Section A6.1.1 Acute Oral Toxicity

Annex Point IIA6.1

Acute oral toxicity in the rat (Acute toxic class Method)
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

Official use only

1 REFERENCE

1.1 Reference

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).

[redacted]

1.2 Data protection

Yes
(indicate if data protection is claimed)

1.2.1 Data owner

Give name of company
[redacted]

1.2.2

1.2.3 Criteria for data protection

Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:
Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

The study report claims compliance with:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity - Acute Toxic Class Method" (adopted 22 March 1996),
- Commission Directive 96/54/EC Method B1 tris Acute Oral Toxicity (Oral - Acute Toxic Class Method)

Review of the study report revealed only minor deviations from OECD Test Guideline 423 (see 2.3).

(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")

2.2 GLP

Yes
(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)

2.3 Deviations

The following minor deviations from OECD Test Guideline 423 (adopted 17 December 2001) were noted:

- Information on the test material including the batch no and the purity were not provided,
- No justification for the choice of vehicle was provided,
- Due to a technical error, the presence of any macroscopic abnormalities for the female animal treated with 2000 mg/kg bw that was killed in extremis was not recorded.

These deviations are not considered to have influenced the outcome or the integrity of the study.

(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")

Section A6.1.1 Acute Oral Toxicity

Annex Point IIA6.1 **Acute oral toxicity in the rat (Acute toxic class Method)**
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

		3 MATERIALS AND METHODS	
		In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.	
3.1	**Test material**	*As given in section 2 or give name used in study report* Copper powder.	
3.1.1	Lot/Batch number	*List lot/batch number if available* Not provided.	
3.1.2	Specification	*As given in section 2, or* *Deviating from specification given in section 2 as follows* *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):* No information was provided on the specification of the sample used in this study.	
3.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Shiny copper coloured powder.	
3.1.2.2	Purity	*Give purity in % of active substance* Not provided.	X
3.1.2.3	Stability	*Describe stability of test material* Test substance: Not provided. Test preparations: Not assessed, however, test substance preparations were freshly prepared.	X
3.2	**Test Animals**	Non-entry field	
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley CD (Cr1: CD® (SD) IGS BR)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and Female.	
3.2.5	Age/weight at study initiation	At the start of the study the males weighed 271 to 299 g and the females weighed 198 to 218 g, and were approximately eight weeks of age.	
3.2.6	Number of animals per group	*Give number* *specify, if there are differences for example for treatment and recovery groups* 3	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral *Fill in respective route in the following, delete other routes*	
3.3.1	Postexposure period	*14 days, 4 weeks or other* 14 days.	
3.3.2	Type	**Oral** *Gavage/in food/in drinking water* Gavage	

Section A6.1.1 Acute Oral Toxicity

Annex Point IIA6.1 **Acute oral toxicity in the rat (Acute toxic class Method)**
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

3.3.3	Concentration	*Gavage* *mg/kg bw* *food, drinking water* *ppm + mg/kg bw* *food consumption per day* *ad libitum/certain amount per day* 200 and 2000 mg/kg bw.
3.3.4	Vehicle	*Moistened with water, aqueous solution, corn oil or other* Arachis oil BP.
3.3.5	Concentration in vehicle	20 and 200 mg/ml at 200 and 2000 mg/kg bw, respectively.
3.3.6	Total volume applied	10 ml/kg.
3.3.7	Controls	*Vehicle, plain diet or other* None.
3.4	**Examinations**	*Clinical observations, necropsy, histopathology or other* Clinical observations The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing subsequently once daily for up to 14 days. Bodyweights Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment. Necropsy At the end of the observation period the surviving animals were killed by cervical dislocation. Animals were subject to a gross necropsy. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained. Due to a technical error, the presence of any macroscopic abnormalities for the female animals treated with 2000 mg/kg bw that was killed in extremis was not recorded. This deviation was considered not to affect the purpose or integrity of the study.
3.5	**Method of determination of LD_{50}**	*Bliss, Litchfield and Wilcoxon, Finney, WEil, Thompson, Miller and Tainter or other* This Acute Toxic Class Method provides a range estimate of the LD_{50} according to a pre-defined scheme outlined in OECD Test Guideline 423.
3.6	**Further remarks**	None

Section A6.1.1 Acute Oral Toxicity

Annex Point IIA6.1

Acute oral toxicity in the rat (Acute toxic class Method)
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

4 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

4.1 Clinical signs

No effects / describe significant effects referring to data in results table

See Table A6.1.1-1 for a summary of the results of this study.

Two animals treated with 2000 mg/kg bw were found dead five days after dosing. One animal treated with 2000 mg/kg bw was killed in extremis eight days after dosing. There were no deaths noted at a dose level of 200 mg/kg bw.

Signs of systemic toxicity noted in animals treated with 2000 mg/kg bw were hunched posture, lethargy, pilo-erection, diarrhoea, decreased respiratory rate, laboured respiration, ataxia, pallor of the extremities, emaciation, tiptoe gait and faeces stained green. Hunched posture was noted during the day of dosing and one day after dosing in one male treated with 200 mg/kg bw. No other signs of systemic toxicity were noted in animals treated with 200 mg/kg bw.

4.2 Pathology

No effects / describe significant effects referring to data in results table

Abnormalities noted at necropsy of the animals treated with 2000 mg/kg bw that died during the study were abnormally red lungs, dark liver, dark kidneys, copper-coloured material present in the stomach, haemorrhagic gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhagic small and large intestines.

No abnormalities were noted at necropsy of animals treated with 200 mg/kg bw.

4.3 Other

Describe any other significant effects

Bodyweight

The surviving animals showed expected gains in bodyweight over the study period.

4.4 LD_{50}

Give LD50 male, females, males + females
State if no lethal effect at maximal dose

The LD_{50} of the test material was estimated to be in the range of 300 - 500 mg/kg bw.

