relevant for being categorized as low-risk active substance according to Regulation (EU) 2017/1432. This is clearly not compatible with the same parameter leading to a classification as CfS under the same Regulation (EC) 1107/2009.
The EUCuTF is of the opinion that Copper compounds should not be considered CfS, and have lodged an action for annulment against Regulation (EU) 2018/1981 and renewing the approval of the active substance Copper compounds as candidate for substitution (case number T-153/19 European Union Task Force v. European Commission).

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

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 as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
4	PL	Strawberry	G	*Marssonina fragariae, Zythia fragariae Mycosphaerella, bacterial disease, Colletotrichum sp.*	Foliar spray	BBCH 13 - BBCH 85	a) 3 b) 3	7	a) 1.33 b) 3.99	a) 1.0 b) 3.0	200 - 800	3								
5	PL	Tomato Eggplant Pepper	G	*Phytophtora spp., Alternaria, Colletotrichum, Bacterial disease (Pseudomonas spp., Xanthomonas spp.).*	Foliar spray	BBCH 15 - BBCH 51	a) 3 b) 3	7	a) 1.33 b) 3.99	a) 1.0 b) 3.0	200-1000	10								
7	PL	Lettuce Scarole	G	*Alternaria, Bremia lactucae Bacterial disease: Erwinia spp., Pseudomonas spp. Xanthomonas spp.*	Foliar spray	BBCH12 - BBCH49	a) 3 b) 3	7	a) 1.33 b) 3.99	a) 1.0 b) 3.0	300-1000	3								
8	PL	Cucumber	G	*Alternaria, Antracnosis, Phytophtora spp.,*	Foliar spray	BBCH 15 - BBCH 89	a) 3 b) 3	7	a) 1.33 b) 3.99	a) 1.0 b) 3.0	200-1000	3								

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

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)

Since application is intended for indoor/greenhouse use, no risk of exposure to Nordox 75 WG is to be expected for birds, mammals and terrestrial vertebrate wildlife.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

In conclusion, acceptable risk to aquatic organisms from the use of Nordox 75 WG was demonstrated for the greenhouse uses.

9.1.1.3 Effects on arthropods other than bees (KCP 10.3.2)

First tier risk assessment demonstrated a low in-field and off-field risk for non-target arthropods other than bees when the product is applied according to GAP.

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

A higher-Tier risk assessment was presented, demonstrating an acceptable risk for application rates of up to ~~8~~ 4 kg Cu/ha/year. Thus, there is no unacceptable risk for non-target soil meso- and macrofauna after exposure to Nordox 75 WG.

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

There is no unacceptable risk for non-target terrestrial plants after exposure to Nordox 75 WG.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

For risk envelopes please refer to each Chapter.

9.1.3 Consideration of metabolites

Copper is an element and therefore the formation of metabolites or breakdown products are not possible. Therefore, conducting metabolite-specific risk assessments for Nordox 75 WG is not required.

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments	The submitted justification was accepted.

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Copper compounds. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on birds of Nordox 75 WG were evaluated as part of the EU assessment of Copper. No new data are submitted with this application. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. The risk assessment is based on the EU agreed endpoints achieved with Copper compounds (EFSA Journal 2018;16(1):5152).

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

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	Copper hydroxide	Acute oral	LD_{50} = 223 mg Cu/kg bw	EFSA, 2018
Coturnix coturnix japonica	Copper hydroxide	Acute oral	LD_{50} = 556 mg Cu/kg bw	EFSA, 2018
Colinus virginianus	Copper hydroxide WP	Acute oral	LD_{50} = 357 mg Cu/kg bw	EFSA, 2018
Colinus virginianus	Copper oxychloride	Acute oral	LD_{50} = 511 mg Cu/kg bw	EFSA, 2018
Coturnix coturnix japonica	**Copper oxychloride WP**	**Acute oral**	**LD_{50} = 173 mg Cu/kg bw**	EFSA, 2018
Colinus virginianus	Bordeaux mixture	Acute oral	LD_{50} > 616 mg Cu/kg bw	EFSA, 2018
Colinus virginianus	Bordeaux mixture WP	Acute oral	LD_{50} >439.9 mg Cu/kg bw	EFSA, 2018
Colinus virginianus	Tribassic Copper sulfate	Acute oral	LD_{50} = 616 mg Cu/kg bw	EFSA, 2018
Colinus virginianus	Tribasic Copper sulfate SC	Acute oral	LD_{50} > 72.4 mg Cu/kg bw	EFSA, 2018
Coturnix coturnix japonica	Tribasic Copper sulfate SC	Acute oral	LD_{50} = 221 mg Cu/kg bw	EFSA, 2018
Coturnix coturnix japonica	Copper oxide	Acute oral	LD_{50} = 1183 mg Cu/kg bw	EFSA, 2018
Coturnix coturnix japonica	Copper oxide WG (Nordox 75 WG)	Acute oral	LD_{50} = 650 mg/kg bw	EFSA, 2018
Colinus virginianus	Copper oxychloride	Short-term	LC_{50} = 1939 mg Cu/kg bw/day LD_{50} = 333 mg Cu/kg feed	EFSA, 2018

Species	**Substance**	**Exposure System**	**Results**	**Reference**
Colinus virginianus	Bordeaux mixture	Short-term	LC_{50} > 1369 mg Cu/kg bw/day LD_{50} > 334.1 mg Cu/kg feed	EFSA, 2018
Colinus virginianus	Copper hydroxide	Short-term	NOEL = 123.6[b] mg Cu/kg bw/day NOEC = 883[c] mg Cu/kg feed	EFSA, 2018
Anas platyrhynchos	Copper hydroxide	Short-term	NOEL = 215.6[b] mg Cu/kg bw/day NOEC = 1053[c] mg Cu/kg feed	EFSA, 2018
Colinus virginianus	Copper hydroxide	Short-term	NOEL = 135.2[b] mg Cu/kg bw/day NOEC = 963[c] mg Cu/kg feed	EFSA, 2018
Anas platyrhynchos	Copper hydroxide	Short-term	NOEL = 190.6[b] mg Cu/kg bw/day NOEC = 963[c] mg Cu/kg feed	EFSA, 2018
Colinus virginianus	Tribasic Copper sulfate	Short-term	NOEL = 89[b] mg Cu/kg bw/day NOEC = 246[c] mg Cu/kg feed	EFSA, 2018
Anas platyrhynchos	Tribasic Copper sulfate	Short-term	NOEL = 176.3[b] mg Cu/kg bw/day NOEC = 530[c] mg Cu/kg feed	EFSA, 2018
Colinus virginianus	Copper oxide	Short-term	NOEL = 32[b] mg Cu/kg bw/day NOEC = 136[c] mg Cu/kg feed	EFSA, 2018
Colinus virginianus	**Copper hydroxide**	**Long-term**	**NOEL = 5.05 mg/kg bw/d** NOEC = 57.5 mg Cu/kg feed	EFSA, 2018
Anas platyrhynchos	Copper hydroxide	Long-term	NOEL = 42.34 mg Cu/kg bw/day NOEC = 288 mg Cu/kg feed	EFSA, 2018
Colinus virginianus	Copper hydroxide	Long-term	NOEL = 25.41 mg Cu/kg bw/day NOEC = 288 mg Cu/kg feed	EFSA, 2018
Anas platyrhynchos	Copper hydroxide	Long-term	NOEL = 50.3 mg Cu/kg bw/day NOEC = 288 mg Cu/kg feed	EFSA, 2018

Values in **bold** used for the risk assessment
b: LD_{50} was not relevant because of food avoidance
c: LC_{50} was not relevant because of food avoidance

9.2.1.1 Justification for new endpoints

The risk assessment is based on the EU agreed endpoints achieved with Copper compounds (EFSA Journal 2018;16(1):5152).

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)

Since application is intended for indoor/greenhouse use, there is no risk of exposure to Nordox 75 WG for birds. Therefore, a risk assessment is not necessary.

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

Not required.

9.2.2.4 Effects of secondary poisoning

Not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not required.

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

Not required.

9.2.4 Overall conclusions

Since application is intended for indoor/greenhouse use, no risk of exposure to Nordox 75 WG is to be expected for birds.

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

zRMS Comments	The submitted justification was accepted.

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with Copper. 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 Nordox 75 WG were evaluated as part of the EU assessment of Copper. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. The risk assessment is based on the EU agreed endpoints achieved with Copper compounds (EFSA Journal 2018;16(1):5152).

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

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

9.3.1.1 Justification for new endpoints

The risk assessment is based on the EU agreed endpoints achieved with Copper compounds (EFSA Journal 2018;16(1):5152).

9.3.2 Risk assessment for spray applications

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

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

Since application is intended for indoor/greenhouse use, there is no risk of exposure to Nordox 75 WG for mammals. Therefore, a risk assessment is not necessary.

9.3.2.2 Higher-tier risk assessment

Not required.

9.3.2.3 Drinking water exposure

Not required.

9.3.2.4 Effects of secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not required.

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

Not required.

9.3.4 Overall conclusions

Since application is intended for indoor/greenhouse use, no risk of exposure to Nordox 75 WG is to be expected for mammals.

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

Since application is intended for indoor/greenhouse use, there is no risk of exposure to Nordox 75 WG for terrestrial vertebrate wildlife. Therefore, a risk assessment is not necessary.

9.5 Effects on aquatic organisms (KCP 10.2)

zRMS Comments	The submitted justification was accepted. The BLM model was not accepted. The endpoints submitted by the Applicant were amended in accordance with LoEP of EFSA, 2018.

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Copper. Full details of these studies are provided in the respective EU DAR.
Effects on aquatic organisms of Nordox 75 WG were evaluated as part of the EU assessment of Copper.

Please note that only the lowest EU agreed endpoint which are relevant for the aquatic risk assessment are presented in the following table.

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Copper oxide	96 h, f	LC_{50} = 0.207 mg TOTAL Cu/L (mm) LC_{50} = 0.0344 mg DISSOLVED Cu/L (mm)	EFSA, 2018
Oncorhynchus mykiss	Copper hydroxide WP	96 h, f	LC_{50} = 0.0165mg TOTAL Cu/L (mm) LC_{50} = 0.0080 mg DISSOLVED Cu/L (mm)	EFSA, 2018
Acipenser transmontanus	Copper sulphate	14 d; 28 d; 53 d, f	53-d EC_{10} (growth) = 0.00112 mg DISSOLVED Cu/L	EFSA, 2018
Daphnia magna	Copper hydroxide	48 h, s	EC_{50} = 0.0266 mg DISSOLVED Cu /L	EFSA, 2018

Species	Substance	Exposure System	Results	Reference
			(mm)	
Daphnia magna	Copper oxychloride	21 d, ss	NOEC = 0.0076 mg TOTAL Cu/L (geometric mean measured)	EFSA, 2018
Chironomus riparius	Tribasic Copper sulphate	28 d, s, spiked sediment	NOEC = 0.5 mg TOTAL Cu/L $_{nom}$ (Water spiked test)	EFSA, 2018
Tubifex tubifex	Copper chloride	28 d, ss, spiked sediment	NOEC = 16.17 mg/kg dry weight normalised to 2.5% OC	EFSA, 2018
Selenastrum capricornutum	Copper hydroxide WP	72 h, s	Growth rate E_rC_{50} = 0.02229 mg TOTAL Cu/L (nom)	EFSA, 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 DISSOLVED Cu/L (mm) (AF = 2 applied)	EFSA, 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 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Nordox 75 WG

Species	Substance	Exposure System	Results	Reference
O. mykiss	Nordox 75 WG	96 h, f	LC_{50} = 0.207 mg TOTAL Cu/L (mm) LC_{50} = 0.0344 mg DISSOLVED Cu/L (mm)	EFSA, 2018
O. mykiss	Nordox 75 WG	96 h, f	LC_{50} = 0.047 mg TOTAL Cu/L (mm) LC_{50} = 0.0106 mg DISSOLVED Cu/L (mm)	EFSA, 2018
O. mykiss	Nordox 75 WG	96 h, ss	LC_{50} = 4.37 mg TOTAL Cu/L (nom)	EFSA, 2018
O. rerio (embryo)	Nordox 75 WG	48 h, ss	NOEC = 1.06 mg TOTAL Cu/L (nom)	EFSA, 2018
D.magna	Nordox 75 WG	48 h, s	LC_{50} = 0.45 mg TOTAL Cu/L (nom)*	EFSA, 2018
D.magna	Nordox 75 WG	21 d, ss	NOEC = 0.0122 mg TOTAL Cu/L (mm)	EFSA, 2018
P. subcapitata	Nordox 75 WG	72 h, s	E_bC_{50} = 0.147 mg TOTAL Cu/L (mm) E_rC_{50} = 0.299 mg	EFSA, 2018

Species	**Substance**	**Exposure System**	**Results**	**Reference**
			TOTAL Cu/L (mm) E_bC_{50} = 0.045 mg DISSOLVED Cu/L (mm) E_rC_{50} = 0.133 mg DISSOLVED Cu/L (mm)	
Higher-tier studies (micro- or mesocosm studies)				
Not available for the formulation.				
s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations				

* The dilution medium used in this study is the Elendt M4 medium which contains EDTA. This chelating agent is known to have an outcome on the biological resulst as it chelates metals such as Copper. Therefore, the results from this study should not be used for the purpose of risk assessment.

