FINAL REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

Product code: FEL02

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

Chemical active substance:

Copper, 200 g/kg

Cymoxanil, 40 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Art. 33 New authorization)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: March 2023; March 2024

MS Finalisation date: November 2023; April 2024

Version history

When	What
March 2023	Part B-Section 6 -Core assessment, Version 01 of applicant
November 2023	Assessment by expert
March 2024	Update by applicant
April 2024	Assessment after the applicant update

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6 Mammalian Toxicology (KCP 7)

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

The product is already approved since several years in other EU member states, mainly in the Southern zone (see details in Part A). Some of the studies included in this dossier have already been evaluated as part of these applications.

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

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

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

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

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

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

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

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

This application follows the data requirements for the active substance laid down in Regulation (EU) No 283/2013 and the data requirements for the plant protection product laid down in Regulation (EU) No 284/2013. Data submitted on the formulated product are owned by the applicant, UPL Europe Ltd. A summary of the data is provided in dRR format.

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

The zonal GAP table presented in this dossier has been prepared in compliance with the renewal regulation of the active substance Copper compounds (Commission Implementing Regulation (EU) 2018/1981 of 13 December 2018) and in line with its specific provisions. The total dose for each use must not exceed 28 kg/ha of Copper metal over 7 years (4 kg/ha/year as a median).

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. **The inappropriateness of current guidelines for the assessment of Copper compounds has been recognised by the EU Commission, EFSA, the RMS and several MS (see comments from DE and IT in the Peer review Report), and this is now fully justified by the documents made available recently by EFSA[1,2] . Those documents confirm that the approaches and methodology suggested by the EUCuTF already during the EU renewal and also presented by its members for Art. 43 and Art. 33 authorizations can be used for transition metals like copper. In addition, and noticeably, the use of the EUCuTF approach is a prerequisite to enable a meaningful assessment and avoid conservative outcomes for copper products.**

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

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

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

- The current GD do not consider bioavailability; for an essential, ubiquitous micronutrient that is a metal it is indispensable to provide assessment methodologies that consider the bioavailability and the potentially toxic fraction in each real-world exposure scenario. Total concentrations do not result in any meaningful outcome.
- Data normalisation to enable comparison of toxicological lab and field data as well as data obtained with different bioavailable fractions is a pre-requisite to allow a realistic assessment of potential risk. Simplistic worst-case scenarios will always indicate a high risk already at naturally occurring concentrations.
- For a homeostatically tightly 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).
Therefore, applicants as well as zRMS are required to deviate from the LoEP and the standard procedures described in the GD. This can now be fully justified by the documents made available recently by EFSA[1,2] Those documents confirm that the approaches and methodology suggested by the EUCuTF already during the EU renewal and also presented by its members for Art. 43 and Art. 33 authorizations will find their way into the evaluation system and can be used for transition metals.

[1] Statement of the PPR Panel on a framework for conducting the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products (PPP) | European Food Safety Authority (europa.eu) EFSA Journal 2021;19(3):6498

[2] Outcome of the Public Consultation on the draft statement of the PPR Panel on a framework for conducting the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products (PPP) - - 2021 - EFSA Supporting Publications - Wiley Online Library EFSA Journal 2021;18(3):EN-6501

6.1 Summary

Table 6.1-1 Information on FEL02*

Product name and code	Copper / Cymoxanil 200 / 40 g/kg WG FEL02
Formulation type	Water dispersible granule [WG]
Active substance(s) (incl. content)	Copper 200 g/kg Cymoxanil 40 g/kg
Function	Fungicide
Product already evaluated as the 'representative formulation' during the approval of the active substance(s)	No
Product previously evaluated in another MS according to Uniform Principles	Italy (as zRMS) (and several other Member states as cMS, see Part A)

* Information on the detailed composition of FEL02 can be found in the confidential dRR Part C.

Justified proposals for classification and labelling

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

Table 6.1-2 Justified proposals for classification and labelling for FEL02 according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Acute Inhalation Toxicity, Cat. 4 Acute Oral Toxicity, Cat. 4 Eye Irritant, Cat. 2 Repro. 2
Hazard pictograms or Code(s) for hazard pictogram(s):	GHS07, GHS08, GHS09
Signal word:	Warning
Hazard statement(s):	H302: Harmful if swallowed H319: Causes serious eye irritation H332: Harmful if inhaled H361fd: Suspected of damaging fertility. Suspected of damaging the unborn child
Precautionary statement(s):	P102: Keep out of reach of children P260: Do not breathe dust/fume/gas/vapour/spray P264: Wash face, hands and any exposed skin thoroughly after handling P270: Do not eat, drink or smoke when using this product P271: Use only outdoors or in a well-ventilated area P280: Wear protective gloves/protective clothing/eye protection/face protection P301+P317: IF SWALLOWED: Get medical help P330: Rinse mouth P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several

	minutes. Remove contact lenses, if present and easy to do. Continue rinsing P337 + P313: IF eye irritation persists: Get medical advice/attention P391: Collect spillage P501: Dispose of contents/ container in accordance with national regulation
Additional labelling phrases:	EUH208 - Contains (Cymoxanil). May produce an allergic reaction EUH401 - To avoid risks to human health and the environment, comply with the instructions for use

Table 6.1-3 Summary of risk assessment for operators, workers, bystanders and residents for FEL02

	Result	PPE / Risk mitigation measures
Operators	Acceptable Tractor mounted:	None
Workers	Acceptable	None
Bystanders	Acceptable	None
Residents	Acceptable	None

No unacceptable risk for operators (tractor mounted applications), workers, bystanders and residents was identified when the product is used as intended.

Table 6.1-4 Critical uses and overall conclusion of exposure assessment-

1	2	3	4	5	6	7	8	9	10			
Use-No.*	Crops and situation (e.g. growth stage of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Application		Application rate		PHI (d)	Remarks: (e.g. safener/synergist (L/ha)) critical gap for operator, worker, bystander or resident exposure based on [Exposure model]	Acceptability of exposure assessment			
			Method / Kind (incl. application technique ***)	Max. number (min. interval between applications) a) per use b) per crop/ season	Max. application rate kg a.s./ha a) Copper b) Cymoxanil	Water L/ha min / max			Operator	Worker	Bystander	Residents
1	Potato	F	LCTM	6 (7)	a) 0.60 b) 0.12	100 - 1000	7	Critical use for operators, workers, residents and bystanders in LC [EFSA Journal 2022;20(1): 7032]				

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
*** e.g. LC: low crops, HC: high crop

Explanation for column 10 “Acceptability of exposure assessment”

A	Exposure acceptable without PPE / risk mitigation measures
R	Further refinement and/or risk mitigation measures required
N	Exposure not acceptable/ Evaluation not possible

Data gaps

Not applicable.

6.2 Toxicological Information on Active Substance(s)

Information regarding classification of the active substances and on EU endpoints and critical areas of concern identified during the EU review are given in **Table 6.2-1**.

Table 6.2-1 Information on active substance(s)

	Copper	**Cymoxanil[3]**
Common Name	Bordeaux Mixture	Cymoxanil
CAS-No.	8011-63-0	57966-95-7
Classification and proposed labelling		
With regard to toxicological endpoints (according to the criteria in Reg. (EC) No 1272/2008, as amended)	Acute Tox Cat. 4; Eye Damage Cat. 1 GHS05; GHS07 Danger H318: Causes serious eye damage; H332: Harmful if inhaled P201, P260, P273, P280, P304 + P340, P305 + P351 + P338, P310	Acute Tox Cat. 4; Skin Sens. 1 A; Repro. 2; STOT RE 2 GHS07; GHS08 Warning H302: Harmful if swallowed; H317: May cause an allergenic skin reaction; H361fd: Suspecting of damaging fertility. Suspected of damaging the unborn child; H373: May cause damage to the organs through prolonged or repeated exposure (blood, thymus) P201, P202, P260, P264, P270, P272, P273, P280, P301 + P312, P330, P302 + P352, P333 + P313, P321, P363, P308 + P313, P391, P501
Additional C&L proposal	Not applicable	RAC opinion (adopted 16 September 2021[4]) **Modify:** H373 (blood system, thymus, eyes) **Add**: oral: ATE = 360 mg/kg bw
Agreed EU endpoints		
AOEL systemic	0.08 mg/kg bw/d (corrected for 50% oral absorption)	0.01 mg/kg bw/d (corrected for 75% oral absorption)
Reference	EFSA Journal 2018;16(1):5152	EFSA Journal 2008;167, 1-116 Proposal for Harmonised Classification and Labelling Report: Cymoxanil, 2011
Conditions to take into account/critical areas of concern with regard to toxicology		
EFSA Conclusion concerns	None	None

[3] 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide

[4] https://echa.europa.eu/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e184568d7e

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for FEL02 is given in the following tables. Full summaries of studies on the product that have not been previously considered within an EU peer review process are described in detail in Appendix 2.

Table 6.3-1 Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for FEL02

of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. (EC) No 1272/2008)	Reference
LD_{50} oral, rat (OECD 425)	1871 mg/kg bw	Yes	**Acute oral toxicity Cat 4 – H302**	[redacted] KCP 7.1.1/01 Supported by [redacted], 1999a KCP 7.1.1/02
LD_{50} percutaneous (dermal), rat (OECD 402)	> 2000 mg/kg bw	Yes	Not to be classified	[redacted] KCP 7.1.2/01 Supported by KCP 7.1.2/02
LC_{50} inhalation, rat (OECD 403)	4623 mg/L	Yes	**Acute inhalation toxicity Cat. 4 – H332**	[redacted] KCP 7.1.3/01
Skin irritation, rabbit (OECD 404)	Not irritating to the skin	Yes	Not to be classified	[redacted] KCP 7.1.4/01 Supported by [redacted] KCP 7.1.4/02
Eye irritation, rabbit (OECD 405)	Slightly irritant to the eye	Yes	**Eye Irrit Cat. 2 - H319**	[redacted] KCP 7.1.5/01 Supported by [redacted] KCP 7.1.5/02
Skin sensitisation, guinea pig (OECD 406, M&K)	Non-sensitising	Yes	Not to be classified	[redacted] KCP 7.1.6/01 Supported by [redacted] KCP 7.1.6/02
Supplementary studies for combinations of plant protection products	No data – not required	Yes	-	-

Studies were performed in 1999 with the product ATO FDH01. Unfortunately, the exact composition of the tested material is no longer available. However, it is thought that the tested composition is sufficiently close to the composition of FEL02, to be used for classification in Europe.
Unfortunately, this was not the case for other regions and some countries outside of Europe have requested to perform a new 6-pack of acute toxicity studies with the correct formulation. These are the studies performed in 2016 (studies sponsored by UPL India Ltd).
The results of both sets of studies are the same and are reported in the table above. An extensive summary is provided only for the studies performed in 2016, since they were done with the correct composition

Table 6.3-2 Additional toxicological information relevant for classification/labelling of FEL02

	Substance (Concentration in product, % w/w)	Classification of the substance (acc. to the criteria in Reg. (EC) 1272/2008)	Reference	Classification of product (acc. to the criteria in Reg. (EC) 1272/2008)
Toxicological properties of active substance(s) (relevant for classification of product)	Bordeaux mixture (20% (w/w))	H332, Acute Tox Cat. 4, LC_{50} = 1.97 mg/L air (WB), H318, Eye Damage Cat. 1	EFSA conclusion (2018)	H332 ~~Based on Chhimwal, 2016a~~ ~~KCP 7.1.3/01~~ H319 ~~Based on and Patel, 2016a and McEwan, Donald, 1999d~~ ~~KCP 7.1.5/01 and 7.1.5/02~~
	Cymoxanil (4% (w/w))	H302, Acute Tox Cat.4, LD_{50} = 960 mg/kg bw; H317, Skin Sens. 1; H361fd, Repro. 2; H373, STOT RE 2	EFSA conclusion (2008)	H302 ~~Based on Verma, 2016a~~ ~~KCP 7.1.1/01~~ H361fd, Repro. 2
Toxicological properties of non-active substance(s) (relevant for classification of product)	Please refer to Part C, no additional classification to be assigned on the basis of the classification of non-active substances.			
Further toxicological information	No data – not required			

6.4 Toxicological Evaluation of Groundwater Metabolites

Copper is an element; no metabolites are possible.

All Cymoxanil metabolite concentrations are predicted to stay below 0.1 µg/L – please refer to dRR Part B, Section 8. No groundwater assessment is required.

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for the active substances Copper and Cymoxanil in FEL02 are presented in the following table.

Table 6.5-1 Dermal absorption rates active substances in FEL02

	Copper		Cymoxanil	
	Value	**Reference**	**Value**	**Reference**
Concentrate	1%	~~New studies reported in 0 (Maas & Brufau Dones 2016a, KCP 7.3/01; Maas& Brufau Dones 2016b, KCP~~	0.42%	New study reported in 0 [redacted], KCP 7.3/05)
Dilution 1:33	9%		22%	

	Copper		Cymoxanil	
	Value	**Reference**	**Value**	**Reference**
		~~7.3/02; Maas, et al. 2016, KCP 7.3/03; Maas 2020a, KCP 7.3/04)~~ EFSA journal 2018		

~~* Dermal absorption based on triple pack calculation approach.~~

6.5.1 Justification for proposed values - Copper

Dermal absorption rates ~~employed~~ proposed by the applicant in the risk assessment for copper are based on three dermal absorption studies (*in vitro* human, *in vitro* rat, and *in vivo* rat) using the triple pack calculation approach.

Table 6.5.1-1 Summary of the results of submitted dermal absorption studies for Copper

Test	Concentrate (350 g Cu/kg)	Spray dilution I (3 g Cu/L)	Spray dilution II (0.3 g Cu/L)	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference
In vitro (human)	0.39%	2.8%	8.7%	Copper hydroxide 53.8 WG containing 53.8% ^{65}Copper hydroxide (equivalent to 35% metallic Copper)	Yes	Yes (see Appendix A 2.10)	Yes	[redacted] KCP 7.3/01
In vitro (rat)	2.6%	3.6%	14%		~~Tes~~ Yes	Yes (see Appendix A 2.10)	Yes	[redacted] KCP 7.3/02
In vivo (rat)	<0.05%	1.0%*	1.9%*		Yes	Yes (see Appendix A 2.10)	Yes	[redacted] KCP 7.3/03

* Based on the mean missing recovery as a worst case.

In support of the registration of Copper products, EuCuTF members conducted a series of *in vitro* dermal absorption studies and performed a literature review. Since the only absorbable species is the hydrated Cu^{2+} ion after complexation, dermal absorption is independent of both the form of Copper (hydroxide, oxychloride, Bordeaux Mixture, tribasic Copper sulphate, and oxide) and of type of formulation (SC, WG or WP). *In vitro* dermal absorption studies are challenging due to the natural Copper content of the skin interfering with the analytical measurements.

Copper in the form of the Cu^{2+} cation is highly soluble in water, but it is known that ions do not penetrate the skin barrier. This is a fact based on the biology of the skin which forms a barrier against charged ions. There is no influence of counter-ions, co-formulants or other active substances in a formulation that may change the impermeability of the skin towards Copper ions (or any other ion). That said, Copper amounts in the epidermis as a result of topical application of an inorganic Copper fungicide are not negligible. However, Copper absorption is tightly controlled by unique homeostatic mechanisms that cannot be replicated in the highly conservative *in vitro* model. Clinical studies indicate that Copper will become available in a gradual way due to homeostatic mechanisms and will not result in high peaks of serum levels of Copper. Indeed, homeostatic mechanisms affect the absorption rate, with Copper absorption shown to decrease as a factor of time, with little or no absorption observed in the final 24 hours of a 72-hour *in vitro* study. Absorption of the SC reservoir will be determined by the desquamation versus diffusion rate, however, any Copper slowly released from the skin will be controlled by efficient homeostatic mechanisms in the body.

All three dermal absorption studies (Maas & Brufau Dones 2016a, KCP 7.3/01; Maas & Brufau Dones 2016b, KCP 7.3/02; Maas et al. 2016, KCP 7.3/03) were conducted with Copper hydroxide 53.8 WG containing 53.8% ^{65}Copper hydroxide (equivalent to 35% metallic Copper). Thus, all three studies are comparable with regards to test material, formulation, vehicle, exposure etc. and all fulfill the criteria for similarity as outlined in the EFSA guidance on der-

mal absorption (2017)[5]. A full summary of all three studies on the dermal absorption of Copper is presented in detail in Appendix 2. A further explanation on the use of dermal absorption studies with copper-containing agrochemical formulations and the biology behind it is also provided in Appendix 2 (Maas, 2020a, KCP 7.3/04).

Dermal absorption rates employed in the risk assessment are based on the triple pack calculation approach and is presented as follows:

Concentrate (350 g/kg):

For the *in vivo* study in rats, absorption from the undiluted concentrate was <0.05% and thus used as a worst-case value.

Therefore, the triple pack calculations for undiluted concentrate would be based on the following values:

- *In vivo* rat: <0.05%
- *In vitro* rat: 2.6%
- *In vitro* human: 0.39%

The dermal absorption value for the concentrate can therefore be calculated as follows:

In vivo human % absorption = *in vivo* rat % absorption / *in vitro* rat % absorption × *in vitro* % human absorption

In vivo human % absorption = <0.05% / 2.6% × 0.39% = 0.0075 or rounded **0.01%**

Therefore, a dermal absorption value of <**0.01%** can be determined for the concentrate from the triple pack approach for copper.

Dilution I (3 g Cu/L)

For the *in vivo* study in rats, mean recovery was 96.08% at 24h, 101.00% at 72h and 99.88% at 144h, which results in a mean recovery of 99.0%, hence the mean missing recovery is 1.0%. It is possible to use this worst-case value of 1.0%, by assuming that all of this amount is in the absorbed fraction.

Therefore, the triple pack calculations for the dilution (3 g Cu/L) would be based on the following values:

- *In vivo* rat: 1.0% (based on mean missing recovery as a worst case)
- *In vitro* rat: 3.6%
- *In vitro* human: 2.8%

The dermal absorption value for the lowest dilution can therefore be calculated as follows:

In vivo human % absorption = *in vivo* rat % absorption / *in vitro* rat % absorption × *in vitro* % human absorption

In vivo human % absorption = 1.0% / 3.6% × 2.8% = **0.8%**

Therefore, a dermal absorption value of **0.8%** can be determined for the lowest dilution (3 g Cu/L) from the triple pack approach.

Dilution II (0.3 g Cu/L)

For the *in vivo* study in rats, mean recovery was 97.41% at 24h, 98.38% at 72h and 97.63% at 144h, which results in a mean over mean recovery of 98.1%, hence the mean missing recovery is 1.9%. It is possible to use this worst-case value of 1.9%, by assuming that all of this amount is in the absorbed fraction.

Therefore, the triple pack calculations for the highest dilution (0.3 g Cu/L) would be based on the following values:

- *In vivo* rat: 1.9% (based on mean missing recovery as a worst case)
- *In vitro* rat: 14%
- *In vitro* human: 8.7%

The dermal absorption value for the highest dilution can therefore be calculated as follows:

In vivo human % absorption = *in vivo* rat % absorption / *in vitro* rat % absorption × *in vitro* % human absorption

In vivo human % absorption = 1.9% / 14% × 8.7% = **1.2%**

[5] European Food and Safety Authority (EFSA), 2017. Guidance on dermal absorption. EFSA Journal 2017;15(6):4873.

However, this position, using the triple pack calculation approach could be acceptable if the formulations used in the triple pack are considered to be comparable to product FEL02. But as FEL02 is a mixture of 2 active substances including cymoxanil which has a profile different to copper compounds, the extrapolation of the dermal absorption values for copper cannot be accepted.

Therefore, dermal absorption rates employed in the risk assessment can be based on the end points for copper (Appendix A of EFSA Journal, 2018).