Section A6.1.1 Acute Oral Toxicity

Annex Point IIA6.1

Acute oral toxicity in the rat (Acute toxic class Method)
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines

This study was performed to assess the acute oral toxicity of the test material following a single gavage administration in the Sprague-Dawley rat. The method meets the requirements of OECD Test Guideline 423 (adopted 17 December 2001). The study was conducted according to GLP.

A group of three fasted females was treated with 2000 mg/kg bw. Based on the results from this dose level further groups of 3 male and 3 female fasted animals were treated at a dose level of 200 mg/kg bw. Dosing was performed sequentially. The test material was administered orally as a suspension in arachis oil BP.

Clinical signs and bodyweight development were monitored during the study. Animals were subjected to gross necropsy.

At the start of the study the males weighed 271 to 299 g and the females weighed 198 to 218 g.

5.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

See Table A6.1.1-1 for a summary of the results of this study.

Two animals treated with 2000 mg/kg bw were found dead five days after dosing. One animal treated with 2000 mg/kg bw was killed in extremis eight days after dosing. There were no deaths noted at a dose level of 200 mg/kg bw.

Signs of systemic toxicity noted in animals treated with 2000 mg/kg bw were hunched posture, lethargy, pilo-erection, diarrhoea, decreased respiratory rate, laboured respiration, ataxia, pallor of the extremities, emaciation, tiptoe gait and faeces stained green. Hunched posture was noted during the day of dosing and one day after dosing in one male treated with 200 mg/kg bw. No other signs of systemic toxicity were noted in animals treated with 200 mg/kg bw.

The surviving animals showed expected gains in bodyweight over the study period.

Abnormalities noted at necropsy of the animals treated with 2000 mg/kg bw that died during the study were abnormally red lungs, dark liver, dark kidneys, copper-coloured material present in the stomach, haemorrhagic gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhagic small and large intestines.

No abnormalities were noted at necropsy of animals treated with 200 mg/kg bw.

5.3 Conclusion

Non-entry field

The acute oral LD_{50} of the test material in the rat was estimated to be in the range of 300 - 500 mg/kg bw.

5.3.1 Reliability

Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4

1

5.3.2 Deficiencies

No

Section A6.1.1 Acute Oral Toxicity

Annex Point IIA6.1

Acute oral toxicity in the rat (Acute toxic class Method)
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)
(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2013
Materials and Methods	Agree with the applicant's version ***Revisions/amendments:*** 3.1.2.2 Purity: <u>The purity of the test material is the responsibility of the sponsor.</u> 3.1.2.3 Stability: <u>The stability of the test material is the responsibility of the sponsor.</u>
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.1-1 Acute oral toxicity study - Summary of findings

Dose [mg/kg bw]	Number dead/ number investigated	Time of death (range)	Observations
2000	3/3 (Female)	Days 5-8	Diarrhoea was seen from 4 h after dosing. Hunched posture, lethargy, pilo-erection, decreased respiratory rate, laboured respiration, ataxia, pallor of the extremities, emaciation, tiptoe gait and faeces stained green were seen from 4 days after dosing. The surviving animals showed expected gains in bodyweight over the study period. Abnormalities noted at necropsy included abnormally red lungs, dark liver, dark kidneys, copper-coloured material present in the stomach, haemorrhagic gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhagic small and large intestines.
200	0/3 (Female)	-	Hunched posture was seen in one male animal on the day of dosing and the day after. The surviving animals showed expected gains in bodyweight over the study period. No abnormalities were noted at necropsy.
200	0/3 (Male)	-	
LD_{50} value	Estimated range: 300 - 500 mg/kg bw.		

Section A6.1.2 Acute Toxicity

Annex Point IIA6.1.2 **Acute Dermal Toxicity Study in the Rat (Limit Test)**
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

			Official use only
		1 REFERENCE	
1.1	**Reference**	*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)* *If necessary, copy field and enter other reference(s).* [redacted]	X
1.2	**Data protection**	Yes *(indicate if data protection is claimed)*	
1.2.1	Data owner	*Give name of company* [redacted]	
1.2.2	Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	The study report claims compliance with: • OECD Guidelines for the Testing of Chemicals No. 402 "Acute Dermal Toxicity" (adopted 24 February 1987), • Commission Directive 92/69/EEC Method B3 Acute Toxicity (Dermal). A review of the study report revealed only minor deviations from the above mentioned guidelines (see point 2.3). *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
2.2	**GLP**	Yes *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
2.3	**Deviations**	The following minor deviations from OECD test Guideline 402 (adopted 24 February 1987), were noted: • No information was provided on the specification of the sample used in this study, • The nature of the clinical observation was not reported. These deviations are not considered to have influenced the outcome or the integrity of the study. *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	
		3 MATERIALS AND METHODS	
		In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.	
3.1	**Test material**	*As given in section 2 or give name used in study report* Copper powder	

Section A6.1.2 Acute Toxicity

Annex Point IIA6.1.2 **Acute Dermal Toxicity Study in the Rat (Limit Test)**
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

3.1.1	Lot/Batch number	*List lot/batch number if available*	
3.1.2	Specification	*As given in section 2, or* *Deviating from specification given in section 2 as follows* *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):* No information was provided on the specification of the sample used in this study.	
3.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Shiny copper coloured powder.	
3.1.2.2	Purity	*Give purity in % of active substance* Not reported.	X
3.1.2.3	Stability	*Describe stability of test material* Not reported.	X
3.2	**Test Animals**	Non-entry field	
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley CD(Crl:CD®(SD)IGS BR)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females.	
3.2.5	Age/weight at study initiation	At the start of the study animals were approximately eight weeks of age: males weighed 257 to 310 g, and the females 221 to 237 g.	
3.2.6	Number of animals per group	*Give number* *specify, if there are differences for example for treatment and recovery groups* One group of 5 males and 5 females.	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal *Fill in respective route in the following, delete other routes*	
3.3.1	Postexposure period	14 days	