9.5.1.1 Justification for new endpoints

The risk assessment is based on the EU agreed endpoints achieved with Copper compounds (EFSA Journal 2018;16(1):5152). For further justifications please see below.

~~**9.5.1.1.1 Use of Biotic Ligand Model**~~

~~Both EFSA and the RMS rejected all novel methods proposed by the notifier for assessing the exposure and risks from the use of Copper, 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. Art.43 submissions will provide an update of the BLM and supporting validation data, as well as justifying cross-species extrapolation.~~

~~**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-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, P, 2019

9.5.1.1.1 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 and 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 ad-verse 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 (anthropo-genic) 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.

Comments of zRMS:	The submitted paper was not evaluated at the zonal level. The paper was not accepted in Poland.

9.5.1.1.2 Aquatic ~~dwelling~~ organisms

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 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_{5-50} 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 MCA-8, point 8.2.8/02 (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) (CA 8.2.8/04), an 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 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 2x 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 □g/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 HC5 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 HC5 cumulative distributions for the different zones, based on the physico-chemical parameters (DOC, pH) from Foregs, is provided in the figure below:

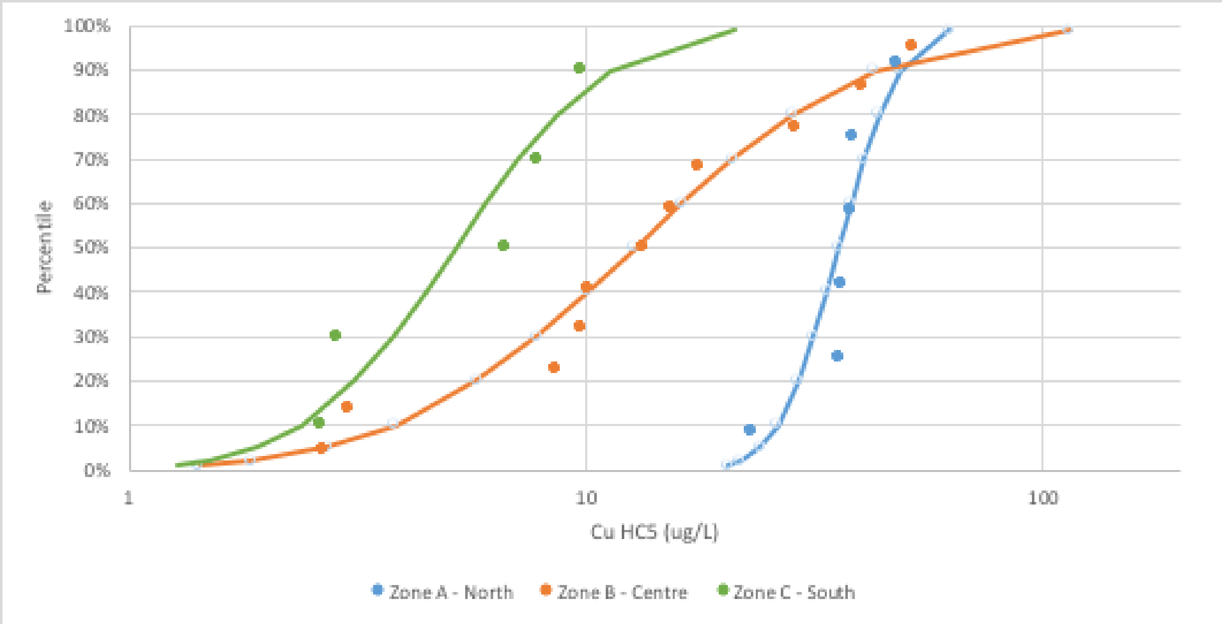


Figure 1: Overview of the Cu HC_5 cumulative distributions for the different PPP zones

From Figure 1 both median (50th %) and realistic worst case (10th %) HC_5 for Cu could be calculated as shown in Table 9.5-3. 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 9.5-3: Overview of the Cu HC_5 values for the different PPP zones

Percentile	**HC_5 – Zone A**	**HC_5 – Zone B**	**HC_5 – 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 HC_5 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, K and Verdonck, F, 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 (CA 8.2.2.1/01) to predict effects of trout populations in realistic conditions. The results of this modelling are summarized below:

Reference:	**KCP 10.2/03, Janssen, S.D., Viaene, K., Van Sprang, P., Deschamphelaere, K., 2019**
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 per-formed 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 under-stand how the effects of a toxicant on individual-level endpoints (i.e. survival, reproduction) trans-late 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 Fraun-hofer test. However, this exposure pattern is not realistic as Funguran-OH is typically applied mul-tiple 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 (EC10 = 1.7 µg Cu/L and EC50 = 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 fac-tor 4 higher for the pulse exposure scenario (7.99 µg/L). Although roughly the same for the contin-uous exposure scenario, the EC50 value for the pulse exposure scenario (9.57 µg/L) was a factor 2 higher compared to the toxicity experiment.

Janssen, S.D. 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

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 will provide 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/gOC. 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 HC_5 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, M, 2019

Comments of zRMS:	The submitted paper was not evaluated at the zonal level. The NOEC for *Tubifex tubifex* with AF = 3 was used in risk assessment.

9.5.2 Risk assessment

9.5.2.1 Risk Assessment for Aquatic ~~Dwelling~~ Organisms

According to the List of Endpoints of Copper, the "Dutch Model" was used for the PEC_{SW} greenhouse calculations.
As discussed above, to achieve a concise risk assessment for aquatic dwelling organisms, an ETO-$_{RACsw;\ ch}$ value of 4.8 µg/L was used as this value was protective of all acute and chronic risks to all relevant aquatic species.

Greenhouse uses

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) based on FOCUS Step 2 maximum PEC_{sw} values for the use of Nordox 75 WG following a single application – greenhouse uses

Group	Aquatic dwelling organisms		
Endpoint [NOEC, µg/L]	4.8		
AF	~~1~~ 2		
ETO RAC_{sw} [µg/L]	~~4.8~~ 2.4		
Uses	**Application rate [kg a.s./ha]**	**“Dutch Model” assuming 0.1 % drift**	
		PECsw [µg/L]	**PEC/RAC**
All uses (worst case application rate)	1.00	0.33	~~0.07~~ 0.14

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

PEC/RAC value is lower than 1 thus indicating no concerns regarding the acute or chronic risks to aquatic organisms from the proposed uses of Nordox 75 WG.

9.5.2.2 Risk Assessment for Sediment Dwelling Organisms

In the following table, the ratios between predicted environmental concentrations in sediment (PEC_{sed}) and the regulatory acceptable concentration (RAC) for sediment dwelling organisms are given per intended use for each FOCUS scenario.
To calculate the PEC sediment accumulation over seven years, the FOCUS step 2 sediment via spray drift and run-off /drainage with a K_{doc} worst case default value of 10,000 mL/g values are added to a **median background level of copper in European sediments of 17 mg/kg**.
For the greenhouse uses, no risk assessment for sediment dwelling organisms is required.

9.5.3 Overall conclusions

In conclusion, acceptable risk to aquatic organisms from the use of Nordox 75 WG was demonstrated for the greenhouse uses.

9.6 Effects on bees (KCP 10.3.1)

Evaluator Comment:	The submitted study reports were accepted (2020) and calculated endpoints were used in risk assessment. The risk assessment for bees considering the standard approach (SANCO, 2002) was

added by the evaluator as SANCO, 2002, as guidance is still in force.

The risk envelope approach is proposed – a maximum of single rate of 1000 g Cu/ha

Intended use	Peas
Active substance	Copper
Application rate (g a.s./ha)	1000

Test design	LD_{50} (µg Cu/bee)	Single application rate (g a.s./ha)	Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$
Oral toxicity	> 116	1000	< 8.62
Contact toxicity	> 82.5		< 12.12

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

The screening step calculations with the formulation endpoints show the hazard quotient for oral and contact exposure of honey bees below the trigger of 50 indicating an acceptable risk.
The further risk refinement is not required.

The new chronic studies were submitted and accepted. The following endpoints were derived:
Adult bees, chronic oral (Colli, 2018):
NOEDD = 0.177 µg Cu/bee/d
LDD_{10} = 0.310 µg Cu/bee/d
LDD_{20} = 0.365 µg Cu/bee/d
LDD_{50} = 0.466 µg Cu/bee/d

NOEC = 6.325 mg Cu/kg diet
EC_{10} = 10.071 mg Cu./kg diet
EC_{20} = 14.099 mg Cu/kg diet
EC_{50} = 23.432 mg Cu/kg diet
The endpoints of the test were calculated with respect to the nominal concentration of the test item.

Larvae, chronic oral (Colli, 2017):
NOED = 14.17 µg Cu/larva
LD_{10} = 5.10 µg Cu/larva
LD_{20} = 8.82 µg Cu/larva
LD_{50} = 20.21 µg Cu/larva

NOEC = 92.0 µg Cu/g diet
EC_{10} = 33.11 µg Cu/g diet
EC_{20} = 57.30 µg Cu/g diet
EC_{50} = 129.67 µg Cu/g diet.
The endpoints of the test were calculated with respect to the nominal concentration of the test item.

An acceptable overall risk to bees is indicated for the intended GAP use.

Risk assessment for bees based on new EFSA guidance, 2013, was not evaluated as the guidance is not in force.

9.6.1 Toxicity data

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 (France, 2017) and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees from exposure to the representative formulations were also evaluated as part of the EU assessment of Copper compounds. Owing to the increased level of toxicity of Copper oxychloride to bees, further studies were conducted with this substance to represent the worst-case exposure to Copper through exposure to any of the supported forms of the active substance.

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

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

Species	**Substance**	**Exposure System**	**Results#**	**Reference**
Apis mellifera	Copper hydroxide technical	Acute	Contact toxicity LD_{50} = 44.46 µg/bee	EFSA, 2018
Apis mellifera	Copper hydroxide WP	Acute	Oral toxicity LD_{50} = 49.0 µg/bee Contact toxicity LD_{50} >57 µg/bee	EFSA, 2018
Apis mellifera	Copper oxychloride	Acute	Oral toxicity LD_{50} = 12.1 µg/bee Contact toxicity LD_{50} = 44.3 µg/bee	EFSA, 2018
Apis mellifera	Bordeaux Mixture WP	Acute	Oral toxicity LD_{50} = 23.3 µg/bee Contact toxicity LD_{50} >25.2 µg/bee	EFSA, 2018
Apis mellifera	Tribasic Copper sulfate SC	Acute	Oral toxicity LD_{50} = 40 µg/bee Contact toxicity LD_{50} >23.5 µg/bee	EFSA, 2018
Apis mellifera	Copper oxide technical	Acute	Contact toxicity LD_{50} > 22.0 µg/bee	EFSA, 2018
Apis mellifera	Copper oxide WG*	Acute	**Oral toxicity** **LD_{50} >116.0 µg/bee** **Contact toxicity** **LD_{50} > 82.5 µg/bee**	EFSA, 2018
Apis mellifera	Copper oxychloride 50 % WP	Adult, chronic oral	**LDD_{50} = 0.466 µg Copper/bee/day** LC_{50} = 10.07 mg Cu/kg food NOEDD = 0.177 µg Cu/bee/day **NOEC =** 6.33 mg Cu/kg food	Colli, 2018 KCP 10.3.1.2/01
Apis mellifera	Copper oxychloride	Larval mortality	**NOED = 14.17 µg**	Colli, 2017

Species	Substance	Exposure System	Results[#]	Reference
	50 % WP	Adult emergence	**Cu/ lava/day** **NOED = 14.17 µg Cu/ larva/day**	KCP 10.3.1.2/02
Higher-tier studies (tunnel test, field studies)				
Two outdoor cages were performed with Copper oxychloride WP and Bordeaux Mixture. No significant effects at rates up to 1.25 kg a.s./ha. A tunnel test wes performed with Copper oxychloride WP on *Phacelia* with single application rates of up to 2.5 kg Cu/ha. A statistically significant reduction (30 %) is observed on flight intensity at a rate of 2.5 kg a.s/ha on Day 1 of application. Total recovery from all transient effects were noted 18 DAA. In conclusion, the application of Copper up to a rate of 2.5 kg/ha has no significant effect on be population and colony development (France, 2018).				