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

Table 6.6-1 Product information and toxicological reference values used for exposure assessment

Product name and code	FEL02	
Formulation type	WG	
Category	Fungicide	
Active substance(s) (incl. content)	**Copper** 200 g/kg	**Cymoxanil** 40 g/kg
AOEL systemic	0.08 mg/kg bw/d	0.01 mg/kg bw/d
Inhalation absorption	100%	100%
Oral absorption	50%	75%
Dermal absorption	Concentrate: 1% Dilution: 9% (Based on product Copper hydroxide 53.8 WG, triple pack approach)	Concentrate: 0.42% Dilution: 22% (0.12 g/L) (Based on product FEL02)

6.6.1 Selection of critical use(s) and justification

The critical GAP use(s) for the exposure assessment of the plant protection product are shown in **Table 6.1-4**. A list of all intended uses within the central zone is given in Part B, Section 0.

Justification

The selected critical uses enable a worst-case scenario for exposure to operators, workers, residents and bystanders according to crop type and maximum application rate. However, since for this article 33 application there is only one use (potato), there is no need to justify a worst-case scenario.

6.6.2 Operator exposure (KCP 7.2.1)

6.6.2.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to Copper and Cymoxanil during application of FEL02 is presented in **Table 6.6.2.1-1**. The outcome of the estimation is presented in **Table 6.6.2.1-2** (longer exposure). Detailed calculations are given in Appendix 3.

Table 6.6.2.1-1 Exposure models for intended uses

Critical use No. 1	Potato, 3 kg f.p./ha (0.6 kg Copper/ha and 0.12 kg Cymoxanil/ha)
EFSA model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2022;20(1): 7032 Calculator version: 0.3.22
	The EFSA Model uses standard figures for different parameters. Models are available for the estimation of operator exposure for liquid pesticide formulations using a tractor-mounted sprayer for application to low crops. The following points are of particular importance when considering the estimates: • The EFSA Model assumes that contamination during mixing/loading and spray application is to the hands, body and head. • Product container size and design are not taken into consideration. • The EFSA Model provides specific dermal exposure values for operators wearing trousers and a long-sleeved shirt during application of the spray. Standard figures are used for the penetration of such clothing. From this basic assumption, the reduction of exposure from the use of protective equipment (e.g. gloves, goggles, headgear, body garment, etc.) can be calculated. Reduction in inhalation exposure may be achieved by additional protection specifically designed to reduce exposure during handling or application. • The EFSA Model estimates exposure for operators with and without protective gloves. When wearing gloves, a different set of exposure values is employed. • It is assumed that 100% of inhaled exposure arising during mixing/loading and spray application is absorbed. Spray volume is not taken into consideration. • The EFSA Model assumes an operator body weight of 60 kg • Operator exposure duration 8 hours

Estimated operator exposure (acute exposure)

An AAOEL was not allocated during the peer review for the renewal of approval of Copper (EFSA, 2018[6]) nor for Cymoxanil (EFSA, 2008[7]). Therefore, estimates of the acute exposure to operators are not required.

Table 6.6.2.1-2 Estimated operator exposure (longer term exposure)

		Copper		**Cymoxanil**	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray application outdoors to low crops					
Application rate		0.6 kg a.s./ha		0.12 kg a.s./ha	
Spray application (AOEM**; 75**th percentile) Body weight: 60 kg	Work wear (arms, body and legs covered) M/L and A	0.011	**14.3**	0.005	**46.5**
Handheld application outdoors to low crops: Not applicable*					

* Since the use of FEL02 is anticipated for professionals in potatoes only, a tractor mounted application would be the standard mode of application, and use with handheld equipment is therefore not anticipated.

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

[7] EFSA Journal 2008;167, 1-116

For tractor mounted application of copper, the operator exposure estimates using a single layer of work clothing, work wear covering arms, body and legs show a safe systemic exposure of 14.3% of the AOEL.

For tractor mounted application of cymoxanil, the operator exposure estimates using a single layer of work clothing, work wear covering arms, body and legs show a safe systemic exposure of 47% of the AOEL.

For the combined exposure to copper and cymoxanil in FEL02 it is referred to 6.6.5.6.6.2.2 Measurement of operator exposure.

For tractor-mounted application, the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mentioned personal protective equipment (PPE), hence a study to provide measurements of operator exposure was not necessary and was therefore not performed.

6.6.3 Worker exposure (KCP 7.2.3)

6.6.3.1 Estimation of worker exposure

Table 6.6.3.1-1 shows the exposure model(s) used for estimation of worker exposure after entry into a previously treated area or handling a crop treated with FEL02 according to the critical use(s). Outcome of the estimation is presented in **Table 6.6.3.1-2** (longer term exposure). Detailed calculations are in Appendix 3.

Table 6.6.3.1-1 Exposure models for intended use

Critical use No. 1	Potato, 6 x 3 kg f.p./ha (max. 6 × 0.6 kg Copper/ha and 6 × 0.12 kg Cymoxanil/ha)
EFSA model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2022;20(1): 7032 calculator version: 0.3.22
	The EFSA Model uses standard figures for different parameters. Models are available for the estimation of worker exposure for liquid pesticide formulations upon re-entry. The following points are of particular importance when considering the estimates: • Dermal transfer factor of 12500 cm²/person per hour (potential exposure) • Dermal transfer factor of 1400 cm²/person per hour (arms, body, legs covered) • Dermal transfer factor of 1250 cm²/person per hour (hands, arms, body, legs covered) • Daily work rate of 2 hours per day (inspection, irrigation) • Dislodgeable foliar residues (default): 3 µg/cm² × kg a.s./ha • Dissipation time (default): 30 days

Estimated worker exposure (acute exposure)

An AAOEL was not allocated during the peer review for the renewal of approval of Copper (EFSA, 2018[8]) nor for Cymoxanil (EFSA, 2008[9]). Therefore, estimates of the acute exposure to operators has not been conducted.

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

[9] EFSA Journal 2008;167, 1-116

Table 6.6.3.1-2 Estimated worker exposure (longer exposure)

		Copper		Cymoxanil	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Inspection, irrigation Outdoor Work rate: 2 hours/day, DT_{50} (default): 30 days DFR (default): 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days					
Number of applications and application rate		6 x 0.6 kg a.s./ha		6 x 0.12 kg a.s./ha	
Body weight: 60 kg	Potential TC: 12500 cm²/person/h	0.3	351	0.1	1373
	Work wear (arms, body and legs covered) TC: 1400 cm²/person/h	0.03	39	0.02	154
	Work wear (arms, body and legs covered) and gloves TC: 1250 cm²/person/h	0.03	**35**	0.01	**137**

For Copper, the potential worker exposure upon re-entry to fields after the application of FEL02 for the use in potato is not safe and exceeds the limits of AOEL at 351%. Also when work wear (arms, body and legs covered) or work wear and gloves (hands, arms, body and legs covered) are worn the worker exposure is safe and further reduced to 39% and 35% of the AOEL, respectively.

For Cymoxanil, the potential worker exposure upon re-entry to fields after the application of FEL02 for the use in potato is not safe and exceeds the limits of AOEL at 1373% AOEL. Also when work wear (arms, body and legs covered) or work wear and gloves (hands, arms, body and legs covered) are worn the worker exposure is not safe and exceeds the limits of AOEL at 154% and 137% AOEL, respectively.

For cymoxanil, higher tier exposure estimations for workers re-entering the field were also performed.

The dissipation time of cymoxanil is low. The DT_{50} value can be estimated using a residue decline study conducted on wheat and peas, a DFR study conducted in grapes, and a DFR study conducted in grapes, tomato and potato. The DT_{50} found in these studies ranged from 0.42 to 1.21 days. In potato the DT_{50} was 0.95 days. Overall, it is reasonable to assume that the cymoxanil DT_{50} value of residues on the raw commodity samples would be approximately 1 day. See Point 6.6.3.2 below and Appendix 4 for further details.

The DT_{50} value of 1 day for cymoxanil is used in the refined calculations below.

Table 6.6.3.1-3 Estimated worker exposure (longer exposure), Refinement DT50

		Cymoxanil	
Model data	**Level of PPE**	**Total absorbed dose [mg/kg bw/day]**	**% of systemic AOEL**
Inspection, irrigation Outdoor Work rate: 2 hours/day, **DT_{50}: 1 day** DFR (default): 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days			
Application rate		6 × 0.12 kg cymoxanil/ha	
Body weight: 60 kg	Potential TC: 12500 cm²/person/h	0.03	333
	Work wear (arms, body and legs covered) TC: 1400 cm²/person/h	0.004	37.3
	Work wear (arms, body and legs covered) and gloves TC: 1250 cm²/person/h	0.003	**33.3**

When the DT_{50} for cymoxanil of 1 day is used, the potential exposure is not safe at 333% of the systemic AOEL. However, when work wear (arms, body and legs covered) is worn, the worker exposure is safe at 37% AOEL, and reduced to 33% AOEL when workwear and gloves are worn.

For the combined exposure to copper and cymoxanil in FEL02 it is referred to 6.6.5.

6.6.3.2 Refinement of generic DT50 value (KCP 7.4)

A position paper (Correia, 2019, see Appendix 4.1) was submitted, in which several residue decline and DFR studies were evaluated. The studies were conducted with a Cymoxanil 45 WG formulation in a residue decline study conducted on wheat and peas, a DFR study conducted in grapes, and a DFR study conducted in grapes, tomato and potato. The DT_{50} found in these studies ranged from 0.42 to 1.21 days. In potato the DT_{50} was 0.95 days (Jullian, 2014). Overall, it is reasonable to assume that the cymoxanil DT_{50} value of residues on the raw commodity samples would be approximately 1 day. See Appendix 4 for further details.

6.6.3.3 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended use and considering above mentioned work clothing, a study to provide measurements of worker exposure was not necessary and was therefore not performed. The risks posed to workers from the application of FEL02 are considered therefore to be acceptable.

6.6.4 Bystander and resident exposure (KCP 7.2.2)

6.6.4.1 Estimation of bystander and resident exposure

The acute exposure assessment for bystanders covers the exposure that a resident could reasonably be expected to incur in a single day. Therefore, there is no need for a separate acute risk assessment for residents.
No bystander risk assessment is required for PPPs that do not have significant acute toxicity or the potential to exert toxic effects after a single exposure. Exposure in this case will be determined by average exposure over a longer duration, and higher exposures on one day will tend to be offset by lower exposures on other days. Therefore, exposure assessment for residents also covers bystander exposure.

Table 6.6.4.1-1 shows the exposure model(s) used for estimation of resident and bystander exposure to Copper and Cymoxanil. The outcome of the estimation is presented in **Table 6.6.4.1-2** (longer term resident exposure). Detailed calculations are given in Appendix 3.

Table 6.6.4.1-1 Exposure models for intended use

Critical use No. 1	Potato, 3 kg f.p./ha (max. 6 × 0.6 kg Copper/ha and 6 × 0.12 kg Cymoxanil/ha)
EFSA model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2022;20(1): 7032 calculator version: 0.3.22 The EFSA Model uses standard figures for different parameters. The following points are of particular importance when considering the estimates: The EFSA Model assumes: • Body weight o Adult resident: 60 kg o Child resident: 10 kg • Transfer coefficients specific to the application procedure and crop are considered, here for application in fields: o Adult resident: 7500 cm²/h (75th percentile), 5980 cm²/h (mean) o Child resident: 2250 cm²/h (75th percentile), 1794 cm²/h (mean) • Multiple application factor is considered taking into account the maximum number of applications, the minimum interval and the DT50 • Air concentration: 0.001 mg/m³ for low volatile substance • Spray drift values specific to the application procedure /crop and distance (buffer strip) are considered, here for application in fields • Dermal exposure (75th percentile) to spray drift, surface deposits and entry into treated crops is considered as well as exposure to spray drift and oral exposure for children (hand to mouth and object to mouth). • For a summary of all pathways the mean values are considered.

Table 6.6.4.1-2 Estimated resident exposure (longer term exposure)

		Copper		Cymoxanil	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray application or handheld application outdoors to low crops Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50} (default): 30 days DFR (default): 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days					
Number of applications and application rate		6 x 0.6 kg a.s./ha		6 x 0.12 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.015	18	0.007	71.6
	Vapour (75th perc.)	0.0008	1	0.0008	8
	Deposits (75th perc.)	0.004	5.3	0.002	19
	Re-entry (75th perc.)	0.04	47	0.02	185
	Sum (mean)	0.04	**53**	0.02	209
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0035	4.4	0.002	17
	Vapour (75th perc.)	0.0003	0.4	0.0003	2.7

	Deposits (75th perc.)	0.0015	1.9	0.0007	7.5
	Re-entry (75th perc.)	0.02	26	0.01	103
	Sum (mean)	0.02	**25**	0.01	98.2

For copper, the estimated exposure for both child and adult residents for the use in potato is safe at 53% and at 25% of the systemic AOEL, respectively.

For cymoxanil, the estimated exposure for both child and adult residents for the use in potato is not safe and exceeds the limits of AOEL at 209% and at 98% of the systemic AOEL, respectively. For cymoxanil, higher tier exposure estimations for child and adult residents were also performed, as this is considered necessary for the risk assessment of the combined exposure to the active substances in FEL02.

The dissipation time of cymoxanil is low. The DT_{50} value can be estimated using a residue decline study conducted on wheat and peas, a DFR study conducted in grapes, and a DFR study conducted in grapes, tomato and potato. The DT_{50} found in these studies ranged from 0.42 to 1.21 days. In potato the DT_{50} was 0.95 days. Overall, it is reasonable to assume that the cymoxanil DT_{50} value of residues on the raw commodity samples would be approximately 1 day.

See Point 6.6.3.2 above and Appendix 4 for further details.

Table 6.6.4.1-3 Estimated resident exposure (longer term exposure), Refinement DT50

		Cymoxanil	
Model data		**Total absorbed dose [mg/kg bw/day]**	**% of systemic AOEL**
Tractor mounted boom spray application or handheld application outdoors to low crops Buffer zone: 2-3 (m) Drift reduction technology: no **DT_{50} : 1 day** DFR (default): 3 µg/cm²/kg a.s./ha Interval between treatments: 7 days			
Number of applications and application rate		6 × 0.12 kg cymoxanil/ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.007	71.6
	Vapour (75th perc.)	0.0008	8
	Deposits (75th perc.)	0.0005	4.6
	Re-entry (75th perc.)	0.004	45
	Sum (mean)	0.009	**86.4**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.002	17
	Vapour (75th perc.)	0.0003	2.7
	Deposits (75th perc.)	0.0002	1.8
	Re-entry (75th perc.)	0.002	25
	Sum (mean)	0.003	**31.9**

When the DT_{50} for Cymoxanil of 1 day is used, the estimated exposure for both child and adult residents for the use in potato is safe at 86% and at 32% of the systemic AOEL, respectively.

For the combined exposure to copper and cymoxanil in FEL02 it is referred to 6.6.5.

Estimated bystander exposure (acute exposure)

No AAOEL for Copper nor Cymoxanil has been set, therefore estimates of the acute bystander exposure was not determined.

6.6.4.2 Measurement of bystander and/or resident exposure

Since the bystander and/or resident exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mentioned risk mitigation measures, a study to provide measurements of bystander/resident exposure was not necessary and was therefore not performed. The risks posed to residents and bystanders from the application of FEL02 are considered therefore to be acceptable.

6.6.5 Combined exposure

The product is a mixture of two active substances.

6.6.5.1 Exposure Assessment of Copper and Cymoxanil in FEL02

Note: The combined toxicological effect of these active substances has not been investigated with regard to repeated dose toxicity.

At the first tier, combined exposure is calculated as the sum of the component exposures without considering the mode of action or mechanism/target of toxicity. Initially, the individual Hazard Quotients (HQ) are calculated for all active substances in the PPP by assessing the exposure according to appropriate models and dividing the individual exposure levels by the respective systemic AOEL. This is equivalent to the predicted exposure as % of systemic AOEL converted to decimal. The Hazard Index (HI) is the sum of the individual HQs.

Table Błąd! W dokumencie nie ma tekstu o podanym stylu.**..5-1: Risk assessment from combined exposure (longer term exposure)**

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ)
Operators – tractor-mounted application	M/L: Workwear App: Workwear	0.6
Workers – Inspection, irrigation	potential	6.8
	Workwear	0.8
	Workwear and gloves	0.7
Resident - child	Drift	0.9
	Vapour	0.09
	Deposits	0.1
	Re-entry	0.9
	Sum of all pathways	1.4
Resident - adult	Drift	0.2
	Vapour	0.03
	Deposits	0.04
	Re-entry	0.5

Application scenario	Active ingredient	Estimated exposure / AOEL (HQ)
	Sum of all pathways	0.6

The Hazard Index is < 1. Thus, combined exposure to both active substances in FEL02 is not expected to present a risk for operators, workers, residents and bystanders. No further refinement of the assessment is required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 7.1.1/01	[redacted]	2016a	ACUTE ORAL TOXICITY STUDY OF COPPER 200 + CYMOXANIL 40 WDG IN RATS [redacted] GLP Unpublished	Y	UPL EU
KCP 7.1.2/01	[redacted]	2016b	ACUTE DERMAL TOXICITY STUDY OF COPPER 200 + CYMOXANIL 40 WDG IN RATS [redacted] GLP Unpublished	Y	UPL EU
KCP 7.1.3/01	[redacted]	2016a	ACUTE INHALATION STUDY OF COPPER 200 + CYMOXANIL 40 WDG IN RATS [redacted] GLP Unpublished	Y	UPL EU
KCP 7.1.4/01	[redacted]	2016a	ACUTE DERMAL IRRITATION STUDY OF COPPER 200 + CYMOXANIL WDG IN RABBITS [redacted] GLP Unpublished	Y	UPL EU
KCP 7.1.5/01	[redacted]	2016b	ACUTE EYE IRRITATION STUDY OF COPPER 200 + CYMOXANIL WDG IN RABBITS [redacted] GLP Unpublished	Y	UPL EU

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 7.1.6/01	[redacted]	2016b	SKIN SENSITISATION STUDY OF COPPER 200 + CYMOXANIL 40 WDG IN GUINEA PIGS [GUINEA PIG MAXIMIZATION TEST] [redacted] GLP Unpublished	Y	UPL EU
KCP 7.3/05	[redacted]	2020b	The In Vitro Percutaneous Absorption of Radiolabelled Cymoxanil in a Concentrate Formulation (FEL02) and an In-Use Dilution through Human Split-Thickness Skin [redacted] GLP Unpublished	N	UPL EU
KCP 7.4/01	Correia, A	2019	Cymoxanil DT_{50} value to use in risk assessment refinement Report No.:ZE/15/003-01 Laboratory: Battelle UK Ltd Owner: Cymoxanil Task Force GLP: No Published: No	N	Cymoxanil Task Force(*)
KCP 7.4/02	Jullian E.	2014	Quantification of dislodgeable foliar residues following Six applications of Vitene Ultra to potato in the United Kingdom, 2013. Report number: S13-01293 Laboratory: Eurofins Agroscience Services, Ltd. Owner: SIPCAM OXON S.p.A GLP: Y Published: N	N	SIPCAM OXON S.p.A(*)

UPL EU = UPL Europe Ltd.
EUCuTF = EU Copper Task Force,
CTF = Cymoxanil task force (DuPont, Sipam Oxon, Belchim, Indofil, UPL),
(*) UPL is a full member of the EU Copper Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of copper compounds
UPL is a full member of the Cymoxanil AIR4 Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of cymoxanil

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 7.1.1/02	[redacted]	1999a	ATO FDH01 – ACUTE ORAL TOXICITY (LIMIT) TEST IN RATS 16786 [redacted] GLP, Unpublished	Y	UPL EU
KCP 7.1.2/02	[redacted]	1999b	ATO FDH01 – ACUTE DERMAL TOXICITY (LIMIT) TEST IN RATS 16634 [redacted] GLP, Unpublished	Y	UPL EU
KCP 7.1.4/02	Xxxxxxxxxx [redacted]	1999c	ATO FDH01 – ACUTE DERMAL IRRITATION TEST IN RABBITS 16603 [redacted] GLP, Unpublished	Y	UPL EU
KCP 7.1.5/02	[redacted]	1999d	ATO FDH01 – ACUTE EYE IRRITATION TEST IN RABBITS 16726 [redacted] GLP, Unpublished	Y	UPL EU
KCP 7.1.6/02	[redacted]	1999	ATO FDH01 – MAGNUSSON-KLIGMAN MAXIMISATION TEST IN GUINEA PIGS 16734 [redacted] GLP, Unpublished	Y	UPL EU
KCP 7.3/01	[redacted]	2016a	*IN VITRO* PERCUTANEOUS ABSORPTION OF COPPER, FORMULATED AS COPPER HYDROXIDE (DPX-GFJ52) 53.8WG (35% AS METALLIC COPPER), THROUGH HUMAN SKIN [redacted] GLP Unpublished	N	EU Copper Task Force(*)
KCP 7.3/02	[redacted]	2016b	*IN VITRO* PERCUTANEOUS ABSORPTION OF COPPER, FORMULATED AS COPPER HYDROXIDE (DPX-GFJ52) 53.8WG (35% AS METALLIC COPPER), THROUGH RAT SKIN	N	EU Copper Task Force(*)

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			[redacted] GLP Unpublished		
KCP 7.3/03	[redacted]	2016	*IN VIVO* PERCUTANEOUS ABSORPTION OF COPPER, FORMULATED AS COPPER HYDROXIDE (DPX-GFJ52) 53.8WG (35% AS METALLIC COPPER), IN RATS [redacted] GLP Unpublished	N	EU Copper Task Force(*)
KCP 7.3/04	[redacted]	2020a	THE FATE OF TEST ITEM RESIDUES IN THE SKIN MEMBRANES *IN VITRO* DERMAL ABSORPTION STUDIES; IMPACT ON THE RISK ASSESSMENT OF INORGANIC COPPER SALTS [redacted] No GLP Unpublished	N	EU Copper Task Force(*)

UPL EU = UPL Europe Ltd.
EUCuTF = EU Copper Task Force,
CTF = Cymoxanil task force (DuPont, Sipam Oxon, Belchim, Indofil, UPL),
(*) UPL is a full member of the EU Copper Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of copper compounds
UPL is a full member of the Cymoxanil AIR4 Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of cymoxanil

Appendix 2 Detailed evaluation of the studies relied upon

A 2.1 Statement on bridging possibilities

In 1999 acute toxicity studies were performed with the formulation ATO FDH01, which is described to contain 20.3% (w/w) Copper and 4.3% (w/w) Cymoxanil. ATO FDH01 is an older version of FEL02, and it is thought that the tested composition is sufficiently close to the composition of FEL02 to be used for classification in Europe. The ATO FDH01 studies were therefore evaluated and accepted by RMS Italy in the authorisation application of FEL02 in the Southern Zone. For clarity the executive summaries of these ATO FDH01 studies are included below.