Section A6.1.2 Acute Toxicity

Annex Point IIA6.1.2 **Acute Dermal Toxicity Study in the Rat (Limit Test)**
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

		Dermal	
3.3.2	Area covered	Approximately 10 % of body surface.	
3.3.3	Occlusion	Semi-occlusive	
3.3.4	Vehicle	Test material was moistened with arachis oil BP.	
3.3.5	Concentration in vehicle	Not specified.	X
3.3.6	Total volume applied	Not specified.	
3.3.7	Duration of exposure	24 h	
3.3.8	Removal of test substance	Treated skin and surrounding hair was wiped with cotton wool moistened with arachis oil BP to remove any residual test material. *(give solvent, detergent)*	
3.3.9	Controls	None	
3.4	**Further remarks**	None	
		4 RESULTS AND DISCUSSION *Describe findings. If appropriate, include table. Sample tables are given below.*	
4.1	**Clinical signs**	*No effects / describe significant effects referring to data in results table* There were no deaths. There were no clinical signs of systemic toxicity. Very slight to well-defined erythema was noted at all treated skin sites one day after dosing with very slight erythema at nine treatment sites two days after dosing. Crust formation was noted at the treatment sites of all males and one female three to six days after dosing with light brown discolouration of the epidermis also noted at the treatment site of one male two to five days after dosing. Treatment sites appeared normal two to seven days after dosing.	
4.2	**Pathology**	*No effects / describe significant effects referring to data in results table* No abnormalities were noted at necropsy.	
4.3	**Other**	*Describe any other significant effects* All animals showed expected gains in bodyweight during the study.	
4.4	**LD_{50}**	*Give LD50 male, females, males + females* *State if no lethal effect at maximal dose* The acute dermal LD_{50} of the test material, in the Sprague-Dawley CD (Cr1:CD® (SD) IGS BR) male and female rat was found to be greater than 2000 mg/kg bw. There were no lethal effects at the maximal dose.	

Section A6.1.2 Acute Toxicity

Annex Point IIA6.1.2 **Acute Dermal Toxicity Study in the Rat (Limit Test)**
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines

This study was conducted according to GLP, and to OECD Test Guideline 402 'Acute Dermal Toxicity' (adopted 24 February 1987). Only minor deviations from the test guideline occurred. These deviations are not considered to have influenced the outcome or the integrity of the study.

Copper powder, moistened with arachis oil BP, was applied to the shaven, intact dorsal skin of 5 male and 5 female Sprague Dawley rats at 2000 mg/kg bw under a semi-occlusive bandage. After a 24 h exposure period the dressing was removed and the treated skin area was wiped with cotton wool moistened with arachis oil BP to remove any residual test material.

At the start of the study animals were approximately eight weeks of age: males weighed 257 to 310 g, and the females 221 to 237 g.

Animals were observed for deaths and overt signs of toxicity 0.5, 1, 2 and 4 hours after test material application and each day thereafter for the remainder of the study. Individual bodyweights were recorded prior to application of the test material, and on day 7 and 14. Observations for dermal irritation were carried out after removal of the dressing and once daily for 14 days. Dermal irritation was measured using the Draize scale.

Animals were killed and necropsied after a 14 day observation period.

5.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

There were no deaths or clinical signs of systemic toxicity in this study.

Signs of skin irritation noted were very slight to well defined erythema, crust formation and light brown discolouration of the epidermis. Treatment sites appeared normal two to seven days after dosing.

All animals showed expected gains in bodyweight during the study. No abnormalities were noted at necropsy.

5.3 Conclusion

Non-entry field

The acute dermal LD_{50} of the test material in the Sprague Dawley rat was found to be greater than 2000 mg/kg bw.

In this study, copper powder does not meet the criteria for classification for acute dermal toxicity according to Annex VI of Commission Directive 2001/59/EC.

5.3.1 Reliability

Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4

1

5.3.2 Deficiencies

No

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

Section A6.1.2 Acute Toxicity

Annex Point IIA6.1.2

Acute Dermal Toxicity Study in the Rat (Limit Test)
Specify section no., heading, route and species as appropriate
Specify type of test (Limit Test, LD_{50}, special investigation)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2013
Materials and Methods	Agree with the applicant's version **Revisions/amendments:** 1.1 Reference: Copper powder: *Acute dermal toxicity (limit test) in the rat.* 3.1.2.2 Purity: The purity of the test material is the responsibility of the sponsor. 3.1.2.3 Stability: The stability of the test material is the responsibility of the sponsor. 3.3.5 Concentration in vehicle: The test material was applied moistened with arachis oil BP at 2000 mg/kg bw.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.2(1)-1 Acute dermal toxicity study - Summary of findings

Dose [mg/kg bw]	Number dead/ number investigated	Time of death (range)	Observations
2000 (Male)	0/5	-	There were no deaths or clinical signs of systemic toxicity in this study.

200 (Female)	0/5	-	Signs of skin irritation noted were very slight to well defined erythema, crust formation and light brown discolouration of the epidermis. Treatment sites appeared normal two to seven days after dosing. All animals showed expected gains in bodyweight during the study. No abnormalities were noted at necropsy.
LD_{50} > 2000 mg/kg bw			

Section A6.1.3 Annex Point A6.1.3	A6.1.3, Acute Inhalation Toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	No acute inhalation toxicity data which conform to current test guidelines are available for granulated copper. Acute inhalation toxicity of granulated copper will be determined to a large extent by particle size, density and solubility. Experimental particle size distribution data showed that the smallest particle diameter in the measured samples was in the region of 1.09 mm (1090 µm). Dense materials with such a large particle size are considered to be neither inhalable nor respirable. In view of this fact, and recognising that unnecessary animal testing should be avoided, it is considered that an inhalation toxicity test with granulated copper should be waived and that the material should not be classified on the basis of inhalation toxicity.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2014	
Evaluation of applicant's justification	Agree with applicant's version However, at the product authorization, if an application by spraying is realised, this point should be assessed. Moreover, the product is often applied in solution and not in form of granulated copper, and the properties are not the same. Remark: It is also important to note that a classification for this endpoint was proposed for coated copper flake.	