* equivalent to Nordox 75 WG
[#]Values in **bold** used for the risk assessment

9.6.1.1 Justification for new endpoints

The acute risk assessment of the effects of Nordox 75 WG on bees was conducted using the EU evaluated endpoints for Copper oxide WG (Nordox 75 WG). The chronic risk assessment is based on new endpoints derived with Copper oxychloride 50 % WP, which represent a worst-case exposure to Copper. Copper oxychloride 50 % WP resulted in a clearly higher acute toxicity to honeybees (please refer to Table 9.6-1).

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the new bee Guidance Document EFSA (2013[1]). But it should be noted that the new EFSA Guidance Document on bees (2013) has not yet been adopted on EU level. Calculations are based on the Bee-Tool v.3.
In accordance with the Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015:EN-924), as no data for bumble bees or solitary bees was available, no risk assessments for these species were performed.

9.6.2.1 Hazard quotients for bees

Acute risk
In the following the acute risk for bees is assessed. To achieve a concise risk assessment, a risk envelope approach is applied: For the Screening assessment an application rate of 1.0 kg a.s./ha is foreseen in all intended uses and covers the risk to bees from all uses.

In the following the acute risk for bees is assessed, results are summarized in the following table:

[1] EFSA (2013: EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees); EFSA Journal 2013;11(7):3295

Table 9.6-2: Screening assessment of the acute risk for bees

Intended use		All uses (use 4-8)			
Active substance		Copper			
Application rate		1.0 kg/ha			
Test design	**LD_{50} (lab.) [µg/bee]**	**Single application rate [g/ha]**	**Risk parameter according to EFSA (2013)**	**Risk value***	**Trigger**
Oral toxicity	> 116.0	1000	HQ	< 12.1	42
Contact toxicity	> 82.5		ETR	< 0.07	0.2

*Values in **bold** indicate unacceptable risks

In conclusion, the presented assessment based on the EU-agreed endpoints for Copper (I) oxide demonstrates an acceptable acute risk to honeybees.

Chronic risk

In the following the chronic risk for bees is assessed. To achieve a concise risk assessment, a risk envelope approach is applied: For the Screening assessment an application rate of 1.0 kg a.s./ha is foreseen in all intended uses and covers the risk to bees from all uses.

Table 9.6-3: Screening assessment of the chronic risk for adult bees

Scenario	**AR** [kg a.s./ha]	**LDD_{50}** [µg a.s./bee/day]	**Shortcut value**	**ETR**	**Trigger value**
Chronic Honey bee adult					
All uses	1.0	0.466	7.6	**16.309**	< 0.03

Values in **bold** indicate unacceptable risks

Table 9.6-4: Screening assessment of the chronic risk for bee larvae

Scenario	**AR** [kg a.s./ha]	**$NOED_{larvae}$** [µg a.s./larvae/ developmental period]	**Shortcut value**	**ETR**	**Trigger value**
Chronic Honey bee larvae					
All uses	1.0	14.17	4.4	**0.31**	< 0.2

Values in **bold** indicate unacceptable risks

In conclusion, the screening assessments of chronic risks to adult and larvae honey bees from the use of Copper indicates that there may be unacceptable risks. Therefore, a 1st tier assessment is presented below.

1st Tier risk assessment

A 1st Tier risk assessment was conducted in accordance with EFSA (2013[2]). Results are presented in the following tables. Please note, for indoor uses only the scenario "foraging on treated crops" is relevant.

[2] EFSA (20139: EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees); EFSA Journal 2013;11(7):3295

Table 9.6-5: Adult chronic - 1st Tier risk assessment

Crop	BBCH	Honeybee	
		ETR	Trigger value
Foraging on treated crops			
Strawberry	10 - 39	**8.96**	0.03
	40 - 69	**8.96**	0.03
	≥ 70	0.00	0.03
Tomato, eggplant	10 - 49	**1.42**	0.03
	50 - 69	**1.42**	0.03
Lettuce	10 - 49	**1.42**	0.03
	50 - 69	**1.42**	0.03
Cucumber	10 - 49	**8.96**	0.03
	50 - 69	**8.96**	0.03
	≥ 70	0.00	0.03

Table 9.6-6: Bee larvae – 1st Tier risk assessment

Crop	BBCH	Honeybee	
		ETR	Trigger value
Foraging on treated crops			
Strawberry	10 - 39	**0.26**	0.2
	40 - 69	**0.26**	0.2
	≥ 70	0.00	0.2
Tomato, eggplant	10 - 49	0.01	0.2
	50 - 69	0.01	0.2
Lettuce	10 - 49	0.01	0.2
	50 - 69	0.01	0.2
Cucumber	10 - 49	**0.26**	0.2
	50 - 69	**0.26**	0.2
	≥ 70	0.00	0.2

The first-tier assessment of the risks to bees from the use of Copper indicates that there may be unacceptable risks, especially regarding adult honeybees. Please refer to Section 9.6.2.2 for further consideration.

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

Two outdoor cage tests were performed with Copper oxychloride WP and Bordeaux Mixture. A comparison of the bee endpoints demonstrates, that Copper oxychloride and Bordeaux Mixture WP are the most toxic products (Table 9.6-1). Thus, it is deemed to be applicable to extrapolate to Nordox 75 WG (Copper (I) oxide). In the outdoor cage tests no significant effects occur at rates up to 1.25 kg Cu/ha (EFSA, 2018).

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, 2018).

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*".

Thus, risks following exposure of Copper to bees are acceptable for single dose rates of up to 2.5 kg a.s./ha.

9.6.3 Effects on bumble bees

No tests submitted on EU level. Based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015:EN-924) not required for product registration.

9.6.4 Effects on solitary bees

No tests submitted on EU level. Based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015:EN-924) not required for product registration.

9.6.5 Overall conclusions

The risks following exposure of Copper to bees are acceptable for single dose rates of up to 2.5 kg a.s./ha.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

Evaluator comment:	The submitted risk assessment was accepted. The justification for non-submission of off-field exposure was accepted. An acceptable risk to non-target arthropods is indicated for the intended GAP use.

9.7.1 Toxicity data

In the framework of the renewal of the active substance 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, 2017) and related documents.

Effects on non-target arthropods of five representative formulations were evaluated as part of the EU assessment of Copper compounds, among the product Copper (I) oxide WG (Nordox 75 WG). The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results [kg Cu/ha]	Reference
Aphidius rhopalosiphi	Copper hydroxide	Laboratory test glass plates (2D)	LR_{50} = 0.05	EFSA, 2018
	Bordeaux Mixture	Laboratory test glass plates (2D)	LR_{50} > 14.7	EFSA, 2018
	Tribasic Copper sulphate	Laboratory test glass plates (2D)	LR_{50} > 0.1344	EFSA, 2018
	Copper oxide (Copper (I) oxide WG; 750 Copper/kg)*	**Laboratory test glass plates (2D)**	**LR_{50} = 39.2**	EFSA, 2018
Typhlodromus pyri	Copper hydroxide	Laboratory test glass plates (2D)	LR_{50} > 14.88	EFSA, 2018
	Copper oxychloride	Laboratory test glass plates (2D)	LR_{50} > 14.89	EFSA, 2018
	Bordeaux Mixture	Laboratory test glass plates (2D)	LR_{50} > 13.2	EFSA, 2018
	Tribasic Copper sulphate	Laboratory test glass plates (2D)	LR_{50} > 0.08	EFSA, 2018
	Copper oxide (Copper (I) oxide WG; 750 Copper/kg)*	**Laboratory test glass plates (2D)**	**LR_{50} > 26.1**	EFSA, 2018
Aphidius rhopalosiphi (adults)	Copper oxychloride WP	Extended laboratory test (3D)	Mortality: 0.0 % at 1.0 0.0 % at 3.97 Fecundity: -22.38 % at 1.0 10.89 % at 3.97	EFSA, 2018
Aphidius rhopalosiphi (adults)	Tribasic Copper sulphate	Extended laboratory test (3D)	Mortality: 0.0 % at 0.00154 2.5 % at 0.00768 2.5 % at 0.0384 5.0 % at 0.192 2.5 % at 0.960 Fecundity: -29.8 % at 0.00154 -72.6 % at 0.00768 -40.4 % at 0.0384 -13.8 % at 0.192 30.5 % at 0.960	EFSA, 2018
Aphidius rhopalosiphi (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 10 % at 3.213 Fecundity: -7.4 % at 3.213	
Typhlodromus pyri (protonymphs)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: -7.4 % at 3.213	EFSA, 2018

Species	**Substance**	**Exposure System**	**Results [kg Cu/ha]**	**Reference**
			Fecundity: 16.9 % at 3.213	
Typhlodromus pyri (protonymphs)	Tribasic Copper sulphate SC	Extended laboratory test (3D)	Mortality: 1.8 % at 0.015 3.5 % at 0.06 13.9 % at 0.25 3.5 % at 1.01 0.0 % at 4.032 Fecundity: -7.3 % at 0.015 -17.1 % at 0.06 -11.0 % at 0.25 12.2 % at 1.01 31.7 % at 4.032	EFSA, 2018
Chrysoperla carnea (larvae)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 1.25 % at 1.922 Fecundity: 0 % at 1.922	EFSA, 2018
Chrysoperla carnea (larvae)	Copper oxychloride WP	Extended laboratory test (3D)	Mortality: 4.8 % at 0.5 21.4 % at 1.0 11.9 % at 2.0 23.8 % at 4.0 40.5 % at 8.0 Fecundity: 1.7 % at 0.5 16.7 % at 1.0 7.9 % at 2.0 15.3 % at 4.0 6.7 % at 8.0	EFSA, 2018
Chrysoperla carnea (larvae)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 55.6 % at 0.56 Fecundity: 71.1 % at 0.56	EFSA, 2018
Trichogramma cacoeciae (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Parasitisation: 6.4 % at 0.59	EFSA, 2018
Trichogramma cacoeciae (adults)	Copper oxychloride WP	Extended laboratory test (3D)	Parasitisation: -42.9 % at 2.02	EFSA, 2018
Diaeretiella rapae (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 14.8 % at 0.59 Parasitisation: 52.5 % at 0.59	EFSA, 2018
Poecilus cupreus (adults)	Copper hydroxide WP	Extended laboratory test (3D)	Mortality: 0 % at 0.59 Predation: 8.0 % at 0.59	EFSA, 2018
Pardosa	Tribasic Copper	Extended laboratory	Mortality:	EFSA, 2018

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

* equivalent to Nordox 75 WG

9.7.1.1 Justification for new endpoints

The risk assessment of the effects of Nordox 75 WG on arthropods other than bees was conducted using the EU agreed endpoints for Copper (I) oxide.

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.

In the review of the data submitted for the approval of Copper compounds, the ecotox experts’ meetings agreed that, to assess the risks to soil dwelling arthropods, the total amount of Copper applied in the season should be used since it cannot be ensured that dissipation occurs between successive applications. Hence a MAF_{soil} of 1 was taken into account.

To achieve a concise risk assessment, the risk envelope approach is applied. An application rate of 1.0 kg a.s./ha is foreseen in all intended uses and is used in the following risk assessment.

9.7.2.1 Risk assessment for in-field exposure

Results of the 1st Tier assessment of the in-field use are presented in the following tables. First, calculations based on a foliar assessment, taking into account the $MAF_{foliage}$ are presented:

Table 9.7-2: First-tier foliar assessment of the in-field risk for non-target arthropods due to the use of Nordox 75 WG, application rate 3 x 1.0 kg a.s./ha (use 4, 5, 7, 8)

Intended use	All uses (use 4-8)		
Active substance/product	Copper (I) oxide / Nordox 75 WG		
Application rate	3 × 1.0 kg a.s./ha, 7 day interval		
MAF (foliage)*	2.7		
Test species Tier I	**LR_{50} (lab.) [kg Cu/ha]**	**$PER_{in-field}$ [kg Cu/ha]**	**$HQ_{in-field}$** criterion: HQ ≤ 2**
Aphidius rhopalosiphi	39.2	2.7	0.07
Typhlodromus pyri	> 26.1		< 0.10

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
*According to ESCORT 2 (Candolfi et al. 2000)
** Criteria values shown in bold breach the relevant trigger.