The applicant has currently no information on the specification of ATO FDH01. The use of the ATO FDH01 studies for classification of FEL02 was not always accepted in other regions, and some countries outside of Europe have requested to perform a new 6-pack of acute toxicity studies with the correct formulation. These are the studies performed in 2016 (studies sponsored by UPL India Ltd).

An extensive summary is provided only for the studies performed in 2016, since they were done with the correct composition.

Comments of zRMS:	N/A

A 2.2 Acute oral toxicity (KCP 7.1.1)

A 2.2.1 Study 1

Comments of zRMS:	**As the oral LD_{50} of Copper 200 + cymoxanil 40 WDG is lower than 2000 mg/kg bw in rats, classification with acute oral toxicity Cat 4 – H302 is required according to Regulation (EC) No. 1272/2008.**

Reference:	KCP 7.1.1/01
Report	Acute oral toxicity study of Copper 200 + Cymoxanil 40 WDG in rats [redacted]
Guideline(s):	OECD 425, OCSPP 870.1100
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes (requested by countries outside Europe, see A 2.1)

Executive Summary

In an acute oral toxicity study (Up and Down Procedure, OECD TG 425) male and female Wistar rats were administrated a single dose of Copper 200 + cymoxanil 40 WDG via oral gavage. The animals were observed at 0.5, 1, 2, 3, 4 and 6 hours post-administration for morbidity and mortality, and then twice daily for a period of 14 days, and 21 days for one rat. Body weights were recorded prior to dosing (day 0) and on days 7, 14 (and day 21 for one rat), and at death. At the end of the 14 days observation period and on day 21 for one rat, surviving rats were euthanized and subjected to gross pathological examination, consisting of external examination and opening of the abdominal and thoracic cavities.

Mortality was observed in all males treated at 2300, 3100, 4100 or 5000 mg Copper 200 + cymoxanil 40 WDG/kg bw. The males treated at 1750 mg Copper 200 + cymoxanil 40 WDG/kg bw survived.

In females, mortality was observed at 4100 and 5000 mg Copper 200 + cymoxanil 40 WDG/kg bw. The females treated at 3100 mg Copper 200 + cymoxanil 40 WDG/kg bw survived.

Clinical signs like lethargy were observed in male rats treated at 2300, 3100, 4100 or 5000 mg Copper 200 + cymoxanil 40 WDG/kg bw and in female rats treated at 3100, 4100 or 5000 mg Copper 200 + cymoxanil 40 WDG/kg bw. Chromodacryorrhea was observed in one female at 3100 mg/kg bw and no clinical signs were observed in females at 1750 mg/kg bw.

Initial body weight loss was seen in the majority of animals surviving the first week. Subsequent body weight gain was observed in the surviving animals.

All the surviving rats at termination and found dead rats were subjected to gross pathological examination. External examination of found dead rats and terminally sacrificed rats did not reveal any abnormality. Visceral examination of found dead rats revealed lesions including congestion in liver and lungs, whereas the terminally sacrificed rats did not reveal any lesion. Lesions observed in the found dead rats could be correlated with the test item used in the present study.

Under the conditions of this study, the acute oral LD_{50} of Copper 200 + cymoxanil 40 WDG was estimated to be 1871 and 3256 mg/kg bw with 95% confidence intervals of 1750 to 2300 and 3100 to 4100 mg/kg bw for male and female rats, respectively (Dixon's maximum likelihood method).

As the oral LD_{50} of Copper 200 + cymoxanil 40 WDG is lower than 2000 mg/kg bw in rats, classification with acute oral toxicity Cat 4 – H302 is required according to Regulation (EC) No. 1272/2008.

MATERIALS AND METHODS

Test material (Lot/Batch No.)	Copper 200 + Cymoxanil 40 WDG (batch No. 15.351.1)
Species	Rat, Wistar (RccHan)
No. of animals (group size)	8 males, 7 female rats
Dose(s)	1750, 2300, 3100, 4100 or 5000 mg/kg bw (males) 2300, 3100, 4100 or 5000 mg/kg bw (females)
Exposure	Once by gavage
Vehicle/Dilution	RO water
Post exposure observation period	14 days (except for 1 male: 21 days)
Remarks	None

RESULTS AND DISCUSSIONS

Table A 2.2.1-1 Results of acute oral toxicity study in rats of Copper 200 + Cymoxanil 40 WDG

Dose [mg/kg bw]	Toxicological results *	Duration of signs	Time of death	LD_{50} [mg/kg bw]
Male rats				
1750	0/0/2		-	Calculated: 1871 mg/kg bw**
2300	3/3/3	2 days	72 h (1 animal), 4-7 days (1 animal), 8-14 days (1 animal)	
3100	1/1/1	3 days	4-7 days	
4100	1/1/1	3 days	8-14 days	
5000	1/1/1	3 days	24 h	

Dose [mg/kg bw]	Toxicological results *	Duration of signs	Time of death	LD_{50} [mg/kg bw]
Female rats				
3100	0/2/2	3 or 8 days	-	Calculated: 3256 mg/kg bw**
4100	3/3/3	1, 2 or 3 days	24 h (1 animal), 4-7 days (2 animals)	
5000	2/2/2	2 or 8 days	48 h (1 animal), 8-14 days (1 animal)	

* Number of animals which died/number of animals with clinical signs/number of animals used.
** Calculated using the Dixon's maximum likelihood method using software (AOT 425 StatPgm). The approximate 95% confidence interval are 1750 to 2300 and 3100 to 4100 mg/kg body weight for male and female rats, respectively.

Table A 2.2.1-2 Summary of findings of acute oral toxicity study in rats of Copper 200 + Cymoxanil 40 WDG

Mortality:	Initially a male rat was tested with a starting dose-level of 5000 mg/kg bw. As this male rat died, subsequently seven additional male rats received a dose of 1750, 2300, 3100 or 4100 mg/kg bw. Both male rats treated at 1750 mg/kg bw survived throughout the 14-day observation period while male rats treated at higher dose levels were found dead. Initially a female rat was tested with a starting dose-level of 5000 mg/kg bw. As this female rat died after 48 hours, subsequently six additional female rats received a dose of 3100, 4100 or 5000 mg/kg bw. Both female rats treated at 3100 mg/kg bw survived throughout the 14 or 21-day observation period while female rats treated at higher dose levels were found dead.
Clinical signs:	No clinical signs were observed in male animals dosed with 1750 mg/kg bw. Lethargy was observed prior to death in all males at 2300, 3100, 4100 and 5000 mg/kg bw, and in all females at 4100 and 5000 mg/kg. The females treated at 3100 mg/kg bw lethargy was observed on days 3-11 (1 female, that also showed chromodacryorrhea on days 8-9) and on days 6-8 in the other female.
Body weight:	Initial body weight loss was seen in the majority of animals surviving the first week. Subsequent body weight gain was observed in the surviving animals. The observation period for one female rat was extended till day 21 as decrease in body weight was observed on days 7 and 14 when compared to day 0 body weight; her body weight increased on day 21 when compared to day 0 body weight.
Macroscopic examination:	External examination of found dead rats and terminally sacrificed rats did not reveal any abnormality. Visceral examination of found dead rats revealed lesions such as congestion in liver and lungs whereas the terminally sacrificed rats did not reveal any lesions.

CONCLUSION

Under the conditions of this study, the acute oral LD_{50} of Copper 200 + cymoxanil 40 WDG was estimated to be 1871 and 3256 mg/kg bw with 95% confidence intervals of 1750 to 2300 and 3100 to 4100 mg/kg bw for male and female rats, respectively.

As the oral LD_{50} of Copper 200 + cymoxanil 40 WDG is lower than 2000 mg/kg bw in rats, classification with acute oral toxicity Cat 4 – H302 is required according to Regulation (EC) No. 1272/2008.

A 2.2.2 Study 2

Previous evaluation dRR FEL02, Southern zone, RMS: Italy (2019)

Comments of zRMS:	**ATO FDH01 has a low toxicity based on the LD_{50} > 2000 mg/kg bw in rats. Consequently, it can be concluded that the LD_{50} of FEL02 is also > 2000 mg/kg bw.**

Reference:	KCP 7.1.1/02
Report	ATO FDH01 Acute Oral Toxicity (Limit) Test in Rats, xxxxxxxxxxxxxxxxxxx. [redacted]
Guideline(s):	OECD Guideline No. 401
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an acute oral toxicity study in accordance to OECD TG 401, one group of 5 male and female Sprague Dawley Rats, between 5 - 7 weeks old were administrated a single dose of 2000 mg/kg bw of ATO FDH01 (batch 980430 HE, 20.3% (w/w) and 4.3% (w/w) Copper and Cymoxanil, respectively) via oral gavage in sterile water. The administration volume was 10 mL/kg bw. The animals were weighed on days 1, 8 and 15. Clinical signs were recorded daily for 14 days.

ATO FDH01 has a low toxicity based on the LD_{50} > 2000 mg/kg bw in rats. Consequently, it can be concluded that the LD_{50} of FEL02 is also > 2000 mg/kg bw.

A 2.3 Acute percutaneous (dermal) toxicity (KCP 7.1.2)

A 2.3.1 Study 1

Comments of zRMS:	**Under the experimental conditions, the dermal LD_{50} of Copper 200 + cymoxanil 40 WDG is higher than 2000 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.**

Reference:	KCP 7.1.2/01
Report	Acute dermal toxicity study of Copper 200 + Cymoxanil 40 WDG in rats xxxxxxxxxxxxxxxxxxxxxxxxxxx
Guideline(s):	OECD 402, OCSPP 870.1200
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	Yes (requested by countries outside Europe, see A 2.1)

Executive Summary

In an acute dermal toxicity study in accordance with OECD guideline No. 402, a group of Wistar rats (5 males and 5 females) was dermally exposed to Copper 200 + cymoxanil 40 WDG for 24 h following application at a limit dose of 2000 mg/kg body weight. The required amount (445.0 to 582.6 mg) of Copper 200 + cymoxanil 40 WDG (pulverised and moistened with 0.2 mL freshly collected RO water) was applied over the clipped area (approximately 7 x 5 cm body surface area) and the rats were observed for a period of 14 days. The test item was held in contact with the skin using porous gauze dressing (not more than 8 ply) and a non-irritating tape.

There were no treatment-related mortality, clinical signs, changes in body weight or necropsy findings recorded.

Under the experimental conditions, the dermal LD_{50} of Copper 200 + cymoxanil 40 WDG is higher than 2000 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

MATERIALS AND METHODS

Test material (Lot/Batch No.)	Copper 200 + Cymoxanil 40 WDG (batch No. 15.351.1)
Species	Rat, Wistar (RccHan)
No. of animals (group size)	5 male and 5 female rats
Dose(s)	2000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	Moistened with RO water
Post exposure observation period	14 days
Remarks	None

RESULTS AND DISCUSSIONS

Table A 2.3.1-1 Results of acute dermal toxicity study in rats of Copper 200 + Cymoxanil 40 WDG

Dose [mg/kg bw]	Toxicological results *	Duration of signs	Time of death	LD_{50} [mg/kg bw] (14 days)
Male rats				
2000	0/0/5	-	14 days	> 2000
Female rats				
2000	0/0/5	-	14 days	> 2000

* Number of animals which died/number of animals with clinical signs/number of animals used.

Table A 2.3.1-2 Summary of findings of acute dermal toxicity study in rats of Copper 200 + Cymoxanil 40 WDG

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of systemic toxicity were observed.

Body weight:	Body weight of all the rats increased on days 7 and 14 when compared to day 0 body weight.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings.

CONCLUSION

Under the experimental conditions, the dermal LD_{50} of Copper 200 + cymoxanil 40 WDG is higher than 2000 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.3.2 Study 2

Previous evaluation: dRR FEL02, Southern zone, RMS: Italy (2019)

Comments of zRMS:	**ATO FDH01 has a low dermal toxicity based on the LD_{50} > 2000 mg/kg in rats. Consequently, it can be concluded that the LD_{50} of FEL02 is also > 2000 mg/kg bw.**

Reference:	KCP 7.1.2/02
Report	ATO FDH01 Acute Dermal Toxicity (Limit) Test in Rats, x[redacted]., [redacted]
Guideline(s):	OECD Guideline No. 402
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an acute dermal toxicity study in accordance to OECD TG 402, one group of 5 male and female Sprague Dawley Rats, between 8 - 10 weeks old were dermally exposed to 2000 mg/kg bw of ATO FDH01 (batch No. 980430 HE, 20.3% (w/w) and 4.3% (w/w) Copper and Cymoxanil, respectively) for 24 h, to an area approx. to about 8% of the total body surface, onto the wetted dorsal skin and spread uniformly over the trunk. The test material was covered by a water moistened semi-occlusive tape. The animals were weighed on days 1, 8 and 15. Clinical signs were recorded daily for 14 days.

ATO FDH01 has a low dermal toxicity based on the LD_{50} > 2000 mg/kg in rats. Consequently, it can be concluded that the LD_{50} of FEL02 is also > 2000 mg/kg bw.

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

A 2.4.1 Study 1

Comments of zRMS:	**Under the experimental conditions, the inhalation LC_{50} of Copper 200 + Cymoxanil 40 WDG is lower than 5.0 mg/L air in rats. Thus, classification with Acute inhalation toxicity Cat. 4 – H332 is required according to Regulation (EC) No. 1272/2008.**

Reference:	KCP 7.1.3/01
Report	Acute inhalation toxicity study of Copper 200 + Cymoxanil 40 WDG in rats [redacted]
Guideline(s):	OECD 403, OCSPP 870.1300
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an acute inhalation toxicity study (OECD TG 403), initially one group of rats consisting of five male and five female rats were exposed with Copper 200 + Cymoxanil 40 WDG and 80% mortality was found, so additionally two groups of rats, consisting of five male and five female rats per group used.

Rats from groups I, II and III were exposed to breathing zone concentrations of 5.396, 2.844 and 4.413 mg Copper 200 + Cymoxanil 40 WDG/L air, respectively. Rats from all the groups were exposed for 4 h followed by observation for a period of 14 days.

Percent mortalities observed (both the sexes combined) were 0, 40 and 80 at the breathing zone concentrations 2.844, 4.413 and 5.396 mg/L air of Copper 200 + Cymoxanil 40 WDG, respectively which indicates dose dependent mortality.

No clinical signs were observed at 2.844 mg/L air of Copper 200 + Cymoxanil 40 WDG. Lethargy was observed at 4.413 and 5.396 mg/L air and abdominal breathing was observed in one male at 4.413 mg/L air.

Initial body weight loss was seen in the majority of animals surviving the first week. Subsequent body weight gain was observed in the surviving animals.

External examination of the found dead and terminally sacrificed rats did not reveal any abnormality of pathological significance. Visceral examination of the found dead rats revealed congestion of liver and lungs whereas terminally sacrificed rats did not reveal any lesion of pathological significance. The lesions observed in the found dead rats could be correlated with the test item used in the present study.

The calculated acute inhalation median lethal concentration (LC_{50}) value of Copper 200 + Cymoxanil 40 WDG were as follows:

- combined sex: 4.623 mg/L air (95% fiducial limits 4.109 - 5.201 mg/L air; y = -3.336 + 12.536 x).
- females: 5.151 mg/L air (95% fiducial limits 4.360 - 6.085 mg/L air; y = -3.924 + 12.536 x).
- males: not possible to calculate, due to mortality pattern

Under the experimental conditions, the inhalation LC_{50} of Copper 200 + Cymoxanil 40 WDG is lower than 5.0 mg/L air in rats. Thus, classification with Acute inhalation toxicity Cat. 4 – H332 is required according to Regulation (EC) No. 1272/2008.

MATERIALS AND METHODS

Test material (Lot/Batch No.)	Copper 200 + cymoxanil 40 WDG (Batch No 15.351.1)
Species	Rat, Wistar
No. of animals (group size)	5 rats/sex/dose
Concentration(s)	2.844, 4.413 and 5.396 mg/L air
Exposure	4 hours (nose only)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

RESULTS AND DISCUSSIONS

Table A 2.4.1-1 Concentration(s) and exposure conditions

Group	Nominal conc. [mg/L air]	Actual conc. [mg/L air]	MMAD * [µm]	GSD ** [µm]
Group II	6.242	2.844 ± 0.044	3.79	1.54
Group III	8.352	4.413 ± 0.06	3.85	1.55
Group I	10.560	5.396 ± 0.11	3.88	1.57

* MMAD = Mass Median Aerodynamic Diameter (50%)
** GSD = Geometric Standard Deviation

Table A 2.4.1-2 Results of acute inhalation toxicity study in rats of Copper 200 + cymoxanil 40 WDG – Mortality

Group	Number animals/sex	Mortality								
		During exposure	After exposure						Number male/ female	Total number (%)
		1-4 h	1 & 2 h	24 h	48 h	72 h	4-7 days	8-14 days		
Group II (2.844 mg/L)	5 M + 5 F	0	0	0	0	0	0	0	0/0	0 (0%)
Group III (4.413 mg/L)	5 M + 5 F	0	0	4	0	0	0	0	3/1	4 (40%)
Group I (5.396 mg/L)	5 M + 5 F	5	0	3	0	0	0	0	5/3	8 (80%)

Table A 2.4.1-3 Results of acute inhalation toxicity study in rats of Copper 200 + cymoxanil 40 WDG – Clinical signs

Group	Number animals/sex	Clinical signs*						
		During exposure	After exposure					
		1-4 h	1 & 2 h	24 h	48 h	72 h	4-7 days	8-14 days
Group II (2.844 mg/L)	5 M 5 F	5/0/0/5 5/0/0/5	5/0/0/5 5/0/0/5	5/0/0/5 5/0/0/5	5/0/0/5 5/0/0/5	5/0/0/5 5/0/0/5	5/0/0/5 5/0/0/5	5/0/0/5 5/0/0/5
Group III (4.413 mg/L)	5 M 5 F	5/0/0/5 5/0/0/5	0/5/0/5 0/5/0/5	0/2/3/5 0/4/1/5	2/0/3/5 4/0/1/5	2/0/3/5 4/0/1/5	2/0/3/5 4/0/1/5	2/0/3/5 4/0/1/5
Group I (5.396 mg/L)	5 M 5 F	0/1/4/5 0/4/1/5	0/1/4/5 0/4/1/5	0/0/5/5 0/2/3/5	0/0/5/5 2/0/3/5	0/0/5/5 2/0/3/5	0/0/5/5 2/0/3/5	0/0/5/5 2/0/3/5

* Number of animals which are normal/number of animals with lethargy/number of animals died/number of total animals.
** Including abdominal breathing in one animal.