Section A6.1.3 **Annex Point A6.1.3**	**A6.1.3, Acute Inhalation Toxicity**
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA6.1.4 **Acute Dermal Irritation study in the rabbit**

Specify section no., heading and species as appropriate

			Official use only
		1 REFERENCE	
1.1	**Reference**	*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)* *If necessary, copy field and enter other reference(s).* [redacted]	
1.2	**Data protection**	Yes *(indicate if data protection is claimed)*	
1.2.1	Data owner	*Give name of company* [redacted]	
1.2.2	Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	The study report claims compliance with: • OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 17 July 1992), • Commission Directive 92/69/EEC Method B4 Acute Toxicity (Skin Irritation). A review of the study report revealed only minor deviations from OECD Guideline No 404 (adopted 24 April 2002) (see point 2.3). *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
2.2	**GLP**	Yes *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
2.3	**Deviations**	The following minor deviations from OECD Test Guideline 404 (adopted 24 April 2002) were noted: The following information was not given in the test report: • The rationale for *in vivo* testing, • Test substance purity (not provided by the sponsor), • Individual animal weight at the conclusion of the test, • Housing conditions. These deviations are not considered to have influenced the outcome or the integrity of the study. *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	X

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA6.1.4 **Acute Dermal Irritation study in the rabbit**

Specify section no., heading and species as appropriate

		3 MATERIALS AND METHODS	
		In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.	
3.1	**Test material**	*As given in section 2 or give name used in study report* Copper powder	
3.1.1	Lot/Batch number	*List lot/batch number if available* Not available.	
3.1.2	Specification	*As given in section 2, or* *Deviating from specification given in section 2 as follows* *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):* No information was provided on the specification of the sample used in this study.	
3.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Shiny copper coloured powder.	
3.1.2.2	Purity	*Give purity in % active substance* Not available.	X
3.1.2.3	Stability	*Describe stability of test material.* Not available.	X
3.2	**Test Animals**	*Non-entry field*	
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White.	
3.2.3	Source	[redacted]	
3.2.4	Sex	One male and 2 females were used in this study.	
3.2.5	Age/weight at study initiation	At the start of the study the animals weighed 2.77 to 3.08 kg and were 12 to 16 weeks old.	
3.2.6	Number of animals per group	*Give number* *specify, if there are differences for example for treatment and recovery groups* 3	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Application	*Non entry field*	

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA6.1.4 **Acute Dermal Irritation study in the rabbit**

Specify section no., heading and species as appropriate

3.3.1.1	Preparation of test substance	A quantity of 0.5 g of the test material was moistened with 0.5 ml of distilled water.
3.3.1.2	Test site and Preparation of Test Site	*State site: dorsal area of the trunk/left/right side of the trunk* *Shaved skin or other* *State skin cleaning method and used agents* On the day before the test each of a group of three rabbits was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.
3.3.2	Occlusion	*semiocclusive or other* Semi-occlusive.
3.3.3	Vehicle	Distilled water.
3.3.4	Concentration in vehicle	50 % w/v.
3.3.5	Total volume applied	ca. 1 ml.
3.3.6	Removal of test substance	*water or solvent* *(give solvent, detergens)* Any residual test material was removed by gentle swabbing with cotton wool soaked in 74% Industrial Methylated Spirits.
3.3.7	Duration of exposure	*24 h or other* 4 h
3.3.8	Postexposure period	*14 days, 4 weeks or other* 72 h
3.3.9	Controls	*solvent or other* None
3.4	**Examinations**	
3.4.1	Clinical signs	*Yes/No* No
3.4.2	Dermal examination	*Yes/No* Yes

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA6.1.4 **Acute Dermal Irritation study in the rabbit**

Specify section no., heading and species as appropriate

3.4.2.1 scoring system

State scoring system

The following scoring system was used:

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Any other skin reactions, if present, were also recorded.

3.4.2.2 Examination time points

60min, /24h, 48h,72h or other

After approximately 60 min and 24, 48 and 72h.

3.4.3 Other examinations

Histopathological examinations, effect of washing or other

The pH of a 10 % w/v aqueous preparation of the test material was determined.

3.5 Further remarks

None

4 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

4.1 Average score

Non-entry field

4.1.1 Erythema

Give average score for all animals at 24, 48, 72 h

0, 0, 0

4.1.2 Edema

Give average score for all animals at 24, 48, 72 h

0, 0, 0

4.2 Reversibility

Yes/No

Name effect and give time for reversion.

Not relevant. No skin irritation was noted in this study.

4.3 Other examinations

Give results

The pH of a 10 % w/v aqueous preparation of the test material was approximately 7.0.

4.4 Overall result

No evidence of skin irritation was noted in this study.