To assess the risks to soil dwelling arthropods, the total amount of Copper applied in the season should be used according to the ecotox expert meeting (for details please see above). Hence in the risk assessment presented below a MAF_{soil} of 1 was taken into account.

Table 9.7-3: First-tier soil assessment of the in-field risk for non-target arthropods due to the use of Nordox 75 WG application rate 3 x 1.0 kg a.s./ha (use 4, 5, 7, 8)

Intended use	Tomato / cucurbits		
Active substance/product	Copper (I) oxide / Nordox 75 WG		
Application rate	3.00 kg Cu/ha		
MAF (soil)	1.0		
Test species Tier I	**LR_{50} (lab.) [kg Cu/ha]**	**$PER_{in-field}$ [kg Cu/ha]**	**$HQ_{in-field}$* criterion: HQ ≤ 2**
Aphidius rhopalosiphi	39.2	3.00	0.08
Typhlodromus pyri	> 26.1		< 0.11

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

The first-tier risk assessment demonstrates a low in-field risk for non-target arthropods other than bees when the product is applied according to the GAP.

9.7.2.2 Risk assessment for off-field exposure

Exposure to non-target arthropods living in off-field areas will mainly be due to spray drift from field applications. In principle, an off-field risk to non-target arthropods from indoor applications is deemed to be unlikely. The in-field risk assessment demonstrated a low in-field risk thus, also an off-field risk to non-target arthropods from indoor applications of Nordox 75 WG according to the GAP can be excluded.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation is needed.

9.7.3 Overall conclusions

First tier risk assessment demonstrates a low risk for non-target arthropods other than bees when the product Nordox 75 WG is applied according to the GAP. No risk mitigation is needed.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

Evaluator comment:	The submitted studies were evaluated and accepted at the EU level and endpoints used in risk assessment were agreed at EU level. The tiered approach was used in risk assessment. Based on information included in LoEP for copper compounds: '*Field study - A field study on earthworm populations has been conducted over 10 years on grassland, with copper applications every year. After 10 years of treatment with copper the NOAEC of the study is the dose rate 4 kg copper/ha/year.'* In accordance with EFSA, 2018, 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 macro-organisms. Taking into account proposed pattern use of maximum 3.0 kg Cu/ha/year in vegetables in greenhouses, the risk assessment non-target soil meso- and macrofauna is acceptable for the intended GAP use.

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 Copper. Full details of these studies are provided in the respective EU DAR (France, 2017) and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Nordox 75 WG were not evaluated as part of the EU assessment of Copper. However, the provision of data on the Nordox 75 WG is not considered essential, because the type of formulation and co-formulant substances used in formulations of different Copper compound variants are not expected to affect the behaviour or the levels of toxicity of the active substance Copper in the environment. As such it is considered that the risks to soil meso-and macrofauna can be assessed for Nordox 75 WG once the exposure and toxicity endpoints are presented in terms of the active substance Copper. This is considered valid without restriction for the purposes of the risk assessment.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Please note that only the lowest EU agreed endpoint which are relevant for the risk assessment are presented in the following table.

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

Species	**Substance**	**Exposure System**	**Results [mg Cu/kg dw]**	**Reference**
Eisenia fetida	Copper oxychloride	Mixed into substrate / 28 d, chronic 3.9 % peat content	NOEC = 8.4	EFSA, 2018
Folsomia candida	Copper chloride	Mixed into substrate 28 d, chronic 1.4-37 % peat content	EC_{10} = 31	EFSA, 2018
Hypoaspis aculeifer	Copper chloride	Mixed into substrate 21 d, chronic 3.9 % peat content	EC_{10} = 179	EFSA, 2018
Field studies				
A 10-year field study on earthworms population has been conducted on grassland with Copper applictaions every year. After 10-years of treatment with Copper, the NOEC of the study is the dose rate 4 kg Cu/ha (EFSA, 2018). For the field study please refer to the higher-tier assessment in Chapter 9.8.2.2. Based on the submitted evaluation a NOEC of 8 kg/ha/year can be concluded (Klein, O., 2019. Addendum 1 to final report, KCP 10.4./02).				
Litter bag test				
Not conducted				

9.8.1.1 Justification for new endpoints

The risk assessment of the effects of Nordox 75 WG on non-target soil meso- and macrofauna was conducted using the EU agreed endpoints.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2. According to the assessment of environmental-fate data, the following was considered for the risk assessment:

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 Chapter 8.7.2.

The PEC_{soil} accumulated values identified for use in risk assessments are 38.0 mg total Cu/kg (based on background level, 90th percentile) and 19 mg total Cu/kg (based on background level, 10th percentile) in arable fields growing tomatoes and cucurbits.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of Nordox 75 WG in arable fields (covering intended greenhouse uses)

Active substance	**NOEC [mg/kg dw]**	**Accumulated PEC_{soil} [mg/kg dw]**	**TER_{lt} (criterion TER ≥ 5)**
Chronic effects on earthworms			
Copper	8.4	38.0 (90th percentile)	**0.22**
Copper	8.4	19.0 (10th percentile)	**0.44**
Chronic effects on *Folsomia candida*			
Copper	31	38.0 (90th percentile)	**0.82**
Copper	31	19.0 (10th percentile)	**1.63**
Chronic effects on *Hypoaspis aculeifer*			
Copper	179	38.0 (90th percentile)	**4.71**
Copper	179	19.0 (10th percentile)	9.42

TER values shown in bold fall below the relevant trigger.

As the long-term first tier TER values for Copper are lower than the trigger, it is concluded that the long-term risk to earthworms and other soil organisms is not acceptable from the proposed uses of the active substances and therefore a higher tier risk assessment is required.

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 *E. fetida* and *E. andrei* but also several species belonging to the invertebrate mesofauna: the springtail *Folsomia candida*, the predatory mite *Hypoaspis aculeifer* (see Table 9.8-1), and the enchytraeid *Enchytraeus crypticus* (see A 2.4.1.1.4 e.g. Caetano et al. 2015[3], KCP 10.4/04). Referring to the information presented above it seems that earthworms are the most sensitive species among those tested so far.

[3] Caetano, A. Luísa; Marques, C. Ribeiro; Gonçalves, F., da Silva, E. Ferreira, and Pereira, R. (2015): Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values. Ecotoxicology 25, 163–177.

However, in higher-tier tests only earthworms and, partly, enchytraeids have been studied (see CA 8.4.2.1/07 Menezes-Oliveira et al. 2011[4], 2013[5]). 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 (Bouché 1977[6]): 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" (Jones et al., 1997[7]). 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[8]), 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 (CA 8.4.1/02. Klein, O. 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 earthworm 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 (Klein, O., 2019. Addendum 1 to final report, KCP 10.4./02). 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

[4] 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. Science of the Total Environment 409, 3370–3374.
[5] 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. Environmental Toxicology and Chemistry 32, 2678–2685.
[6] Bouché, M.B. (1977): Strategies lombriciennes. In: Lohm, U., Persson, T. (Eds.), Soil Organisms as Components of Ecosystems, pp. 122–132.
[7] Jones, C. G., J. H. Lawton, and M. Shachak (1997): Positive and negative effects of organisms as physical ecosystem engineers. Ecology 78:1946-1957.
[8] Van Groenigen, J. W., I. M. Lubbers, H. M. J. Vos, G. G. Brown, G. B. De Deyn and K. J. van Groenigen (2014): Earthworms increase plant production: a meta-analysis. – Scientific Reports 4: 6365; DOI: 10.1038/srep06365.

    - procedure recommended by ISO (ISO 11268-3, ISO 2014) and by De Jong et al. (2006)[9]
- 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)[10] 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)[11], 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.

[9] De Jong, F.M.W., Van Beelen, P., Smit, C.E. & Montforts, M.H.M.M. (2006) Guidance for summarising earthworm field studies – A guidance document of the Dutch platform for the assessment of higher tier studies. RIVM, The Netherlands, 47 pp. SAS INSTITUTE INC. 2002-2008 (2008) SAS® Proprietary Software 9.2; Cary, NC, USA.
[10] 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.
[11] ENVIRONMENT CANADA (2005) Guidance Document on Statistical Methods. EPS, l/RM/46. Ottawa, ON, Canada.

According to the RMS, additional statistical analysis using the LMM provided in the study report (CA 8.4.1/02. Klein, O. 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 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 (CA 8.4.1/02. Klein, O. 2015), a long-term laboratory study with earthworms and soil from the investigated sites of the field study was performed (Wagenhoff, 2018; see A 2.4.1.1, KCP 10.4/01). 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 top soils (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. water 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; see A 2.4.1.1.3, KCP 10.4/03). 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 (CA 8.4.1/02. Klein, O. 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[12]). For detailed summary please refer to KCP 10.4/05.

These RACs are based on an evaluation of a quality-screened database on chronic toxicity of Cu to earthworms (CA 8.4.1/01. Oorts, K. 2015c). 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/EC10 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/EC10 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.

[12] 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. 19 pp.

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 10th, 50th (median) and 90th percentiles, together with minimum and maximum values, of the Copper RAC data are reported (Tables 3 and 4 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 (CA 8.4.1/02. Klein, O. 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 9.8-3: Distributions of regulatory acceptable concentrations (RAC) for Cu in soil (mg Cu/kg soil dw) in the whole of Europe and major regulatory zones. (for detailed summary please refer to KCP 10.4/05)

Zone	# of data points	Min	P10	Median	P90	Max
EU	21980	46	**111**	215	374	1158
North	5129	46	**94**	175	367	1158
Centre	8133	46	**107**	219	383	1034
South	8609	46	**132**	230	369	753

Table 9.8-4: Distributions of regulatory acceptable concentrations (RAC) for Cu in soil for the land coverages under scrutiny (for detailed summary please refer to KCP 10.4/05)

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

9.8.3 Overall conclusions

A higher-Tier risk assessment was presented, demonstrating an acceptable risk for application rates of up to 8 kg Cu/ha/year. Thus, there is no unacceptable risk for non-target soil meso- and macrofauna after exposure to Nordox 75 WG.

9.9 Effects on soil microbial activity (KCP 10.5)

Evaluator comment:	The justification considering risk assessment was accepted.

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with Copper compounds. Full details of these studies are provided in the respective EU DAR (France, 2017) and related documents.
Effects on soil microorganisms of six representative formulations were evaluated as part of the EU assessment of Copper compounds, among the product Copper oxide WP (Nordox 50 WP). For a bridging statement please refer to Part C. No new data were submitted with this application.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Endpoint	**Substance**	**Exposure System**	**Results**	**Reference**
N-mineralisation	Copper hydroxide WP	62 d, aerobic soil type	No effect at day 62 at 12.5 kg Cu/ha	EFSA, 2018
	Copper oxychloride WP	28 d, aerobic soil type	No effect at day 28 at 12.4 kg Cu/ha	EFSA, 2018
	Copper oxychloride WP	28 d, aerobic soil type	No effect at day 28 at 18.1 kg Cu/ha	EFSA, 2018
	Bordeaux mixture WP	28 d, aerobic soil type	No effect at day 28 at 20.0 kg Cu/ha	EFSA, 2018
	Tribastic copper sulfate SC	28 d, aerobic soil type	No effect at day 28 at 11.6 kg Cu/ha	EFSA, 2018
	Copper oxide WP*	**28 d, aerobic soil type**	**No effect at day 28 at 15 kg Cu/ha**	**EFSA, 2018**
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 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 evolved CO_2 and 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.			

* Equivalent to Nordox 50 WP. For a bridging statement please refer to Part C.

9.9.1.1 Justification for new endpoints

The risk assessment of the effects of Nordox 75 WG on soil microbial activity was conducted using the EU agreed endpoints.