Table A 2.4.1-4 Summary of findings of acute inhalation toxicity study in rats of Copper 200 + cymoxanil 40 WDG

Mortality:	Percent mortalities observed (both the sexes combined) were 0, 40 and 80 at the breathing zone concentrations 2.844, 4.413 and 5.396 mg/L air of Copper 200 + Cymoxanil 40 WDG, respectively which indicates dose dependent mortality.

Group (mg/L)	Males (mortality/total)	Females (mortality/total)
2.844	0/5	0/5
4.413	3/5	1/5
5.396	5/5	3/5

Clinical signs:	No clinical signs were observed at 2.844 mg/L air of Copper 200 + Cymoxanil 40 WDG. Lethargy was observed at 4.413 and 5.396 mg/L air and abdominal breathing was observed in one male at 4.413 mg/L air.
Body weight:	Initial body weight loss was seen in the majority of animals surviving the first week. Subsequent body weight gain was observed in the surviving animals.
Macroscopic examination:	External examination of the found dead and terminally sacrificed rats did not reveal any abnormality of pathological significance. Visceral examination of the found dead rats revealed lesions including congestion in the lungs and the liver, whereas terminally sacrificed rats did not reveal any lesion of pathological significance. The lesions observed in the found dead rats could be correlated with the test item used in the present study.

CONCLUSION

The calculated acute inhalation median lethal concentration (LC_{50}) value of Copper 200 + Cymoxanil 40 WDG were as follows:

- combined sex: 4.623 mg/L air (95% fiducial limits 4.109 - 5.201 mg/L air; y = -3.336 + 12.536 x).
- females: 5.151 mg/L air (95% fiducial limits 4.360 - 6.085 mg/L air; y = -3.924 + 12.536 x).
- males: not possible to calculate, due to mortality pattern

Under the experimental conditions, the inhalation LC_{50} of Copper 200 + Cymoxanil 40 WDG is lower than 5.0 mg/L air in rats. Thus, classification with Acute inhalation toxicity Cat. 4 – H332 is required according to Regulation (EC) No. 1272/2008.

A 2.5 Skin irritation (KCP 7.1.4)

A 2.5.1 Study 1

Comments of zRMS:	**Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is not a skin irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.**

Reference:	KCP 7.1.4/01
Report	Acute dermal irritation study of Copper 200 + Cymoxanil 40 WDG in rabbits [redacted]
Guideline(s):	OECD 404, OCSPP 870.2500
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes (requested by countries outside Europe, see A 2.1)

Executive Summary

In an acute dermal irritation study (compliant with OECD TG 404), adult male New Zealand White rabbits were dermally exposed to 500 mg Copper 200 + Cymoxanil 40 WDG (pulverized and moistened with 0.5 mL distilled water), for 4 h (day 0), applied to approximately 6 cm^2 area of skin. Initially one rabbit was tested with a single patch applied evenly to the intact skin for a period of 4 h. Based on the observations at 24 h post patch removal, two additional rabbits were tested simultaneously to confirm the irritation response. The control skin site of each rabbit was untreated. The treated and the control sites were covered with a gauze patch that was secured at the margins by non-irritating tape for a period of 4 h. At the end of the 4 h exposure period, the residual test item was removed with cotton soaked in distilled water. The skin reactions of each rabbit were observed at 1, 24, 48 and 72 h post patch removal. Irritation was scored according to OECD 404.

There were no signs of systemic adverse effect observed in any treated rabbit.

Erythema was evident at 1 h in all the rabbits which resolved by 24 h post patch removal. The mean dermal irritation scores at 24, 48 and 72 h post-patch removal for rabbit N° 1, 2 and 3 were 0.00, 0.00, 0.00 for erythema and 0.00, 0.00, 0.00 for oedema, respectively.

Under the experimental conditions, Copper 200 + cymoxanil 40 WDG is not a skin irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

MATERIALS AND METHODS

Test material (Lot/Batch No.)	Copper 200 + Cymoxanil 40 WDG (Batch No 15.351.1)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 Males
Initial test using one animal	Yes
Exposure	0.5 g (4 hours, non-occlusive dressing)

Vehicle/Dilution	Moistened with 0.5 mL distilled water
Post exposure observation period	72 hours
Remarks	None

RESULTS AND DISCUSSIONS

Table A 2.5.1-1 Skin irritation of Copper 200 + cymoxanil 40 WDG

Animal No.		Scores after treatment *			Mean scores (24 - 72 h)	Reversible [day]
		24 h	48 h	72 h		
1	Erythema Oedema	0 0	0 0	0 0	0 0	- -
2	Erythema Oedema	0 0	0 0	0 0	0 0	- -
3	Erythema Oedema	0 0	0 0	0 0	0 0	- -

* Scores in the range of 0 to 4.

Clinical signs:	At 1 hour post patch removal, the treated skin site of all three rabbits revealed very slight erythema (barely perceptible); the treated skin site of all three rabbits recovered completely and appeared to be normal at 24 hours post patch removal. No symptoms of systemic toxicity were found.

CONCLUSION

Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is not a skin irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.5.2 Study 2

Previous evaluation	dRR FEL02, Southern zone, RMS: Italy (2019)

Comments of zRMS:	**In this study ATO FDH01 was non-irritating to the skin. Consequently, it can be concluded that FEL02 is also not irritating to the skin.**

Reference:	KCP 7.1.4/012
Report	ATO FDH01 Acute Dermal Irritation Test in Rabbits, [redacted]
Guideline(s):	OECD Guideline No. 404
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a primary dermal irritation study in accordance to OECD Guideline No.404, 3 male New Zealand white rabbits, between 2.42 - 2.97 kg were dermally exposed to 0.5 g of ATO FDH01 (batch No. 980430 HE, 20.3% (w/w) and 4.3% (w/w) Copper and Cymoxanil, respectively). The test material was applied on to the wetted dorsal trunk under a water moistened semi occlusive tape for 4 hours. The untreated skin of each animal served as the control. Skin irritation was assessed at 1, 24, 48 and 72 hours after patch removal.

In this study ATO FDH01 was non-irritating to the skin. Consequently, it can be concluded that FEL02 is also not irritating to the skin.

A 2.6 Eye irritation (KCP 7.1.5)

A 2.6.1 Study 1

Comments of zRMS:	**Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is an eye irritant. Thus, classification with Eye Irrit Cat. 2 - H319 is required according to Regulation (EC) No. 1272/2008.**

Reference:	KCP 7.1.5/01
Report	Acute eye irritation study of Copper 200 + Cymoxanil 40 WDG in rabbits [redacted]
Guideline(s):	OECD 405, OCSPP 870.2400
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	Yes (requested by countries outside Europe, see A 2.1)

Executive Summary

In an acute eye irritation study (compliant with OECD TG 405), 3 adult female New Zealand White rabbits were given a single ocular application of 0.1 mL Copper 200 + Cymoxanil 40 WDG (pulverised) in the right eye of the rabbit while the contralateral eye remained untreated and served as the control. Initially one rabbit was tested. Based on the results obtained at 24 h post-test item application (TIA), the irritation response was confirmed by testing two additional rabbits, simultaneously. Observations were made at 1, 24, 48 and 72 h and on days 7, 14 and 21 post TIA. General health status, including body weight development was also checked.

Conjunctival effects were evident at 1, 24, 48 and 72 h and on days 7 and 14 which were resolved on day 21 in all three rabbits post-TIA.

The individual animal mean eye irritation scores of the 24, 48 and 72 h post TIA observation were 1.00, 1.00, 1.00 for corneal opacity, 0.00, 0.00, 0.00 for iris effects, 2.00, 2.67, 2.67 for conjunctival redness and 2.00, 2.00, 2.00 for conjunctival chemosis for rabbit N° 1,2 and 3, respectively.

Examination with fluoresce in dye and cobalt blue filter post TIA revealed, diminishing corneal epithelium damage (90 to 10% of surface involvement) at 24, 48 and 72 h and on days 7 and 14 which were resolved on day 21 in all three rabbits.

The control eye did not show any abnormal reaction during the study. Moreover, there were no sign of systemic toxicity in any animal observed.

Other than eye irritation, no signs of systemic toxicity including clinical observation and body weight were observed in the rabbits throughout the experimental period.

In summary, Copper 200 + Cymoxanil 40 WDG showed severe eye irritation lesions which were recovered within the normal observation period of 21 days post TIA. Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is an eye irritant. Thus, classification with Eye Irrit Cat. 2 - H319 is required according to Regulation (EC) No. 1272/2008.

MATERIALS AND METHODS

Test material (Lot/Batch No.)	Copper 200 + Cymoxanil 40 WDG (Batch No 15.351.1)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 females
Initial test using one animal	Yes
Exposure	0.1 mL Copper 200 + Cymoxanil 40 WDG (pulverised)
Vehicle/Dilution	None
Post exposure observation period	21 days
Remarks	None

RESULTS AND DISCUSSIONS

TABLE A 2.6.1-1 EYE IRRITATION OF COPPER 200 + CYMOXANIL 40 WDG

Animal No.		**Scores after treatment ***	**Mean scores (24 - 72 h)**	**Reversible [day]**

		24 h	48 h	72 h		
1	Corneal opacity	1	1	1	1.00	21
	Iritis	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2.00	21
	Chemosis conjunctivae	2	2	2	2.00	21
2	Corneal opacity	1	1	1	1.00	21
	Iritis	0	0	0	0.00	-
	Redness conjunctivae	3	3	2	2.67	21
	Chemosis conjunctivae	2	2	2	2.00	14
3	Corneal opacity	1	1	1	1.00	21
	Iritis	0	0	0	0.00	-
	Redness conjunctivae	3	3	2	2.67	21
	Chemosis conjunctivae	2	2	2	2.00	14

* Scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis.

Clinical signs:	No clinical signs of systemic toxicity were observed.

CONCLUSION

Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is an eye irritant. Thus, classification with Eye Irrit Cat. 2 - H319 is required according to Regulation (EC) No. 1272/2008.

A 2.6.2 Study 2

Previous evaluation dRR FEL02, Southern zone, RMS: Italy (2019)

Comments of zRMS:	**The test item ATO FDH01 showed slight to obvious, reversible eye irritating characteristics in the rabbit. Based on these results, it is concluded that ATO FDH01 and consequently FEL02 are subject to classification as irritating to the rabbits' eye. Thus, classification as Eye Irritant Cat. 2 and labelling with the hazard statement H319 (Causes serious eye irritation) is warranted according to Regulation (EC) No 1272/2008.**

Reference:	KCP 7.1.5/02
Report	ATO FDH01 Acute Eye Irritation Test in Rabbits, [redacted]
Guideline(s):	OECD Guideline No. 405
Deviations:	No deviation with impact on quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a primary eye irritation study in accordance to OECD Guideline No. 405, 3 male New Zealand White rabbits, between 2.42 - 2.73 kg were treated with weight equivalent 0.1 mL of ATO FDH01 (batch No. 980430 HE, 20.3% (w/w) and 4.3% (w/w) Copper and Cymoxanil, respectively) instilled into the conjunctival sac of the right eye for an hour. The non-treated left eye served as a control. The animals were then observed at 1, 24, 48, 72 hours after instillation of the test substance. Observation was extended up to day 28 in order to fully evaluate the reversibility of reactions.

Corneal responses covering up to three quarters of the cornea were noted in two animals only up to 72 h after instillation of the test material. Mild to moderate conjunctival redness and chemosis were noted in all three animals. A slight to moderate discharge was noted up to 15 days after instillation of the test material in 1 animal, day 7 in another and up to 48 h in the remaining animal.

The test item ATO FDH01 showed slight to obvious, reversible eye irritating characteristics in the rabbit. Based on these results, it is concluded that ATO FDH01 and consequently FEL02 are subject to classification as irritating to the rabbits' eye. Thus, classification as Eye Irritant Cat. 2 and labelling with the hazard statement H319 (Causes serious eye irritation) is warranted according to Regulation (EC) No 1272/2008.

A 2.7 Skin sensitisation (KCP 7.1.6)

A 2.7.1 Study 1

Comments of zRMS:	**Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is not a skin sensitiser. Thus, no classification is required according to Regulation (EC) No. 1272/2008**.

Reference:	KCP 7.1.6/01
Report	Skin sensitisation study of Copper 200 + Cymoxanil 40 WDG in guinea pigs [Maximisation Test Method] [redacted]
Guideline(s):	OECD 406, OCSPP 870.2600
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	Yes (requested by countries outside Europe, see A 2.1)

Executive Summary

In this skin sensitization study (compliant with OECD TG 406), fifteen Hartley strain male guinea pigs were randomly divided into two groups. The control group comprised 5 guinea pigs and the treatment group comprised 10 guinea pigs. Based on the results of the pilot study, 5.0% (w/v) Copper 200 + Cymoxanil 40 WDG in distilled water was selected for intradermal injection during induction exposure on day 0. As Copper 200 + Cymoxanil 40 WDG was found to be non-irritant when applied topically, on day 6 clipped sites was applied with 0.5 mL 10% (w/v) sodium lauryl sulphate in vaseline to augment the local skin irritation. An amount of 100 mg of Copper 200 + Cymoxanil 40 WDG moistened with 0.2 mL of distilled water was selected for topical application during induction on day 7 and for challenge exposure on day 21.

The skin reactions of the guinea pigs were recorded post induction (intradermal injections/topical application) following the Draize method (Draize et al., 1944) and at 24 and 48 h post challenge treatment following the Magnusson and Kligman grading scale (Magnusson and Kligman, 1969),

In the induction phase, well-defined erythema and very slight to slight oedema were observed in all animals of the treatment group following intradermal injection. Very slight erythema and very slight oedema were observed following topical induction. No skin reactions were observed in the control group during the induction phase.

Visual observation of the skin following challenge exposure revealed a negative skin response at 24 and 48 h post patch removal in the guinea pigs belonging to the treatment group as well as in the control group.

No clinical signs related to treatment other than skin irritation were observed during the course of the study in guinea pigs. The mean body weight of the treatment group guinea pigs remained comparable to the control group.

A sensitisation rate of zero percent at 24 and 48 h post patch removal was observed using an adjuvant.

Under the experimental condition, Copper 200 + Cymoxanil 40 WDG is not a skin sensitiser. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

MATERIALS AND METHODS

Test material (Lot/Batch No.)	Copper 200 + Cymoxanil 40 WDG (Batch No 15.351.1)
Species	Guinea pig, Hartley strain
No. of animals (group size)	Pilot study: Intradermal injection test with 0.2 mL Copper 200 + Cymoxanil 40 WDG at 5.0%, 2.5%, 1.0% and 0.5% (w/v) in distilled water (2 males and 2 females) Topical irritancy test with 100, 75, 50 and 25 mg Copper 200 + Cymoxanil 40 WDG moistened with 0.2 mL distilled water (2 males and 2 females). Main study (Maximisation Test): Test substance group: 10 males Negative control groups: 5 males Positive control groups: 10 (5M + 5F) control and 20 (10M + 10F) test animals
Range finding:	Yes
Exposure (concentration(s), no. of applications)	Intradermal induction: 5.0% Topical induction: Undiluted Challenge: Undiluted
Vehicle	Moistened with 0.2 mL distilled water
Pretreatment prior to topical application	Yes (sodium lauryl sulfate)

Reliability check	α-Hexylcinnamaldehyde
Remarks	None

RESULTS AND DISCUSSIONS

In the induction phase, well-defined erythema (in 10/10 guinea pigs) and very slight oedema (in 1/10 guinea pigs) to slight oedema (in 9/10 guinea pigs) were observed on day 1 in the treatment group following intradermal injection. Very slight erythema (in 8/10 guinea pigs) and very slight oedema (in 5/10 guinea pigs) were observed on day 10 on the left flank of the treatment group guinea pigs following topical application on day 7. No skin reactions were observed in the guinea pigs from the control group (days 1 and 10).

Table A 2.7.1-1 Results of skin sensitisation study of Copper 200 + Cymoxanil 40 WDG

	Skin reactions following challenge application*		
	24 hours	**48 hours**	**Animals affected [%]****
	After challenge		
Test Vehicle Control Group	0/5	0/5	0%
Copper 200 + cymoxanil 40 WDG	0/10	0/10	0%
Positive control	9/20	8/20	45%

* Number of animals with positive dermal response (scores of 1-3) /number of animals in dose group
** A response of at least 30% is considered as positive in adjuvant method test such as the Maximisation Test.

Clinical signs:	No clinical signs of systemic toxicity were observed.

CONCLUSION

Under the experimental conditions, Copper 200 + Cymoxanil 40 WDG is not a skin sensitiser. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.7.2 Study 2

Previous evaluation dRR FEL02, Southern zone, RMS: Italy (2019)

Comments of zRMS:	**No positive responses were observed in the test group or remaining control group animals following challenge with 75% ATO FDH01. In accordance with Regulation (EC) No 1272/2008, ATO FDH01 is not considered to be a sensitizer in guinea pigs. Consequently, it can be concluded that FEL02 is also not sensitizing.**

Reference:	KCP 7.1.6/02
Report	ATO FDH01 Magnusson-Kligman Maximisation Test in Guinea Pigs, x [redacted]
Guideline(s):	OECD Guideline No. 406
Deviations:	Yes, some animals were outside the weight range stated in the protocol (300 – 500 g), however, this deviation was considered not to have affected the outcome of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a dermal sensitization study in accordance to OECD Guideline No. 406, 20 female albino Guinea Pigs between 292 - 328 g were subjected to sensitisation procedures with ATOFDH01 (batch No. 980430HE, 20.3% (w/w) and 4.4% (w/w) Copper and Cymoxanil, respectively) according to Magnusson and Kligman in comparison with a negative control group (10 guinea pigs) exposed to vehicle, distilled water.

The maximum slight to moderate irritant concentration determined by intradermal administration and used during the primary induction phase was 0.1% (w/v) in water for injection or Freund's Complete Adjuvant (FCA) (50/50 (v/v)) applied on day 1. The intradermal induction was followed by a topical induction (0.5 mL test item at 75%) on day 7. A total of 10 concurrent control animals were treated with the vehicle during sensitisation and with ATO FDH01 at 75%. On day 14, the all animals received two occlusive topical applications each at concentrations of 75% and 0% ATO FDH01. After 24 h the occlusive dressing was removed, and the skin was rinsed with distilled water.

No positive responses were observed in the test group or remaining control group animals following challenge with 75% ATO FDH01. In accordance with Regulation (EC) No 1272/2008, ATO FDH01 is not considered to be a sensitizer in guinea pigs. Consequently, it can be concluded that FEL02 is also not sensitizing.

A 2.8 Supplementary studies for combinations of plant protection products (KCP 7.1.7)

There are no supplementary studies on FEL02 to be considered.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.9.1 Material safety data sheet for each co- formulant

Information regarding material safety data sheets of the co-formulants can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.9.2 Available toxicological data for each co-formulant

Available toxicological data for each co-formulant can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.10 Studies on dermal absorption (KCP 7.3)

FEL02 was not evaluated as part of the EU review of active substance Copper compounds.

Dermal absorption rates employed in the risk assessment of copper are based on three dermal absorption studies using the triple pack calculation approach.

All three studies are comparable with regards to test material, formulation, vehicle, exposure etc. and fulfills all the criteria for similarity as outlined in the EFSA guidance on dermal absorption (2017)[10]. Summaries of the studies are given below.

Dermal absorption rates employed in the risk assessment of cymoxanil are based on a dermal absorption study in human skin *in vitro* with Cymoxanil in FEL02 (Maas, 2020), see A.2.10.5.