See Table A.6.1.4-1 for dermal irritation scores.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA6.1.4 **Acute Dermal Irritation study in the rabbit**

Specify section no., heading and species as appropriate

5.1	**Materials and methods**	*Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines*	
		This study was conducted according to GLP, and to OECD Test Guideline 402 'Acute Dermal Irritation/Corrosion' (adopted 24 April 2002). Only minor deviations from test guideline occurred. These deviations are not considered to have influenced the outcome or the integrity of the study.	X
		An amount of 0.5 g of copper powder, moistened with 0.5 ml of distilled water, was applied to the shaven, intact dorsal skin of 3 New Zealand White rabbits under an occlusive bandage. After a 4 h exposure period the dressing was removed and any excess test article was removed by swabbing with cotton wool soaked in 74 % Industrial Methylated Spirits.	X
		Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the scale outlined under point 3.4.2.1.	
		At the start of the study the animals weighed 2.77 to 3.08 kg and were 12-16 weeks old.	
5.2	**Results and discussion**	*Summarize relevant results; discuss dose-response relationship.*	
		No skin irritation was noted in this study.	
5.3	**Conclusion**	A single 4 hr, semi-occluded application of the test material to the intact skin of three rabbits produced no evidence of skin irritation.	
		In this study, copper powder does not meet the criteria for classification for dermal irritation according to Annex VI of Commission Directive 2001/59/EC.	
5.3.1	Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4*	
		1	
5.3.2	Deficiencies	No	
		(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2014

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA6.1.4 **Acute Dermal Irritation study in the rabbit**

Specify section no., heading and species as appropriate

Materials and Methods	Agree with the applicant's version **Revisions/amendments:** 2.3 Deviation: *Test substance purity* and batch number *(not provided by the sponsor)* 3.1.2.2 Purity: The purity of the test material is the responsibility of the sponsor. 3.1.2.3 Stability: The stability of the test material is the responsibility of the sponsor.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version **Revisions/amendments:** 5.1 Materials and methods: *This study was conducted according to GLP, and to OECD Test Guideline ~~402~~* 404 *'Acute Dermal Irritation/Corrosion'.* (…) *3 New Zealand White rabbits under an* semi-*occlusive bandage.*
Reliability	1
Acceptability	acceptable
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A.6.1.4-1 Dermal irritation scores

Skin reaction	Reading (hours)	Animal/Sex		
		34/Female	35/Male	36/Male
Erythema/ Eschar formation	**1**	0	0	0
	24	0	0	0
	48	0	0	0
	72	0	0	0
Mean scores (24, 48 and 72 h)		**0**	**0**	**0**
Oedema Formation	**1**	0	0	0
	24	0	0	0
	48	0	0	0
	72	0	0	0
Mean scores (24, 48 and 72 h)		**0**	**0**	**0**

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

Official use only

1 REFERENCE

1.1 Reference

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).

[redacted]

1.2 Data protection Yes

(indicate if data protection is claimed)

1.2.1 Data owner

Give name of company

[redacted]

1.2.2 Criteria for data protection

Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:

Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

The study report claims compliance with:

- OECD Guidelines for the Testing of Chemicals No. 405 "Acute Eye Irritation/Corrosion" (adopted 24 February 1987),
- Commission Directive 92/69/EEC Method B5 Acute Toxicity (Eye Irritation).

A review of the study report revealed only minor deviations from OECD Test Guideline No 405 (adopted 24 April 2002) (see point 2.3).

(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")

2.2 GLP Yes

(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)

2.3 Deviations

The following minor deviations from OECD Test Guideline 405 (adopted 24 April 2002) were noted:

- The test report does not provide a rational for *in vivo* testing,
- Test material information including purity and batch no is not available.

These deviations are not considered to have influenced the outcome or the integrity of the study.

(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

		3 MATERIALS AND METHODS	
		In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.	
3.1	**Test material**	*As given in section 2 or give name used in study report* Copper powder	
3.1.1	Lot/Batch number	*List lot/batch number if available* Not available.	
3.1.2	Specification	*As given in section 2, or* *Deviating from specification given in section 2 as follows* *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):* No information was provided on the specification of the sample used in this study.	
3.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Shiny copper coloured powder.	
3.1.2.2	Purity	*Give purity in % active substance* Not available.	X
3.1.2.3	Stability	*Describe stability of test material* Not available.	X
3.2	**Test Animals**	Non-entry field	
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White.	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	At the start of the study the animals weighed 2.0 to 3.5 kg and were 12 to 16 weeks old.	
3.2.6	Number of animals per group	*Give number* *specify, if there are differences for example for treatment and recovery groups* 3	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Ocular	
3.3.1	Preparation of test substance	Test substance was used as delivered.	
3.3.2	Amount of active substance instilled	0.1 ml (approximately 72 mg).	
3.3.3	Exposure period	The test material was placed into the conjuctival sac of the right eye. The upper and lower lids were held together for ca. 1 second after treatment. Apparently eyes were not rinsed.	
3.3.4	Postexposure period	Up to 14 days.	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	Yes Examination of the eye was facilitated by the use of the light source from a standard ophthalmoscope.	
3.4.1.1	Scoring system	*state scoring system and give time table of examinations, describe the terms slight, moderate, etc., if these terms are used* See Appendix A6.1.4-1.	
3.4.1.2	Examination time points	1, 24, 48 and 72 h. Additional observations were made on Days 7 and 14 to assess the reversibility of the ocular effects.	
3.4.2	Other investigations	*for example: effect of rinsing* None	X
3.5	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	*Describe findings. If appropriate, include table. Sample tables are given below. No effects / describe significant effects referring to data in results table* Copper-coloured staining of the eyelids and fur around the treated eye was noted in all animals during the study.	
4.2	**Average score**	Non-entry field	
4.2.1	Cornea	*Give average score for all animals at 24, 48, 72 h* 0, 1, 2	X
4.2.2	Iris	*Give average score for all animals at 24, 48, 72 h* 0, 0.7, 1	X
4.2.3	Conjunctiva	Non-entry field	
4.2.3.1	Redness	*Give average score for all animals at 24, 48, 72 h* 1.7, 1.7, 2	X
4.2.3.2	Chemosis	*Give average score for all animals at 24, 48, 72 h* 0.7, 1, 1.7	X
4.3	**Reversibility**	Yes. All findings were reversible within 14 days. *Name effect and give time for reversion.*	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

4.4 Other

Describe any other significant effects

None.