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 maximum annual application of Copper to soil from the use of Nordox 75 WG is 3.00 kg Cu/ha. Given that there were no long-term effects on soil microflora up to 15 kg Cu/ha, applied as a single application, the risks to soil microflora are expected to be very low following the use of Nordox 75 WG. Besides, a field study was already EU-evaluated, demonstrating no significant effects on evolved CO_2 and nitrogen nitrification after a 28-day incubation, based on soils contaminated with Copper hydroxide WP at 16 kg and 48 kg Cu/ha. Copper hydroxide WP (500 g Copper/kg) was used in these field tests and is considered to be representative of Nordox 75 WG. This is justified on the basis that effects of Copper over longer periods of time in soil will not be greatly influenced by the particular Copper salt. Influence from the salt or formulation is likely to occur only over short periods of time (EU DAR; France, 2017).

Based on laboratory and field data it can be concluded that an annual application of 6.0 kg Cu/ha, in the form of Nordox 75 WG is not expected to cause adverse effects on soil microbial function and so the risks following the proposed use of Nordox 75 WG are acceptable.

9.9.3 Overall conclusions

There is no unacceptable risk for soil microorganisms after exposure to Nordox 75 WG.

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

Evaluator comment:	The justification and risk assessment was accepted. No mitigation measures are required.

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with Copper. Full details of these studies are provided in the respective EU DAR (France, 2017) and related documents.
Effects on non-target terrestrial plants of five representative formulations were evaluated as part of the EU assessment of Copper compounds, among the product Nordox WG 75. No new data were submitted with this application.

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

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

Species	Substance	Exposure System	Results [kg Cu/ha]	Reference
6 species	5 different Copper based test items	21 d Vegetative vigour	ER_{50} > 2	EFSA, 2018

9.10.1.1 Justification for new endpoints

The risk assessment of the effects of Nordox 75 WG on non-target terrestrial plants was conducted using the EU agreed endpoints.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.
Exposure to non-target plants will mainly be due to spray drift from field applications. In principle, a risk from indoor applications is deemed to be unlikely. However, to exclude a risk, calculations were conducted taking into account a drift value of 0.1 % (according to "Dutch Model").

To achieve a concise risk assessment, the risk envelope approach is applied. The assessment is based on the highest yearly application rate of 3 kg a.s./ha (relevant for all intended uses).
It cannot be ensured that dissipation occurs between successive applications. Thus, a MAF_{soil} of 1 was taken into account.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of Nordox 75 WG in tomato, cucurbits

Intended use	All uses (use 4-8)			
Active substance/product	Copper			
Application rate	1 × 3 kg a.s./ha			
MAFsoil	1			
Test species	**ER_{50} [kg/ha]**	**Drift rate***	**$PER_{off-field}$ [kg/ha]**	**TER criterion: TER ≥ 5**
All	> 2.0	0.1 %	0.005	> 666

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

The presented risk assessment demonstrates that a risk to non-target plants can be excluded.

9.10.2.3 Higher-tier risk assessment

Not required.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

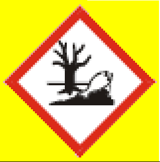
9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

Relevant toxicity	**Active substance Copper (I) Oxide**
Classification and labelling according to Regulation 1272/2008	
Hazard symbol	GHS09 
Signal word	Warning
Hazard statement	H400 Aquatic Acute 1 H410 Aquatic chronic cat.1

The proposed classification is based on ecotoxicological studies for formulation.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
~~KCP 10.2/01~~	~~Van Sprang, P.~~	~~2019~~	~~Response to EFSA comments on the aquatic effects assessment for Cu – extension~~ ~~GLP: N~~ ~~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~~ ~~CuPPP20170705~~ ~~GLP: N~~ ~~Published: No~~	~~N~~	~~EUCuTF~~
~~KCP 10.2/03~~	~~Janssen, S.D., Viaene, K., Van Sprang, P., Deschamphelaere, K.~~	~~2019~~	~~Modelling of the Funguran-OH Effects on *Onchorhynchus mykiss* Populations~~ ~~GLP: N~~ ~~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~~ ~~GLP: N~~ ~~Published: No~~	~~N~~	~~EUCuTF~~
KCP 10.3.1.2/01	Colli, M.	2018	Chronic oral effects of Copper oxychloride 50% WP to adult worker honeybees *Apis mellifera* L., 10-day feeding laboratory test BT215/17	N	EUCuTF

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
			Biotechnologie BT srl, Italy GLP: Y Published: No		
KCP 10.3.1.2/02	Colli, M.	2017	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
KCP 10.4/01	Wagenhoff, E.	2018	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 Report no. S18-00119 GLP: Y Published: No	N	EUCuTF
KCP 10.4/02	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. 20031343/G1-NFEw Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: N Published: No	N	EUCuTF
KCP 10.4/03	Amossé et al.	2018	Short-term effects of two fungicides on enchytraeid and earthworm communities under field conditions. Ecotoxicology GLP: N Published: Yes, DOI https://doi.org/10.1007/s10646-018-1895-7	N	-
KCP 10.4/04	Caetano et al.	2015	Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values. Ecotoxicology GLP: N	N	-

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Published: Yes, DOI 10.1007/s10646-015-1577-7		
KCP 10.4/05	Oorts K. and Peeters B.	2019	Distribution of RAC values for effect of Cu to soil invertebrates in Eu-rope. ARCHE Consulting, Belgium. Research report submitted to the European Copper Task Force. GLP: N Published: No	N	EUCuTF

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

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

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was evaluated at EU/zonal level in 2020. The following endpoints were calculated: LDD_{50} = 0.466 µg Cu/bee/day and LC_{50} = 23.432 mg copper/kg food. LDD_{10} = 0.310 µg Cu/bee/d LDD_{20} = 0.365 µg Cu/bee/d LDD_{50} = 0.466 µg Cu/bee/d NOEDD = 0.177 µg copper/bee/day the NOEC = 6.325 mg a.s./kg food; EC_{10} = 10.071 mg Cu./kg food EC_{20} = 14.099 mg Cu/kg food EC_{50} = 23.432 mg Cu/kg food

Reference:	KCP 10.3.1.2/01
Report:	Chronic oral effects of Copper oxychloride 50% WP to adult worker honeybees *Apis mellifera* L., 10-day feeding laboratory test, Colli, M (2018) 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 hrs/day this deviation is not considered to adversely affect the results of the study.
GLP:	Yes
Acceptability:	Yes

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.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Copper oxychloride 50% WP
	Batch no.:	183538
	Purity:	50.6% as Copper
	Date of expiry:	July 2018
2.	**Toxic Reference:**	Dimethoate
	Batch no.:	779155
	Purity:	99.5%
	Test concentrations:	Control (50 % w/v aqueous sucrose solution) 3.16, 6.33, 12.65, 25.30, 50.60 mg Copper/kg feeding solution (f.s) 1 mg dimethoate/kg feeding solution (f.s)
3.	**Test organism:**	Worker honey bees *Apis mellifera* L.
	Source:	Hives no. 4 and 7 of the Biotecnologie BT S.r.l.breeding colonies
	Age:	Max. 2 days old – young worker bees
	Housing:	Disposable cardboard cages 5×9.5×6.5 cm with a frontal transparent lid, 10 bees/cage.
	Feeding:	*ad libitum* via feeders (plastic syringes, tips removed).
4.	**Environmental conditions**	
	Temperature:	26.3 – 33.6 °C (average 32.4ºC)
	Relative humidity:	28.5 – 66.6 % (average 52.6%)
	Light:	24 hours dark, except during observations

B. STUDY DESIGN AND METHODS

1. In-life phase: 13-27 September 2017

2. Test organism assignment and 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.

3. Dose preparation

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).

4. Measurements and 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 according to the following categories:

- M: moribund
- A: affected
- C: cramps
- Ap: apathy
- V: vomiting

The amount of consumed feeding solution was determined daily by weighing each feeder with a calibrated precision balance before and after administration.

5. 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.

II. RESULTS AND DISCUSSION

A. 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- 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)

B. 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%).

C. Toxicity Endpoints

The LC_{50} and NOEC, based on nominal concentration, and the LDD_{50} and NOEDD, based on the mean uptake of test item per bee are presented in the following table. Calculated values of $LC_{20,10}$ and $LDD_{20,10}$ are also presented.

Table A 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; $\alpha = 0.05$; one sided greater)

III. CONCLUSION

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 LC_{50} 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

Comments of zRMS:	The study was evaluated at EU/zonal level in 2020. The following endpoints were calculated: NOED = ~~15.17~~ 14.17 µg Cu/bee/day LD_{10} = 5.10 µg Cu/larva LD_{20} = 8.82 µg Cu/larva LD_{50} = 20.21 µg Cu/larva NOEC = 92.00 µg a.s./g food; EC_{10} = 33.11 µg Cu/g food EC_{20} = 57.30 µg Cu/g food EC_{50} = 129.67 µg Cu/g food

Reference:	KCP 10.3.1.2/02
Report:	Effects of Copper oxychloride 50% WP to honeybees *Apis mellifera* L. Larval toxicity test, repeated exposure, Colli, M (2017) Report No: BT216/17
Guideline(s):	OECD 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

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.

	Larval Mortality (D8) [µg Copper/lava]	Adult Emergence (D22) [µg Copper/larva]
LC_{10}/ED_{10}	17.39	5.09
LC_{20}/ED_{20}	21.65	8.82
LC_{50}/ED_{50}	30.16	20.21
NOED	14.17	14.17

No developmental or behavioral abnormality was observed during the study.

I. MATERIALS AND METHODS

A. MATERIALS

1 Test materials:

Test item:	Copper oxychloride 50% WP
Batch No:	20151202003
Purity:	50.6% as Copper
Date of expiry:	July 2018
Toxic Reference:	Dimethoate
Batch No:	779155
Purity:	99.5%
Date of expiry	February 2022

2 Test concentrations:

Test item:	11.636, 29.091, 72.727, 181.818, 454.545 mg test item/kg diet
Toxic reference:	47.99 mg dimethoate/kg diet

3 Test organisms:

Species	*Apis mellifera* L.
Source	Biotechnologie BT
Age	3-day old larvae
Housing	Crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm.
Feeding	**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

4 Environmental conditions:

Temperature	33 – 35ºC
Relative humidity	49.3 – 96.8 %
Photoperiod	24 hours dark, except during observations

B. STUDY DESIGN AND METHODS

1. In-life phase: 25 July 2016 – 15 August 2016

2. Test organism assignment and 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.

In the analytical phase of the study, the concentration of the active substance in the test item stock solutions as well as in each final diet of the test item group was determined.

3. Dose preparation

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.

4. Measurements and 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 was 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.

5. 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 EDx/LDx and ECx/LCx values.

The software ToxRat Professional 3.2.1 was used to perform the statistics.

II. RESULTS AND DISCUSSION

A. Food consumption and mortality

The following table show the mean larval mortality, the pupal mortality and the effects on the emergence of adults:

Table A 2- 3: Mean mortality *Apis mellifera*

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	Corrrected	Absolute	Corrrected	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.

B. 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), in the study BT215/17. 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 analyzed by an ICP-MS (Inductively Coupled Plasma Mass Spectrometry). 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, according to the following formula:

% Recovery = (Measured content/Nominal content) x 100

C. 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%).

III. 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

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 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.

Comments of zRMS:	The study was evaluated at EU/zonal level. 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*.

Reference:	KCP 10.4/01
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., 2018, Report no. S18-00119
Guideline(s):	Yes/No (If yes, give guidelines; If no, give justification, e.g., “ no guidelines available” or “ methods used comparable to guideline(s) xxx”)
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 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 x 2 x 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.

A. MATERIALS

1	**Test materials:**	
	Test item:	Copper
	Batch No:	
	Purity:	
	Date of expiry:	
2	**Test concentrations:**	

	Test item:	Niefern: (control) 26.5 and 135.2 mg/kg soil dw); Heiligenzimmern: (control) 25.9 and 142.2 mg/kg soil dw
3	**Test organisms:**	
	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).
4	**Environmental conditions:**	
	Temperature	17.4 – 19.6 ºC
	Relative humidity	Start: 46.1 – 60.4 %, End: 38 – 53.6 %
	Photoperiod	16 hours light / 8 hours dark

B. STUDY DESIGN AND METHODS

1. In-life phase: 08 November 2017 – 23 March 2018

2. 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 organisms 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.

3. 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.

4. 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 $CaCl_2$ 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.

5. 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 pairwise 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 percentages 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.