A 2.10.1 Copper: Study 1 – Dermal absorption, *in vitro* using human skin

Previous evaluation	fRR FAP13, Central zone, RMS: Poland (2022) Comment zRMS Poland : Dermal absorption values can be for Copper hydroxide in the WG 53.8 formulation: • 0.39% for the neat formulation (350 g/kg) • 2.8% for the intermediate dose (3 g/L) • 8.7% for the low dose (0.3 g/L)

Comments of zRMS:	**Therefore, the following dermal absorption values can be proposed for Copper hydroxide in the WG 53.8 formulation:** **• 0.39% for the neat formulation (350 g/kg)** **• 2.8% for the intermediate dose (3 g/L)** **• 8.7% for the low dose (0.3 g/L)**

Reference	KCP 7.3/01
Report	*In vitro* percutaneous absorption of copper, formulated as Copper Hydroxide (DPX-GFJ52) 53.8WG (35% as metallic copper), through human skin [redacted]
Guideline(s):	OECD 428 (2004); OECD Assessment No 28 (2004); EFSA Guidance on Dermal Absorption (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

MATERIALS AND METHODS

[10] European Food and Safety Authority (EFSA), 2017. Guidance on dermal absorption. EFSA Journal 2017;15(6):4873.

Test material	Name (Lot/Batch No.)	
	Non-radiolabelled	Copper hydroxide
	Radiolabelled	^{65}Copper hydroxide
	Formulation	Copper hydroxide 53.8 WG containing 53.8% ^{65}Copper hydroxide (equivalent to 35% metallic Copper)*

* The amount of copper was determined at mass 65 *m/z* and mass 63 *m/z* using a double focusing high resolution inductively coupled plasma spectrometry (HR-ICP-MS) in medium mode (resolution, 40000) in all collected samples.

Test system		
Diffusion cell	Cell type	Dynamic
	(if dynamic) Flow rate	1.2 mL/h
	Exposed skin area	-
	Cover	-
Membrane	Skin type	Human, dermatomed
	Skin thickness range	200 to 400 µm
	Skin donors age	-
	Skin donors sex	Female
	Location	Abdomen and/or breast
	Source	TNO Triskelion
	Integrity test	Yes (Permeability coefficient)
Receptor	Receptor medium	Phosphate-buffered saline (PBS) containing 0.01% sodium azide (w/v), supplemented with 6% poly-oxy-ethylene 20-oleyl glycol (PEG) (w/v), pH 7.2
	Solubility in receptor medium	Yes
Sample Time	Exposure time	Single: 6 hours exposure
	Observation time	48 hrs
Sampling	Sample intervals	0-1 h, 1-2 h, 2-4 h, 4-6 h, 6-12 h and 12-24 h after application
Washing		Following 6 hours' exposure; second skin wash was performed at 24 hours post-application
Final Procedure	Tape stripping	Yes (pooled as 1, 2, 3, 4, 5, 6, 6-10 and 11-15)
	TS1-2 analysed separately	Yes
Remarks:		

Tested doses	**Concentrate****	**Spray dilution 1**	**Spray dilution 2**
Target concentration	350 g/kg	3 g ^{65}Copper/L	0.3 g ^{65}Copper/L
No. of donors	4	4	4
No of cells used/valid cells*	8/8	8/8	8/8

* Justification for excluded cells, if applicable
** The undiluted concentrate (powder) was distributed over the skin and wetted using a minimal volume of artificial sweat (to mimic conditions on the skin under occlusive clothing conditions).

RESULTS AND DISCUSSIONS

Copper was demonstrated to be sufficiently soluble in the receptor fluid to avoid any risk of back diffusion.

Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable.

Mean recovery was above 95% in all cases, being approximately 107% for the concentrate (350 g/kg) and both spray dilutions (3 and 0.3 g/L). Therefore, no adjustments for low recovery were required.

The study results are presented in the following Tables:

Table A 2.10.1-1 Distribution of radioactivity at 24 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (undiluted concentrate) to human skin samples

	Distribution of radioactivity (% dose applied)								**N = 8** **K N° = 0.84**
Donor N°	**1**	**1**	**2**	**2**	**3**	**3**	**4**	**4**	
Sex	F	F	F	F	F	F	F	F	
Cell N°	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	Mean ± SD
Skin wash 6 h	97.2	96.0	87.9	108.7	110.4	101.7	128.3	114.8	105.6 ± 12.7
Skin wash 24 h	0.6	0.4	0.4	0.1	0.2	0.3	0.1	0.1	0.3 ± 0.2
Tape strip 1	0.84	0.42	0.10	0.06	0.42	0.73	0.04	0.05	0.33 ± 0.32
Tape strip 2	0.09	0.06	0.03	0.02	0.11	0.06	0.02	0.02	0.05 ± 0.04
Total tape strips 1 + 2	0.93	0.48	0.13	0.08	0.53	0.79	0.06	0.07	0.38 ± 0.35
Donor chamber	0.16	0.04	0.37	0.01	0.11	0.10	0.01	0.00	0.101 ± 0.124
TOTAL NON-ABSORBED	**98.89**	**96.92**	**88.8**	**108.89**	**111.24**	**102.89**	**128.47**	**114.97**	**106.38 ± 12.30**
Stripped skin	0.36	0.19	<0.00	0.00	0.33	0.30	0.04	0.04	0.16 ± 0.15
Tape strip 3	0.03	0.02	0.00	0.01	0.05	0.03	0.01	0.02	0.02 ± 0.01
Tape strip 4	0.01	0.01	0.00	0.00	0.03	0.01	--	--	0.01 ± 0.01
Tape strip 5	0.02	0.01	<0.00	<0.00	0.02	0.01	--	--	0.01 ± 0.01
Tape strip 6-10	--	0.06	0.01	<0.00	0.05	0.05	--	--	0.03 ± 0.03
Tape strip 11-15	--	--	<0.00	<0.00	0.05	0.01	--	--	0.02 ± 0.02
TOTAL Tape strips 3+ [a]	0.06	0.1	0.01	0.01	0.2	0.07	0.01	0.02	0.07 ± 0.07
TOTAL DOSE SITE	**0.78**	**0.48**	**0.01**	**0.01**	**0.91**	**0.68**	**0.09**	**0.1**	**0.22 ± 0.21**
Receptor fluid (0 – 12 h)	0.0008	0.0006	0.0011	<0.0006	<0.0008	0.0007	0.0067	<0.0006	0.0015 ± 0.0021
Receptor fluid (0 – 24 h)	<0.0013	<0.0011	0.0023	<0.011	0.0023	0.0013	0.0073	<0.0011	0.0022 ± 0.0021
%Ratio receptor 12 h/24 h	61	55	48	5	35	54	92	55	**68**
Receptor chamber wash	0.000	0.000	<0.000	<0.000	0.000	<0.000	<0.000	<0.000	<0.00 ± 0.00
TOTAL DIRECT	**0.0021**	**0.0017**	**0.0034**	**0.0116**	**0.0031**	**0.002**	**0.014**	**0.0017**	**0.0050 ± 0.0049**
POTENTIAL (dose site + receptor)	**0.7821**	**0.4817**	**0.0134**	**0.0216**	**0.9131**	**0.682**	**0.104**	**0.1017**	**0.3875 ± 0.3710**
POTENTIAL (skin + receptor)	0.3621	0.1917	0.0034	0.0116	0.3331	0.302	0.054	0.0417	0.1625 ± 0.1529
TOTAL RECOVERY	**99.6721**	**97.4017**	**88.8134**	**108.9116**	**112.1531**	**103.572**	**128.574**	**115.0717**	**106.771 ± 12.240**
	Evaluation according to EFSA Guidance (2017)								
	Absorption >75% within half of the study duration?					No (include tape strip values except 1 & 2)			
	Recovery <95%?					No			
	Total % potentially absorbable (adjusted)					0.22 ± 0.1; k = 0.84			

		= 0.22 + (0.84 × 0.21) = **0.39%**

[a] tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.

Table A 2.10.1-2 Distribution of radioactivity at 24 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (Dilution I) to human skin samples

	Distribution of radioactivity (% dose applied)								**N= 8** **K N° = 0.84**
Donor N°	**1**	**1**	**2**	**2**	**3**	**3**	**4**	**4**	
Sex	F	F	F	F	F	F	F	F	
Cell N°	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	Mean ± SD
Skin wash 6 h	80.7	99.9	102.4	108.2	99.2	106.8	126.2	102.9	103.3 ± 12.5
Skin wash 24 h	2.0	1.5	0.4	1.9	1.3	1.1	2.5	1.5	1.5 ± 0.6
Tape strip 1	0.12	<0.11	<0.11	0.31	<0.11	<0.11	0.48	0.20	0.19 ± 0.14
Tape strip 2	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	0.45	<0.12	<0.15 ± 0.12
Total tape strips 1 + 2	0.23	0.22	0.22	0.42	0.22	0.22	0.93	0.32	0.35 ± 0.25
Donor chamber	0.03	0.02	0.03	0.19	0.03	0.02	0.05	0.04	0.05 ± 0.06
TOTAL NON-ABSORBED	**82.96**	**101.64**	**103.05**	**110.71**	**100.75**	**108.14**	**129.68**	**104.76**	**105.21 ± 12.93**
Stripped skin	4.2	2.0	<0.1	0.2	0.7	1.2	1.1	0.6	1.3 ± 1.3
Tape strip 3	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	0.14	<0.12	<0.11 ± 0.01
Tape strip 4	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	0.26	<0.12	<0.13 ± 0.05
Tape strip 5	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	0.14	<0.12	<0.11 ± 0.01
Tape strip 6-10	--	0.15	<0.11	0.14	0.15	<0.11	--	<0.12	<0.13 ± 0.02
Tape strip 11-15	--	--	--	--	<0.11	<0.11	--	--	<0.11 ± 0.00
TOTAL Tape strips 3+ [a]	0.33	0.48	0.44	0.47	0.59	0.55	0.54	0.48	0.49 ± 0.08
TOTAL DOSE SITE	**4.53**	**2.48**	**0.44**	**0.67**	**1.29**	**1.75**	**1.64**	**1.08**	**1.74 ± 1.30**
Receptor fluid (0 – 12 h)	<0.0006	<0.0007	0.0009	0.0021	0.0010	<0.0005	0.0017	0.0026	0.0013 ± 0.0008
Receptor fluid (0 – 24 h)	<0.0011	<0.0012	0.0022	0.0027	0.0016	0.014	0.0022	0.0031	0.0019 ± 0.0007
%Ratio receptor 12 h/24 h	54	58	50	78	63	36	77	84	**68**
Receptor chamber wash	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	0.003	<0.001 ± 0.001
TOTAL DIRECT	**0.0017**	**0.0019**	**0.0031**	**0.0048**	**0.0026**	**0.0145**	**0.0039**	**0.0087**	**0.0052 ± 0.0044**
POTENTIAL (dose site + receptor)	**4.5317**	**2.4819**	**0.4431**	**0.6748**	**1.2926**	**1.7645**	**1.6439**	**1.0887**	**1.7402 ± 1.2981**
POTENTIAL (skin + receptor)	4.2017	2.0019	0.1031	0.2048	0.7026	1.2145	1.1039	0.6087	1.2677 ± 1.3318
TOTAL RECOVERY	**87.49**	**104.12**	**103.49**	**111.38**	**102.04**	**109.90**	**131.32**	**105.85**	**106.95 ± 12.23**
	Evaluation according to EFSA Guidance (2017)								
	Absorption >75% within half of the study duration?					No (include tape strip values except 1 & 2)			
	Recovery <95%?					No			
	Total % potentially absorbable (adjusted)					1.7402 ± 1.2981; k = 0.84 = 1.7402 + (0.84 × 1.2981) = **2.8%**			

[a] tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.

Table A 2.10.1-3 Distribution of radioactivity at 24 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (Dilution II) to human skin samples

	Distribution of radioactivity (% dose applied)								**N= 8 K N° = 0.84**
Donor N°	**1**	**1**	**2**	**2**	**3**	**3**	**4**	**4**	
Sex	F	F	F	F	F	F	F	F	
Cell N°	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	Mean ± SD
Skin wash 6 h	93.1	77.0	91.0	98.3	97.0	88.5	90.0	86.4	90.1 ± 6.7
Skin wash 24 h	9.8	10.0	5.7	5.1	7.7	6.6	7.3	7.7	7.49 ± 1.76
Tape strip 1	<1.07	<1.07	<1.07	<1.07	<1.07	<1.09	<1.36	<1.37	<1.15 ± 0.13
Tape strip 2	<1.07	<1.07	<1.07	<1.07	<1.07	<1.09	<1.36	<1.37	<1.15 ± 0.13
Total tape strips 1 + 2	2.14	2.14	2.14	2.14	2.14	2.18	2.72	2.74	2.29 ± 0.27
Donor chamber	0.30	0.34	0.28	0.21	0.36	0.22	0.63	0.56	0.36 ± 0.15
TOTAL NON-ABSORBED	**105.34**	**89.48**	**99.12**	**105.72**	**107.2**	**97.5**	**100.65**	**97.4**	**100.31 ± 5.83**
Stripped skin	2.4	1.5	<1.0	<1.0	<1.0	<1.1	2.8	3.8	1.83 ± 1.06
Tape strip 3	<1.07	<1.07	<1.07	<1.07	1.23	<1.09	<1.36	<1.37	<1.17 ± 0.13
Tape strip 4	<1.07	<1.07	<1.07	<1.07	<1.07	<1.09	<1.36	<1.37	<1.15 ± 0.13
Tape strip 5	<1.07	<1.07	<1.07	<1.07	<1.07	<1.09	<1.36	<1.37	<1.15 ± 0.13
Tape strip 6-10	<1.07	<1.07	<1.07	<1.07	<1.07	<1.09	<1.36	<1.37	<1.15 ± 0.13
Tape strip 11-15	<1.07	<1.07	<1.07	<1.07	<1.07	<1.09	--	<1.37	<1.12 ± 0.11
TOTAL Tape strips 3+ [a]	5.35	5.35	5.35	5.35	5.51	5.45	5.44	6.85	5.58 ± 0.52
TOTAL DOSE SITE	**7.75**	**6.85**	**6.35**	**6.35**	**6.51**	**6.55**	**8.24**	**10.65**	**7.41 ± 1.48**
Receptor fluid µg (0 – 12 h)	0.0008	0.0010	0.0007	0.0008	0.0010	0.0007	0.0011	<0.0006	0.0008 ± 0.0002
Receptor fluid µg (0 – 24 h)	0.0018	0.0016	<0.0013	<0.0014	<0.0016	<0.0013	<0.0017	<0.0011	<0.0015 ± 0.0002
%Ratio receptor 12 h/24 h	44	63	54	57	63	54	65	55	**53**
Receptor fluid (%)	0.07	0.06	<0.05	<0.05	<0.06	<0.05	<0.08	<0.06	<0.06 ± 0.01
Receptor chamber wash	<0.004	<0.004	0.008	<0.003	<0.004	0.013	<0.003	<0.003	<0.005 ± 0.003
TOTAL DIRECT	**0.074**	**0.064**	**0.058**	**0.053**	**0.064**	**0.063**	**0.083**	**0.063**	**0.0653 ± 0.0093**
POTENTIAL (dose site + receptor)	**7.824**	**6.914**	**6.408**	**6.403**	**6.574**	**6.613**	**8.323**	**10.713**	**7.4715 ± 1.4872**
POTENTIAL (skin + receptor)	2.474	1.564	1.058	1.053	1.064	1.163	2.883	3.863	1.9 ±1.1064
TOTAL RECOVERY	**113.16**	**96.39**	**105.53**	**112.12**	**113.77**	**104.11**	**108.97**	**108.11**	**107.8 ± 5.8**
	Evaluation according to EFSA Guidance (2017)								
	Absorption >75% within half of the study duration?					No (include tape strip values except 1 & 2)			
	Recovery <95%?					No			
	Total % potentially absorbable (adjusted)					7.4715 ± 1.4872; k = 0.84 = 7.4715 + (0.84 × 1.4872) = **8.7%**			

[a] tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.

CONCLUSION/ENDPOINT

The dermal penetration through human dermatomed skin of ^{65}Copper hydroxide in a WG 53.8 formulation was investigated at three nominal concentrations corresponding to the neat product (350 g /kg) and to two representative spray dilutions of 3 g/L and 0.3 g/L.

Concentrate

The mean percentage of ^{65}Copper hydroxide in the WG 53.8 formulation that was considered to be potentially absorbable for the neat formulation applying the EFSA guidance (2017) to the study data was 0.39%.

Intermediate Dose level (Spray dilution at 3 g/L)

The mean percentage of ^{65}Copper hydroxide in the WG 53.8 formulation that was considered to be potentially absorbable for the spray dilution at 3 g/L applying the EFSA guidance (2017) to the study data was 2.8%.

Low Dose level (Spray dilution at 0.3 g/L)

The mean percentage of ^{65}Copper hydroxide in the WG 53.8 formulation that was considered to be potentially absorbable for the spray dilution at 0.3 g/L applying the EFSA guidance (2017) to the study data was 8.7%.

Therefore, the following dermal absorption values can be proposed for Copper hydroxide in the WG 53.8 formulation:

- 0.39% for the neat formulation (350 g/kg)
- 2.8% for the intermediate dose (3 g/L)
- 8.7% for the low dose (0.3 g/L)

Although the above values have been determined in accordance with the EFSA guidance on dermal absorption (2017), it is noted that this is overly conservative in the case of copper, as the amount retained in the stratum corneum and stripped skin is not likely to be available for systemic absorption over time.

A 2.10.2 Copper: Study 2 – Dermal absorption, *in vitro* using rat skin

Previous evaluation	fRR FAP13, Central zone, RMS: Poland (2022) Comment zRMS Poland : Dermal absorption values can be for Copper hydroxide in the WG 53.8 formulation: • 2.6% for the neat formulation (350 g/kg) • 3.6% for the intermediate dose (3 g/L) • 14% for the low dose (0.3 g/L)
Comments of zRMS:	**Therefore, the following dermal absorption values can be proposed for Copper hydroxide in the WG 53.8 formulation:** **• 2.6% for the neat formulation (350 g/kg)** **• 3.6% for the intermediate dose (3 g/L)** **• 14% for the low dose (0.3 g/L)**
Reference	KCP 7.3/02
Report	*In vitro* percutaneous absorption of copper, formulated as Copper Hydroxide (DPX-GFJ52) 53.8WG (35% as metallic copper), through rat skin [redacted]
Guideline(s):	OECD 428 (2004); OECD Assessment No 28 (2004); EFSA Guidance on Dermal Absorption (2017)
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

MATERIALS AND METHODS

Test material	Name (Lot/Batch No.)	
	Non-radiolabelled	Copper hydroxide
	Radiolabelled	^{65}Copper hydroxide
	Formulation	Copper hydroxide 53.8 WG containing 53.8% ^{65}Copper hydroxide (equivalent to 35% metallic Copper)*

* The amount of copper was determined at mass 65 *m/z* and mass 63 *m/z* using a double focusing high resolution inductively coupled plasma spectrometry (HR-ICP-MS) in medium mode (resolution, 40000) in all collected samples.

Test system		
Diffusion cell	Cell type	Dynamic
	(if dynamic) Flow rate	1.2 mL/h
	Exposed skin area	-
	Cover	-
Membrane	Skin type	Rat, dermatomed
	Skin thickness range	200 to 400 µm
	Skin donors age	10-12 weeks old Wistar (HsdCpb:Wu) rats
	Skin donors sex	Male Wistar rats
	Location	Dorsal region
	Source	Harlan, Horst, The Netherlands
	Integrity test	Yes (Permeability coefficient)
Receptor	Receptor medium	Phosphate-buffered saline (PBS) containing 0.01% sodium azide (w/v), supplemented with 6% poly-oxy-ethylene 20-oleyl glycol (PEG) (w/v), pH 7.2
	Solubility in receptor medium	Yes
Sample Time	Exposure time	Single: 6 hours exposure
	Observation time	48 hrs
Sampling	Sample intervals	0-1 h, 1-2 h, 2-4 h, 4-6 h, 6-12 h and 12-24 h after application
Washing		Following 6 hours' exposure; second skin wash was performed at 24 hours post-application
Final Procedure	Tape stripping	Yes (pooled as 1, 2, 3, 4, 5, 6, 6-10 and 11-15)
	TS1-2 analysed separately	Yes
Remarks:		

Tested doses	Concentrate**	Spray dilution 1	Spray dilution 2
Target concentration	350 g/kg	3 g ^{65}Copper/L	0.3 g ^{65}Copper/L
No. of donors	4	4	4
No of cells used/valid cells*	8/7	8/8	8/8

* Concentrate: replicate A-5 was excluded from the calculations owing to a deviating absorption profile caused by damaged membrane integrity.
** The undiluted concentrate (powder) was distributed over the skin and wetted using a minimal volume of artificial sweat (to mimic conditions on the skin under occlusive clothing conditions).