4.5 Overall result

See Table A6.1.4-1 for ocular scores.

Average scores after 24, 48 and 72 hours are presented for each animal:

Mean scores (24, 48, 72 h)	Cornea opacity	Iris lesion	Conjunctivae	
			redness	chemosis
Animal No. 1 (110)	0	0	1.7	0.7
Animal No. 2 (174)	1	0.7	1.7	1
Animal No. 3 (176)	2	1	2	1.7

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines

This study was conducted according to GLP, and to OECD Test Guideline 405 'Acute Eye Irritation/Corrosion' (adopted 24 April 2002). Only minor deviations from test guideline occurred. These deviations are not considered to have influenced the outcome or the integrity of the study.

Eye irritation potential of copper powder was investigated in 3 female New Zealand White rabbits.

Initially, a single rabbit was treated. A volume of 0.1 ml of copper powder (ca.72 mg) was placed into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about one second immediately after treatment, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes. After consideration of the ocular response in the first animal 2 further animals were treated in the same manner.

Assessment of ocular damage/irritation was made approximately 1 hour and 24, 48 and 72 h following treatment, according to the numerical evaluation given in Appendix A6.1.4-1. Any other ocular effects were also noted. Examination of the eye was facilitated by the use of the light source from a standard ophthalmoscope. Additional observations were made on Days 7 and 14 to assess the reversibility of the ocular effects.

At the start of the study the animals weighed 2.0 to 3.5 kg and were 12 to 16 weeks old.

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

5.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

See Table A6.1.4-1 for ocular scores.

Copper-coloured staining of the eyelids and fur around the treated eye was noted in all animals during the study.

Diffuse or translucent corneal opacity was noted in two treated eyes at the 24, 48 and 72-hour observations. Translucent corneal opacity persisted in one treated eye at the 7-day.

Iridial inflammation was noted in two treated eyes at the 24 and 48-hour observations and persisted in one treated eye at the 72-hour and 7-day observations.

Minimal to moderate conjunctival irritation was noted in all treated eyes one hour after treatment with moderate conjunctival irritation at the 24 and 48-hour observations and minimal to moderate conjunctival irritation at the 72-hour observation. Minimal conjunctival irritation was noted in two treated eyes at the 7-day observation.

One treated eye appeared normal at the 7-day observation and two treated eyes appeared normal at the 14-day observation.

Average scores after 24, 48 and 72 hours are presented for each animal:

Mean scores (24, 48, 72 h)	Cornea opacity	Iris lesion	Conjunctivae	
			redness	chemosis
Animal No. 1 (110)	0	0	1.7	0.7
Animal No. 2 (174)	1	0.7	1.7	1
Animal No. 3 (176)	2	1	2	1.7

5.3 Conclusion

In this study the test material showed a slight potential to induce eye irritation. However, copper powder does not meet the criteria for classification for eye irritation according to Annex VI of Commission Directive 2001/59/EC.

5.3.1 Reliability

Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4

1

5.3.2 Deficiencies

No

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2014
Materials and Methods	Agree with the applicant's version **Revisions/amendments:** 3.1.2.2 Purity: The purity of the test material is the responsibility of the sponsor. 3.1.2.3 Stability: The stability of the test material is the responsibility of the sponsor. 3.4.2 Other investigations: Any other ocular effects were also noted.
Results and discussion	Agree with the applicant's version **Revisions/amendments:** 4.2.1 Cornea: ~~*0,1,2*~~ Animal No. 1 (110) = 0 Animal No. 2 (174) = 1 Animal No. 3 (176) = 2 4.2.2 Iris: ~~*0, 0.7,1*~~ Animal No. 1 (110) = 0 Animal No. 2 (174) = 0.7 Animal No. 3 (176) = 1 4.2.3.1 Redness: ~~*1.7, 1.7, 2*~~ Animal No. 1 (110) = 1.7 Animal No. 2 (174) = 1.7 Animal No. 3 (176) = 2 4.2.3.2 Chemosis: ~~*0.7, 1, 1.7*~~ Animal No. 1 (110) = 0.7 Animal No. 2 (174) = 1 Animal No. 3 (176) = 1.7
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4 **Acute Eye Irritation study in the rabbit**

Specify section no., heading and species as appropriate

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.4-1 Ocular scores (individual and total)

Rabbit Number and Sex	IPR= 2 110 Female					IPR = 2 174 Female						IPR = 2 176 Female					
Time After Treatment	1 hour	24 hours	48 hours	72 hours	7 days	1 hour	24 hours	48 hours	72 hours	7 days	14 days	1 hour	24 hours	48 hours	72 hours	7 days	14 days
CORNEA																	
E = Degree of Opacity	0	0	0	0	0	0	1	1	1	0	0	0	2	2	2	2	0
F = Area of Opacity	0	0	0	0	0	0	2	2	1	0	0	0	2	2	2	1	0
Score (E x F) x 5	0	0	0	0	0	0	10	10	5	0	0	0	20	20	20	10	0
IRIS																	
D	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1	1	0
Score (D x 5)	0	0	0	0	0	0	5	5	0	0	0	0	5	5	5	5	0
CONJUNCTIVAE																	
A – Redness	1	2	2	1	0	1	2	2	1	1	0	1	2	2	2	1	0
B = Chemosis	1	1	1	0	0	1	2	1	0	0	0	1	2	2	1	0	0
C – Discharge	1Sf	2Sf	1Sf	1Sf	0	2Sf	1Sf	1Sf	1Sf	0	0	1Sf	2Sf	1Sf	1Sf	1	0
Score (A + B + C) x 2	6	10	8	4	0	8	10	8	4	2	0	6	12	10	8	4	0
Total Score	6	10	8	4	0	8	25	23	9	2	0	6	37	35	33	19	0