II. RESULTS AND DISCUSSION

A. 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).

Table A 2- 4 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	2.0	4.0
Niefern/ Copper	89.0	5.3	5.9	12.9	79.4	7.8	silt loam	3.0	5.1	1.9	3.9
Heiligen zimmern / control	77.0	6.8	7.2	17.8	52.1	30.1	silty clay loam	4.2	7.2	7.8	15.5
Heiligen zimmern / Copper	80.5	6.6	7.2	14.2	47.1	38.8	silty clay loam	3.9	6.7	7.7	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

B. 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- 5).

Table A 2- 5 Copper residues in the tests 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

C. 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- 6 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	Heiligenzimmern	0.0	0.0	0.0	10.0
Niefern	Copper	Niefern	0.0	5.0	5.0	5.0
Niefern	Copper	Heiligenzimmern	0.0	0.0	0.0	0.0
Heiligenzimmern	Control	Heiligenzimmern	0.0	0.0	0.0	0.0
Heiligenzimmern	Control	Niefern	0.0	0.0	0.0	0.0
Heiligenzimmern	Copper	Heiligenzimmern	0.0	0.0	0.0	0.0
Heiligenzimmern	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.

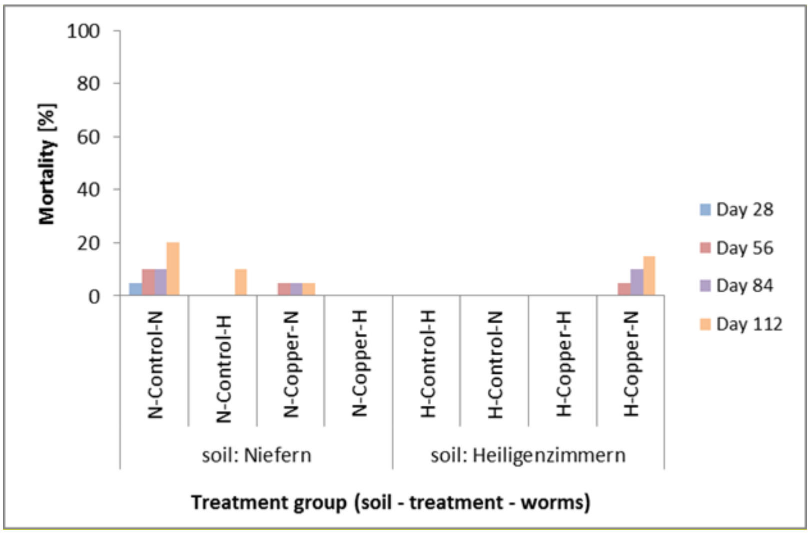


Figure 1. Mortality of adult *Aporrectodea caliginosa* for each of the eight treatment groups after 28, 56, 84 and 112 days of exposure to the test soils (naming of treatment groups: N-Control-N: Niefern soil, control treatment, Niefern worms, etc.)

D. 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- 7). 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- 7 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**	**Heiligenzimmern**	0 (0 %)	0 (0 %)	3 (15 %)	**7 (35 %)**
Heiligenzimmern	Control	Heiligenzimmern	0 (0 %)	0 (0 %)	0 (0 %)	13 (65 %)
Heiligenzimmern	Control	Niefern	0 (0 %)	0 (0 %)	5 (25 %)	4 (20 %)
Heiligenzimmern	**Copper**	**Heiligenzimmern**	0 (0 %)	1 (5 %)	1 (5 %)	**17 (85 %)**
Heiligenzimmern	Copper	Niefern	0 (0 %)	1 (5 %)	6 (33 %)	8 (47 %)
all soils combined			0 (0 %)	3 (2 %)	25 (16 %)	72 (48 %)

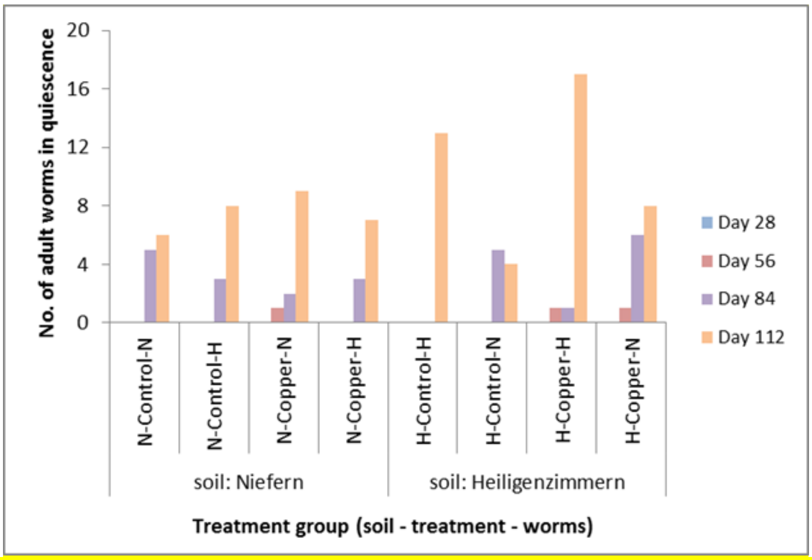


Figure 2. Appearance of the quiescence stage in adult *Aporrectodea caliginosa* after 28, 56, 84 and 112 days of exposure to the test soils (naming of treatment groups: N-Control-N: Niefern soil, control treatment, Niefern worms, etc.)

E. 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, α = 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: $p > 0.05$).

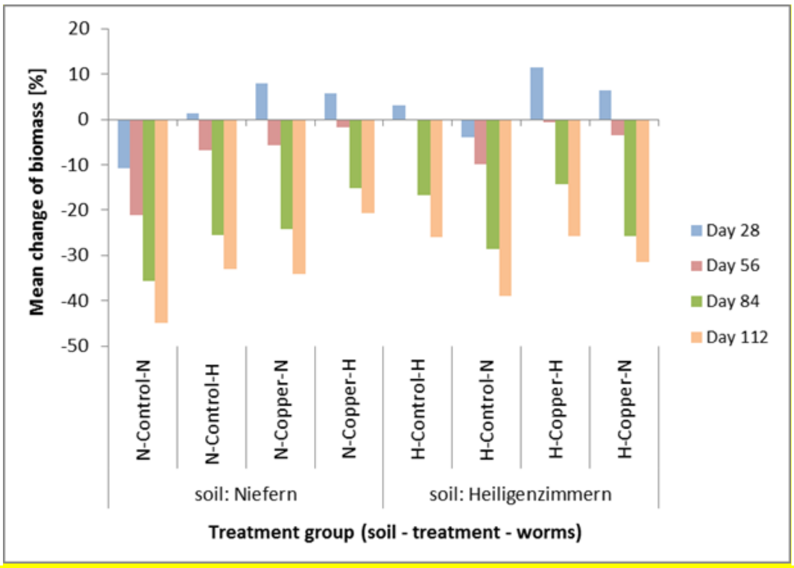


Figure 3. Biomass development (mean % change per treatment group) of adult *Aporrectodea caliginosa* after 28, 56, 84 and 112 days of exposure to the test soils (naming of treatment groups: N-Control-N: Niefern soil, control treatment, Niefern worms, etc.)

F. 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:

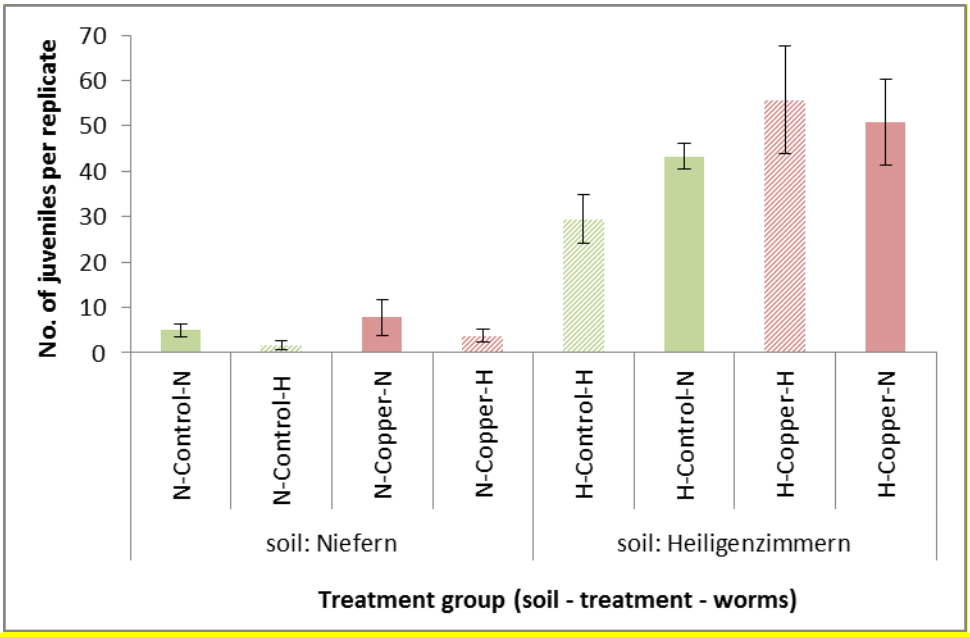


Figure 4. Number of juveniles per replicate (mean ± sd) of *Aporrectodea caliginosa* after 112 days of exposure to the test soils (green columns: control soils, red columns: Copper-treated soils; filled columns: Niefern worms, dashed columns: Heiligenzimmern worms; naming of treatment groups: N-Control-N = 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).

III. CONCLUSION

A. 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.

B. 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.

C. 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).

D. 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.

E. 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.

F. 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 2.4.1.1.2 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

Comments of zRMS:	The study was evaluated at EU/zonal level. The conclusion considers that after 10 years of treatment with copper the NOEC of the study is of the dose rate T2 (8 kg copper/ha/year).

Reference:	KCP 10.4/02
Report:	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. Testing facility: Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany. 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).

A. 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- 8 : 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 treatment: Permutation test for test item treatments

(Copper treatments and toxic reference)	[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

B. Explanation of the applied statistical methods

B.1 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).

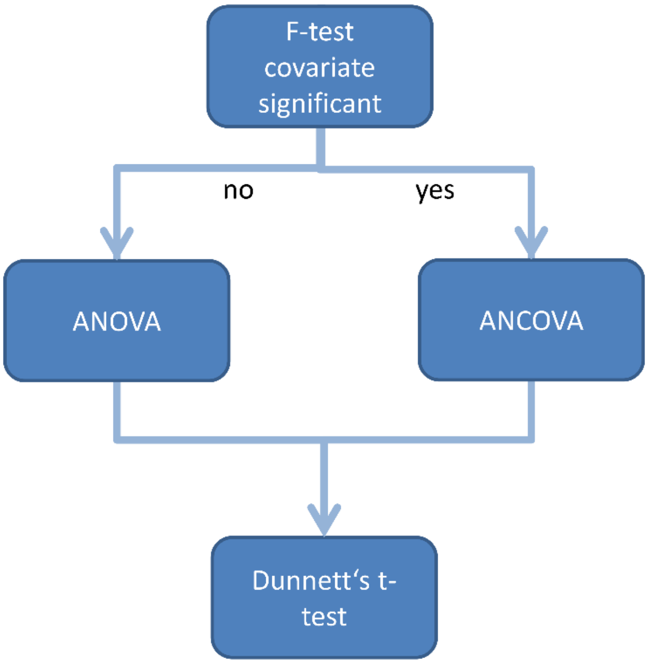


Figure 1: Decision tree for ANOVA/ANCOVA test procedure

A 2.4.1.1.3 Short-term effects of two fungicides on enchytraeid and earthworm communities under field conditions.

Comments of zRMS:	The study was evaluated at zonal level and used as an additional information.

Reference:	KCP 10.4/03
Report:	Short-term effects of two fungicides on enchytraeid and earthworm communities under field conditions. Ecotoxicology, J. Amossé et al., 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.

A. MATERIALS

1	**Test materials:**	
	Test item:	Cuprafor Micro
	Source:	Industrias Quimicas del Valles
	Purity:	500 g/kg Copper oxychloride, $Cu_2Cl(OH)_3$
	Date of expiry:	
2	**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).
3	**Test organisms:**	
	Species	*Lumbricidae* and *Enchytraeidae*
	Source	Not applicable
	Age	Not applicable
	Feeding	
4	**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_{H2O} 7.5 and Cu_{tot} 25.2 mg kg−1)

B. STUDY DESIGN AND METHODS

1. In-life phase: April – May 2016

2. 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.

3. 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.

4. 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).

5. 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 backtransformation 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).

II. RESULTS AND DISCUSSION

A. 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 (Supplementary table 1). 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- 9).

Table A 2- 9: 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)

B. 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 A 2- 9).

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.