RESULTS AND DISCUSSIONS

Copper was demonstrated to be sufficiently soluble in the receptor fluid to avoid any risk of back diffusion. Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable. The study results are presented in the following Tables:

Table A 2.10.2-1 Distribution of radioactivity at 24 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (undiluted concentrate) to rat skin samples

	Distribution of radioactivity (% dose applied)								N = 7 K N° = 0.92
Donor N°	**1**	**1**	**2**	**2**	**3***	**3**	**1**	**2**	
Sex	M	M	M	M	M	M	M	M	
Cell N°	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	Mean ± SD
Skin wash 6 h	118.2	108.3	116.1	101.7	99.2	98.5	108.6	97.8	107.0 ± 8.1
Skin wash 24 h	1.5	1.2	1.7	1.9	1.1	0.9	1.0	0.9	1.3 ± 0.4
Tape strip 1	0.20	0.13	0.16	0.16	0.08	0.14	0.13	0.17	0.16 ± 0.03
Tape strip 2	0.10	0.10	0.06	0.07	0.06	0.05	0.06	0.10	0.08 ± 0.02
Total tape strips 1 + 2	0.3	0.23	0.22	0.23	0.14	0.19	0.19	0.27	0.23 ± 0.04
Donor chamber	0.01	0.00	0.00	0.03	0.03	0.01	0.00	0.00	0.008 ± 0.010
TOTAL NON-ABSORBED	**120.01**	**109.73**	**118.02**	**103.86**	**100.47**	**99.6**	**109.79**	**98.97**	**108.57 ± 8.34**
Stripped skin	1.42	0.38	2.47	1.43	1.61	0.57	1.73	1.26	1.32 ± 0.70
Tape strip 3	0.04	0.13	0.10	0.22	0.02	0.04	0.29	0.29	0.16 ± 0.11
Tape strip 4	0.09	0.20	0.08	0.08	0.01	0.06	--	--	0.10 ± 0.05
Tape strip 5	0.12	0.16	0.07	0.06	0.01	0.06	--	--	0.09 ± 0.05
Tape strip 6-10	--	0.15	0.23	0.12	0.05	0.07	--	--	0.14 ± 0.07
Tape strip 11-15	--	--	0.33	0.15	0.02	0.05	--	--	0.18 ± 0.14
TOTAL Tape strips 3+ [a]	0.25	0.64	0.81	0.63	0.11	0.28	0.29	0.29	0.46 ± 0.23
TOTAL DOSE SITE	**1.67**	**1.02**	**3.28**	**2.06**	**1.72**	**0.85**	**2.02**	**1.55**	**1.78 ± 0.81**
Receptor fluid µg (0 – 12 h)	0.004	0.020	0.115	0.129	15.826	0.057	1.356	0.032	0.24 ± 0.49
Receptor fluid µg (0 – 24 h)	0.008	0.068	0.399	0.133	15.829	0.077	2.163	0.034	0.41 ± 0.78
%Ratio receptor 12 h/24 h	50	29	29	97	97	74	63	94	**56**
Receptor fluid (%)	0.001	0.004	0.027	0.008	1.161	0.005	0.131	0.002	0.025 ± 0.047
Receptor chamber wash	0.00	0.00	0.001	0.001	0.309	0.003	0.00	0.00	0.001 ± 0.001
TOTAL DIRECT	**0.001**	**0.004**	**0.028**	**0.009**	**1.47**	**0.008**	**0.131**	**0.002**	**0.0261 ± 0.0471**
POTENTIAL (dose site + receptor)	**1.671**	**1.024**	**3.308**	**2.069**	**3.19**	**0.858**	**2.151**	**1.552**	**1.8047 ± 0.8203**
POTENTIAL	1.421	0.384	2.498	1.439	3.08	0.578	1.861	1.262	1.3490 ± 0.7221

(skin + receptor)									
TOTAL RECOVERY	**121.68**	**110.75**	**121.33**	**105.93**	103.66	**100.46**	**111.94**	**100.52**	**110.37 ± 8.81**
	Evaluation according to EFSA Guidance (2017)								
	Absorption >75% within half of the study duration?					No (include tape strip values except 1 & 2)			
	Recovery <95%?					No			
	Total % potentially absorbable (adjusted)					1.80 ± 0.82; k = 0.92 = 1.80 + (0.92 × 0.82) = **2.6%**			

[a] tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.
* Replicate A-5 was excluded from the calculations owing to a deviating absorption profile caused by damaged membrane integrity.

Table A 2.10.2-2 Distribution of radioactivity at 24 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (Dilution I) to rat skin samples

	Distribution of radioactivity (% dose applied)								**N = 6 K N° = 1**
Donor N°	**1**	**1**	**2**	**2**	3*	**3**	3*	**1**	
Sex	M	M	M	M	M	M	M	M	
Cell N°	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	Mean ± SD
Skin wash 6 h	97.8	91.5	78.3	81.8	67.4	84.9	78.0	103.7	89.7 ± 9.8
Skin wash 24 h	6.1	9.5	10.7	9.6	4.6	8.4	10.9	11.4	9.3 ± 1.9
Tape strip 1	1.18	4.66	3.74	5.32	2.73	2.33	5.24	4.14	3.56 ± 1.54
Tape strip 2	0.74	2.09	3.63	2.62	1.24	2.19	2.90	2.94	2.37 ± 0.97
Total tape strips 1 + 2	1.92	6.75	7.37	7.94		4.52		1.08	5.93 ± 2.29
Donor chamber	0.04	0.05	0.04	0.04	0.05	0.05	0.06	0.08	0.05 ± 0.01
TOTAL NON-ABSORBED	**105.86**	**107.8**	**96.41**	**99.38**		**97.87**		**116.26**	**103.93 ± 7.55**
Stripped skin	1.6	0.5	2.6	2.9	2.4	1.2	2.7	1.5	1.7 ± 0.9
Tape strip 3	0.46	1.40	1.46	1.39	0.97	1.50	2.54	2.06	1.38 ± 0.52
Tape strip 4	0.67	1.51	1.36	0.52	1.17	1.55	0.93	1.20	1.14 ± 0.44
Tape strip 5	0.31	0.92	4.02	0.64	0.46	0.93	0.83	0.78	1.27 ± 1.37
Tape strip 6-10	--	1.21	3.31	0.58	2.37	1.64	--	0.70	1.49 ± 1.10
Tape strip 11-15	--	--	--	--	0.79	0.47	--	--	0.47
TOTAL Tape strips 3+ [a]	1.44	5.04	10.15	3.13		6.09		4.74	5.10 ± 2.96
TOTAL DOSE SITE	**3.04**	**5.54**	**12.75**	**6.03**		**7.29**		**6.24**	**6.82 ± 3.23**
Receptor fluid µg (0 – 12 h)	0.01	0.07	0.28	0.20	5.97	0.15	1.75	0.04	0.12 ± 0.10
Receptor fluid µg (0 – 24 h)	0.01	0.09	0.41	0.20	5.97	0.16	2.22	0.04	0.15 ± 0.14
%Ratio receptor 12 h/24 h	100	77	68	100	100	94	79	100	**80**
Receptor fluid (%)	0.039	0.365	1.593	0.775	23.126	0.613	8.597	0.174	0.59 ± 0.56
Receptor chamber wash	0.001	<0.001	0.006	0.036	0.303	0.002	0.007	0.008	0.009 ± 0.014
TOTAL DIRECT	**0.04**	**0.366**	**1.599**	**0.811**		**0.615**		**0.182**	**0.6022 ± 0.5632**
POTENTIAL (dose site + receptor)	**3.08**	**5.906**	**14.349**	**6.841**		**7.905**		**6.422**	**7.4172 ± 3.7605**
POTENTIAL (skin + receptor)	1.64	0.866	4.199	3.711		1.815		1.682	2.3188 ± 1.3194
TOTAL RECOVERY	**108.94**	**113.71**	**110.76**	**106.22**		**105.78**		**122.68**	**111.35 ± 6.29**
	Evaluation according to EFSA Guidance (2017)								
	Absorption >75% within half of the study duration?					Yes (exclude all tape strips)			

	Recovery <95%?	No
	Total % potentially absorbable (adjusted)	2.3188 ± 1.3194; k = 1 = 2.3188 + (1 × 1.3194) = **3.6%**

[a] tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.
* Replicates B-5 and B-7 were excluded from the calculations owing to a deviating absorption profile caused by damaged membrane integrity.

Table A 2.10.2-3 Distribution of radioactivity at 24 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (Dilution II) to rat skin samples

	Distribution of radioactivity (% dose applied)								**N = 6 K N° = 1**
Donor N°	**1**	**1**	**2**	**2**	**3***	**3**	**2***	**3**	
Sex	M	M	M	M	M	M	M	M	
Cell N°	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	Mean ± SD
Skin wash 6 h	91.0	84.5	73.4	86.4	85.1	78.0	88.2	35.8	74.9 ± 20.1
Skin wash 24 h	12.5	10.1	10.0	8.1	7.8	9.0	6.6	11.7	10.24 ± 1.67
Tape strip 1	<0.90	0.91	<0.90	<0.90	<0.90	<0.89	2.97	1.61	<1.02 ± 0.29
Tape strip 2	<0.90	<0.90	<0.90	<0.90	<0.90	<0.89	<0.90	<0.91	<0.90 ± 0.00
Total tape strips 1 + 2	1.8	1.81	1.8	1.8		1.78		2.52	1.92 ± 0.29
Donor chamber	0.91	0.93	0.85	0.91	0.90	0.86	0.23	0.98	0.91 ± 0.05
TOTAL NON-ABSORBED	**106.21**	**97.34**	**86.05**	**97.21**		**89.64**		**51**	**87.91 ± 19.39**
Stripped skin	2.1	3.2	4.5	3.5	5.6	5.2	11.9	8.6	4.50 ± 2.27
Tape strip 3	<0.90	<0.90	<0.90	<0.90	<0.90	<0.89	<0.90	<0.91	<0.90 ± 0.00
Tape strip 4	<0.90	<0.90	<0.90	<0.90	<0.90	<0.89	<0.90	<0.91	<0.90 ± 0.01
Tape strip 5	<0.90	<0.90	<0.90	<0.90	<0.90	<0.89	<0.90	<0.91	<0.90 ± 0.00
Tape strip 6-10	3.97	2.03	4.17	3.73	2.20	1.75	2.58	2.33	3 ± 1.08
Tape strip 11-15	<0.90	3.11	0.95	1.87	2.52	2.28	-	2.43	1.93 ± 0.87
TOTAL Tape strips 3+ [a]	7.57	7.84	7.82	8.3		6.7		7.49	7.62 ± 0.53
TOTAL DOSE SITE	**9.67**	**11.04**	**12.32**	**11.8**		**11.9**		**16.09**	**12.14 ± 2.15**
Receptor fluid µg (0 – 12 h)	0.007	0.058	0.291	0.171	2.504	0.116	0.736	0.040	0.114 ± 0.105
Receptor fluid µg (0 – 24 h)	0.009	0.065	0.326	0.173	2.507	0.120	0.885	0.042	0.123 ± 0.115
%Ratio receptor 12 h/24 h	78	89	89	99	100	97	83	95	**93**
Receptor fluid Total	0.36	2.58	12.82	6.85	99.26	4.68	34.73	1.69	**4.83 ± 4.54**
Receptor chamber wash	<0.009	0.016	0.019	<0.008	<0.008	0.017	0.075	0.016	0.014 ± 0.004
TOTAL DIRECT	**0.369**	**2.596**	**12.839**	**6.858**		**4.697**		**1.706**	**4.8442 ± 4.5370**
POTENTIAL (dose site + receptor)	10.039	13.636	25.159	18.658		16.597		17.796	16.9808 ± 5.0967
POTENTIAL (skin + receptor)	**2.469**	**5.796**	**17.339**	**10.358**		**9.897**		**10.306**	**9.3608 ± 5.0206**
TOTAL RECOVERY	**116.25**	**110.98**	**111.21**	**115.87**		**106.24**		**68.80**	**104.89 ± 18.06**
	Evaluation according to EFSA Guidance (2017)								
	Absorption >75% within half of the study duration?					Yes (exclude all tape strips)			
	Recovery <95%?					No			
	Total % potentially absorbable (adjusted)					9.3608 ± 5.0206; k = 1 = 9.3608 + (1 × 5.0206)			

		= **14%**

[a] tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.
** Replicates C-5 and C-7 were excluded from the calculations owing to a deviating absorption profile caused by damaged membrane integrity.

CONCLUSION/ENDPOINT

The dermal penetration through rat dermatomed skin of ^{65}Copper hydroxide in a WG 53.8 formulation was investigated at three nominal concentrations corresponding to the neat product (350 g /kg) and to two representative spray dilutions of 3 g/L and 0.3 g/L.

Concentrate

The mean percentage of ^{65}Copper hydroxide in the WG 53.8 formulation that was considered to be potentially absorbable for the neat formulation applying the EFSA guidance (2017) to the study data was 2.6%.

Intermediate Dose level (Spray dilution at 3 g/L)

The mean percentage of ^{65}Copper hydroxide in the WG 53.8 formulation that was considered to be potentially absorbable for the spray dilution at 3 g/L applying the EFSA guidance (2017) to the study data was 3.6%.

Low Dose level (Spray dilution at 0.3 g/L)

The mean percentage of ^{65}Copper hydroxide in the WG 53.8 formulation that was considered to be potentially absorbable for the spray dilution at 0.3 g/L applying the EFSA guidance (2017) to the study data was 14%.

Therefore, the following dermal absorption values can be proposed for Copper hydroxide in the WG 53.8 formulation:

- 2.6% for the neat formulation (350 g/kg)
- 3.6% for the intermediate dose (3 g/L)
- 14% for the low dose (0.3 g/L)

Although the above values have been determined in accordance with the EFSA guidance on dermal absorption (2017), it is noted that this is overly conservative in the case of copper, as the amount retained in the stratum corneum and stripped skin is not likely to be available for systemic absorption over time.

A 2.10.3 Copper: Study 3 – Dermal absorption, *in vivo* in rats

Previous evaluation	fRR FAP13, Central zone, RMS: Poland (2022) Comment zRMS Poland : The amount of the applied dose of 65Cu in the stripped skin was very low; the largest amount being observed in dilution II at 24 hours (0.3%). The amount present in the stratum corneum showed no clear decrease over time and the majority remained in the upper layers. Therefore, for all 3 dose levels the amount of 65Cu located in the stratum corneum was not available for absorption under the conditions of the study.

Comments of zRMS:	**For all 3 dose levels used, the amount of 65Cu located in the stratum corneum was not available for absorption under the study conditions. The study is accepted**

Reference	KCP 7.3/03
Report	*In vivo* percutaneous absorption of copper, formulated as Copper Hydroxide (DPX-GFJ52) 53.8WG (35% as metallic copper), in rats [redacted]

Guideline(s):	OECD 428 (2004); OECD Assessment No 28 (2004); EFSA Guidance on Dermal Absorption (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Test material	Name (Lot/Batch No.)	
	Non-radiolabelled	Copper hydroxide
	Radiolabelled	^{65}Copper hydroxide
	Formulation	Copper hydroxide 53.8 WG containing 53.8% ^{65}Copper hydroxide (equivalent to 35% metallic Copper)*

* The amount of copper was determined at mass 65 *m/z* and mass 63 *m/z* using a double focusing high resolution inductively coupled plasma spectrometry (HR-ICP-MS) in medium mode (resolution, 40000) in all collected samples.

Tested doses	**Concentrate (Group A)**	**Spray dilution 1 (Group B)**	**Spray dilution 2 (Group C)**
Target concentration	350 g/kg	3 g ^{65}Copper/L	0.3 g ^{65}Copper/L

Test system		
Test animals	Species	Rat
	Strain	Wistar (HsdCpb:Wu)
	Number, sex	36 males
	Age	9-10 weeks
	Weight at dosing	296-332g (group A; concentrate), 295-330g (group B; dilution I), 286-333g (group C; dilution II)
	Source	Harlan, Horst, The Netherlands
Treatment	Treatment groups	The dermal absorption of ^{65}Copper hydroxide was investigated in 3 groups of male rats, each comprising 3 subgroups of 4 animals as follows: Group A, undiluted concentrate, comprising time groups AT1 (24 h), AT2 (72 h) & AT3 (144 h) Group B, spray dilution I, comprising time groups BT1 (24 h), BT2 (72 h) & BT3 (144 h) Group C, spray dilution II, comprising time groups CT1 (24 h), CT2 (72 h) & CT3 (144 h) The extended time periods after the test material was washed off the skin at 6 hours, provided additional information on the bioavailability of the test substance after passing the epidermis and entering the systemic circulation.
	Treatment conditions	At least 24-hours prior to dosing, each animal had an area of 20

		cm^2 clipped between the dorsal and shoulder region (care was taken to avoid skin damage). The area was swabbed with acetone and checked for abrasions; only rats with intact skin were used. Following shaving and skin wash the animals were moved to metabolism cages. In all groups, the test substance was applied via dermal application on a dorsal area of approximately 10 cm^2 limited by an 'O' ring under semi-occlusive conditions (plastic cover and permeable tape) using the following methods: Group A: the skin was moistened with physiological saline and an appropriate amount of weighed test substance was applied to the skin and evenly spread within the 'O' ring. Groups B & C: the formulations were vortexed and checked for homogeneity. 100 µL of test substance was applied to the area with a pipette and evenly spread within the 'O' ring. The exposure period was 6-hours for all animals and the rats were subsequently maintained until 24, 72 and 144 hours post-dose (18, 66 & 138 hours post exposure).
	Pilot study	Due to the fact that Copper is abundantly available from various sources, a pilot study was undertaken to determine the background levels of copper in various matrices and samples of untreated rats and to ascertain the feasibility of analysing copper in the various matrices.
Sampling	Skin washing	6 hours following initiation of exposure, the 'O' ring was removed and retained, and the skin washed 9 times with mild soap solution.
	Excreta	Urine and faeces were collected at 24-hour intervals until sacrifice. After each 24-hour collection the cages were washed, and faeces was mixed with 3 parts water and weighed and homogenised ready for analysis.
	Sacrifice	Animals were sacrificed by exsanguination following anaesthesia. At sacrifice the following samples were collected; 'O' ring and protective device, skin wash at sacrifice, individual surface tape strips (stratum corneum) to a maximum of 15, post-stripping application site, non-treated skin, whole blood, plasma, GI-tract, and residual carcass.
Controls		Urine, faeces, and tissues were collected from untreated control animals. In addition, blank materials and cage wash solutions were collected.
Remarks:		

RESULTS AND DISCUSSIONS

Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable. The actual administered doses for the concentrate were slightly higher than intended, recoveries for this group ranged from 94.6% to 96.5%.

There were no deaths or clinical signs of toxicity in the animals. Body weights at sacrifice were only slightly lower compared with the start of treatment with no dose response and were considered to be secondary to housing and devices used in the study and not a treatment related effect.

Absorption and excretion

For the undiluted concentrate, after 24-hours, <0.051% of the applied dose was absorbed and the recovery was comparable after 72 hours (<0.042%) and 144 hours (<0.047%), indicating the amount present in the stratum corneum and stripped skin was not systemically available. This is supported by the fact that the amount present in the stratum corneum or stripped skin did not decrease over time.

It was not possible to determine the levels of Cu for the 2 spray dilutions because of high amounts of endogenous copper in the urine, faeces, cage wash, blood, GI tract and carcass; therefore, absorption was only determined in the unabsorbed fractions as well as control skin, stripped skin and tape strips. Based on the very low absorption found for the concentrate formulation and considering the good recoveries for the field dilutions, it is concluded that only a negligible amount of applied copper was absorbed from either dilution.