IPR= initial pain reaction

Sf = copper staining of eyelids and fur

Appendix A6.1.4-1 Draize Scale for Scoring Ocular Irritation

1. CONJUNCTIVAE

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs a considerable area around the eye	3

THE TOTAL SCORE = (A + B + C) x 2 **MAXIMUM TOTAL = 20**

2. IRIS

(D) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

THE TOTAL SCORE = D x 5 **MAXIMUM TOTAL = 10**

3. CORNEA

(E) Degree of Opacity (most dense area used)

No opacity	0
Scattered or diffuse areas, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris not discernible through the opacity	4

(F) Area of Cornea Involved

One quarter (or less) but not zero	1
Greater than one quarter but less than half	2
Greater than half but less than three quarters	3
Greater than three quarters, up to whole area	4

THE TOTAL SCORE = (E x F) x 5 **MAXIMUM TOTAL = 80**

MAXIMUM TOTAL SCORE POSSIBLE = 110

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

Official use only

1 REFERENCE

1.1 Reference

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).

[redacted]

1.2 Data protection

Yes

(indicate if data protection is claimed)

1.2.1 Data owner

Give name of company

[redacted]

1.2.2 Criteria for data protection

Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:

Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

The study report claims compliance with:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin sensitisation" (adopted 17 July 1992),
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin sensitisation).

Review of the study report revealed only minor deviations from OECD Test Guideline 406 (adopted 17 July 1992) (see 2.3).

(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")

2.2 GLP

Yes

(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)

2.3 Deviations

The following minor deviations from OECD Test Guideline 406 (adopted 17 July 1992) were noted:

- Information on the test material including the batch no and the purity were not provided,
- No justification is given for the choice of vehicle.

These deviations are not considered to have influenced the outcome or the integrity of the study.

(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")

3 MATERIALS AND METHODS

In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

3.1	**Test material**	*As given in section 2 or give name used in study report* Copper powder	
3.1.1	Lot/Batch number	*List lot/batch number if available* Not provided.	
3.1.2	Specification	*As given in section 2, or* *Deviating from specification given in section 2 as follows* *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):* No information was provided on the specification of the sample used in this study.	
3.1.2.1	Description	Shiny copper coloured powder. *If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)*	
3.1.2.2	Purity	*Give purity in % of active substance* Not provided.	X
3.1.2.3	Stability	*Describe stability of test material* Active substance: not provided. Dosing preparations: no stability analysis was conducted; however dosing preparations were freshly prepared.	X
3.1.2.4	Preparation of test substance for application	*a) for induction: used as delivered or other; state solvent* *b) for challenge: used as delivered or other; state solvent* For both induction and challenge the test material was freshly prepared in acharis oil BP.	
3.1.2.5	Pretest performed on irritant effects	*Yes/No* Yes	
3.2	**Test Animals**	Non-entry field	
3.2.1	Species	*Guinea pigs or other* *state reason for non-standard species* Guinea Pig	
3.2.2	Strain	Albino Dunkin Hartley	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	At the start of the main study the animals weighed 340 to 436 g, and were approximately eight to twelve weeks old.	
3.2.6	Number of animals per group	*10 or other(GPMS)* *20 or other (Buehler test)* *Specify, if there are differences e. g. for treatment and recovery groups* 6 sighting animals 10 test animals 5 control animals	
3.2.7	Control animals	Yes	

Section A6.1.5		**Skin sensitisation**
Annex Point IIA6.1.5		**Guinea pig maximisation test (GPMT)** *Specify type of study:* *Guinea pig maximisation test (GPMT), Buehler Test or other*
3.3	**Administration/ Exposure**	*State study type:* *Adjuvant / Non-Adjuvant* Adjuvant study.
3.3.1	Induction schedule	*day 0 – day –xx – day xxx* *see table in appendix* Day 0: Intradermal induction Day 7: Topical induction See Table A6.1.5-1 for an outline of the treatment schedule.
3.3.2	Way of Induction	*Intradermal or topical* Intradermal induction followed by a Topical (occlusive) induction.
3.3.3	Concentrations used for induction	*µg test substance / ml* *(causing mild to moderate irritation)* Intradermal induction : 0.1 % w/w in arachis oil BP. Topical induction : 50 % w/w in arachis oil BP.
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	*state concentration and vehicle (for GPMT only):* *10 % or other* *in water or physiological saline* Freund's Complete Adjuvant plus distilled water in the ratio of 1:1.
3.3.5	Challenge schedule	*day y; see table in appendix* Day 21: Topical challenge. See Table A6.1.5-1 for an outline of the treatment schedule.
3.3.6	Concentrations used for challenge	*µg test substance / ml* *(usually maximum non-irritant concentration)* 50 and 25 % w/w in arachis oil BP.
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	24 and 48h after challenge.
3.3.9	Removal of the test substance	*give time and solvent (water or other)* The challenge sites were swabbed with cotton wool soaked in diethylether to remove residual material.
3.3.10	Positive control substance	2-Mercaptobenzothiazole.

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

3.4	**Examinations**	Non-entry field
3.4.1	Pilot study	The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows: Selection of Concentration for Intradermal Induction Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of four guinea pigs, using a range of concentrations (0.1, 0.5, 1 and 5 % w/w in arachis oil BP). The degree of erythema at the injection sites was assessed approximately 24, 48 and 72 hours, and 7 days after injection. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The animals treated with the 0.5, 1 and 5 % concentrations were killed for humane reasons after the 24-hour observation, due to the severity of the reactions noted. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study. Selection of Concentration for Topical Induction Two guinea pigs (intradermally injected with Freund's Complete Adjuvant ten days earlier) were treated with four preparations of the test material (50, 25, 10 and 5 % w/w in arachis oil BP). The 50 % w/w concentration was the maximum attainable concentration. Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. One animal was found dead approximately 48 hours after dosing. The absence of this animal for the 48-hour observation was considered not to affect the purpose or integrity of the study. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study. Selection of Concentration for Topical Challenge Four preparations of the test material (50, 25, 10 and 5 % w/w in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study.
3.5	**Further remarks**	The scale for evaluation of skin reactions is given in Appendix A6.1.5-1.