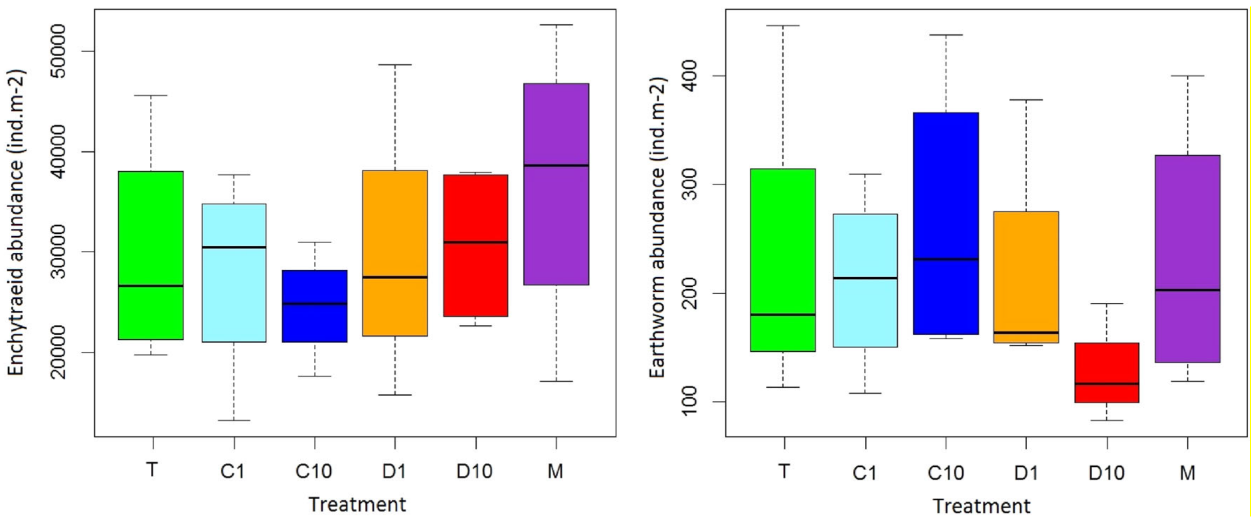


Figure 2. Total densities of enchytraeids (on the left) and earthworms (on the right) per treatment. Treatments are: control (T), Cuprafor Micro at 0.75 kg Cu ha−1 (C1) and 7.5 kg Cu ha−1 (C10), Swing Gold® at one (D1) and at ten (D10) times the recommended dose, and a mixture of Cuprafor Micro 0.75 kg Cu ha^{-1} and Swing Gold at the recommended dose (M)

C. 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.

D. 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).

III. CONCLUSION

A. Enchytraeids

It was found in the study that enchytraeids were not affected by a pesticide formulation with Copper (Cuprafor Micro) whatever the fungicide concentrations.

B. 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.

C. 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.

D. 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%).

A 2.4.1.1.4 Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values.

Comments of zRMS:	The study was evaluated at zonal level and used as an additional information.

Reference:	KCP 10.4/04
Report:	Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values. Ecotoxicology, Caetano et al., 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).

A. MATERIALS

1 Test materials:

Test item:	Copper (II) sulfate pentahydrate ($CuSO_4*5H_2O$)
Source:	Merck Ensure
Purity:	
Date of expiry:	

2 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

3	**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.
4	**Environmental conditions:**	
	Air temperature	
	Soil temperature	
	Relative humidity	
	Photoperiod	
	Soil	

B. STUDY DESIGN AND METHODS

1. In-life phase:

2. 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 defaunated 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 defaunated 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.

3. 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 soildw corresponded no the negative controls; Table A 2- 10). 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 ($CaSO_4$*$2H_2O$) were additionally performed at 2303.2, 366.3 mg of $CaSO_4$*$2H_2O$K/g soildw for *Eisenia andrei* and *Enchytraeus crypticus*, respectively.

Table A 2- 10: 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

4. 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.

5. 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 noobserved-effect-concentration (NOEC) and low-observedeffect-concentration (LOEC) values were determined based on the outcomes of the post hoc tests. The metal concentration producing a 20 % (EC20) and a 50 % (EC50) 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.

II. 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 soildw).

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 (Fig. 2; Table A 2- 11).

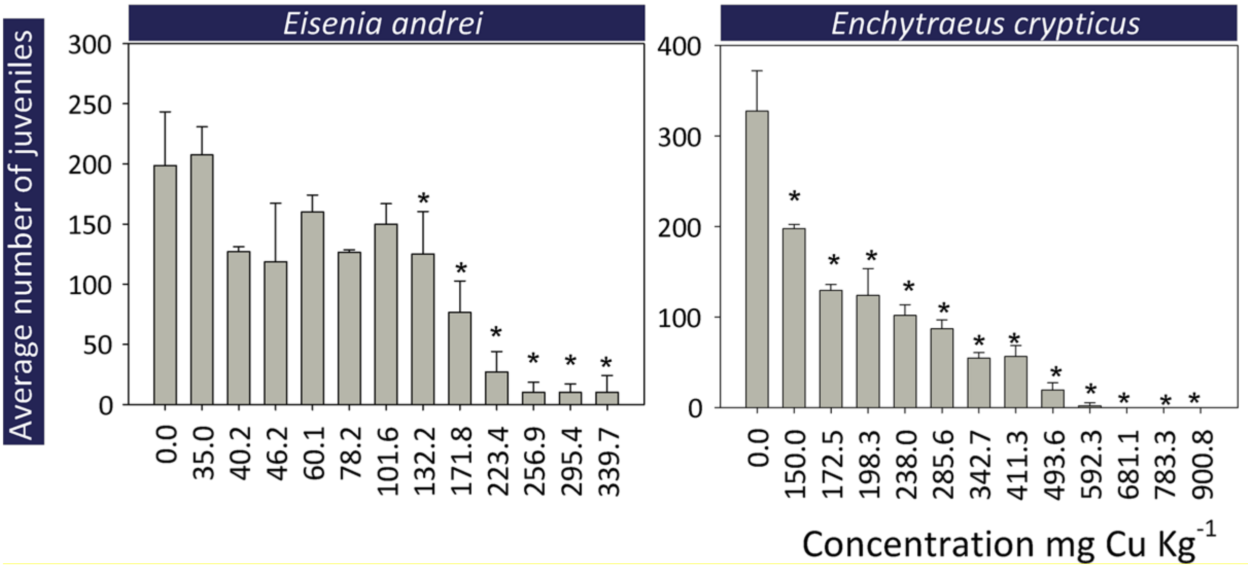


Fig. 1 Reproductive output of *Eisenia andrei*, *Enchytraeus crypticus* exposed to the natural soil PTRS1 spiked with increasing Copper concentrations. Error bars indicate the standard error and asterisks sign out significant differences between the treatment and the control (0.0 mg Cu Kg/dw) (p=0.05)

III. CONCLUSION

The results accomplished in this study strengthened the toxicity of Cu reported in the literature for different soil organisms and endpoints. The estimated EC20 (65.8 to \150.0 mg Kg-1 soil dw) and EC50 (130.9–191.6 mg/Kg soil dw) values were similar for both invertebrates (Table A 2- 11). 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- 11 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 2.4.1.1.5 Distribution of RAC values for effects of Cu to soil invertebrates in Europe

Comments of zRMS:	The study was accepted as an additional information. The proposed median RAC values for other soil macro-fauna are slightly lower than for earthworms and vary between 25 and 920 mg Cu/kg dw with a median of 179 mg Cu/kg dry weight. Median RAC values for both groups of organisms are higher in the Southern zone than in the Central and Northern zone, which is explained by the observed differences in soil properties affecting the bioavailability and toxicity of Cu in soil. Copper RAC values for soils with vineyards, olive or fruit orchards show a similar range as the RAC values for the Southern zone, with median values between 227 and 252 mg Cu/kg dry soil for earthworms and between 190 and 212 mg Cu/kg dry soil for other soil invertebrates. These data use in further risk assessment could be decided at cMS level.

Reference:	KCP 10.4/05
Report:	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. 19 pp
Guideline(s):	No (not applicable)
Deviations:	No (not applicable)
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

A sound risk assessment for trace elements in soil must preferentially take into account the inherent variation in both exposure (ambient metal concentration) and effects (through differences in bioavailability across soil types). In the case of Copper, soil properties strongly affect the bioavailability and toxicity to soil organisms and the cation exchange capacity of the soil is the best predictor of toxicity to soil invertebrates. The strong spatial variability of soil properties should therefore be considered for making conclusions on the relevant regulatory acceptable concentration (RAC) of Copper in the soil.

The Land Use and Cover Area frame Statistical survey (LUCAS) topsoil database provides soil properties for 22000 sites across the European Union, together with information on land use and crop cover. All data are georeferenced, and therefore the LUCAS dataset also allows mapping of the regional distribution of predicted RAC values for Cu in soil.

I. MATERIALS AND METHODS

A. LUCAS Database

The Land Use and Cover Area frame Statistical survey (LUCAS) aims to collect harmonised data about the state of land use and land cover across the European Union (EU) (Ballabio et al. 2016). This is done by field observation of geographically referenced points. LUCAS is co-ordinated by The Statistical Office of the European Commission (Eurostat) and its data is used for deriving land cover and land use statistics at European level, for monitoring changes in agro-environment, for landscape monitoring, for analysing the soil quality, etcetera (Lucas Technical reference document C1, 2009).

Data collection was done in two instances: in the 2009 LUCAS survey, topsoil samples were collected from 19,969 locations (approximately 10 % of the total LUCAS observations) from 25 out of 28 EU countries, except for Romania, Bulgaria and Croatia (Figure 1; Table 1; Toth et al., 2013; Orgiazzi et al., 2018). In the 2012 LUCAS survey, a further 2034 topsoil samples were collected from Bulgaria and Romania following the standard proce- dure of 2009. The sampling density of this pan-EU soil survey is approximately one topsoil sample every 196 km^2 (Panagos et al., 2013), which equates to a sample about every 14 km × 14 km (Ballabio et al. 2016).

LUCAS soil points are representative for the land use and topography within each country, to different degree, depending on the heterogeneity of land use and topography of the country. Areas above 1,000 m in elevation were not covered. Samples were taken predominantly of agricultural land, followed by grasslands and woodlands. soil samples may over represent properties from the more heavily sampled conditions whiles under- representing others e.g. rough grazing and wetlands (Figure 1; Table 1). This bias may limit the spatial extrapolation of the data to heavily sampled land cover classes. The applicability of the LUCAS soil survey for soil mapping is possi- bly problematic, because the survey was designed to allocate sampling points with similar densities in each country, rather than to allocate sampling points according to soil heterogeneity in different regions in the EU. LU-CAS data are thus representative on regional to country level. They are, however, not representative of local conditions and certainly not of specific field conditions (Toth et al., 2013).

B. Calculation of site specific RAC values

The cation exchange capacity (CEC) data for all LUCAS topsoils was used as input to calculate site specific regulatory acceptable concentrations (RAC) for effect of Cu to earthworms and to soil macro-fauna other than earthworms according to Oorts, 2015b and 2015c.

In short, 62 reliable EC_{10} or NOEC values were retrieved for chronic effects of Cu on 6 earthworm species (*Ei- senia andrei, Eisenia fetida, Lumbricus rubellus, Aporrectodea caliginosa, Dendrobaena rubida* and *Octolasion cyaneum*), representative of compost worms, epigeic and endogeic species (Oorts, 2015b). The selected endpoints were all relevant to assess effects at population level: mortality, growth and reproduction. For *Lumbricus rubellus*, data for a functional parameter (litter breakdown) are also available. Data are all derived from tests with chronic exposure, with duration of exposure being up to 90 days for *Dendrobaena rubida* and 110 days for *Lumbricus rubellus*. The NOEC/EC_{10} data were derived from tests performed in a variety of natural/artificial soils reflecting the variability in physico-chemical conditions in European arable soils. According to OECD (2016) and Smolders et al. (2009), bioavailability corrections were applied to the toxicity data derived from laboratory experiments in order to increase their comparability and relevance for field conditions. The chronic toxicity data were corrected for the higher toxicity observed in soils freshly spiked with a soluble Cu salt compared to toxicity tests in field contaminated soils, by a lab-to-field correction factor of 4 (Ruyters et al., 2013, Oorts, 2012 and Oorts, 2015a). Subsequently, the NOEC/EC_{10} values with sufficient information on the physico-chemical properties of the substrate tested were normalized towards physico-chemical properties of the LUCAS topsoils samples using the regression model developed for reproduction of *Eisenia fetida* according to the models derived by Criel et al. (2008). 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 is selected as the regulatory acceptable concentration (RAC) for effects of Cu on earthworms. Based on the conservative nature of the correction factors, the large amount of high-quality data available and most importantly the field validation, it is considered that an additional assessment factor should not be applied to the lowest geometric mean normalized NOEC/EC_{10} value for earthworms for a direct comparison with the exposure concentrations (Oorts 2015b).