Tape stripping

Tape stripping in all groups revealed that most of the applied ^{65}Cu was concentrated in the upper layers of the stratum corneum at 144 hours post application.

In the undiluted concentrate group, the amount of ^{65}Cu in tape strips 3+ after 24 hours was 0.056% of the administered dose and decreased only very slightly to 0.022% at 144 hours following dosing, confirming that little to no absorption from the application site occurs, resulting in very low systemic uptake (below the limit of detection [LoD]).

In the spray dilution groups, the amount in tape strips 3+ after 24 hours was 0.61% and 2.50% for dilutions I and II, respectively. Again, decreasing only slightly to 0.46% and 1.90% respectively after 144 hours. The total amount in all tape strips (1+) was constant across the time groups, indicating that little to no ^{65}Cu becomes systemically available over time.

Stripped skin

After 24 hours the amount in the stripped skin was 0.009% of the administered concentrate, after 72 and 144 hours, 0.002% and <0.001% was found. For spray dilution I the majority of stripped skin (except one animal) was below the LoD resulting in < 0.04% and < 0.02% of the administered dose being recovered at 24 and 144 hours. Similarly, for dilution II all of the stripped skin except one animal was below the LoD, resulting in <0.29% and 0.17% of the administered dose being recovered at 24 and 144 hours. This indicates that little to no ^{65}Cu form the stripped skin compartment becomes systemically available over time.

Carcass and blood

For the concentrate, 0.019%, 0.014% and <0.005% were found in the residual blood at 24, 72 and 144 hours. It was not possible to determine the levels of ^{65}Cu in the blood or residual carcass because of the high background levels of endogenous copper present in these matrices.

The results are presented in the tables below:

Table A 2.10.3-1 Distribution of radioactivity at 24, 72 and 144 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (undiluted concentrate) to rats

	AT1 (24 h)		AT2 (72 h)		AT3 (144 h)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Urine (total)	<0.001	-	<0.001	-	<0.002	-
Faeces (total)	<0.001	-	<0.018	-	<0.035	-
Cage wash	<0.001	-	<0.003	-	<0.002	-
Blood	<0.001	-	<0.001	-	<0.001	-
Control skin	<0.001	-	<0.001	-	<0.001	-
GI tract	<0.002	-	<0.002	-	<0.002	-
Carcass	<0.025	-	<0.017	-	<0.007	-
Stripped skin	0.009	0.012	0.002	0.002	<0.001	<0.001
Absorbed	**<0.051**	**0.044**	**<0.042**	**0.027**	<0.047	**0.005**
Total skin wash[1]	94.31	0.86	96.32	1.70	94.71	1.66
O-ring/cover	0.17	0.12	0.11	0.08	0.12	0.08
Tape strips (3+)[2]	0.06	0.04	0.03	0.04	0.02	0.01
Not absorbed	**94.54**	**0.95**	**96.46**	**1.63**	**94.85**	**1.57**

Recovery	**94.59**	**0.94**	**96.50**	**1.61**	**94.90**	**1.57**

[1] Including tape strips 1 & 2 (values below the LoD were considered as the LoD for the calculations.
[2] Tape strip 3 to the final tape strip taken (up to 15).

Table A 2.10.3-2 Distribution of radioactivity at 24, 72 and 144 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (Dilution I) to rats

	BT1 (24 h)		BT2 (72 h)		BT3 (144 h)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Urine (total)	n.m.	-	n.m.	-	n.m.	-
Faeces (total)	n.m.	-	n.m.	-	n.m.	-
Cage wash	n.m.	-	n.m.	-	n.m.	-
Blood	n.m.	-	n.m.	-	n.m.	-
Control skin	0.01	-	0.01	-	0.01	-
GI tract	n.m.	-	n.m.	-	n.m.	-
Carcass	n.m.	-	n.m.	-	n.m.	-
Stripped skin	<0.04	-	<0.02	-	<0.02	-
Absorbed	**n.a**	**-**	**n.a**	**-**	**n.a**	**-**
Total skin wash[1]	95.24	3.19	100.41	2.56	99.17	1.30
O-ring/cover	0.18	0.05	0.12	0.05	0.22	0.06
Tape strips (3+)[2]	0.61	0.22	0.45	0.39	0.46	0.21
Not absorbed	**96.04**	**3.16**	**100.98**	**2.26**	**99.85**	**1.47**
Recovery	**96.08**	**3.15**	**101.00**	**2.26**	**99.88**	**1.47**

[1] Including tape strips 1 & 2 (values below the LoD were considered as the LoD for the calculations.
[2] Tape strip 3 to the final tape strip taken (up to 15).
n.m = not measured; n.a. = not applicable

Table A 2.10.3-3 Distribution of radioactivity at 24, 72 and 144 hours after dose application of ^{65}Copper hydroxide in a WG 53.8 formulation (Dilution II) to rats

	CT1 (24 h)		CT2 (72 h)		CT3 (144 h)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Urine (total)	n.m.	-	n.m.	-	n.m.	-
Faeces (total)	n.m.	-	n.m.	-	n.m.	-
Cage wash	n.m.	-	n.m.	-	n.m.	-
Blood	n.m.	-	n.m.	-	n.m.	-
Control skin	<0.10	-	<0.10	-	<0.10	-
GI tract	n.m.	-	n.m.	-	n.m.	-
Carcass	n.m.	-	n.m.	-	n.m.	-
Stripped skin	<0.29	-	<0.17	-	<0.17	-
Absorbed	**n.a**	**-**	**n.a**	**-**	**n.a**	**-**
Total skin wash[1]	94.75	94.89	95.80	1.56	94.78	1.29
O-ring/cover	0.65	0.77	1.20	0.28	0.95	0.30

Tape strips (3+)[2]	2.50	1.11	1.37	0.18	1.90	0.59
Not absorbed	**97.41**	**0.85**	**98.38**	**1.37**	**97.63**	**1.23**
Recovery	**97.80**	**0.76**	**98.64**	**1.38**	**97.89**	**1.23**

[1] Including tape strips 1 & 2 (values below the LoD were considered as the LoD for the calculations.
[2] Tape strip 3 to the final tape strip taken (up to 15).
n.m = not measured; n.a. = not applicable

CONCLUSION/ENDPOINT

The *in vivo* dermal penetration in rats of ^{65}Copper hydroxide in a WG 53.8 formulation was investigated at three nominal concentrations corresponding to the neat product (350 g /kg) and to two representative spray dilutions of 3 g/L and 0.3 g/L.

Concentrate

After 24 hours, < 0.051% of the applied dose was absorbed (sum of excreta, GI tract, Stripped skin, and carcass). The absorption was comparable after 72 and 144 hours (< 0.042% and < 0.047%).

Mean recovery within the time groups was 94.59% to 96.50%.

Intermediate Dose level (Spray dilution at 3 g/L)

It was not possible to determine an increase in ^{65}Cu in the spray dilutions owing to the high levels of background endogenous copper in the matrices. However, owing to the small absorption for the concentrate and good recovery for the spray dilutions it is concluded that only a negligible amount of copper would be absorbed.

Mean recovery across the time groups was 96.08% to 101.0%.

Low Dose level (Spray dilution at 0.3 g/L)

It was not possible to determine an increase in ^{65}Cu in the spray dilutions owing to the high levels of background endogenous copper in the matrices. However, owing to the small absorption for the concentrate and good recovery for the spray dilutions it is concluded that only a negligible amount of copper would be absorbed.

Mean recovery across the time groups was 97.80% to 98.64%.

The absorption of ^{65}Cu from the undiluted concentrate was <0.05% over 144 hours. For both spray dilutions, a worst-case assumption for absorption was determined from the non-absorbed fractions (owing to the high background levels of endogenous copper preventing measurement in some matrices). Based on the low absorption for the concentrate and the good recoveries for both dilutions from the non-absorbed fractions, it is concluded that very little absorption of applied copper had occurred in both field dilutions.

The amount of the applied dose of ^{65}Cu in the stripped skin was very low; the largest amount being observed in dilution II at 24 hours (0.3%). The amount present in the stratum corneum showed no clear decrease over time and the majority remained in the upper layers.

Therefore, for all 3 dose levels the amount of ^{65}Cu located in the stratum corneum was not available for absorption under the conditions of the study.

A 2.10.4 Copper: Study 4 – Dermal absorption, paper on residues in skin membrane and impact on risk assessment of inorganic copper salts

Previous evaluation	fRR FAP13, Central zone, RMS: Poland (2022) Comment zRMS Poland : Based on a generally accepted triple-pack approach, a dermal absorption value of 0.01% for the concentrate and 1% for diluted products containing inorganic Copper compounds, is considered adequately worst-case and has been used in the risk assessment for FAP13. Acceptable

Comments of zRMS:	**The following position of the Applicant is accepted** **The applicant proposes a dermal absorption of 0.01% for the concentrate and 1% for the dilution, based on the triple-pack approach, and taking into account the advices provided in this paper (Maas, 2020a**

Reference	KCP 7.3/04
Report	The fate of the test item residues in the skin membranes in in vitro dermal absorption studies; impact on the risk assessment of inorganic copper salts [redacted]
Guideline(s):	OECD 428 (2004); OECD Assessment No 28 (2004); EFSA Guidance on Dermal Absorption (2017)
Deviations:	Not relevant
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

In this paper information is provided on which experimental data should be used in the risk assessment of copper-containing agrochemical formulations, based on the biology behind dermal absorption and experimental data from various in vitro and in vivo studies performed in recent years at various (GLP) testing laboratories.

Diffusion (and therefore dermal absorption) requires the movement of a chemical across a concentration gradient from a high concentration to a low concentration across a semi-permeable membrane, like the *stratum corneum*. Once a chemical has crossed the stratum corneum, depending on its phys-chem properties, it may then be retained in the skin or taken away by the systemic circulation. Highly lipophilic molecules or metal complexes etc. have an affinity for the stratum corneum which can retain considerable masses of these potential penetrants. Physico-chemically, these test items do not partition, to a great extent, away from the lipophilic stratum corneum into the hydrophilic receptor fluid, even when suitable lipophilic acceptors such as BSA or PEG are added. This is also the case for these materials in human skin in vivo.

The five forms of "Copper compounds" currently authorized as fungicides are all of inorganic nature. Copper containing inorganic salts have no lipophilic characteristics, although they do have a very limited solubility in water. In contrast to lipophilic compounds, copper containing inorganic salts will not show a preference for partitioning into a lipophilic skin fraction from which they would then slowly be released. As a general rule, solids/solid compounds will not be absorbed through the skin at all, since there is no concentration gradient present as driving force and the particles of solid are too large. This has been demonstrated for copper oxide even for nanoparticles. Any copper that is measured in the (stripped) skin in vitro should, therefore, be Cu(-containing) ions that are (to a limited extent) formed from

the copper salts, either upon adding water to a concentrate formulation (to optimize skin contact during exposure and to mimic sweat on the skin surface or occlusive conditions under clothing), or after adding water to the concentrate formulation to prepare the field dilution for testing. For ions in general, water solubility (and consequently absorption into the receptor fluid in vitro) will not be an issue, and they will follow the water pathway of absorption through skin. When no copper is measured in the receptor fluid, this can be considered not to be the result of inadequate solubility, but a confirmation that copper was not at the concentration present in the skin to partition into receptor fluid (and thus the blood in vivo).

No notable absorption into the receptor fluid was observed following the application of various inorganic Copper-containing agrochemical formulations to human skin *in vitro.* A repeated dose study showed that, despite (slightly) raised skin levels of copper following repeated application, still no absorption into the receptor fluid occurred. Studies using a stable isotope of copper (^{65}Cu), that allows to distinguish between natural copper being present in the skin and copper present from an exogenous source, actually show that upon application of the diluted product, in absolute numbers the increase in copper levels is within the range of natural copper levels present.

The rat *in vivo* study using ^{65}Cu, which studied the absorption of copper until 144 hours, again confirmed that no absorption of Copper into the blood occurs following application of a Copper hydroxide-containing concentrate formulation, while absorption from the diluted product (i.e., 1.9%) could only be, very conservatively, estimated based on the "missing" recovery.

For compounds such as Copper, which are naturally present, it makes more sense to consider absolute numbers rather than % of applied dose in order to adequately judge the biological/toxicological relevance of the data.

The paper proposes dermal absorptions values for products containing inorganic copper compounds, based on the generally accepted triple-pack approach.

Note from the applicant: No detailed calculations are provided in the position paper above. It is therefore unclear why the authors propose a dermal absorption of 0.1% for the dilution. The applicant proposes a dermal absorption of 0.01% for the concentrate and 1% for the dilution, based on the triple-pack approach, and taking into account the advices provided in this paper (Maas, 2020a).

A 2.10.5 Cymoxanil: Study 1 –Dermal absorption, *in vitro* using human skin

Comments of zRMS:	**The dermal penetration of cymoxanil formulated as FEL02 through human dermatomed skin was determined in vitro. The amount of applied dose penetrating within 24 hours was determined to be 0.421% (mean ± standard deviation) and 21.930% for the formulation concentrate and the 1:333 spray dilution (0.12 g/L), respectively. The dermal penetration estimates to be used for risk assessment were set at 0.42% and 22% for the formulation concentrate and the 1:333 spray dilution (0.12 g/L) based on the EFSA guidance criteria.**

Reference	KCP 7.3/05
Report	The In Vitro Percutaneous Absorption of Radiolabelled Cymoxanil in a Concentrate Formulation (FEL02) and an In-Use Dilution through Human Split-Thickness Skin, [redacted]
Guideline(s)	Yes OECD 428
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

Test material	Name (Lot/Batch No.)	[acetyl-2- 14C]Cymoxanil (10863CEO001-3)
	Test preparation	radioformulation
	Specific activity	-
	Radiochemical purity	96.8%
Product	Name (Lot/Batch No.)	FEL02
	Company code	-
	Concentration a.s.	40 g/kg cymoxanil
	Formulation type	WG
Blank product	Name (Lot/Batch No.)	Cymoxanil blank of FEL02 (043 SDC)
	Concentration a.s.	0 g/kg

Test system		
Diffusion cell	Cell type	dynamic
	(if dynamic) Flow rate	1.5 ml/h
	Exposed skin area	1 cm²
	Cover	open
Membrane	Skin type	dermatomed
	Skin thickness range	ca. 200-400 µm
	Skin donors age	49-65 years
	Skin donors sex	Female
	Location	Breast / abdomen
	Source	surgery
	Integrity test	yes
Receptor	Receptor medium	Phosphate buffered saline (PBS) containing polyoxyethylene 20 oleyl ether (6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL)
	Solubility in receptor medium	yes
Sample Time	Exposure time	8 h
	Observation time	24 h
Sampling	Sample intervals	0-1h, 1-2h, followed by 2 h intervals until 24 hours post dose
Washing		post exposure
Final Procedure	Tape stripping	y
	TS1-2 analysed separately	y
Remarks:		

Tested doses	Concentrate	Spray dilution
Target concentration	40 mg/g	0.12 mg/mL
Area dose	200 µg/cm²	1.2 µg/cm²
Total dose	200 µg/cm²	1.2 µg/cm²
Specific activity [kBq/ml]	-	-
No. of donors	4	4
No of cells used/valid cells*	8/8	8/8

* Justification for excluded cells, if applicable

Results and discussions

Table A 1: In-vitro dermal penetration of active substance 1 formulated as product code/name through human skin - Recovery data

Dose group		Formulation concentrate		Spray dilution 1:333	
Target concentration	[mg/mL]	40		0.12	
Target dose	[µg/cm²]	200 µg/cm²		1.2 µg/cm²	
Mean actual applied dose	[µg/cm²]	206.5 µg/cm²		1.2 µg/cm²	
		Recovery [%]		Recovery [%]	
		Mean	S.D.	Mean	S.D.
Dislodgeable dose					
e.g. Skin washing after 8 h		91.28	7.52	80.78	5.65
e.g. Skin washing after 24 h					
Donor chamber wash		0.09	0.11	0.03	0.01
Dose associated to skin					
Tape strips: 1st sample, strips 1 + 2		0.01	0.01	0.04	0.01
Tape strips: 2nd sample; strips 3 - n		0.03	0.02	0.39	0.13
Skin preparation		0.01	0.00	0.18	0.08
Absorbed dose					
Receptor fluid		0.33	0.09	17.41	5.09
Receptor chamber wash		0.01	0.01	0.01	0.00
Total recovery[1]					
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]		Yes		Yes	
If no: Absorption estimates = absorbed dose + skin preparation + tape strips sample 2)[2]					
If yes: Absorption estimates = absorbed dose + skin preparation		0.34	0.09	17.6	5.16
Absorption estimate normalised[3]		No		No	
Relevant absorption estimate[4]		0.421		21.930	
Absorption estimates used for risk assessment[5]		**0.42**		**22**	

[1] Values may not calculate exactly due to rounding of figures

[2] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.6.2-1) Finally, the skin preparation is also considered potentially absorbable.

[3] According to the EFSA Guidance on Dermal Absorption, cells with insufficient recovery (< 95%) can be corrected by normalisation of absorption estimate to 100% recovery; explanation should be included.

[4.] In accordance with the EFSA Guidance on Dermal Absorption, one standard deviation was added to the mean% dermal penetration in cases where the standard deviation was ≥ 25% of the mean value.

[5] Relevant absorption estimate was rounded to the required number of significant figures.

N/A: not applicable

Remarks

The relatively low mean recovery for the concentrate was caused by four out of eight membranes showing a recovery 95%).The missing recovery is considered not to be associated with the absorbed fractions. For example, Cells 22 and 23 show an overall recovery of 99.4% and 88.1%, respectively, but the dermal delivery is comparable (i.e. 0.29% and 0.55%, respectively) or Cells 24 and 25 that show an overall recovery of 102.7% and 85.7%, respectively, and a comparable dermal delivery of 0.32% and 0.31%, respectively. The lower recovery values are very likely related to losses caused by technical difficulties involved with accurately weighing low amounts of solid formulation.

Conclusion/endpoint:

The dermal penetration of cymoxanil formulated as FEL02 through human dermatomed skin was determined in vitro. The amount of applied dose penetrating within 24 hours was determined to be 0.421% (mean ± standard deviation) and 21.930% for the formulation concentrate and the 1:333 spray dilution (0.12 g/L), respectively. The dermal penetration estimates to be used for risk assessment were set at 0.42% and 22% for the formulation concentrate and the 1:333 spray dilution (0.12 g/L) based on the EFSA guidance criteria.

A 2.11 Other/Special Studies

There are no further studies required.