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

4 RESULTS AND DISCUSSION

4.1 Results of pilot studies

Describe findings. If appropriate, include table. Sample tables are given below. give information on dose selection, i.e. maximum non irritant concentration, if available

Selection of Concentration for Intradermal Induction

The animals treated with 0.5, 1 and 5 % w/w in arachis oil BP were killed for humane reasons after the 24-hour observation, due to the severity of the reactions noted.

The animal receiving 0.1 % showed mild to moderate skin irritation; this concentration was tolerated systemically and was selected for the intradermal induction stage of the main study.

Selection of Concentration for Topical Induction

One of the two sighting study animals was found dead approximately 48 hours after dosing. The absence of this animal for the 48-hour observation was considered not to affect the purpose or integrity of the study.

The highest concentration (50 % w/w) produced only mild to moderate dermal irritation and was selected for the topical induction stage of the main study.

Selection of Concentration for Topical Challenge

The highest non-irritant concentration (50 % w/w) of the test material and one lower concentration (25 % w/w) were selected for the topical challenge stage of the main study.

4.2 Results of test

4.2.1 24h after challenge

Number of animals with signs of allergic reactions / number of animals

0/10 animals with allergic reactions.

4.2.2 48h after challenge

Number of animals with signs of allergic reactions / number of animals

0/10 animals with allergic reactions.

4.2.3 Other findings

None.

4.3 Overall result

Under the conditions of this test, copper powder produced a 0 % (0/10) sensitisation rate.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines

This study was conducted according to GLP, and to OECD Test Guideline 406 'Skin sensitisation' (adopted 17 July 1992). Only minor deviations from test guideline occurred. These deviations are not considered to have influenced the outcome or the integrity of the study.

In a skin sensitisation study by the maximisation method of Magnusson and Kligman, 5 control and 10 treated male albino Dunkin Hartley guinea pigs were tested according to the dosing regime described below:

Induction of the Test Animals

Shortly before treatment on Day 0 the hair was removed from an area approximately 40 x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line into a 20 x 40 mm area. The injections

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5

Guinea pig maximisation test (GPMT)

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

were:

a. Freund's Complete Adjuvant plus distilled water in the ratio 1:1
b. a 0.1 % w/w formulation of the test material in arachis oil BP
c. a 0.1 % w/w formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites was evaluated.

On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A filter paper patch saturated with the test material formulation (50 % w/w in arachis oil BP) was applied to the prepared skin and held in place with a strip of surgical adhesive tape covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale described in Appendix 6.1.5-1.

Any other reactions were also recorded.

Induction of the Control Animals

The intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection ‘b’ was therefore the vehicle alone, injection ‘c’ was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

Challenge

Shortly before treatment on Day 21, an area of approximately 50 x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch, saturated with the test material formulation at the maximum non-irritant concentration (50 % w/w in arachis oil BP) was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 25 % w/w in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was removed. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified.

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

5.2	**Results and discussion**	*Summarize relevant results; discuss dose-response relationship.* <u>Induction</u> Skin reactions noted at the intradermal induction sites of test group animals included intense erythema and swelling, green/brown coloured dermal necrosis, eschar and focal eschar. Staining precluded evaluation of erythema at the topical induction sites of test group animals. <u>Challenge</u> Brown/grey coloured staining was noted at the topical challenge sites of all test and control group animals at the 24 and 48-hour observations. The staining did not affect evaluation of skin responses. No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations. Non-concurrent positive control studies with 2-mercaptobenzothiazole, showing 90-100 % incidence of sensitisation, confirmed the sensitivity of this assay. See also Table A.6.1.5-2.
5.3	**Conclusion**	Under the conditions of this test, copper powder produced a 0 % (0/10) sensitisation rate. Copper powder did not meet the criteria for classification as a sensitiser by skin contact according to labelling regulations outlined in Annex VI of Commission Directive 2001/59/EC.
5.3.1	Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 1
5.3.2	Deficiencies	No *(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)*

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1.5 **Guinea pig maximisation test (GPMT)**

Specify type of study:
Guinea pig maximisation test (GPMT), Buehler Test or other

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2014
Materials and Methods	Agree with the applicant's version **Revisions/amendments:** 3.1.2.2 Purity: The purity of the test material is the responsibility of the sponsor. 3.1.2.3 Stability: The stability of the test material is the responsibility of the sponsor.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.5-1 Treatment schedule

Treatment	GPMT		Observations/Remarks *give information on irritation effects*
	day of treatment	application	
Induction 1	0	intradermal	Skin reactions at the intradermal induction sites of the test group animals included intense erythema and swelling, green/brown coloured dermal necrosis, eschar and focal eschar.
Induction 2	7	topical	Staining precluded evaluation of erythema.
Challenge	21	topical	No skin reactions were noted at the challenge sites of the test or control group animals at 24 and 48 hour observations.

Table A6.1.5-2 Result of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group	
	Negative control	**Test group**
scored after 24h	**0 / 5**	**0 / 10**
scored after 48h	**0 / 5**	**0 / 10**

Appendix A6.1.5-1. Scales for Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No erythema	0
Barely perceptible erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre extending beyond the area of exposure)	4

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† From: Draize, J H (1977) 'Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC, p31.

Section A6.1.1