For soil invertebrates other than earthworms, 71 reliable EC_{10} or NOEC values were retrieved for chronic effects of Cu on 9 soil species belonging to 5 different families and representing different living and feeding conditions: *Cognettia sphagnetorum*, *Enchytraeus albidus* and *Enchytraeus crypticus* (Enchytraedae), *Plectus acuminatus* (Plectidae), *Folsomia candida*, *Folsomia fimetaria* and *Isotoma viridis* (Isotomidae), *Hypoaspis aculeifer* (Lael- apidae) and *Platynothrus peltifer* (Camisiidae) (Oorts 2015c). The data are all derived from tests with chronic exposure (duration of exposure being between 21 and 70 days for all studies). The NOEC/EC_{10} data were ex- tracted from tests performed in a variety of natural and artificial soils, covering more than 90 % of the variation in soil characteristics of arable land in Europe. As for the earthworms, the chronic toxicity data were corrected for the higher toxicity observed in soils freshly spiked with a soluble Cu salt compared to toxicity tests in field contaminated soils, by a lab-to-field correction factor of 4. Subsequently, the NOEC/EC_{10} values with sufficient information on the physico-chemical properties of the substrate tested were normalized towards physico-chemical properties of the LUCAS topsoil samples using the regression models developed for reproduction of *Eisenia fetida* (for soft-bodied organisms) and *Folsomia candida* (fo hard-bodied organisms) (Criel et al., 2008). The geometric mean normalized NOEC/EC_{10} value for the most sensitive endpoint was calculated for each species. Aged and normalized species mean values NOEC/EC_{10} values for effect of Cu on soil macro fauna in a reason- able worst-case soil (eCEC of 8 cmolc/kg) vary between 99 mg Cu/kg dw for reproduction of *Plectus acuminatus* and 884 mg Cu/kg dw for reproduction of *Enchytraeus albidus*. The median HC_5 (HC_{5-50}) was derived from a log-normal species sensitivity distribution. A comparison with field data illustrates that the HC_{5-50} value from the SSD for soil macro fauna can be considered protective for effects of Cu on soil invertebrates under field conditions. Therefore, no additional assessment factor is applied on the HC_{5-50} in order to derive an appropriate regulatory acceptable concentration (RAC) for risk assessment for effects of Cu on soil macro fauna other than earthworms.

C. Data analyses

Distribution of RAC values

Soil properties (pH, organic carbon content, clay content, CEC) are strongly variable among soils, resulting in a wide range in RAC values across Europe. Distributions of RAC values for the EU and the regulatory zones (North, Centre and South) are 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. In addition, the distribution of RAC values for specific land cover types (Fruit trees, Vineyards and Olive groves) is also calculated. These land cover types of interest are not evenly distributed across Europe (Table 4). Because the limited locations sampled in the Northern and Central zones, the distribution of RAC values was only calculated for the total European Union.

Kriging

To allow area-based distributions and graphical presentation of Copper RAC data on a European scale, the Kriging technique was used. Kriging is a group of geostatistical techniques to interpolate the value of a random field (e.g. RAC value as a function of geographic location) at an unobserved location from observations of its value at nearby locations. This method produces visually appealing maps from irregularly spaced data.

QGIS v3.4.3

The datasets acquired with the Kriging method as described above, where then imported in QGIS v3.4.3. QGIS functions as geographic information system (GIS) software, allowing users to analyse and edit spatial information, in addition to composing and exporting graphical maps. QGIS supports both raster and vector layers; vector data is stored as either point, line, or polygon features. Multiple formats of raster images are supported, and the soft- ware can georeference images (QGIS website, 2018). Data can be added as layers on top of one another. In this way, a representation of the desired data on a customised map of Europe could be made.

II. RESULTS AND DISCUSSION

A. Distribution of soil properties

The number of sampling data, the minimum value, the maximum value and the 10th, 50th (median) and 90th percentiles of general soil properties (CEC, pH, organic matter and clay content) were summarized for the whole EU and the major regulatory zones (Table 5) and per relevant land cover for use of Copper fungicides (Table 6).

Across all soil samples taken all over the EU, the effective cation exchange capacity (CEC) values range from 0 to 234 cmol(+)/kg, pH $CaCl_2$ ranges from 2.6 to 9.3, organic carbon content from 0.1 to 58.7 % and the clay con- tent varied between 1 % and 96 %. Ranges for the regulatory zones did show clear differences with increasing pH, decreasing organic carbon content, increasing clay content and generally increasing CEC from North to South. This corresponds with the general observation of varying soil types and land uses in Europe, with more acid sandy forest soils in the Northern zone.

When these parameters were filtered for land coverage, it is noticeable that the ranges are rather similar for all three different crop types. Ranges and median values for the soil properties observed for all three land covers correspond well with the overall data for the Southern zone. This is easily explained by the fact that the majority of the vineyards, olive groves and fruit orchards are located in the Southern zone of Europe.

Table 5: General soil characteristics for the whole European Union and major regulatory zones

Zone	# of data points	Min	P10	Median	P90	Max
		CEC (cmol(+)/kg)				
EU	21980	1	4.3	13.5	34.5	234
North	5129	1	3.3	9.5	33.3	234
Centre	8133	1	4.1	13.9	35.8	193.1
South	8609	1	5.9	15.1	33.6	112.8
		pH (in $CaCl_2$)				
EU	22001	2.6	3.7	5.8	7.4	9.3
North	5129	2.6	3.2	4.1	6.3	7.4
Centre	8154	2.6	3.9	5.7	7.2	9.3
South	8609	2.7	4.6	6.9	7.5	8.8
		Organic Carbon (%)				
EU	21988	0.1	0.9	2.0	8.2	58.7
North	5129	0.1	1.4	3.9	39.9	58.7
Centre	8141	0.1	0.9	2.0	5.7	56.4

South	8609	0.1	0.7	1.5	4.2	47.2
		Clay (%)				
EU	21990	1	3	17	41	96
North	5129	1	1	6	25	79
Centre	8143	1	4	18	46	96
South	8609	1	9	22	42	95

Table 6: General soil characteristics for the land coverages under scrutiny

Land cover	# of data points	Min	P10	Median	P90	Max
		CEC (cmol(+)/kg)				
Vineyards	338	1	8.5	16.3	30.9	52.4
Olive groves	416	1	8.7	17.6	29.9	61.9
Fruit trees	304	1	7.1	15.4	34.2	66.8
		pH (in $CaCl_2$)				
Vineyards	338	3.9	6.1	7.4	7.6	7.8
Olive groves	416	4.0	5.7	7.4	7.6	8.1
Fruit trees	306	3.8	4.9	7.2	7.6	7.9
		Organic Carbon (%)				
Vineyards	338	0.1	0.5	1.1	2.2	14.3
Olive groves	416	0.1	0.6	1.4	2.7	8.2
Fruit trees	304	0.2	0.7	1.7	3.2	7.5
		Clay (%)				
Vineyards	338	4	11	24	42	75
Olive groves	416	1	11	25	45	64
Fruit trees	306	2	9	23	43	72

B. Distribution of RAC values across European soils

Table 7 reports the number of sampling data, the minimum value, the maximum value and the 10th, 50th (median) and 90th percentiles of the calculated Copper RAC values for soil in the EU and the major regulatory zones. The distribution of the RAC values for the relevant land covers for use of Copper fungicides is reported in Table 8.

The LUCAS dataset allows calculating 21980 RAC values of Copper for both earthworms and soil macro-fauna other than earthworms. Individual RAC earthworm values range from 46 to 1158 mg Cu/kg dw (difference factor 25) across soils in the EU, with 10th, 50th and 90th percentiles of 111, 215 and 374 mg Cu/kg dry weight, respectively. Predicted RAC values for other soil macro-fauna are slightly lower than for earthworms and vary between 25 and 920 mg Cu/kg dw (difference factor 37) across soils in the EU, with 10th, 50th and 90th percentiles of 83, 179 and 319 mg Cu/kg dry weight, respectively.

Median RAC values for both groups of organisms are higher in the Southern zone than in the Central and Northern zone, which is explained by the observed differences in soil properties affecting the bioavailability and toxicity of Cu in soil (Table 5). Copper RAC values for soils with vineyards, olive or fruit orchards show a similar range as the RAC values for the Southern zone (Table 8), with median values between 227 and 252 mg Cu/kg soil for earthworms and between 190 and 212 mg Cu/kg soil for other soil invertebrates.

Table 7: Distributions of regulatory acceptable concentrations (RAC) for Cu in soil in the whole of Europe and major regulatory zones

Zone	# of data points	Min	P10	Median	P90	Max
	Cu RAC for earthworms (mg Cu/kg)					
EU	21980	46	111	215	374	1158
North	5129	46	94	175	367	1158
Centre	8133	46	107	219	383	1034
South	8609	46	132	230	369	753
Cu RAC for soil macro-fauna other than earthworms (mg Cu/kg)						
EU	21980	25	83	179	319	920
North	5129	25	67	142	312	920
Centre	8133	25	79	182	326	831
South	8609	25	102	192	314	622

Table 8: 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
	Cu RAC for earthworms (mg Cu/kg)					
Vineyards	326	46	164	239	345	468
Olive groves	409	46	165	252	345	528
Fruit trees	279	46	143	227	369	523
Cu RAC for soil macro-fauna other than earthworms (mg Cu/kg)						
Vineyards	326	25	132	200	294	397
Olive groves	409	25	133	212	294	446
Fruit trees	279	25	113	190	314	442

The median RAC values as based on an area-based distribution were calculated to account for the potential bias due to the spatial heterogeneity of sampling density. Comparison of the values in Table 7 and Table 9 show that area-based median values are slightly higher than the median values calculated based on the sites sampled. Differences for the Central and Southern zone are however negligible (< 3 %), while differences for the Northern zone are approximately 12 %.

Table 9: Area-based median regulatory acceptable concentrations (RAC) for Cu in soil of major regulatory zones

Zone	Cu RAC (mg Cu/kg)	
	Earthworms	Macro-fauna other than earthworms
North	196	159
Centre	223	185
South	236	197

The spatial variation of Cu RAC values across Europe is shown in Figure 4 (RAC earthworms) Figure 5 (RAC soil macro-fauna other than earthworms) as based on the interpolations using the Kriging technique. Dark purple/black zones have low predicted RAC values, while the orange and yellow zones have high RAC values. Both RAC values show very similar distributions because they depend on the same soil properties (CEC). The lowest RAC values are generally found in Scandinavia, Poland, Portugal and Western Spain. The highest RAC values are found in Central and Southern Europe. The break between Northern and Southern Europe coincides with the maximum extent of the last ice age. Romania and Bulgaria show clear higher RAC values than the rest of Europe. Although sampling and analytics followed the same protocol as for the other countries, these distinct differences may however be due to differences in analyses because data for both countries were not part of the initial data- base (Tóth et al., 2013).

III. CONCLUSION

Data from the LUCAS project allows calculation of site-specific regulatory acceptable concentrations (RAC) for effects of Cu to earthworms and soil macro-fauna other than earthworms in 21980 soil samples taken from across the European Union. The individual Cu RAC values for earthworms across the EU vary from 46 to 1158 mg Cu/kg dw with a median value of 215 mg Cu/kg dry weight. Predicted RAC values for other soil macro-fauna are slightly lower than for earthworms and vary between 25 and 920 mg Cu/kg dw with a median of 179 mg Cu/kg dry weight.

Median RAC values for both groups of organisms are higher in the Southern zone than in the Central and Northern zone, which is explained by the observed differences in soil properties affecting the bioavailability and toxicity of Cu in soil. Copper RAC values for soils with vineyards, olive or fruit orchards show a similar range as the RAC values for the Southern zone, with median values between 227 and 252 mg Cu/kg dry soil for earthworms and between 190 and 212 mg Cu/kg dry soil for other soil invertebrates.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

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 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

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

A 2.8 KCP 10.8 Monitoring data