Appendix 3 Exposure calculations for operator, worker, resident and bystander (KCP 7.2)

Product name	FEL02
Formulation type	Wettable granules, soluble granules
Product category	Other
Name of active substance	Copper
Concentration of active substance [g a.s./l or kg]	200
AOEL [mg/kg bw/day]	0.08
AAOEL [mg/kg bw]	
Inhalation absorption [%]	100
Oral absorption [%]	50
Dermal absorption [%] (concentrate)	1
Dermal absorption [%] (dilution) 0.33 [g a.s./l or kg]	9
Name of active substance	Cymoxanil
Concentration of active substance [g a.s./l or kg]	40
AOEL [mg/kg bw/day]	0.01
AAOEL [mg/kg bw]	
Inhalation absorption [%]	100
Oral absorption [%]	75
Dermal absorption [%] (concentrate)	0.42
Dermal absorption [%] (dilution) 0.12 [g a.s./l or kg]	22

1. Assessed uses

Uses	Crops	Max. application rate of the product [l or kg/ha]	Unit	Max. no. of applications	Interval between multiple applications [days]	Min. volume water [l/ha]	Max. volume water [l/ha]	Indoor/outdoor	Application method	Type of cultivation	Application technique	Drift reduction [%]
Use 1	Low vegetables	3	kg/ha	6	7	100	1000	Outdoor	Downward spraying	Normal	Vehicle-mounted	0

A 3.1 Exposure calculations without refinement

A 3.1.1 Operator exposure calculations (KCP 7.2.1.1)

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
Low vegetables/Outdoor/Downward spraying/Vehicle-mounted/Drift reduction: 0 %/75th percentile Crop density: Normal			
Copper	Number of applications and application rate: 6 x 0.6 kg a.s./ha Dermal absorption (concentrate): 1 % Dermal absorption (in-use dilution): 9 %		
	M/L: Workwear App: Workwear	0.01	14.3
Cymoxanil	Number of applications and application rate: 6 x 0.12 kg a.s./ha Dermal absorption (concentrate): 0.42 % Dermal absorption (in-use dilution): 22 %		
	M/L: Workwear App: Workwear	0.005	46.5
Combined exposure			Hazard index
	M/L: Workwear App: Workwear		0.6

A 3.1.2 Worker exposure calculations (KCP 7.2.3.1)

Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL	Re-entry restriction [days]
		Inspection, irrigation (All) / Outdoor Work rate: 2 hours/day Interval: 7 days Body weight: 60 kg TC (potential): 12500 cm²/h TC (workwear (arms, body and legs covered)): 1400 cm²/h TC (workwear (arms, body and legs covered) and gloves): 1250 cm²/h TC (gloves): NA cm²/h	
Copper		Number of applications & application rate: 6 x 0.6 kg a.s./ha Dermal absorption: 9 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days	
Potential	0.3	351	55
Workwear	0.03	39.3	0
Workwear and gloves	0.03	35.1	0
Hands covered, no workwear			
Cymoxanil		Number of applications & application rate: 6 x 0.12 kg a.s./ha Dermal absorption: 22 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50 Foliar: 30 days DT50 Air: 30 days DT50 Soil: 30 days	
Potential	0.1	1373	114
Workwear	0.02	154	19
Workwear and gloves	0.01	137	14
Hands covered, no workwear			
Combined		Hazard index	
potential		17.2	124
Workwear		1.9	29
Workwear and gloves		1.7	24
Hands covered, no workwear			0

A 3.1.3 Bystander and resident exposure calculations (KCP 7.2.2.1)

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
			Season: Not relevant Buffer zone: 2-3 m Drift reduction technology: 0 % Interval between treatments: 7 days Minimum volume of water: 100 l
Copper			Number of applications and application rate: 6 x 0.6 kg a.s./ha Dermal absorption: 9 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days
Resident child Body weight: 10 kg	Drift (75th perc.)	0.01	18.4
	Vapour (75th perc.)	0.0008	1
	Deposits (75th perc.)	0.004	5.4
	Re-entry (75th perc.)	0.04	47.4
	Sum (mean)	0.04	52.8
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.003	4.4
	Vapour (75th perc.)	0.0003	0.3
	Deposits (75th perc.)	0.002	1.9
	Re-entry (75th perc.)	0.02	26.3
	Sum (mean)	0.02	24.8
Cymoxanil			Number of applications and application rate: 6 x 0.12 kg a.s./ha Dermal absorption: 22 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days
Resident child Body weight: 10 kg	Drift (75th perc.)	0.007	71.6
	Vapour (75th perc.)	0.0008	8
	Deposits (75th perc.)	0.002	19
	Re-entry (75th perc.)	0.02	185
	Sum (mean)	0.02	209
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.002	17
	Vapour (75th perc.)	0.0003	2.7
	Deposits (75th perc.)	0.0007	7.5
	Re-entry (75th perc.)	0.01	103
	Sum (mean)	0.01	98.2
Combined exposure			Hazard index
Resident child Body weight: 10 kg	Drift (75th perc.)		0.9
	Vapour (75th perc.)		0.09
	Deposits (75th perc.)		0.2
	Re-entry (75th perc.)		2.3

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
	Sum (mean)		2.6
Resident adult Body weight: 60 kg	Drift (75th perc.)		0.2
	Vapour (75th perc.)		0.03
	Deposits (75th perc.)		0.09
	Re-entry (75th perc.)		1.3
	Sum (mean)		1.2

A 3.2 Exposure calculations with refinement

A refinement of the calculations is needed. Therefore a DT50 of 1 day is used (see also appendix 4)

A 3.2.1 Operator exposure calculations (KCP 7.2.1.1)

No refinement needed.

A 3.2.2 Worker exposure calculations (KCP 7.2.3.1)

Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL	Re-entry restriction [days]
		Inspection, irrigation (All) / Outdoor Work rate: 2 hours/day Interval: 7 days Body weight: 60 kg TC (potential): 12500 cm²/h TC (workwear (arms, body and legs covered)): 1400 cm²/h TC (workwear (arms, body and legs covered) and gloves): 1250 cm²/h TC (gloves): NA cm²/h	
Copper		Number of applications & application rate: 6 x 0.6 kg a.s./ha Dermal absorption: 9 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days	
Potential	0.3	351	55
Workwear	0.03	39.3	0
Workwear and gloves	0.03	35.1	0
Hands covered, no workwear			
Cymoxanil (refined dt50 foliar)		Number of applications & application rate: 6 x 0.12 kg a.s./ha Dermal absorption: 22 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50 Foliar: 1 days DT50 Air: 30 days DT50 Soil: 30 days	
Potential	0.03	333	2
Workwear	0.004	37.3	0
Workwear and gloves	0.003	33.3	0
Hands covered, no workwear			
Combined		Hazard index	
potential		6.8	55
Workwear		0.8	0
Workwear and gloves		0.7	0
Hands covered, no workwear			0

A 3.2.3 Bystander and resident exposure calculations (KCP 7.2.2.1)

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
			Season: Not relevant Buffer zone: 2-3 m Drift reduction technology: 0 % Interval between treatments: 7 days Minimum volume of water: 100 l
Copper		Number of applications and application rate: 6 x 0.6 kg a.s./ha Dermal absorption: 9 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days	

Model data	Level of PPE	Total absorbed dose [mg/kg bw per day]	% of systemic AOEL
Resident child Body weight: 10 kg	Drift (75th perc.)	0.01	18.4
	Vapour (75th perc.)	0.0008	1
	Deposits (75th perc.)	0.004	5.4
	Re-entry (75th perc.)	0.04	47.4
	Sum (mean)	0.04	52.8
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.003	4.4
	Vapour (75th perc.)	0.0003	0.3
	Deposits (75th perc.)	0.002	1.9
	Re-entry (75th perc.)	0.02	26.3
	Sum (mean)	0.02	24.8
Cymoxanil	Number of applications and application rate: 6 x 0.12 kg a.s./ha Dermal absorption: 22 % DFR: 3 µg/cm² foliage per kg a.s./ha DT50: 30 days		
Resident child Body weight: 10 kg	Drift (75th perc.)	0.007	71.6
	Vapour (75th perc.)	0.0008	8
	Deposits (75th perc.)	0.002	19
	Re-entry (75th perc.)	0.02	185
	Sum (mean)	0.02	209
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.002	17
	Vapour (75th perc.)	0.0003	2.7
	Deposits (75th perc.)	0.0007	7.5
	Re-entry (75th perc.)	0.01	103
	Sum (mean)	0.01	98.2
Combined exposure			Hazard index
Resident child Body weight: 10 kg	Drift (75th perc.)		0.9
	Vapour (75th perc.)		0.09
	Deposits (75th perc.)		0.2
	Re-entry (75th perc.)		2.3
	Sum (mean)		2.6
Resident adult Body weight: 60 kg	Drift (75th perc.)		0.2
	Vapour (75th perc.)		0.03
	Deposits (75th perc.)		0.09
	Re-entry (75th perc.)		1.3
	Sum (mean)		1.2

Appendix 4 Detailed evaluation of exposure and/or DT50 studies relied upon (KCP 7.2, KCP 7.2.1.1, KCP 7.2.2.1, KCP 7.2.3.1)

A 4.1 Study 1 – DT50

Comments of zRMS:	**It is acceptableto assume that the cymoxanil DT50 value of residues on the raw commodity samples would be approximately 1 day.**

Reference	KCP 7.4/01
Report	Position paper: Cymoxanil DT_{50} value to use in the risk assessment refinement. A. Correia 2019, Study number ZE/15/003-01
Guideline(s)	Not applicable
Deviations	No
GLP	No
Acceptability	Yes
Duplication (if vertebrate study)	No

Executive summary

The aim of the position paper is to define a cymoxanil DT_{50} value for non-dietary risk assessment refinement.

The DT_{50} value can be estimated using a residue decline study conducted on wheat and peas, a DFR study conducted in grapes, and a DFR study conducted in grapes, tomato and potato.

Overall, it is reasonable to assume that the cymoxanil DT_{50} value of residues on the raw commodity samples would be approximately 1 day.

MATERIALS AND METHODS

The proposed cymoxanil DT50 value relied on the following experimental studies:

- a residue decline study conducted on wheat and peas
- a DFR study conducted in grapes,
- a DFR study conducted in grapes, tomato and potato

Test Substance	Matrix	Endpoints	Author, year
Cymoxanil 45 WG	Wheat & pea	DT50 (Wheat) = 1.20 days DT50 (Pea) = 1.00 days	Amossé, J. (2019a)
		DT50 (Wheat) = 1.21days DT50 (Pea) = 1.05 days	Amossé, J. (2019b)
		DT50 (Wheat) = 0.82 days DT50 (Pea) = 0.67 days	Andrews, G., Bills, K. (2019a)
		DT50 (Wheat) = 0.42 days DT50 (Pea) = 1.18 days	Andrews, G., Bills, K. (2019b)

	Grape, tomato, potato	DT50 (Grape)= 0.93 days DT50 (Tomato) = 0.83 days DT50 (Potato)= 0.95 days	Jullian E. (2014 a,b,c)
	Grape	DT50 (Grape)= 0.91 days	Wilson A. (2011)

RESULTS

- Amossé, J. (2019a): From the residue trials conducted in monocotyledon and dicotyledon plants it was demonstrated the rapid decline of the residues over the sampling period to below the LOQ. It is not possible to accurately calculate the DT50 of the residues because of lack of enough data points to derive a valid decline curve. However, it was clearly observed that in all cases, the measured residues have declined to < 50% of the initial value within 24 hours. Therefore, it is reasonable to assume that the DT50 value of residues on the raw commodity samples would be approximately 1.2 days for wheat and 1.0 day for peas. A similar pattern was observed in other crops which all indicate very quick degradation of Cymoxanil in plant material. These data can be used to support a DT50 of 1.0 day for grapes, potato, and tomato, which are all dicotyledon plants. Also, a DT50 of 1 day was used for the refinement of the ecotoxicology risk assessment.
- Amossé, J. (2019b): Residues of the active substance, cymoxanil, were shown to rapidly decline with DT50 for residue dissipation of cymoxanil in wheat plants of 1.21 days using a single first order kinetic (SFO, Chi^2 error: 3.1%, t-test: $p<0.01$). The DT50 for residue dissipation of cymoxanil in pea plants was 1.05 days using a single first order kinetic (SFO, Chi^2 error: 11.9%, t-test: $p<0.01$).
- Andrews, G., Bills, K. (2019a): Residues of the active substance, cymoxanil, were shown to rapidly decline with DT50 values of 0.82 and 0.67 days being determined for wheat and peas, respectively, and using a single first order kinetic curve.
- Andrews, G., Bills, K. (2019b): Residues of the active substance, cymoxanil, were shown to rapidly decline with DT50 values of 0.42 and 1.18 days being determined for wheat and peas, respectively, and using a single first order kinetic curve.
- Jullian E. (2014a,b,c): These studies encompasses 2 trials on grape (average DT50 = 0.93 days), 1 trial on tomato (DT50 = 0.86 days), and 1 trial on potato (DT50 = 0.95 days). And, all together these 4 trials provide very consistent data which support DT50 < 1 day; it is to be noted that the values obtained from DFR trials are more relevant for refining worker exposure than the DT50 obtained from residue trials, because they measure the residue on the leaf surface and not the residue in the whole plant.
- Wilson A. (2011): This study was conducted after six applications of cymoxanil 5% WG to the grape. Overall, and although this study is not used to determine a DFR value, the data contained in the study report can be used to determine the DT50 of residues on grape leaves. First order kinetic plot indicates that the DT50 of residues on vine leaves is 0.91 days.

CONCLUSION

Overall, it is reasonable to assume that the cymoxanil DT_{50} value of residues on the raw commodity samples would be approximately 1 day.

A 4.1 Study 2 – DT50

Comments of zRMS:	**The highest DFR value of 0.2982 µg/cm2 per 148.5 g a.s./ha, corresponding to about 2 µg/cm2 kg a.s./ha was obtained just after the second application, to be used in re-entry evaluation. DT_{50} was 0.9535 days and will be approximated to 1 for risk assessment purpose.** **The statement is acceptable**

Reference	KCP 7.4/02
Report	Cymoxanil - Quantification of dislodgeable foliar residues following six applications of Vitene Ultra to potato in the United Kingdom, 2013, Jullian E, 2014, Study number S13-01293
Guideline(s)	EU 1999: 1607/VI/97, OECD Test Guideline 504; SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1; Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 (for residue studies); OECD Series on Testing and Assessment No. 9 ”Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application ”, Paris 1997. OCDE/GD(97)148; U.S. EPA Series 875.2100 Occupational and Residential Exposure Test Guidelines. Foliar Dislodgeable Residue Dissipation
Deviations	No
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Executive summary

The objective of the study was to quantify the amount of cymoxanil residue that can be dislodged from potato leaves following six applications of Vitene Ultra. One dislodgeable foliar residue trial was conducted on potato during 2013 in the UK (S13-01293-01). Six applications of Vitene Ultra (cymoxanil 225 g/L) were applied at 0.66 L product/ha, diluted with water immediately prior to application to a target spray volume of 300 L/ha.

Residues of cymoxanil were undetectable (less than 0.01 µg/cm2) in all untreated leaf washing specimens. Cymoxanil showed a rapid degradation with residues falling below the detection limit starting from five days after the last application. DT 50 based on SFO kinetics were calculated resulting in 0.771 and 1.08 days for S12-01291-01 (Northern France) and S13-1291-02 (Italy) trials respectively.

DT_{50} was 0.9535 days and will be approximated to 1 for risk assessment purpose.

MATERIALS AND METHODS

The objective of the study was to quantify the amount of cymoxanil residue that can be dislodged from potato leaves following six applications of Vitene Ultra.

One dislodgeable foliar residue trial was conducted on potato during 2013 in the UK (S13-01293-01). Six applications of Vitene Ultra (cymoxanil 225 g/L) were applied at 0.66 L product/ha, diluted with water immediately prior to application to a target spray volume of 300 L/ha.

Following each application of Vitene Ultra, an SC formulation containing 225 g/L cymoxanil, leaf disc samples were taken at pre-defined sampling times pre and post application. The foliar residues were dislodged using an aqueous solution of a surfactant and the dislodged solution specimens were analyzed for cymoxanil.

Leaf disc specimens were collected from the untreated and treated plots and were taken using a birkestrand precision leaf punch, with leaf punch diameter of 2.523cm and leaf surface area of 10cm2 (5cm2 x 2 surfaces). Leaf disc specimens were collected from the untreated plot, before the first and the last application, and 14 days after the last application. Leaf disc specimens were collected from the treated plot after the first application, before and after each other applications and 1, 2, 3, 5, 7, 10, and 14 days after the last application

After sampling, the leaf discs were transported to a field lab where the foliage was mechanically shaken for 15 minutes with two sequential 100mL washes with an aqueous solution of 0.01% Aerosol OT100 (dislodging solution). Following each wash and shake, the wash solution was transferred into a single glass 500mL jar of ‘Total washings’ and the remaining leaf disc foliage discarded. Before freezing 1mL of 1% formic acid was added to each ‘Total washings’ specimens.

The analytical method applied in this analytical phase was validated under GLP compliance in the Huntingdon Life science Project Identity LRP0003 (30th November 2009 – Author Howard Harper – Sponsor Oxon Italia S.p.a.). The whole frozen sample was de-frozen at room temperature and mixed using a glass rod, a portion of the homogeneous sample (20mL) was extracted with dichloromethane, concentrated to dryness and reconstituted with a mixture water/acetone/formic acid 75/25/0.05 v/v/v prior to be analysed by an HPLC system coupled with a triple quadrupole mass analyser (LC-MS/MS).

The limit of quantitation for cymoxanil was set at 0.01 µg/mL / 0.005 µg/cm2.Procedural recoveries run concurrently with test specimen at levels of 0.01, 0.10 and 1.0 µg/mL gave an overall mean recovery of 86.04%.

RESULTS

Residues of cymoxanil were undetectable (less than 0.01 µg/cm2) in all untreated leaf washing specimens. Cymoxanil showed a rapid degradation with residues falling below the detection limit starting from five days after the last application. DT 50 based on SFO kinetics were calculated resulting in 0.771 and 1.08 days for S12-01291-01 (Northern France) and S13-1291-02 (Italy) trials respectively.

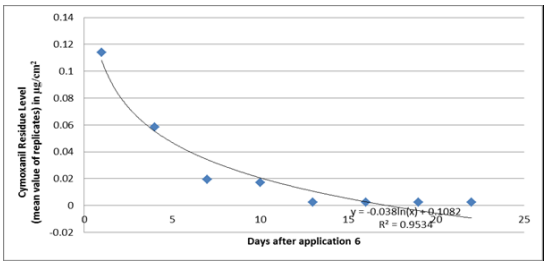
Summary of Cymoxanil Residues in Leaf Washing Specimens

Sampling code	Sample timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm2)**
S2	0DAA1	P2	L13-01293-01-022	0.5602	0.2801
			L13-01293-01-023		
			L13-01293-01-024		
S3	0DBA2	P2	L13-01293-01-028	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-029		
			L13-01293-01-030		
S4	0DAA2	P2	L13-01293-01-034	0.5964	0.2982
			L13-01293-01-035		
			L13-01293-01-036		
S5	0DBA3	P2	L13-01293-01-040	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-041		
			L13-01293-01-042		
S6	0DAA3	P2	L13-01293-01-046	0.0804	0.0402
			L13-01293-01-047		
			L13-01293-01-048		
S7	0DBA4	P2	L13-01293-01-052	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-053		
			L13-01293-01-054		
S8	0DAA4	P2	L13-01293-01-058	0.2855	0.1428
			L13-01293-01-059		
			L13-01293-01-060		
S9	0DBA5	P2	L13-01293-01-064	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-065		
			L13-01293-01-066		
S10	0DAA5	P2	L13-01293-01-070	0.1403	0.0702
			L13-01293-01-071		
			L13-01293-01-072		
S11	0DBA6	P2	L13-01293-01-076	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-077		
			L13-01293-01-078		
S12	0DAA6	P2	L13-01293-01-100	0.2281	0.1141
			L13-01293-01-101		
			L13-01293-01-102		
S13	1DAA6	P2	L13-01293-01-106	0.1165	0.0583
			L13-01293-01-107		
			L13-01293-01-108		

Sampling code	Sample timing	Plot	Specimen code	Cymoxanil Residue Level Mean Value of Replicates	
				(µg/mL)	(µg/cm2)**
S14	2DAA6	P2	L13-01293-01-112	0.0390	0.0195
			L13-01293-01-113		
			L13-01293-01-114		
S15	3DAA6	P2	L13-01293-01-118	0.0339	0.0170
			L13-01293-01-119		
			L13-01293-01-120		
S16	5DAA6	P2	L13-01293-01-124	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-125		
			L13-01293-01-126		
S17	7DAA6	P2	L13-01293-01-130	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-131		
			L13-01293-01-132		
S18	10DAA6	P2	L13-01293-01-136	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-137		
			L13-01293-01-138		
S19	14DAA6	P2	L13-01293-01-142	<0.01 (n.d.)	<0.005 (n.d.)
			L13-01293-01-143		
			L13-01293-01-144		

Note:DBA = days before application ;DAA = days after application; n.d. = not determined
** µg/cm2: data calculated considering that 200mL of washing solution were used to wash 400 cm2 of leaves, therefore µg/cm2= (µg/mL)*200mL / 400cm2

Dissipation curve for trial S13-01291-03 is shown below.



CONCLUSION

The highest DFR value of 0.2982 µg/cm2 per 148.5 g a.s./ha, corresponding to about 2 µg/cm2 kg a.s./ha was obtained just after the second application, to be used in re-entry evaluation. DT_{50} was 0.9535 days and will be approximated to 1 for risk assessment purpose.

Jullian E. (2014)